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Research Article

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Multivariate analysis of the salinity-induced alterations in morphology, physiology, nodulation, and yield in two contrasting mungbean varieties

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Abstract

Changes were estimated in the morphology, physiology, photosynthesis, nodulation, and yield in two mungbean varieties 'PKV AKM 12-28' and 'VBN (Gg)3' under salt stress (0, 75, 100, and 125 mM NaCl) for 15, 30, and 45 days. Multivariate modelling was used to analyse results to explore complex data and to visualize time and concentration-dependent modulations. Principal component analysis showed modulations in morpho-physiological attributes such as shoot length, root length, the number of secondary branches, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and leaf area; photosynthetic attributes such as chlorophyll 'a', chlorophyll 'b', total chlorophyll, total carotene and total anthocyanine content; nodulation attributes such as nodules per plant, size of the nodule, and fresh weight per nodule, and yield attributes such as number of pods per plant, fresh weight per pod, and seed characteristics such as the number of seeds per pod and fresh weight per 1000 seeds are key traits affected by salt stress and can be used as indicators. Discriminant analysis identified modulations in morpho-physiological attributes such as root length, leaf area, root fresh weight, shoot fresh weight, shoot dry weight, shoot length and photosynthetic attributes such as chlorophyll-a content, and mean nodule weight as discriminating variables at different salt concentrations. Besides, it identified modulations in morpho-physiological attributes such as root length, root fresh weight, photosynthetic attributes such as total anthocyanin content and total chlorophyll content, nodulation attribute such nodule size and nodule weight, and yield attributes such as pod number and number of seeds per pod are discriminating variables at various durations of salt stress. Principal component analysis and discriminant analysis identified 'PKV-AKM 12-28' as salt-tolerant and 'VBN (Gg)3' as salt-susceptible varieties. Multiple correlation analysis identified significant correlations among morphological, physiological, photosynthetic, nodulation and yield parameters.

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Keywords: mungbean; nodulation; physiological; salt stress; yield

Abbreviations: Chla: Chlorophyll 'a' content, Chlb: Chlorophyll 'b' content, DPT: day post salt treatment, FW-1000: fresh weight per 1000 seeds, FWP: fresh weight per pod, HCA: hierarchical cluster analysis, , LA: leaf area, MCA: multiple correlation analysis, NN: number of nodules per plant, NP: number of pods per plant, NS: size of the nodule, NSP: number of seeds per pod, NW: fresh weight per nodule, PCA: principal component analysis, RDW: Root dry weight, RFW: root fresh weight, RL: root length, RTWC%: Root tissue water content, SB: secondary branches, SDW: shoot dry weight, SFW: shoot fresh weight, SL: shoot length, STI: salt tolerance index, STWC%: shoot tissue water content, TAC: total anthocyanin content, TCC: total carotene content, TChl: Total chlorophyll content

Introduction

Mungbean, *Vigna radiata* (L.) R. Wilczek (Fabaceae) is one of the valuable dietary pulse crops playing a vital role in fulfilling the food requirements of the ever-increasing population in the world and especially in India (Ram and Singh, 1993). In India, 3.72 million hectares of agricultural land is under mungbean cultivation, giving 1.56 million tons of production (Ali and Gupta, 2012). Mungbean is used as food, fodder, and green manure and also in cosmetics and pharmaceuticals industries (Tang *et al*., 2014). It is an important source of proteins, vitamins, antioxidants, and minerals (Randhir *et al*., 2004) and has a significant role in ensuring the nutrition security of developing countries such as India (Dhingra *et al*., 1991).

Soil salinity is one of the significant abiotic environmental stress factors responsible for limiting agricultural productivity in many regions of the world (Panta *et al*., 2014). Salinity has already affected 20–50% of agricultural land, and it is increasing by nearly 10% annually (Xu *et al*., 2011; Shrivastava and Kumar, 2015). FAO (2015) reported that 800 million hectares of land and 32 million hectares of agricultural land are under salinity worldwide. Saline soils are dominated mainly by Na^+ and Cl^- ions; both are toxic to plants and are considered the most important ions (Hasegawa, 2013). Salinity limits plant growth and development by disrupting the osmotic and ionic balance in the form of water stress, nutritional stress, oxidative stress, and ion toxicity (Arif *et al*., 2020).

Salinity affects the shoot and root length, fresh and dry biomass, and leaf area in most crops (Sarabi *et al*., 2016; Raza *et al*., 2017; Shelke *et al*., 2017). Moreover, it adversely affects many essential cellular and metabolic processes like photosynthesis (Hamani *et al*., 2020). Furthermore, reduction in photosynthetic capacity under salinity depends on salinity type, duration of treatment, species, and plant age (Sultana *et al*., 1999; Steduto *et al*., 2000; Hester *et al*., 2001; Koyro, 2006). Salinity also limits plants' growth and development, which ultimately affects yield parameters such as pod and seed characters in most of the crops (Ahmed, 2009). Also, salinity limits the plant productivity in legumes by hampering the nodulation process by affecting nodule number, nodule size, and fresh nodule mass (Elahi *et al*., 2004).

The effect of NaCl stress in plants has been studied generally through morphological and physiological responses in plants and conventional visual perception of their variations or changes (Elahi *et al*., 2004; Ghosh *et al*., 2015; Sehrawat *et al*., 2015; Muchate *et al*., 2016; Shelke *et al*., 2017; Rahneshan *et al*., 2018). However, interpretations and conclusions based on conventional approaches are less conclusive because of the complicated nature of morphological and physiological responses and their interrelationships. Moreover, traditional data analysis can extract only quantitative data characteristics and does not interpret conceptual descriptions of dependencies among data variables and the underlying reasons (Michalski and Kaufman, 1997). Multivariate analysis tools such as principal component analysis (PCA), discriminant analysis (DA), Pearson's multiple correlation analysis (MCA) enables the accurate analysis and interpretation of vast and complex datasets. These tools adequately analyse and interpret complex interrelationships among parameters in environmental, biological, chemical, and ecotoxicological studies (Mujunen *et al*., 1996; Simeonov *et al*., 2003; Singh *et al*., 2004; Sinha *et al*., 2009a, 2009b; Shelke *et al*., 2017).

In the present study, the effects of salt stress levels and exposure time on morpho-physiological, photosynthetic, nodulation, and yield parameters in previously screened salt-tolerant 'PKU-AKM 12-28' and susceptible 'VBN (Gg)3' mungbean varieties were evaluated. Furthermore, the effect of these changes on yield under salinity was also evaluated.

Materials and Methods

Plant materials, growth, and salt treatment

Certified and disease-free seeds of mungbean [*Vigna radiata* (L.) R. Wilczec] 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, 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 Charholi village in Pune district (MS). Plants were grown in non-perforated 35 cm × 20 cm plastic pots. Each pot contained a 15 kg soil and farmyard manure in a 3:1 ratio.

Fifteen seeds were sown in each pot. Thinning of plants was done fifteen days after sowing to maintain six plants per pot. The salt stress was given to fifteen-day seedlings through Hoagland nutrient medium (Hoagland and Arnon, 1950) containing 0.75, 100, and 125 mM NaCl (equivalent to 0.3, 7, 8, and 9 dsm-2 EC, respectively). To maintain the desired EC of the potting mixture, 300 ml respective salt solution was added to each pot on every alternate day until the experiments were concluded. Each treatment was replicated in three pots. The following data was collected/analyses were performed on two plants per pot on the 15th, 30th, and 45th day after the salt stress treatments began.

Morpho-physiological growth analysis

The data was collected on the plant morphological parameters that included shoot length (SL), root length (RL), the number of secondary branches (SB), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), shoot tissue water content (STWC%), root tissue water content (RTWC%), and leaf area (LA) (Shelke *et al*., 2017).

Photosynthetic pigments analysis

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.

Nodulation parameters analysis

After harvesting the pods, plants were uprooted. The number of nodules per plant (NN), size of the nodule (NS), and fresh weight per nodule (NW) were measured (Elahi *et al*., 2004).

Yield parameters analysis

After completion of the plant's life cycle, plant pod characteristics such as the number of pods per plant (NP), fresh weight per pod (FWP), and seed characteristics such as the number of seeds per pod (NSP) and fresh weight per 1000 seeds (FW-1000) were measured (Ahmed, 2009).

Statistical analyses

All the experiments were performed with three replicates in a completely randomized block design (CRD). The data were presented as a mean ± standard deviation (Table 1a-1d). For multivariate modelling, the morpho-physiological dataset consisted of 12 variables, photosynthetic datasets consisted of 5 variables, the nodulation dataset consisted of 3 variables, and the yield dataset consisted of 4 variables. These data sets were subjected to multivariate modelling through principal component analysis (PCA) in the PAST statistical package (Hammer *et al*., 2001). The discriminant analysis (DA) was performed in Statistica V 10.0 software using the standard, forward stepwise and, backward stepwise modes (Sinha *et al*., 2009a). The correlations between morpho-physiological and photosynthetic, nodulation, and yield parameters of the NaCl stressed plants at different salt stress levels and durations were determined using Pearson's correlation method in SPPS V 20 software (Chunthaburee *et al*., 2015; Shelke *et al*., 2017).

