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Full Length Research Paper

Genetic analysis of Myanmar *Vigna* species in responses to salt stress at the seedling stage

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Twelve (12) *Vigna* genotypes were investigated for the evaluation of their tolerance levels in responses to four concentrations of NaCl (0, 75, 150 and 225 mM) at seedling stage. In the investigation, salt stress inhibited almost all the growth parameters as well as relative water content; however, the degree of reduction was highly dependent on different genotypes and salinity levels. Generally, the control plants showed higher degree of all measured parameters than those of salt stress plants. Analysis of the heredity parameters based on the 12 investigated genotypes showed different genotypic variance of the salt tolerance index (STI) values. Salinity stress induced two new bands between 45 and 22 kDa, respectively, in salt tolerant genotypes. Furthermore, band intensity of the salt treated genotypes was higher than the control plants. Ward's clustering technique was clearly divided into two clusters, A and B, according to their levels of salt tolerance. Considering their STI values of growth parameters, two genotypes V7 and V4 were identified as salt tolerant, whereas, V2, V6, V9, V8, V11 and V1 were recognized as salinity susceptible genotypes. These results suggest that, the genetically diverse accessions resistant to salt stresses within the *Vigna* genotypes can be of considerable practical value for studying the mechanism of salt tolerance and for the provision of genetic resources for salinity breeding program.

Key words: Cluster analysis, heritability, salt tolerance, SDS-PAGE, *Vigna*.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilezek) and black gram (*Vigna mungo* L. Hepper) belonging to the subgenus *Ceratotropis* in the genus *Vigna* are economically important cultigens in Asia. Myanmar has become the second largest beans and pulses exporter in the world after Canada and topped beans exporter in Asia (Xinhua, 2008). Being the major pulses in Myanmar, mungbean and black gram account for 45% of the total food legume area (2.5 million ha). In Myanmar, Ayeyawaddy and Bago divisions are one of the five major pulses growing areas. The climatic condition of these areas (delta region) is tropical wet due to monsoon from the Bay of Bengal. Major problem of pulses production in delta regions is the intrusion of sea water.

Salinity, one of the most important abiotic stresses, is a global problem and out of 230 million hectare irrigated land of the world, 45 million hectare was salt affected. Approximately 400 million ha throughout the world are affected by salinity (FAO, 2005). Soil salinity is notorious, reduces crop yield drastically and has serious detrimental effect on productivity. Legumes are among the most sensitive plants to salinity (Rogers et al., 2005). The threshold and slope of mungbean (*V. radiata*) are 1.8

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dSm^{-1} and 20.7% per dSm^{-1} of saturated soil extract (ECe), respectively (Maas, 1990). Salt stress can affect plant height, plant survival and affect the capacity of plants to collect water and nutrients (Jaleel et al., 2007). Every aspect of the morphological, physiological and biochemical pathway is strongly related with soil salinity which affects plants yield. Limited knowledge of heritability and the genetic mode of salinity tolerance had resulted in slower progress in crop improvement for salt tolerance because few studies have yet been conducted in these areas. This study analyzed the heredity parameters of 12 *Vigna* genotypes based on their STI values which are the ratios between the observed values with and without salt treatment. It is an indicator of salt tolerance in many crops and can be used as a reliable criterion for ranking genotypes for their salinity tolerance. In this study, critical information about salt tolerance in different cultivars of *Vigna* accessions was obtained based on the comparison of genetic parameters. There are multiple genes that seem to act in concert to increase NaCl tolerance and certain proteins involved in salinity stress protection have been recognized (Bohnert and Jensen, 1996; Hare et al., 1996). Biochemical genetic marker such as SDS-PAGE was substantially involved in drought and salinity stresses (Rahman et al., 2007).

In order to make effective utilization of salt affected soils, it is necessary to select ideal legume genotypes, which may be tolerant to salt stress and produce substantial yield under saline environment. Shannon (1997) reported that considerable inter- and intra-crop diversity in salt tolerance emphasizes the need to identify crop genotypes that are adaptable to saline conditions. The most common approach to identify sources of variability for salt tolerance breeding has been investigation among primitive cultivars, landraces, wild species and world collections for those which exhibit characteristics for salt tolerance. It is believed that *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* are ancestors of mungbean and black gram, respectively (Arora et al., 1973; Chandel, 1984; Miyazaki, 1982). Myanmar has the huge potential for legume production through sustainable use of wide genetic resources which have tolerance to abiotic and biotic stress on potential land. This study is the first report on the determination of genetic analysis of salt tolerance among the Myanmar *Vigna* genotypes collected over a wide geographical range at seedling stage. Although, the salt tolerance level depends on the different growth stage, screening and selection for any characters are desired at the earliest developmental stage if possible (Murillo-Amador et al., 2001). The objective of this study was to find the salt tolerance levels of twelve (12) *Vigna* genotypes at different levels of NaCl concentration.