Results

Effect of salt stress on morpho-physiological parameters

The variations in the morpho-physiological parameters in 'PKU-AKM 12-28' and 'VBN (Gg)3' at different concentrations and exposure of NaCl stress are presented in Table 1a. The analysis of variance (ANOVA) showed a differential response of mungbean varieties to NaCl.

At 15 DPT (day post salt treatment), increase in salt concentration significantly reduced plants SL, RL, LA, SB, SFW, RFW, RDW, SDW, STWC and RTWC. Noteworthy similar trends were observed for 30 and 45 DPT. At 15 DPT in 'VBN(Gg)3' SL (18%, 22% and 25%), RL (14%, 18% and 30%), LA (33%, 42% and 49%), SB (0%, 5% and 33%), SFW (2%, 23% and 31%), RFW (11%, 40% and 66%), SDW (42%, 56% and 81%), RDW (27%, 40% and 61%), STWC (11%, 12% and 21%), and RTWC (8%, 0% and 9%), were significantly reduced under 75, 100 and 125 mM NaCl respectively compared to control. While, at 30 DPT SL (11%, 23%, and 27%), RL (11%, 14%, and 40%), LA (17%, 20% and 32%), SB (0%, 13% and 13%), SFW (24%, 25% and 41%), RFW (20%, 33% and 44%), SDW (12%, 58% and 67%), RDW (27%, 43% and 61%), STWC (5%, 13% and 13%), and RTWC (3%, 5% and 8%) were decreased under 75, 100 and 125 mM NaCl respectively. Further 45 DPT, the salt's effect was more pronounced resulting in reduced SL (22%, 28%, and 37%), RL (11%, 17%, and 39%), LA (39%, 33% and 52%), SB (23%, 28% and 37%), SFW (12%, 27% and 32%), RFW (23%, 32% and 42%), SDW (39%, 62% and 73%) RDW (19%, 71% and 72%), STWC (21%, 32% and 40%), and RTWC (5%, 46% and 42%) under 75, 100 and 125 mM NaCl respectively.

Effect of salt stress on photosynthetic parameters

Photosynthetic pigment content was affected in both the varieties with increasing salinity and exposure duration (Table 1b). At 15 DPT in 'VBN(Gg)3', Chl 'a' (26%, 28% and 45%), Chl 'b' (33%, 40% and 30%), TChl (28%, 32% and 40%), TCC (56%, 35% and 42%), TAC (2%, 6% and 9%), was significantly reduced under 75, 100 and 125 mM NaCl respectively compared to control. While, at 30 DPT even more reduction in Chl 'a' (24%, 52% and 60%), Chl 'b' (25%, 44% and 64%), TChl (24%, 49% and 59%), TCC (26%, 35% and 57%), TAC (25%, 36% and 41%) was observed under 75, 100 and 125 mM NaCl. At 45 DPT, the salt's effect was more pronounced and profound reduction in Chl 'a' (49%, 72% and 74%), Chl 'b' (55%, 69% and 79%), TChl (51%, 71% and 75%), TCC (57%, 69% and 89%), TAC (44%, 59% and 69%) was observed under 75, 100 and 125 mM NaCl.

Effect of salt stress on nodulation parameters

Increasing salinity and exposure duration also affected nodulation parameters in both varieties (Table 1c). At 15 DPT in 'VBN(Gg)3' NN (52%, 76% and 100%), NS (20%, 37% and 100%), NW (46%, 61% and

100%) was reduced significantly under 75, 100 and 125 mM NaCl respectively compared to control. While, at 30 DPT, further reduction in NN (28%, 48% and 66%), NS (36%, 58% and 100%), NW (49%, 59% and 100%) was observed. After 45 DPT, the effect was more pronounced and severe reduction in NN (43%, 64% and 87%), NS (20%, 51% and 65%), NW (30%, 47% and 70%) was observed under 75, 100 and 125 mM NaCl stress. However, both genotypes differed significantly for nodulation parameters under 75, 100 and 125 mM NaCl at 15, 30 and 45 DPT compared with their controls. The 'PKU-AKM 12-28' was less harmed for these parameters than 'VBN(Gg)3' at all salt concentrations and exposure durations.

Thus, though both genotypes differed significantly in their responses on morpho-physiological, photosynthetic, and nodulation parameters at all salt concentrations and exposure durations, these parameters were less affected in 'PKU-AKM 12-28' than 'VBN(Gg)3'.

Table 1a. Effect of NaCl stress on morpho-physiological traits in Vigna radiata varieties 'PKU AKM 12-28' and 'VBN (Gg)3'

Variety	NaCl (mM)	Exposure duration (days)	Coding	Shoot length (cm)	Root length (cm)	Number of Secondary branches	Leaf area (cm^2)	Shoot fresh Weight (gm)	Root fresh Weight (gm)	Shoot dry Weight (gm)	Root dry Weight (gm)	Shoot tissue water content (%)	Root tissue water content (%)
PKU AKM 12-28	0	15	PC0E1	34.60 ± 2.28	23.10 ± 2.61	4.00 ± 0.00	33.94 ± 4.66	6.79 ± 0.59	0.414 ±0.050	1.419 ± 0.126	0.158 ± 0.019	79.09 ± 0.93	61.11 ± 8.80
		30	PC0E2	37.20 ± 1.56	28.12 ± 2.37	5.20 ± 1.10	40.34 ± 9.95	8.59 ± 0.88	1.104 ±0.229	2.111 ± 0.202	0.250 ± 0.032	75.08 ± 4.72	76.67 ± 5.45
		45	PC0E3	43.93 ± 2.11	31.40 ± 3.86	7.20 ± 0.45	62.34 ± 4.06	9.61 ± 0.92	1.689 ± 0.262	3.650 ± 0.243	0.710 ± 0.027	61.83 ± 3.34	57.33 ± 5.15
	75	15	PC1E1	34.76 ± 2.93	28.82 ± 2.00	4.00 ± 0.00	28.97 ± 6.20	6.42 ± 0.89	0.733 ± 0.045	1.287 ± 0.121	0.179 ± 0.021	79.90 ± 2.83	75.52 ± 2.84
		30	PC1E2	36.92 ± 2.40	32.16 ± 1.26	4.80 ± 0.45	42.87 ± 6.95	9.26 ± 1.03	1.496 ± 0.153	2.101 ± 0.221	0.430 ± 0.013	77.10 ± 3.49	71.02 ± 3.00
		45	PC1E3	41.22 ± 1.26	34.26 ± 2.06	6.40 ± 0.55	49.45 ± 3.06	9.48 ± 0.76	1.787 ± 0.254	3.151 ± 0.156	0.957 ± 0.029	66.59 ± 3.17	45.54 ± 8.41
	100	15	PC2E1	33.28 ± 3.25	18.72 ± 2.72	4.40 ± 0.55	28.35 ± 6.48	5.57 ± 1.15	0.486 ± 0.994	1.147 ± 0.292	0.146 ± 0.018	78.14 ± 9.14	69.53 ± 5.96
		30	PC2E2	36.54 ± 1.40	26.72 ± 1.16	4.40 ± 0.55	36.06 ± 1.92	7.74 ± 1.77	0.994 ± 0.197	1.183 ±0.197	0.309 ± 0.011	84.24 ± 3.62	68.01 ± 6.25
		45	PC2E3	39.06 ± 1.39	27.26 ± 1.47	5.20 ± 0.45	42.24 ± 3.68	8.51 ± 0.87	1.527 ± 0.271	3.064 ± 0.137	0.545 ± 0.025	63.67 ± 4.49	63.14 ± 8.18
	125	15	PC3E1	31.32 ± 0.31	19.90 ± 3.63	3.75 ± 0.50	23.71 ± 2.82	4.49 ± 0.78	0.324 ± 0.089	0.995 ± 0.189	0.137 ± 0.035	77.27 ± 6.08	54.42 ±19.73
		30	PC3E2	35.66 ± 1.59	21.78 ± 1.20	4.20 ± 0.45	32.11 ± 1.96	6.40 ± 1.20	0.756 ± 0.120	1.295 ± 0.119	0.154 ± 0.012	79.17 ± 4.38	79.12 ± 4.73
		45	PC3E3	35.54 ± 1.09	23.26 ± 2.98	5.40 ± 0.55	37.49 ± 1.76	7.69 ± 0.53	1.287 ± 0.173	1.607 ± 0.278	0.274 ± 0.026	78.90 ± 4.75	78.38 ± 3.57
VBN (Gg)3	0	15	VC0E1	30.54 ± 1.96	21.20 ± 2.63	3.60 ± 0.55	31.18 ± 2.12	5.80 ± 1.08	0.443 ± 0.061	1.296 ± 0.146	0.146 ± 0.018	77.30 ± 2.76	66.91 ± 3.50
		30	VC0E2	34.40 ± 2.90	26.16 ± 1.07	4.60 ± 0.55	37.00 ± 3.56	8.73 ± 0.85	0.969 ± 0.100	1.980 ± 0.178	0.234 ± 0.017	77.02 ± 4.18	75.62 ± 3.61
		45	VC0E3	43.92 ± 1.65	28.90 ± 2.14	7.00 ± 0.00	54.99 ± 4.38	9.39 ± 1.72	1.560 ± 0.110	3.554 ± 0.237	0.695 ± 0.019	60.68 ±10.46	55.23 ± 4.07
	75	15	VC1E1	24.88 ± 1.04	18.20 ± 1.80	3.60 ± 0.55	21.01 ± 5.61	5.64 ± 1.38	0.394 ± 0.056	0.746 ± 0.061	0.107 ± 0.011	86.14 ± 3.53	72.64 ± 3.05
		30	VC1E2	30.54 ± 2.59	23.20 ± 4.82	4.60 ± 0.55	30.65 ± 3.40	6.62 ± 1.03	0.774 ± 0.101	1.747 ± 0.160	0.169 ± 0.014	72.97 ± 5.88	77.92 ± 2.24
		45	VC1E3	34.26 ± 0.89	25.64 ± 2.27	5.40 ± 0.55	33.29 ± 2.00	8.25 ± 0.59	1.207 ± 0.191	2.176 ± 0.312	0.562 ± 0.035	73.56 ± 4.04	52.32 ± 9.60
	100	15	VC2E1	23.99 ± 1.07	17.30 ± 2.10	3.40 ± 0.55	17.95 ± 1.78	4.46 ± 0.83	0.264 ± 0.035	0.572 ± 0.041	0.087 ± 0.008	86.68 ± 3.51	66.92 ± 3.00
		30	VC2E2	26.62 ± 1.61	22.48 ± 2.57	4.00 ± 0.00	29.69 ± 3.20	6.52 ± 0.62	0.644 ± 0.075	0.834 ± 0.071	0.132 ± 0.012	87.07 ± 1.86	79.16 ± 3.41
		45	VC2E3	31.72 ± 1.10	23.90 ± 1.70	5.00 ± 0.00	36.92 ± 2.22	6.85 ± 0.92	1.053 ± 0.148	1.339 ± 0.148	0.199 ± 0.018	80.09 ± 3.89	80.86 ± 2.95
	125	15	VC3E1	22.94 ± 1.26	14.88 ± 1.54	2.40 ± 0.55	15.89 ± 2.62	3.98 ± 0.82	0.148 ± 0.026	0.240 ± 0.044	0.057 ± 0.013	93.79 ± 1.43	60.58 ± 12.78
		30	VC3E2	25.18 ± 1.31	15.56 ± 0.99	4.00 ± 0.00	25.28 ± 2.66	5.18 ± 0.71	0.543 ± 0.120	0.647 ± 0.094	0.092 ± 0.012	87.39 ± 1.86	82.11 ± 5.96
		45	VC3E3	27.56 ± 1.34	17.66 ± 2.49	4.40 ± 0.55	26.27 ± 3.19	6.38 ± 0.59	0.899 ± 0.177	0.948 ± 0.111	0.191 ± 0.021	85.12± 1.10	78.31 ± 3.76

Table 1b. Effects of NaCl stress on photosynthetic pigments in *Vigna radiata* varieties 'PKU AKM 12-28' and 'VBN (Gg)3'

Variety	NaCl (mM)	Exposure Duration (days)	Coding	Chlorophyll 'a' content (µg/gm FW)	Chlorophyll 'b' content (µg/gm FW)	Total Chlorophyll content (µg/gm FW)	Total caroteonoid content (µg/gm FW)	Total anthocyanine content (µg/gm FW)
PKU AKM 12-28	0	15	PC0E1	25.62 ± 0.38	13.19 ± 0.32	39.43 ± 0.53	9.67 ± 0.28	0.479 ± 0.011
		30	PC0E2	32.70 ± 0.61	17.02 ± 0.47	50.51 ± 1.10	13.12 ± 0.33	0.843 ± 0.056
		45	PC0E3	36.98 ± 2.62	15.99 ± 2.43	53.85 ± 4.91	20.77 ± 0.34	1.383 ± 0.046
	75	15	PC1E1	25.54 ± 0.69	13.20 ± 0.83	39.36 ± 1.54	9.15 ± 0.42	0.568 ± 0.010
		30	PC1E2	28.73 ± 0.61	14.35 ± 1.00	43.76 ± 1.62	12.56 ± 0.44	0.635 ± 0.037
		45	PC1E3	25.06 ± 0.64	11.51 ± 1.08	37.17 ± 1.74	14.52 ± 0.34	0.980 ± 0.065
	100	15	PC2E1	26.53 ± 0.73	14.08 ± 0.27	41.25 ± 1.01	7.59 ± 0.37	0.424 ± 0.010
		30	PC2E2	26.56 ± 0.69	14.24 ± 0.63	41.45 ± 1.15	11.95 ± 0.50	0.732 ± 0.041
		45	PC2E3	19.33 ± 0.72	9.94 ± 0.63	29.74 ± 1.26	9.76 ± 0.41	0.676 ± 0.035
	125	15	PC3E1	19.93 ± 0.30	9.11 ± 0.96	29.51 ± 1.20	7.27 ± 0.39	0.458 ± 0.028
		30	PC3E2	24.86 ± 0.47	13.87 ± 0.50	39.32 ± 0.82	7.66 ± 0.43	0.660 ± 0.045
		45	PC3E3	17.68 ± 0.46	8.19 ± 1.19	26.29 ± 1.64	6.72 ± 0.35	0.738 ± 0.027
VBN (Gg)3	0	15	VC0E1	24.25 ± 0.35	12.97 ± 0.74	37.80 ± 1.03	9.43 ± 0.49	0.464 ± 0.036
		30	VC0E2	29.41 ± 0.64	16.92 ± 0.63	47.04 ± 1.08	11.76 ± 0.41	0.781 ± 0.039
		45	VC0E3	36.08 ± 1.58	15.64 ± 1.24	52.59 ± 2.86	18.77 ± 0.57	1.143 ± 0.035
	75	15	VC1E1	18.03 ± 0.70	8.67 ± 0.90	27.13 ± 1.61	4.14 ± 0.25	0.474 ± 0.016
		30	VC1E2	22.32 ± 0.57	12.72 ± 1.37	35.58 ± 1.95	8.69 ± 0.57	0.585 ± 0.026
		45	VC1E3	18.27 ± 0.74	6.98 ± 0.88	25.68 ± 1.64	8.02 ± 0.50	0.641 ± 0.049
	100	15	VC2E1	17.55 ± 0.32	7.71 ± 0.70	25.68 ± 1.03	6.08 ± 0.51	0.490 ± 0.036
		30	VC2E2	14.00 ± 0.19	9.41 ± 0.72	23.75 ± 0.91	7.68 ± 0.25	0.495 ± 0.031
		45	VC2E3	10.09 ± 0.72	4.78 ± 0.54	15.12 ± 1.13	5.71 ± 0.36	0.466 ± 0.026
	125	15	VC3E1	13.28 ± 0.98	9.04 ± 2.42	22.64 ± 1.77	5.49 ± 0.22	0.421 ± 0.026
		30	VC3E2	12.96 ± 0.40	6.00 ± 0.64	19.28 ± 0.40	5.04 ± 0.30	0.463 ± 0.019
		45	VC3E3	9.46 ± 0.46	3.26 ± 0.41	12.94 ± 0.74	2.04 ± 1.45	0.356 ± 0.020

The data represented mean ± standard deviation

Table 1c. Effect of NaCl stress on nodulation traits in *Vigna radiata* varieties 'PKU AKM 12-28' and 'VBN (Gg)3'