MATERIALS AND METHODS

Plant materials

A pot experiment was conducted to assess the different levels of salt tolerance among the *Vigna* genotypes at different levels of salt concentration. Twelve (12) *Vigna* genotypes including 5 accessions each of mungbean (*Vigna radiata*), black gram (*Vigna mungo*) and one each of their wild relatives, *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* were used in this study (Table 1). The *Vigna* genotypes used in this study included genetically distinct approved varieties and promising advanced lines obtained from seed bank, Myanmar. Furthermore, all these genotypes have been widely cultivated in Myanmar. Seeds were surface sterilized using 70% ethanol for 2 min. Then, seeds were rinsed thoroughly in sterilized water. Ten seeds were sown in each earthen pot (18 x 20 cm) filled with commercial peat soil. They were germinated in a green house located at Tokyo University of Agriculture and Technology, under natural conditions; a daytime temperature of 24 to 30°C. Thinning was done one week after crop emergence leaving 5 seedlings per pot. At the appearance of the first trifoliate leaf, seedlings were subjected to salt stress by the addition of 0, 75, 150 and 225 mM NaCl for 21 days. There were three replications per NaCl treatment and the control (no treatment with NaCl, using tap water).

Growth measurement

After 21 days of treatment application, the data were collected. Each individual plant was measured for its shoot length, root length, plant height and leaf number. The leaf area was determined with an automatic area meter (AAM-8, Hayashi Denko Co., Japan). Shoot and root fresh weight was determined and plant was subjected to oven drying (70°C for 72 h) to the get dry weight.

Chlorophyll content

After 21 days of stress, the relative chlorophyll content of the second leaf was measured using a SPAD (Soil plant analysis development) analyzer (Minolta, by Hydro Agri, Dülmen, Germany) which measures transmission of wavelengths absorbed by chlorophylls in intact leaves (mid position). Each replication was measured 30 times and the mean value was used for analysis.

Measurement of plant water status

Leaf water relations were measured after 21 days of treatment application. Relative water content (RWC) and water uptake capacity (WUC) were readily determined by obtaining the fresh weight or field weight of fresh leaf (the second leaf) and then, measuring its turgid weight after equilibration (floating tissue on water or placing it on water-saturated polyurethane foam in a moist chamber) for a prescribed period of time. The same tissue was oven-dried to a constant weight and the leaf water statuses were determined by using the following equations;

Relative water content (RWC %) = [(fresh weight – dry weight) / (turgid weight – dry weight)] x 100

Water uptake capacity (WUC) = Turgid weight – fresh weight / dry weight

Salt tolerance index (STI)

Following Zeng et al. (2002), all the data were converted to salt tolerance indices before the cluster analysis to allow comparisons among the genotypes for salt tolerance by using the measured growth parameters. A salt tolerance index was defined as the observation at salinity divided by the average of the controls.

Table 1. Cultivar name and origin of *V.radiata*, *V. mungo* and their wild relatives.

Name	Cultivar name	Code no.	Origin
V1	Yezin 6 (*V. radiata*)	004180	Myanmar
V2	Yezin 9 (*V. radiata*)	004182	Myanmar
V3	Yezin 2 (*V. radiata*)	004143	Myanmar
V4	VC5805A (*V. radiata*)	004183	Myanmar
V5	Local (Sagaing) (*V. radiata*)	7666	Myanmar
V6	*V. radiata* (Var. *sublobata*)	107875	National Institute of Agricultural Science (NIAS), Japan (India origin)
V7	U Taung-2 (*V.mungo*)	003917	Myanmar
V8	Mut Pe Khaing To (*V. mungo*)	003919	Myanmar
V9	Mut Pe Lone Gyi (*V. mungo*)	003920	Myanmar
V10	Min Hla Tun (local) (*V.mungo*)	003935	Myanmar
V11	Min Hla Lone Gyi (*V. mungo*)	007341	Myanmar
V12	*V. mungo* (Var. *silvestris*)	107874	National Institute of Agricultural Science (NIAS), Japan (India origin)4

Estimation of genetic parameters for STI values

Genetic parameters for STI values were estimated by using the following formulae (Singh and Chaudhary, 1977);

$$\text{Genotypic coefficient variation (GCV \%)} = \text{genetic variance } (\delta g2) \,/\, \text{total mean value } (\bar{x})$$

$$\text{Phenotypic coefficient variation (PCV \%)} = \sqrt{\delta p2}/\bar{x}$$

$$\text{Phenotypic variance} = \text{genetic variance } (\delta g2) + \text{environmental variance } (\delta e2)$$

$$\text{Broad heritability } (h2\ \%) = (\delta g2) \,/\, (\delta p2)$$

$$\text{Genetic gain} = i\sqrt{(\delta p2.\ \sqrt{h2}};$$

$$\text{Relative genetic gain} = i\sqrt{(\delta p2.\ \sqrt{h2}/\bar{x}}$$

SDS-PAGE analysis

Protein extraction was performed using upper young leaves from the treated plants. Protein electrophoresis and SDS polyacrylamide gel electrophoresis was performed in 12% acrylamide slab gels according to Laemmli (1970). For gel analysis, gel was photographed, scanned and analyzed using Gel Doc 2000 Bio Rad system.

Statistical analysis of data

All analyses were completely randomized. Data were analyzed statistically following the ANOVA technique to determine the effects of the treatments. Ward's minimum variance clustering method was used to classify accessions into discrete clusters (Romersburg, 1988).

RESULTS

Effect of salt stress on morphological traits

The growth parameters of the 12 *Vigna* genotypes exhibited differential responses to different levels of the imposed salinity stress in this study. Generally, plant height, leaf number, shoot and root length, chlorophyll content, shoot and root fresh weight, shoot and root dry weight and leaf area decreased with increasing salinity. Shoot and root fresh weights of the control plants were commonly higher than those for salt stress plants. Compared with the control plants, relatively higher amounts of shoot fresh weight was observed in V4, V7 and V5. Varieties V4, V7 and V11 showed higher amounts of root fresh weight among the 12 varieties at different levels of NaCl. Chlorophyll contents of leaves were significantly responsive to different levels of salt stress in all genotypes. Salinity stress on root dry weight was sharper than those of the shoot dry weight in all genotypes. The relative salt tolerance indices (STI) for all the measured parameters varied among the genotypes (Table 2). Salt tolerance index value of plant height ranged from 0.93 to 0.62, at low salinity and from 0.80 to 0.40 at the highest salinity. At low salinity, reduction of chlorophyll content was not shown significantly, but it varied significantly from 0.90 to 0.65 at the highest salinity among the genotypes. Salt tolerance indices ranged from 0.94 to 0.44 for shoot fresh weight and from 0.88 to 0.48 for root fresh weight at low salinity and from 0.73 to 0.23 for shoot fresh weight and from 0.74 to 0.21 for root fresh weight at the highest salinity.

Effect of salt stress on leaf water status

The effect of salt stress on leaf water status varied with cultivars and the parameters measured (Figure 1). The relative water contents (RWC) were decreased in the leaves of *Vigna* accessions grown at a high salinity when compared with the unstressed control plants. All cultivars showed similar RWC in each control (0% NaCl). Under the highest salinity stress, V12 showed the highest RWC, while V3 was observed as the lowest. As opposite to RWC, water uptake capacities (WUC) were increased by increasing salinity in all treatments (Figure 1b).

Heredity parameters of the STI values

In order to compare the behavior of genetic heritability

Table 2. Salt tolerance indices of agronomic parameters in 12 *Vigna* species under different salinity levels.