Variety	NaCl (mM)	Exposure Duration (days)	Codings	Number of nodules/ Plant	Size of nodule (mm)	Fresh weight / Nodule (mg)
PKU AKM 12-28	0	15	PC0E1	11.00 ± 1.22	2.65 ± 0.32	12.41 ± 1.84
		30	PC0E2	14.20 ± 0.84	3.11± 0.54	15.79 ± 0.94
		45	PC0E3	25.80 ± 1.30	5.28 ± 0.18	18.24 ± 0.72
	75	15	PC1E1	11.00 ± 2.45	2.22 ± 0.25	10.94 ± 1.63
		30	PC1E2	12.00 ± 2.24	2.94 ± 0.33	13.22 ± 0.99
		45	PC1E3	18.60 ± 2.07	4.34 ± 0.25	16.29 ± 0.89
	100	15	PC2E1	9.40 ± 1.82	1.53 ± 0.28	7.40 ± 0.71
		30	PC2E2	11.60 ± 2.07	2.68 ± 0.05	10.91 ± 0.57
		45	PC2E3	15.80 ± 0.84	3.53 ± 0.28	14.69 ± 1.14
	125	15	PC3E1	5.40 ± 0.55	1.23 ± 0.29	6.15 ± 0.16
		30	PC3E2	11.00 ± 1.58	1.85 ± 0.27	8.67 ± 0.46
		45	PC3E3	12.20 ± 0.84	2.62 ± 0.34	11.71 ± 1.29
VBN (Gg)3	0	15	VC0E1	9.20 ± 0.45	2.17 ± 0.18	13.30 ± 1.93
		30	VC0E2	11.20 ± 1.30	2.79 ± 0.20	14.09 ± 0.81
		45	VC0E3	22.80 ± 0.84	4.89 ± 0.35	17.82 ± 0.72
	75	15	VC1E1	4.40 ± 0.55	1.73 ± 0.14	7.11 ± 0.77
		30	VC1E2	8.00 ± 0.71	1.78 ± 0.26	7.16 ± 1.30
		45	VC1E3	13.00 ± 0.71	3.91 ± 0.44	12.53 ± 0.78
	100	15	VC2E1	2.20 ± 0.45	1.37 ± 0.10	5.24 ± 0.56
		30	VC2E2	5.80 ± 1.30	1.16 ± 0.29	5.71 ± 0.97
		45	VC2E3	8.20 ± 0.84	2.38 ± 0.60	9.44 ± 0.71
	125	15	VC3E1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		30	VC3E2	3.80 ± 0.45	0.00 ± 0.00	0.00 ± 0.00
		45	VC3E3	3.00 ± 1.00	1.72 ± 0.21	5.25 ± 0.76

The data represented mean ± standard deviation

Table 1d. Effect of NaCl stress on pod and seed traits in *Vigna radiata* varieties 'PKU AKM 12-28' and 'VBN (Gg)3' at 45 days after salt treatment

Variety	NaCl (mM)	Coding	Number of pods/Plant	Fresh weight /Pod (gm)	Number of seeds/Pod	Fresh weight of 1000 seeds (gm)
PKU AKM 12-28	0	PC1	4.00±0.71	1.21±0.24	11.20±0.84	58.83±7.73
	75	PC2	3.20±0.45	1.16±0.10	10.80±0.45	58.60±10.33
	100	PC3	2.80±0.45	0.70±0.06	11.20±0.84	59.02±6.35
	125	PC4	2.00±0.00	0.41±0.17	7.00±1.58	50.67±7.69
VBN (Gg)3	0	VC1	3.80±0.45	0.97±0.07	5.62±2.00	52.12±8.21
	75	VC2	2.40±0.55	0.44±0.13	6.02±0.55	37.80±6.39
	100	VC3	1.40±0.55	0.28±0.02	4.29±1.00	31.23±4.88
	125	VC4	1.20±0.45	0.04±0.01	0.00±0.00	0.00±0.00

The data represented mean ± standard deviation

Effect of salt stress on yield parameters

In 'PKU-AKM 12-28' at 45 DPT of 75, 100, and 125 mM NaCl stress (Table 1d), NP was reduced by 20, 30, and 50% respectively, whereas in 'VBN (Gg)3', these reductions were by 36.84, 63.16, and 68.42% respectively. At 75 mM NaCl, FWP was drastically decreased in VBN (Gg)3 by 54.34%. As the salt concentration increased to 125 mM NaCl, FWP was reduced by 65.72% and 95.46% in 'PKU-AKM 12-28' and 'VBN (Gg)3', respectively.

Seed parameters such as NSP and FW-1000 were affected differently in these two mungbean varieties under salinity. At 75 mM NaCl, SPP and SW-1000 were decreased significantly in 'VBN (Gg)3' (16 and 22.47% respectively). As the salt concentration increased to 125 mM NaCl, SPP, and SW-1000 were decreased by 37.50% and 13.86%, respectively in 'PKU-AKM 12-28'. Moreover, seed formation did not occur in 'VBN (Gg)3' at 125 mM NaCl. Thus, 'PKU-AKM 12-28' was less affected by salt stress than 'VBN(Gg)3'.

Principal component analysis

PCA was performed on the normalized dataset to evaluate: a) the plant-NaCl interactions, b) differences in responses on parameters under NaCl stress levels, and c) dependence among variables and the factors that influence them.

PCA of the entire morphophysiological data set (Table 1a) produced ten PCs, with the first two PCs explaining about 90.69% of the total variance in the morphophysiological data set. The loadings and scores of the first two PCs (PC1 vs. PC2) are presented in Figure 1. The first two PCs represent maximum variance and reflect the main groupings in the data set. PC1 accounting for 80.87% of the total variance was positively correlated (loading > 90%) with SL, RL, SB, LA, SFW, RFW, SDW, and RDW. PC2 accounting for 9.82% of the total variance was positively correlated (loading > 86%) with RTWC. The scores show a visible differentiation between the tissue responses at different salinity levels and exposure time in both varieties. Scores of the PC1 in PKU-AKM 12-28 were higher than those of 'VBN(Gg)3'. The score in PC1 was highest in 'PKU-AKM 12-28' for 75 mM NaCl exposure for 45 days. On the contrary, it was least in 'VBN (Gg)3' exposed to all NaCl concentrations for 45 days. It indicates 'PKU-AKM 12-28' has a higher tolerance level than 'VBN (Gg)3' under salinity. Thus, differences in changes in the morphological variables under all salt concentrations and exposure durations suggest intrinsic differences in two mungbean varieties.

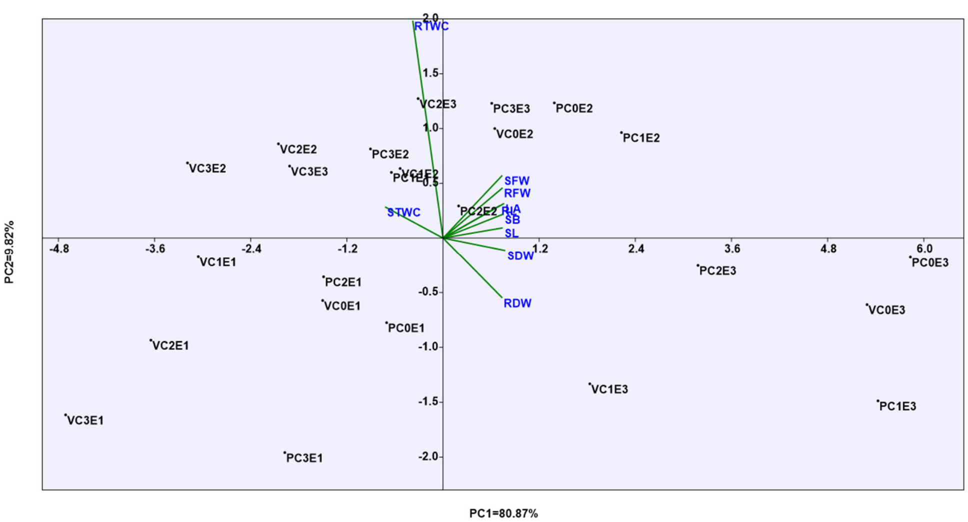

Figure 1. PCA scores and loadings of the first two PCs obtained from the morpho-physiological dataset of mungbean varieties

PCA of the photosynthetic dataset (Table 1b) gave five PCs, with the first two PCs explaining 98.03% of the total variance. Figure 2 shows the photosynthetic data sets' loadings and scores of the first two PCs (PC1 vs. PC2). The PC1 (86.76% variance) showed high positive loadings (>90%) on Chl-a, Chl-b, TChl, and TCC and positive loadings (>83%) on TAC. On the other hand, the second PC accounted for 11.27% of the total variance. The corresponding scores in the plot, along with the loadings, suggest that at all NaCl concentrations for a short exposure (E1, 15 days), Chl-a, Chl-b, TChl, and TCC were affected and reduced by a greater extent in 'VBN (Gg)3' as compared to 'PKU-AKM 12-28'. However, both varieties showed increased TAC at 75 mM NaCl and decreased at 125 mM NaCl. Moderate exposure (E2, 30 days) brought significant changes in Chl-a, Chl-b, TChl, and TCC. These parameters were more affected in 'VBN (Gg)3' than 'PKU-AKM 12-28'. Likewise, the TChl was more affected in 'VBN (Gg)3' at 125 mM NaCl at moderate exposure (E2). Exposure for a longer duration (E3, 45 days) and higher salt concentration was associated with a remarkable reduction in all photosynthetic parameters in 'VBN (Gg)3' than 'PKU-AKM 12-28'. Hence, scores of 'VBN (Gg)3' (VC1E3, VC2E3, VC3E3) lay more on the negative side as compared to scores of 'PKU-AKM 12-28' (PC1E3, PC2E3, PC3E3) for PC1. Moreover, the scores of 'VBN (Gg)3' were more negative compared to 'PKU-AKM 12-28' at all salt concentrations and exposure durations. It indicates that 'PKU-AKM 12-28' has a higher salinity tolerance than 'VBN (Gg)3'.