Genotype	Salinity levels (mM NaCl)	Plant height	Leaf number	Root length	Shoot length	Chlorophyll content	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight	Leaf area
V1	75	0.71	0.83	0.99	0.78	0.94	0.68	0.49	0.61	0.41	0.54
	150	0.67	0.78	0.81	0.72	0.85	0.38	0.37	0.56	0.41	0.48
	225	0.62	0.67	0.91	0.65	0.86	0.25	0.32	0.34	0.36	0.45
V2	75	0.91	0.69	0.74	0.75	1.01	0.48	0.86	0.60	0.93	0.52
	150	0.86	0.65	0.59	0.60	0.86	0.43	0.78	0.43	0.71	0.43
	225	0.58	0.62	0.55	0.43	0.68	0.45	0.47	0.38	0.40	0.31
V3	75	0.93	0.81	0.98	0.90	0.99	0.59	0.50	0.85	0.95	0.70
	150	0.78	0.74	0.71	0.86	0.90	0.42	0.37	0.71	0.55	0.59
	225	0.66	0.59	0.80	0.65	0.90	0.31	0.44	0.57	0.44	0.45
V4	75	0.84	1.17	0.80	0.96	1.00	0.80	0.94	0.94	1.17	0.93
	150	0.73	0.75	0.79	0.81	0.94	0.77	0.78	0.83	0.83	0.90
	225	0.72	0.67	0.78	0.75	0.87	0.74	0.65	0.78	0.73	0.80
V5	75	0.87	0.77	0.99	0.85	0.99	0.76	0.73	0.89	1.00	0.37
	150	0.85	0.62	0.92	0.81	0.96	0.41	0.56	0.71	0.74	0.22
	225	0.80	0.62	0.75	0.79	0.86	0.47	0.50	0.58	0.70	0.26
V6	75	0.89	0.86	0.71	0.73	0.96	0.66	0.78	0.60	0.59	0.89
	150	0.77	0.73	0.66	0.46	0.92	0.63	0.62	0.56	0.56	0.48
	225	0.63	0.62	0.60	0.39	0.85	0.47	0.48	0.48	0.49	0.39
V7	75	0.85	0.75	0.92	0.88	1.01	0.88	0.92	0.88	1.05	0.82
	150	0.76	0.69	0.90	0.81	0.99	0.84	0.81	0.72	0.95	0.76
	225	0.68	0.63	0.72	0.81	0.80	0.72	0.73	0.67	0.83	0.71
V8	75	0.62	0.78	0.76	0.59	0.94	0.65	0.47	0.50	0.43	0.53
	150	0.55	0.73	0.68	0.51	0.90	0.47	0.45	0.47	0.42	0.34
	225	0.40	0.64	0.55	0.43	0.79	0.22	0.27	0.26	0.26	0.26
V9	75	0.74	0.85	0.67	0.75	0.93	0.62	0.53	0.51	0.71	0.59
	150	0.72	0.64	0.61	0.69	0.91	0.47	0.52	0.33	0.51	0.60
	225	0.60	0.57	0.47	0.56	0.84	0.28	0.40	0.32	0.52	0.35
V10	75	0.83	0.75	0.95	0.85	1.01	0.65	0.68	0.68	0.93	0.47
	150	0.81	0.64	0.91	0.69	0.94	0.61	0.56	0.44	0.63	0.24
	225	0.66	0.63	0.74	0.65	0.89	0.41	0.45	0.41	0.47	0.24
V11	75	0.62	0.78	0.96	0.73	1.01	0.85	0.59	0.89	0.64	0.47
	150	0.61	0.66	0.88	0.68	0.93	0.66	0.47	0.49	0.43	0.37
	225	0.46	0.60	0.78	0.59	0.88	0.39	0.33	0.17	0.28	0.24
V12	75	0.77	0.94	0.88	0.65	0.96	0.56	0.44	1.03	0.97	0.86
	150	0.69	0.66	0.79	0.65	0.90	0.54	0.45	0.86	0.78	0.26
	225	0.52	0.51	0.70	0.56	0.65	0.21	0.23	0.32	0.30	0.28

parameters between the treatment with and without salt stress, estimation of the heredity parameters of STI values for 12 traits in 12 *Vigna* accessions at 75 mM of NaCl was observed (Table 3). High genotypic coefficient of variation (GCV %) were observed in plant height, shoot fresh weight and shoot length, whereas, shoot dry weight, chlorophyll content and leaf number showed low genotypic coefficient of variation. The value of the phenotypic coefficient of variation (PCV %) varied from 90.23 (plant height) to 23.10 (shoot dry weight). The higher value of heritability means more heterogeneous and higher variability of the population. The highest broad heredity of STI values were observed in plant height, chlorophyll content, root length, leaf area, shoot fresh weight and root dry weight. The genetic gains were calculated at a selection intensity of 10 and 50% for all the measured parameters. The genetic gain ($K_{0.01}$) calculated from (selection-population mean) plant height was 1.50 and