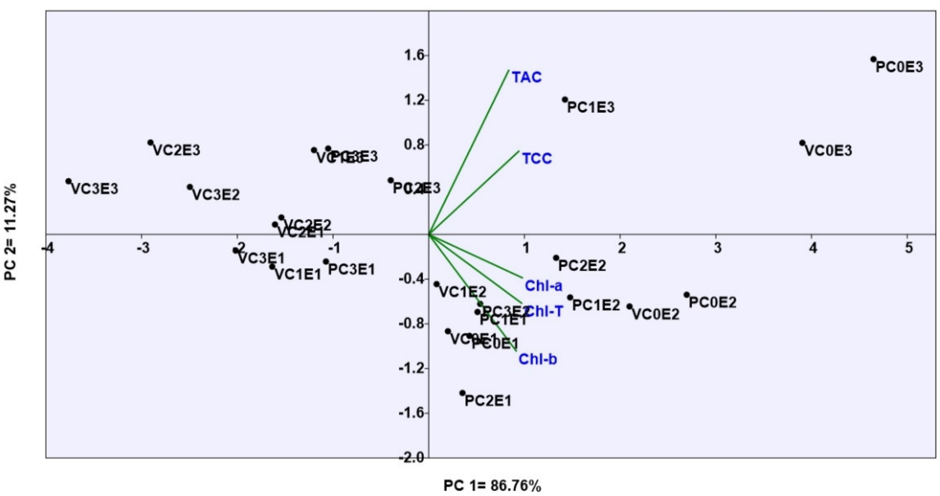

Figure 2. PCA scores and loadings of the first two PCs obtained from the photosynthetic dataset of mungbean varieties

PCA of nodulation dataset (Table 1c) yielded three PCs, with the first two PCs explaining 98.26% of the total variance. The loadings and scores of the first two PCs (PC1 vs. PC2) of the nodulation data set are given in (Figure 3). It may be noted that PC1 (95.11% variance) showed high positive loadings (>97%) on all nodulation parameters studied in the present investigation. On the other hand, the second PC accounted for 3.15% of the total variance. The corresponding scores in combination with the loadings suggested that at short exposure (E1), at 75 mM NaCl, NN and NW was drastically decreased in 'VBN (Gg)3' compared to 'PKU-AKM 12-28'. Moreover, at 125 mM, nodules were not formed in 'VBN (Gg)3' at a short exposure period. Hence, scores of 'VBN (Gg)3' (VC1E1, VC2E1, VC3E1) lie more on the negative side compared to scores of 'PKU-AKM 12-28' (PC1E1, PC2E1, PC3E1) for PC1. However, the moderate exposure (E2) brought significant changes in NN, NS, and NW. The NS and NW were negligible in 'VBN (Gg)3' compared to 'PKU-AKM 12-28'. Exposure to higher duration (E3) and higher salt concentration was associated with remarkable changes in NN, NS, and NW, which was more reduced in 'VBN (Gg)3' than 'PKU-AKM 12-28'. Thus, 'PKU-

AKM 12-28' showed a higher tolerance level than VBN (Gg)3 under saline conditions, resulting in better nodulation.

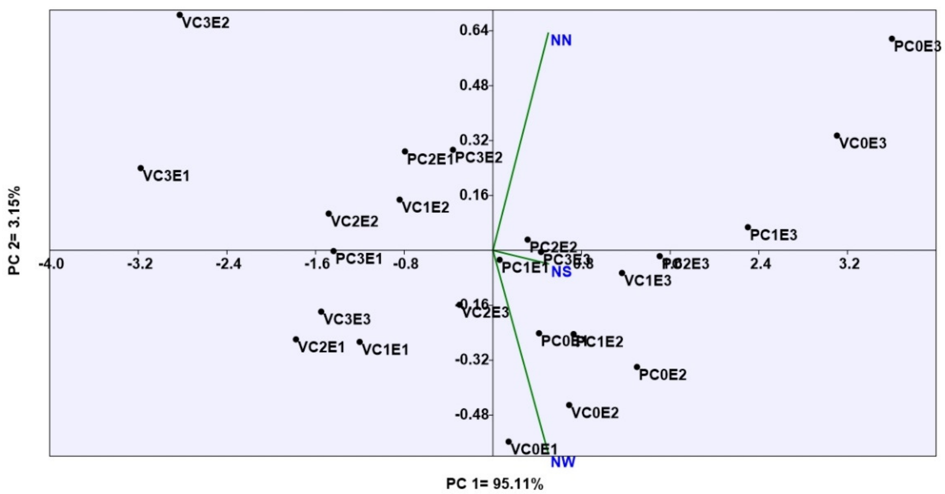

Figure 3. PCA scores and loadings of the first two PCs obtained for the nodulation dataset of mungbean varieties

At 45 days exposure time (E3), PCA was performed on the normalized yield dataset (Table 1d) that gave four PCs, with the first two PCs explaining about 97.01% of the total variance in the yield data set. The loadings and scores of the first two PCs (PC1 vs. PC2) of yield data set given in (Figure 4) The PC1 (87.48%) showed high positive loadings (>91%) on all yield parameters studied in the present investigation. On the other hand, the second PC accounted for 9.53% of the total variance. The corresponding scores in combination with the loadings suggested that at more prolonged exposure (E3), salinity influenced all yield parameters as salt concentration increased. However, at 75 and 100 mM NaCl, all yield parameters were significantly reduced in 'VBN (Gg)3' than 'PKU-AKM 12-28'. Hence, the scores of (VC3 and VC2) and (PC2 and PC3) are present at negative and positive quadrant with respect to PC1. In contrast, a higher salt concentration (125 mM) was associated with a remarkable reduction in all yield parameters in 'VBN (Gg)3'. Hence, the score (VC4) is present in the negative quadrant with respect to PC1 and PC2. Thus, for yield output, 'PKU-AKM 12-28' has a higher salinity tolerance than 'VBN (Gg)3'.

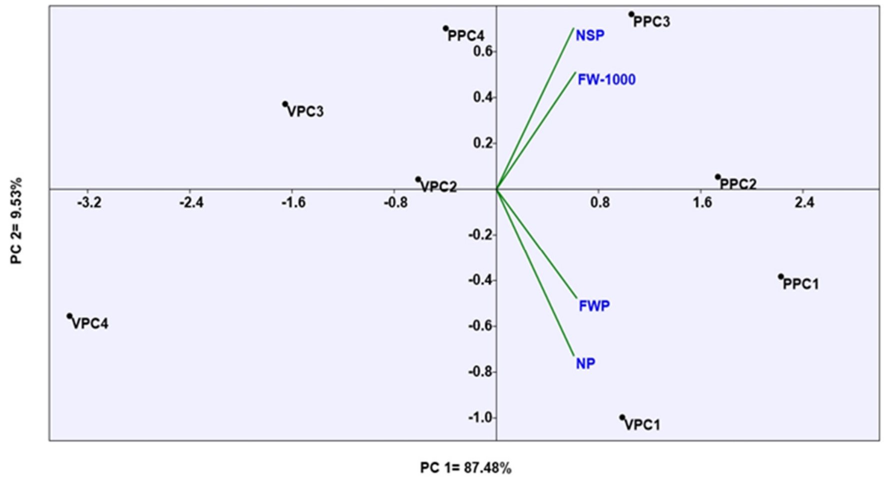

Figure 4. PCA scores and loadings of the first two PCs obtained for the yield dataset of mungbean varieties

Discriminant analysis

Variations in morpho-physiological, photosynthetic, nodulation, and yield parameters at different NaCl concentrations in mungbean varieties were further investigated through discriminant analysis (DA).

Discriminant analysis at different NaCl concentrations

The category variables (Y) were four concentrations of NaCl to which mungbean varieties were exposed. Standard, forward and backward stepwise modes of DA were performed, and the discriminant functions (DFs) (Table 2a) and classification matrices were obtained (Table S1a). For morphophysiological parameters, the standard and forward stepwise DA constructed DFs that included all ten parameters and six parameters, respectively, and rendered the corresponding CMs assigning 91.66% and 83.83% cases correctly. DA with forward stepwise mode includes variables obtained from more significant to insignificant changes. In contrast, backward stepwise mode removes the beginning with less significant changes until no considerable changes are observed. Forward stepwise DA showed RL, LA, RFW, SFW, SDW, and SL. Thus, the DA results suggest that RL, LA, RFW, SFW, SDW, and SL are the most significant parameters to differentiate among the four sets of the plant responses observed at the four NaCl-induced stress levels. Both CA and DA identified morphological parameters that significantly changed under different salt stress levels. The box and whisker plots of selected parameters (forward step mode) showing different responses identified by DA are presented in (Figure S1a). All the chosen parameters showed observable variations in morpho-physiological characters at different NaCl concentrations. The RL and RFW showed a similar variation pattern that increased at 75 mM NaCl and again decreased at 100 and 125 mM NaCl. LA, SFW, SDW, and SL also showed a similar pattern of variation.