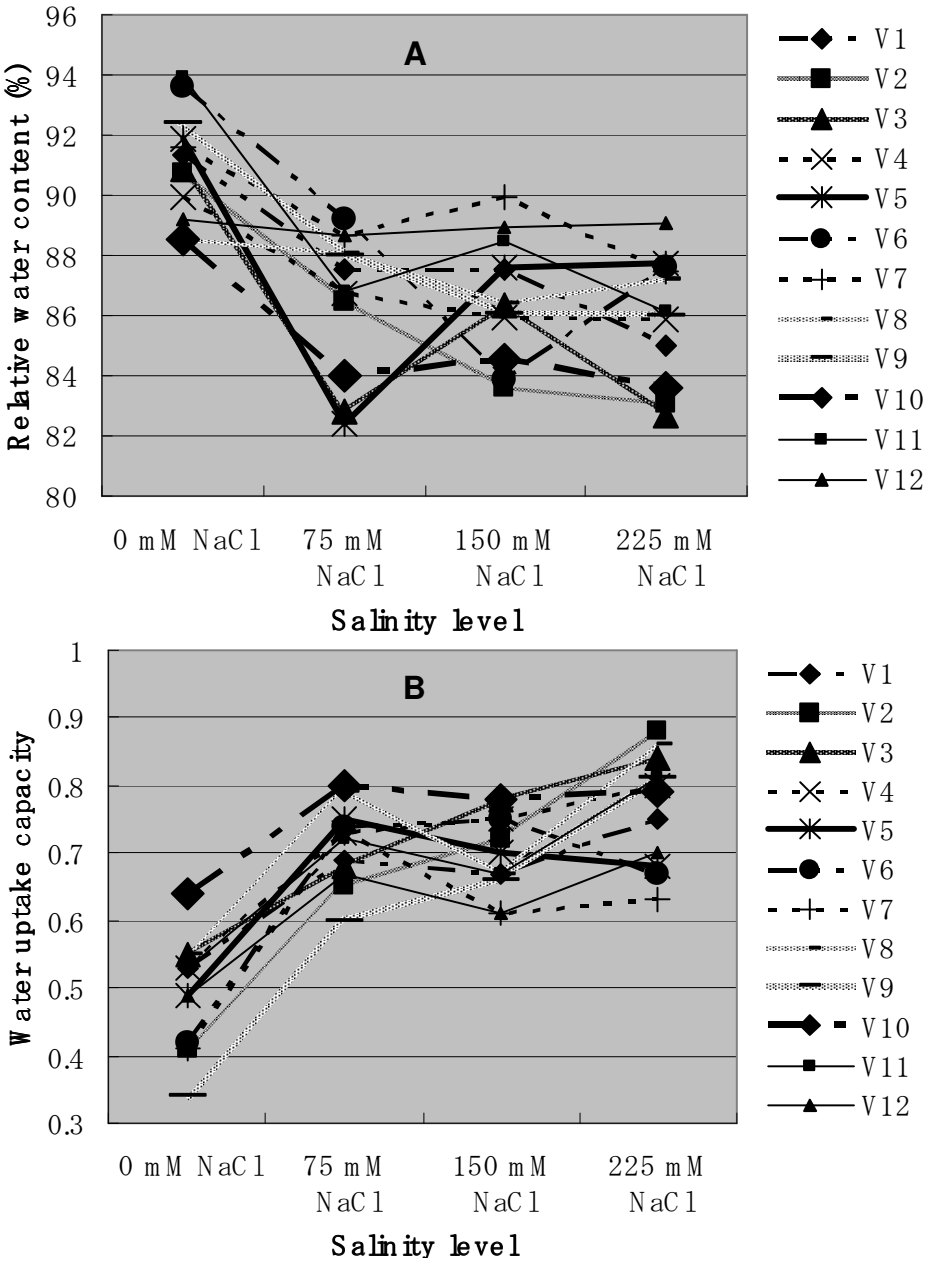

Figure 1. Effect of salinity stress on leaf water status; (A) relative water content %; (B), water uptake capacity of 12 *Vigna* genotypes.

0.31 in shoot dry weight.