For photosynthetic parameters, the standard and forward stepwise DA modes constructed DFs (Table 2b) that included all five and only one parameters, respectively, and rendered the corresponding CMs (Table S1b), assigning 62.50% and 50% cases correctly. These results suggest that Chla is a significant parameter to discriminate the four sets of the plant responses corresponding to the four NaCl concentrations. Figure S1b shows box and whisker plots of the selected parameters showing responses at different NaCl concentrations identified by DA. Chl 'a' showed significant variation under different salinity levels.

For nodulation parameters, the standard and forward stepwise DA modes constructed DFs (Table 2c), including all three, and one parameter, respectively, and rendered the corresponding CMs (Table S1c), assigning 50% and 58.33% cases correctly. Thus, the DA results suggest that the NW variable is a significant parameter to discriminate between the four sets of the plant responses corresponding to different NaCl concentrations. As identified by DA, box and whisker plots of selected parameters showing different responses to NaCl concentration are given in (Figure S1c). NW showed variations in nodulation under salinity at different NaCl concentrations.

For yield parameters, the standard and forward stepwise DA modes constructed DFs (Table 2d), including all four, and two parameters, respectively, and rendered the corresponding CMs (Table S1d), assigning 87.50% and 75% cases correctly. Thus, the DA results suggest that NP and NSP variables are significant parameters to discriminate between the four sets of the plant responses corresponding to different NaCl concentrations. As identified by DA, box and whisker plots of selected parameters showing different NaCl concentration responses are presented in (Figure S1d). NP and NSP showed variations in nodulation changes under salinity at different NaCl concentrations.

Table 2a. Classification functions for discriminant analysis (DA) of NaCl stress levels and morpho-physiological parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups			
	Coefficient [a]			
	C0	C1	C2	C3
SL	24.09	24.31	25.47	26.84
RL	4.06	5.25	3.38	1.98
SB	95.04	95.70	92.42	90.80
LA	-11.30	-12.90	-12.29	-12.48
SFW	-75.71	-82.88	-87.28	-93.62
RFW	-363.18	-325.42	-296.43	-261.47
SDW	711.44	692.20	690.40	691.55
RDW	-223.60	-168.99	-196.43	-211.50
STWC	54.47	53.54	53.99	54.67
RTWC	4.27	4.68	4.44	4.32
Constant	-2902.09	-2804.36	-2804.61	-2837.42
Forward DA mode				
RL	1.051	1.817	0.554	-0.429
LA	2.537	1.136	1.429	1.125
RFW	-198.965	-144.388	-128.863	-100.970
SFW	40.750	31.071	28.189	23.523
SDW	19.767	13.535	5.836	-0.906
SL	-0.580	-0.062	0.811	1.788
Constant	-146.082	-98.446	-87.125	-72.126
Backward DA mode				
No variables in the model				

[a] Discriminant function coefficient for different concentrations of NaCl

Table 2b. Classification functions for discriminant analysis (DA) of NaCl stress levels and photosynthetic parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups			
	Coefficient [a]			
	C0	C1	C2	C3
Chl-a	-156.233	-25.4380	-223.567	44.4849
Chl-b	-153.414	-25.3860	-218.584	43.5800
Chl-T	153.607	25.6656	218.931	-42.9237
TCC	-1.034	-1.4601	-1.180	-2.1963
TAC	14.550	18.6754	16.735	28.7089
Constant	-18.310	-11.7295	-9.381	-9.6374
Forward DA mode				

Chl-a	1.0141	0.7560	0.62507	0.53799
Constant	-17.0231	-10.0771	-7.32761	-5.78752
Backward DA mode				
No variables in the model				

[a]Discriminant function coefficient for different concentrations of NaCl

Table 2c. Classification functions for discriminant analysis (DA) of NaCl stress levels and nodulation parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups			
	Coefficients [a]			
	C0	C1	C2	C3
NN	-0.2851	-0.47787	-0.29071	-0.02407
NS	-2.2465	0.52571	-0.50152	-0.90021
NW	2.1207	1.30428	1.17356	0.67328
Constant	-11.4343	-6.76885	-4.79501	-2.54175
Forward DA mode				
NW	1.1444	0.83974	0.66667	0.39683
Constant	-10.1268	-6.09233	-4.35242	-2.43724
Backward DA mode				
No variables in the model				

[a]Discriminant function coefficient for different concentrations of NaCl

Table 2d. Classification functions for discriminant analysis (DA) of NaCl stress levels and yield parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups			
	Coefficients [a]			
	C0	C1	C2	C3
NP	41.9003	26.3729	18.4692	19.5117
FWP	-6.7078	-8.3712	-14.2649	-13.9229
NSP	-4.0862	-2.0917	-0.9653	-1.2877
FWS-1000	-0.1388	-0.0944	-0.0283	-0.0428
Constant	-58.4046	-23.8890	-12.9079	-12.6333
Forward DA mode				
NP	38.9620	23.5019	14.9170	15.8907
NSP	-4.7786	-2.7054	-1.5894	-1.9453
Constant	-57.2681	-22.9129	-10.8943	-10.6946
Backward DA mode				
No variables in the model				

[a]Discriminant function coefficient for different concentrations of NaCl

Discriminant analysis at different salt exposure periods

The effect of the duration of salt stress in the mungbean varieties was also analysed through discriminant analysis. The category variables (Y) were the three exposure durations (E1, E2, and E3). For morphophysiological parameters, the standard and forward stepwise DA modes constructed DFs (Table 3a) that included all ten parameters of standard DA mode, three parameters of forward DA mode, and two parameters backward DA mode and rendered the corresponding CMs assigning 100%, 95.83%, and 87.50% cases correctly (Table S2a). Backward stepwise DA showed that the two variables RL and RFW followed RFW, RL and, RTWC in the forward stepwise DA. The box whisker plots of selected parameters showing responses at three durations of salt stress are given in Figure S2a. The RL and RFW showed observable variations in the plants exposed to NaCl stress for different durations. These results suggest higher salt stress tolerance in 'PKU-AKM 12-28' than 'VBN (Gg)3'. For photosynthetic parameters, the standard, forward, and backward stepwise DA modes constructed DFs, including all 5, 3, and 2 parameters, respectively (Table 3b) and rendered the corresponding CMs (Table S2b), assigning 87.50%, 87.50%, and 79.16% cases correctly. Thus, the DA results revealed that TAC and Chl-T (Figure S2b) variables are the most critical parameters to distinguish the plant's responses at different salt stress durations

For nodulation parameters, DA modes constructed DFs, including all 3 and 2 parameters, respectively (Table 3c), and rendered the corresponding CMs (Table S2c), assigning 70.83% and 70.83% cases correctly. Thus, the DA results revealed that NS and NW (Figure S2c) variables are the most critical parameters to discriminate between the three sets of exposure duration.

Table 3a. Classification function for discriminant analysis (DA) of NaCl stress exposure duration and morpho-physiological parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups		
	Coefficient [a]		
	E1	E2	E3
SL	27.82	27.24	26.61
RL	14.59	13.26	11.85
SB	76.85	78.42	85.09
LA	-16.73	-16.59	-16.69
SFW	-106.03	-102.05	-103.13
RFW	-412.56	-383.93	-336.05
SDW	679.07	668.86	663.07
RDW	60.77	66.44	39.23
STWC	53.60	53.08	52.76
RTWC	7.25	7.45	6.98
Constant	-2896.19	-2863.60	-2803.71
Forward DA mode			
RFW	-52.9685	-24.8113	7.9188
RL	4.7909	3.7403	2.007
RTWC	1.2531	1.4998	1.3714
Constant	-80.3519	-92.8168	-76.9834
Backard DA mode			
RL	4.0180	2.8152	1.1612
RFW	-60.4579	-33.7754	-0.2775
Constant	-29.6968	-20.2488	-16.3136

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

Table 3b. Classification function for discriminant analysis (DA) of NaCl stress exposure duration and photosynthetic parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups		
	Coefficient [a]		
	E1	E2	E3
TCC	-2.0453	-2.150	-1.7515
TAC	15.5846	28.852	43.5428
Chl-T	77.6689	98.227	89.2248
Chl-b	-76.3725	-95.990	-89.2785
Chl-a	-79.1170	-100.831	-91.1556
Constant	-8.8371	-11.723	-10.9816
Forward DA mode			
TAC	-3.98361	8.32545	26.84189
Chl-b	1.07203	2.03613	-0.19708
Chl-a	-0.05132	-0.75577	-0.21431
Constant	-5.50456	-8.05605	-8.55061
Backward DA mode			
TAC	-5.89820	3.58689	26.82064
Chl-T	0.33578	0.21606	-0.20508
Constant	-5.22112	-6.32339	-8.55060