Cluster analysis based on the STI values

Ward's clustering technique clearly defined the cluster based on the 12 measuring parameters at different levels of salinity. Cluster analysis for 12 *Vigna* accessions based on STI values at different salinity levels was illustrated by the dendrogram (Figure 2). All the *Vigna* accessions studied were grouped into mainly two groups; A and B, on the basis of Ward's distance ranges. The latter was further divided into two sub-clusters; B1 and B2. The cluster A was composed of 2 genotypes and that of cluster B consisted of 10 genotypes. According to the reduction percentage in the 12 *Vigna* genotypes' parameters, clustering analysis showed that V9, V6, V2, V8, V11 and V1 grouped in sub-clusters B2 had reduction in some parameters as a result of salt stress, whereas, V4 and V7 (group A) showed the highest values of all the measured parameters at different levels of salinity. In group B, genotypes V10, V5, V12 and V3 of the sub-

Table 3. Estimation of heredity parameters for salt tolerance indices for different traits in 12 *Vigna* genotypes at 75 mM salinity levels.

Trait	Mean	Range	h^2%	GCV (%)	PCV (%)	Gg		RGg	
						$k_{0.05}$=2.06	$K_{0.01}$=2.64	$k_{0.05}$=2.06	$K_{0.01}$=2.64
Plant height	0.80	0.61-0.92	79.07	80.23	90.23	1.17	1.50	1.46	1.88
Leaf no.	0.84	0.69-1.16	68.85	24.40	29.40	0.35	0.44	0.41	0.53
Root length	0.86	0.67-0.99	75.00	25.48	29.41	0.39	0.50	0.45	0.58
Shoot length	0.79	0.59-0.96	67.50	36.03	43.85	0.48	0.61	0.60	0.78
Chlorophyll content	0.98	0.93-1.01	79.41	23.71	26.61	0.42	0.54	0.43	0.55
Root fresh weight	0.68	0.48-0.88	64.42	29.04	35.11	0.32	0.41	0.48	0.61
Shoot fresh weight	0.66	0.44-0.95	72.57	54.00	63.38	0.62	0.80	0.94	1.21
Root dry weight	0.75	0.50-1.03	71.93	27.00	31.83	0.35	0.45	0.47	0.60
Shoot dry weight	0.81	0.40-1.17	62.86	18.31	23.10	0.24	0.31	0.29	0.38
Leaf area per plant	0.64	0.37-0.93	75.00	29.65	34.23	0.33	0.43	0.52	0.67
RWC	0.95	0.82-1.03	57.01	25.99	34.43	0.30	0.38	0.31	0.40
WUC	1.46	1.12-2.18	69.09	26.70	32.13	0.46	0.58	0.31	0.40