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

Table 3c. Classification function for discriminant analysis (DA) of NaCl stress exposure duration and nodultion parameters in the mungbean varieties under salinity

Standard DA mode	Linear discriminant functions for groups		
	Coefficients [a]		
	E1	E2	E3
NN	-0.22221	0.03228	-0.27848
NS	0.18621	-0.12711	7.62617
NW	0.54314	0.42797	-0.75725
Constant	-2.64157	-3.14643	-7.67020
Forward DA mode			
NS	-0.68209	-0.00097	6.53798
NW	0.50589	0.43338	-0.80393
Constant	-2.52641	-3.14400	-7.48934
Backward DA mode			
No variables in the model			

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

Multiple correlations

Variations in the Morphophysiological, photosynthetic and nodulation parameters in mungbean varieties exposed to different levels and durations of NaCl stress were evaluated through Pearson's correlation (Table S3a and Figure 5). Variation in SL was positively correlated with LA (r=0.91**), SFW (r=0.85**) and SDW (r=0.89**) while variation in RL was positively correlated with SB (r=0.75**), SFW (r=0.89**). The SB showed a positive correlation (r=0.90**) with LA, RFW, and SDW. LA was positively correlated with SFW (r=0.90**) and SDW (r=0.93**). Furthermore, SFW was positively correlated with RFW (r=0.93**). Very high positive correlations were observed among variations in Chl-a, Chl-b, and TChl. Variation in TCC was positively correlated with Chl-a (r=0.88**), Chl-b (r=0.76**) and TChl (r=0.85**). The variation in TAC was positively correlated (r=0.90**) with TCC. The Photosynthetic parameters significantly correlated with SL, RL, SB, LA, SFW, RFW, SDW, and RDW. More than (r=0.90**) strong positive correlation was observed in variation of NN, NS, and NW under salinity. NN, NS, and NW were positively correlated with all morphological and photosynthetic parameters except RTWC and STWC, which are negatively correlated.

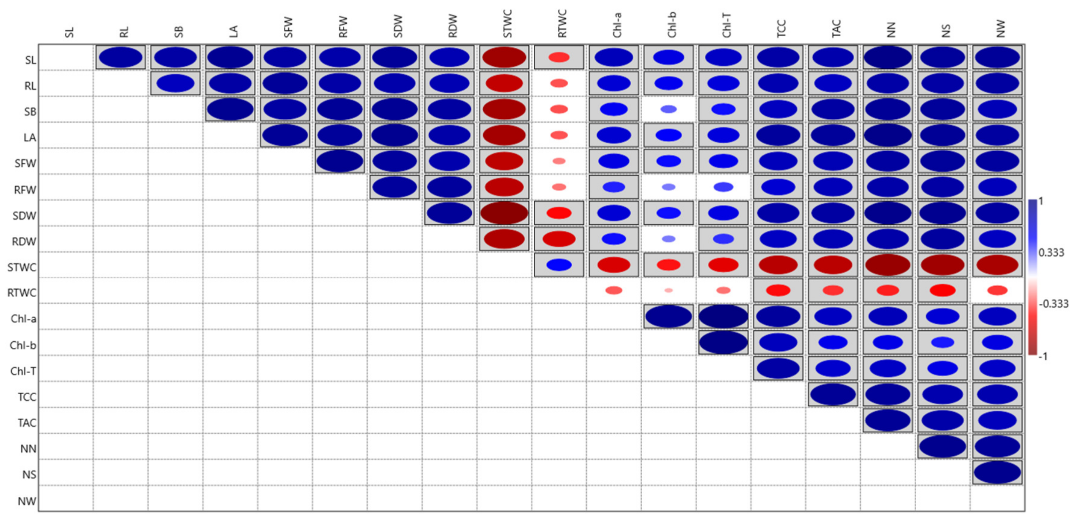

Figure 5. Pearson's correlation among variations in the morpho-physiological, photosynthetic and nodulation parameters in mungbean plants exposed to different concentrations NaCl for different durations. (Correlations significant at $p<0.05$ are boxed.)

At 45 days (E3) exposure time (Table S3b and Figure 6), the variations in NP and FWP were correlated significantly positively with each other by more than (r=0.90**) under salt stress. While variation in NSP and SW-1000 was significantly positively correlated with each other by more than (r=0.90**) under salt stress. Variations in seed parameters NP and FWP were significantly positively correlated with seed parameters by more than 72 and 80% under salt stress, respectively. Moreover, more than (r=0.90**) positive correlation was observed in NN, NS, NW, NP and FWP. Variation in NSP showed positive correlation with variation in NN (r=0.75*), NS (r=0.68), NW (r=0.80*), NP (r=0.71*) and FWP (r=0.80*) under salinity. Moreover, variation in FW-1000 was positively correlated with NN (r=0.83**), NS (r=0.75*), NW (r=0.90**). Changes in all pod and seed parameters showed a significant positive correlation with changes in morphological, photosynthetic, and nodule parameters except RTWC and STWC, which were negatively correlated under salinity. Thus, a decrease in morpho-physiological, photosynthetic, and nodulation parameters directly affect yield parameters.

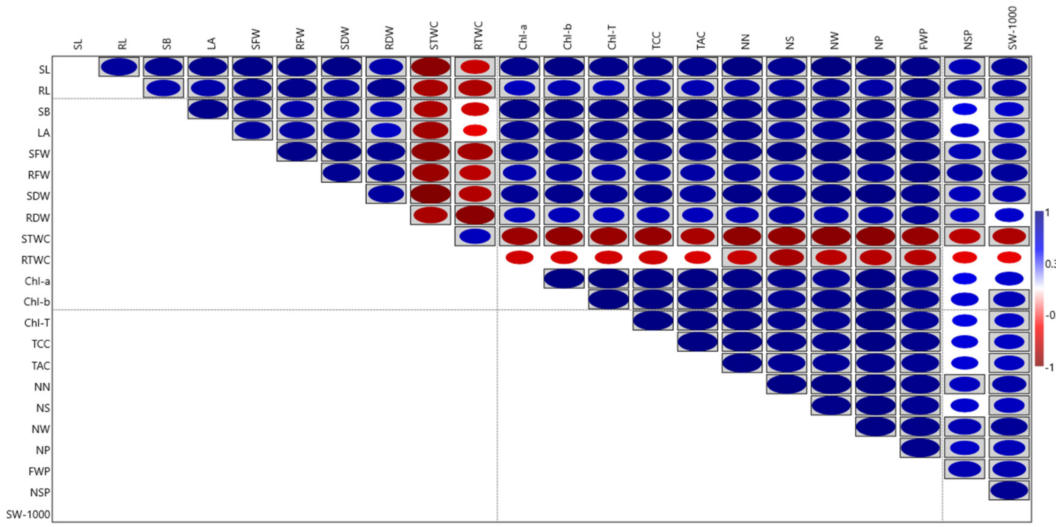

Figure 6. Pearson's correlation among variations in the morpho-physiological, photosynthetic and nodulation and yield parameters in mungbean plants exposed to different concentrations NaCl at 45 days after salt treatment. Correlation is significant at $p<0.05$ are boxed.

Discussion

Soil salinity limits the plant's growth and development through osmotic and ionic stress and reduces productivity (Arif *et al*., 2020). Mungbean [*Vigna radiata* (L.) R. Wilczek], one of India's economically important and significant dietary crop plants is susceptible to salt stress (Ghosh *et al*., 2015). Its production in the last decades was influenced mainly by its susceptibility to different biotic and abiotic stresses at various stages of growth, including the soil salinity (Sehrawat *et al*., 2015). The salinity equivalent to about 50 mM NaCl can reduce the yield by more than 60% (Abd-Alla *et al*., 1998). It is also suspected that increasing soil salinity will result in ~50% loss of arable land by the mid-21st century (Hasanuzzaman *et al*., 2012). Soil salinity mainly alters various morphological, photosynthetic, nodulation, and yield parameters, the extent of which varies with severity and duration of stress, and ultimately limits crop productivity (Elahi *et al*., 2004; Munns 2005; Ahmed 2009; Shelke *et al*., 2017). Differential responses to salinity at the varietal level were observed for morphology, photosynthesis, nodulation, and yield (Rao *et al*., 2002; Singla and Garg, 2005; Kumar and Singh, 2012; Chunthaburee *et al*., 2015; Sarabi *et al*., 2016; Shelke *et al*., 2017). Therefore, the present study was aimed to investigate morpho-physiological, photosynthetic, nodulation, and yield modulation in two mungbean varieties, 'PKV AKM 12-28' and 'VBN (Gg)3', under various salt regimes and exposure durations.