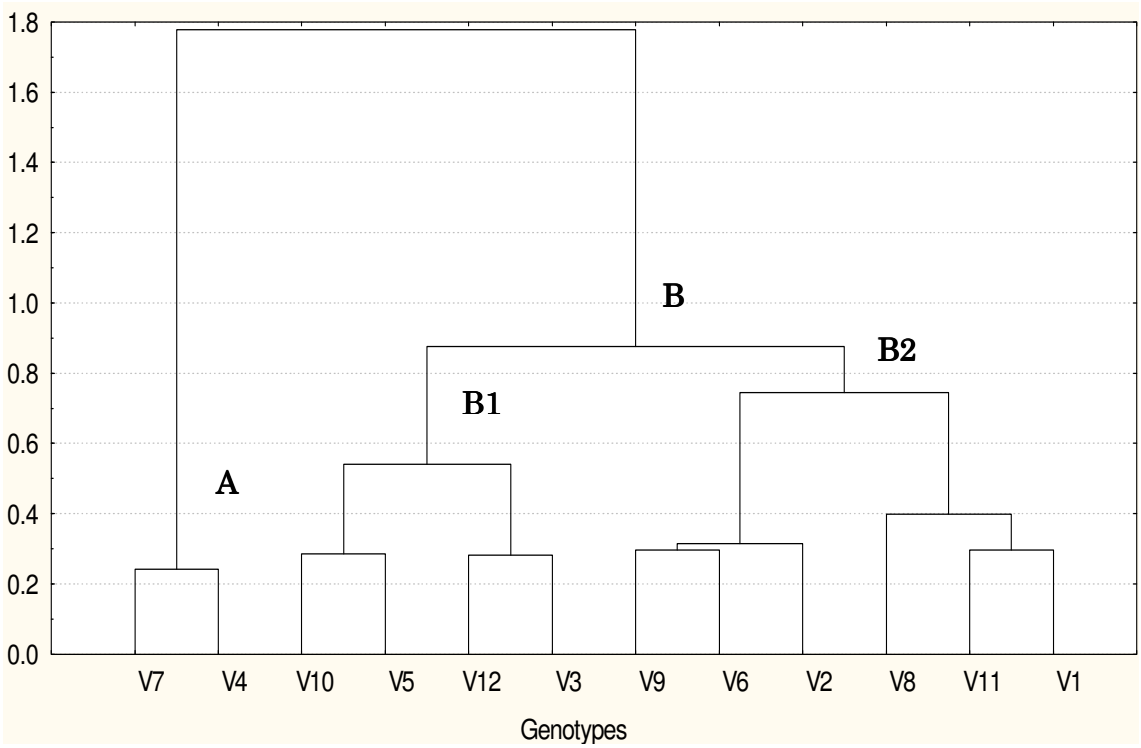

Figure 2. Dendrogram of the 12 *Vigna* genotypes clustered using salt tolerance indices of Ward's distances.

cluster B1 had intermediate level of salt resistance and were regarded as moderate.

Changes in protein profiles

The electrophoretic patterns of *Vigna* accessions studied in this investigation were used to detect the differentiation among the accessions and the treatments of NaCl. The pattern bands of 45 kDa on the gels were stained more intensely in all the *Vigna* accessions studied (Figure 3). Salinity stress induced 2 new bands between 45 and 22 kDa, respectively in salt tolerant genotypes (V4 and V7) and some moderate genotypes (V12). No new band was detected between the controls and salt treated plants of salt sensitive genotypes (V9). Besides, band intensity of the salt treated genotypes was higher than the control plants in all genotypes.

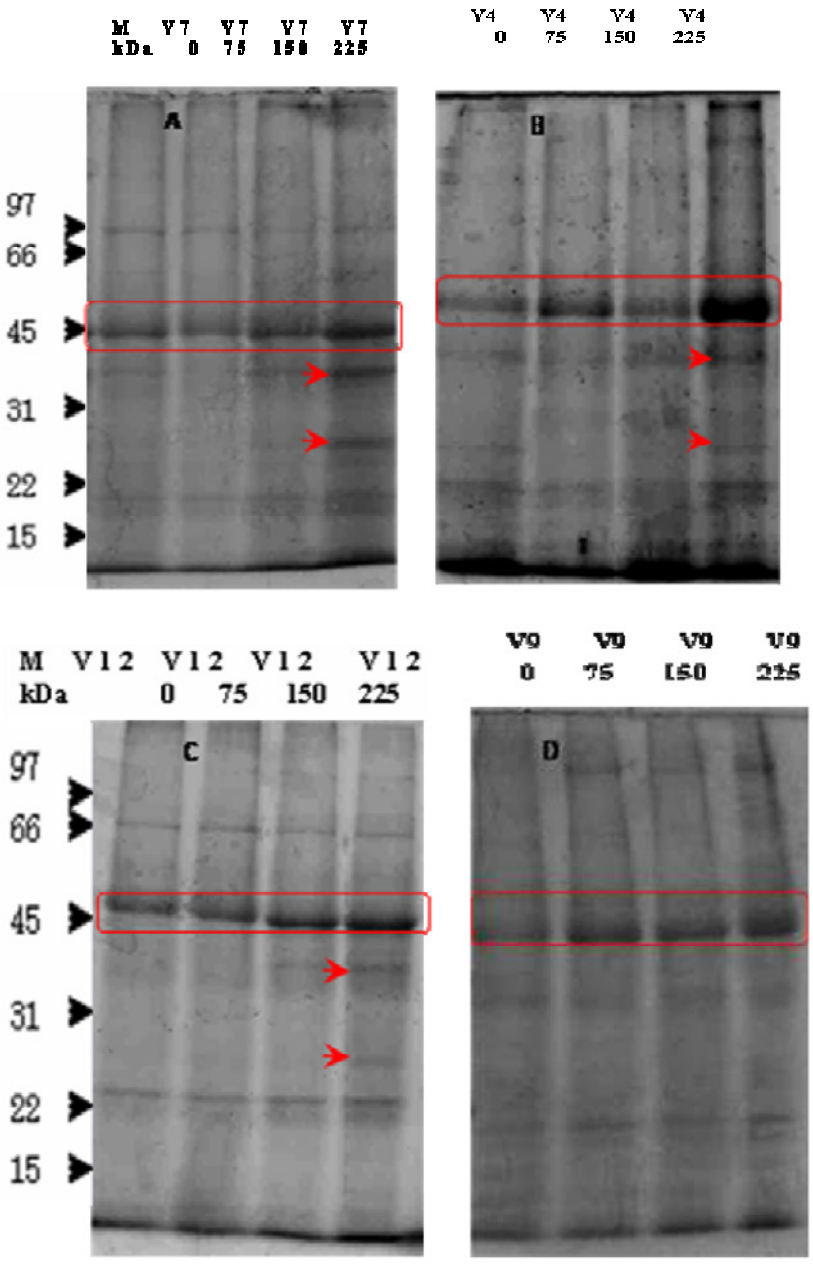

Figure 3. Protein profile of the control and salt treated *Vigna* genotypes. (A) and (B) salt tolerant genotypes;(C) ,moderate genotypes; (D), salt sensitive genotypes.