Soil salinity has significantly affected the morphological parameters such as shoot and root length, total biomass, plant height, and leaf growth in many crops (Dolatabadian *et al*., 2011; Morales *et al*., 2012). In the present investigation, modulations in SL, RL, SB, SFW, RFW, SDW, RDW, STWC%, RTWC%, and LA with the increasing NaCl concentrations of 75, 100, and 125 mM were observed. Principal component analysis (PCA) of the morphological dataset suggests that the parameters with significant loadings in the first principal component (PC1) are related to plant stress and may be considered 'stress factor.' A close association of SL, RL, SB, LA, SFW, RFW, SDW, and RDW showed a nearly 90% positive correlation with PC1, which indicates a reduction in these parameters under salinity. PCA finds structure in a multivariate dataset, identifies the most relevant parameters, uncovers the variance of a large dataset of inter-correlated variables and transform them into a smaller set of (uncorrelated) independent variables (principal components), and can discriminate samples of diverse biological groups (Singh *et al*., 2004; Sinha *et al*., 2009b; 2009a; Chunthaburee *et al*., 2015; Shelke *et al*., 2017). A simultaneous interpretation of the scores and loadings suggests significant changes in morphological parameters at all levels of salt stress. However, the plant's defense system is suppressed at higher salt concentrations and more prolonged exposures.

It was observed that 'VBN(Gg)3' is more affected under salinity stress than 'PKU-AKM 12-28'. The reduction SL, RL, SB, LA, SFW, RFW affects the absorption and transport of water and nutrient allocation from root to shoot, ultimately affecting plants' growth and development (Satti and Lopez, 1994; Sherif *et al*., 2007). Our results are in line with those of (Kamrul *et al*., 2018) and (Rahman *et al*., 2016). The DA is used to identify the variables which discriminate between two or more naturally occurring groups. It constructs a discriminant function (DF) for each group by analysing raw data (Singh *et al*., 2004; Sinha *et al*., 2009a; 2009b). In the present investigation, the DA results indicate RL, LA, RFW, SFW, SDW, and SL to be the most significant parameters to discriminate the four sets of the plant responses corresponding to four concentrations of NaCl. In contrast, the RL and RFW were the most critical parameters to distinguish among the three levels of stress exposure durations.

Salinity impairs the synthesis of plant pigments (Taïbi *et al*., 2016). Also, it reduces photosynthesis either due to a reduction in green pigments or inhibition of their synthesis (Najar *et al*., 2019). In PCA, a highly positive correlation of Chl-a, Chl-b, Chl-T, TCC, and TAC loadings to PC1 indicate that these photosynthetic parameters are closely related and affected under the increasing concentration of NaCl. The distribution pattern of scores and loading plots suggests a remarkable reduction in the photosynthetic parameters during moderate and more prolonged salt stress exposures. During the moderate exposure duration (30 days), significant induction in the defense mechanism was observed, worsened by prolonged exposure of 45 days. These results suggest an impaired stress defense mechanism with increasing levels of salt stress. These results align with those in *P. vulgaris* (Turan *et al*., 2007) and *Vigna subterranean* (Taffouo *et al*., 2010). An increase in the anthocyanin content was observed in the present investigation at a low salinity level. It could induce an active protective response under saline stress (Chutipaijit *et al*., 2009). Salinity also affected carotenoid contents in both varieties. These results corroborate with reports in P. vulgaris (Gadallah, 1999) and maize and wheat genotypes (Singh *et al*., 2008). The DA results indicate Chl-a to be the most significant attribute to discriminate the four sets of the plant responses corresponding to four concentrations of NaCl stress. These results also reveal the TAC and Chl-T as the most critical parameter to discriminate the three levels of stress exposure durations. In the present study, the photosynthetic pigments viz., chlorophyll a, b, and total chlorophyll, carotenoid, and anthocyanins decreased with increasing salinity. A more significant reduction was observed in 'VBN (Gg)3' as compared to 'PKU AKM 12-28'.

A reduction in nodulation parameters was observed with increasing salinity. Leguminous plants can fix atmospheric nitrogen through symbiotic association with soil bacteria (*Rhizobium* spp.) which form nodules on the roots of these plants. It is essential to understand the optimum conditions required for nitrogen fixation to provide full benefits to the plant (Kijne *et al*., 1995). Legume-Rhizobium symbiosis may get affected under salinity by inhibiting the bacterial infection process, reducing the survival of rhizobia, disrupting nodule development and function, or decreasing plant growth (Singleton and Bohlool, 1984). In the PCA analysis of the nodulation dataset, a close association of NN, NS, and NW was observed in PC1, which is indicative of a decrease in nodule number, nodule size, and nodule weight under NaCl stress. Scores indicated that salinity significantly affects plants at moderate and highest salinity and exposure time. However, it was affected more in 'VBN (Gg)3' than 'PKU-AKM 12-28'. These results corroborate earlier reports in other plant species. (Elsheikh and Wood, 1995) reported adverse effects of salinity on growth and nodulation in soybean and that nodulation was more sensitive than plant growth under saline conditions. A significant reduction was observed in the nodule number, nodule size, and nodule biomass under salinity in soybean (Singleton and Bohlool, 1984), Sesbania sesban (Mahmood *et al*., 2008). Mirza and Tariq (1993a,1993b) also reported adversely affected nodulation of *Cicer arietinum* and *Trifolium alexandrinum* under salt stress. The DA results in the present investigation indicate NW to be the most significant parameters to discriminate between the four sets of the plant responses corresponding to four concentrations of NaCl stress. These results also reveal that NS and NW are the most critical parameters to discriminate the three levels of stress exposure durations.

Our results revealed a decrease in yield (pod and seed) parameters as salt concentration increased. Salinity adversely affects the economic yield of the crop (Sarin, 1975). Reduced pod number may be one of the main parameters to measure the quantitative yield. PCA revealed that studied yield parameters had a close association with each other and showed more than 90% positive correlation with PC1 under salinity, thus indicating that all these parameters are significantly reduced under salinity. Furthermore, nodulation in 'VBN (Gg)3' was affected more than 'PKV-AKM 12-28'. Gill (1979) observed similar results in barley yield under salinity. Reduction in yield under salinity has been reported in many crops such as rice, cotton, bean, barley, and wheat (Keating and Fisher, 1985). Reduced dry matter and grain yield were reported in sorghum cultivars by Maas *et al*. (1986). Elahi *et al*. (2004) also reported a reduction in pod number and pod fresh mass under increasing salinity levels in mungbean. The DA results indicate NP and NSP to be the most significant parameters to discriminate between the four sets of the plant responses corresponding to four concentrations of NaCl stress.

Furthermore, MCA revealed a significant positive correlation between photosynthetic and growth parameters. Taiz and Zeiger (1998) have suggested that plant growth depends on photosynthesis. Therefore, environmental stresses affecting photosynthesis, in turn, reduce growth. Fisarakis *et al*. (2001) remarked that a decline in photosynthesis under salinity inhibits vegetative growth. Significant Positive correlations were observed among photosynthetic and yield parameters. Less green leaves, leaf expansion, production, and senescence result in less photosynthetic activity, which may be why the yield is reduced under salinity (Ahmed, 2009). There was observed a positive correlation between nodulation and yield parameters. It indicates that the effects of salinity on nodulation can ultimately reduce yield and production. Our result corroborates with the observations of Rao *et al*. (2002), who suggested that the grain production in legumes is reduced because of their low salt tolerance in combination with the high sensitivity of the symbiotic nitrogen fixation process under stress.

Applying a multivariate modelling technique to analyse the effects of salt stress on morpho-physiological, photosynthetic nodulation, and yield parameters in two mungbean varieties thus demonstrated the grouping of variables and their interrelationship. This technique also identified significant differences in variables responsible for differential behaviour. It also identified significant parameters responsible for differential behaviour. Such an interpretation is not possible by using conventional methods.

Conclusions

These analytical tools revealed differential patterns for morpho-physiological, photosynthetic, nodulation, and yield changes in the mungbean varieties 'PKU-AKM 12-28' and 'VBN(Gg)3'. These tools extracted the patterns of variations in significant morpho-physiological, photosynthetic, nodulation, and yield parameters and their inter-relationships under NaCl stress. The multivariate modelling approach identified morpho-physiological (RL, LA, RFW, SFW, SDW, SL, RL, RFW) and photosynthetic (Chl-a, Chl-T, and TAC), nodulation (NN, NS, and NW), and yield (NP, FWP, NSP and FW-1000) as critical parameters that can discriminate tolerant varieties. This analysis also identified positive correlations among yield and other morpho-physiological, photosynthetic, nodulation parameters. Moreover, it also revealed the level of salt tolerance in selected mungbean varieties and confirmed 'PKU-AKM 12-28' as salt-tolerant and 'VBN(Gg)3' as salt susceptible variety. This multivariate modelling approach can be used to understand the complex datasets on nodulation and yield, their interrelationships, and visualization of relationships among other variables.

Authors' Contributions

GDM: Performed the experiments, collected and analysed the data and prepared draft manuscript, URW: Analysed data and prepared draft manuscript, KBR: Performed the experiments and collected data, DBS: Analysed data and prepared draft manuscript, PSM and MLA: 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.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

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

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