DISCUSSION

Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994). Some grain and legume crops such as sorghum, maize, barley, rice, cowpea and wheat, are salinity tolerant at germination, but sensitive at the seedling and early vegetative growth stages, but again become tolerant at maturity (Akbar and Yabuno, 1977; Ashraf, 1994). It is a good reason to screen the germplasm accessions and breeding material for salt tolerance where the plant is only sensitive at one particular growth stage. The twelve (12) genotypes of *Vigna* displayed distinct responses to a prolonged salt stress. The three salinity levels retarded markedly plant height, leaf number, shoot length, root length, chlorophyll content, shoot fresh weight, root fresh weight, root dry weight, shoot dry weight and leaf area, as well as the percentage of water content of *Vigna* plants. This research was in agreement with the works of other researchers who stated that a progressive gradual decrease in seed germination, plant

height, shoot and root length, dry matter, biomass, root, stem and leaf weights were observed with progressive increase in salinity stress of mungbean plant (Misra et al., 1996; Maity et al., 2000; Misra and Dwivedi, 2004; Rabie, 2005; Raptan et al., 2001; Yupsanis et al., 2001). In this study, the different degree of salinity tolerances among the 12 *Vigna* accessions at the three levels of NaCl confirmed the significant differences among the *Vigna* accessions in all the growth parameters. Varieties differ considerably in their susceptibilities to salinity (Castro and Sabado, 1977). The presence of variation in salt tolerance had been reported in different crops; in wheat (Ashraf and Shahbaz, 2003; El-Hendawy et al., 2005; Ali et al., 2007), tomato (Turhan et al., 2009; Alian et al., 2000), green gram (Misra and Dwivedi, 2004) cowpea (Murillo-Amador et al., 2006), pepper (Aktas et al., 2006) and rice (Mohammadi-Nejad et al., 2008; Bhowmik et al., 2009). At higher level of salinity (225 mM), chlorophyll content of all genotypes showed serious decrease symptoms. The decrease of chlorophyll content by salt stress has been well recognized in many plants (Hernandez et al., 1995; Mitsuya et al., 2002; Hasan et al., 2005) and was considered as one of the indicators of salinity stress (Chen et al., 1998). In this study, RWC dramatically was decreased and WUC increased when NaCl was applied at different rates. Similar results were reported by Kabir et al. (2004). The decreased RWC under saline condition was also reported by Nandwal et al. (2000) in mungbean. It was suggested that the increase of water uptake capacity under the salt stress, promoted the plant that has been suffering water stress at a greater degree. The low solute potential in the cell sap might pull more water to reach turgidity under saline condition. Two major factors might be involved in soil-water salinity which inhibits plant growth and development. Firstly, salt particles reduce the capacity of water potential in the cell sap and this leads to slower growth and development. Secondly, salt concentration inside the plant cell causes toxicity effect which retards plant growth. Plants initially adjust to saline conditions by decreasing tissue water content through osmotic adjustment (Marschner, 1995). Therefore, water status is highly sensitive to salinity and is dominant in determining the plant responses to stress (Stepien and Klobus, 2006).

Knowledge of the genetic variability of traits is greatly important and essential for selection and breeding in crop improvement. In this study, plant height, shoot fresh weight and shoot length showed high genotypic coefficient of variation and relative genetic gains. Breeding and selection for these traits is feasible for the stability of salt tolerant cultivars to pass them into generation to generation. The quantification of the variability and estimates of genetic parameters are highly important, since they reveal more about the genetic structure of a population, aiding in appropriate decisions making on the selection methods to be chosen. In contrast, root length, root dry and chlorophyll content were observed as low genotypic and phenotypic variances but high heredity. Mistrol et al. (2004) stated that the genotypic coefficient of variation (GCV %), which express the amount of genetic variation in percentage of the general mean, are of great importance for genetic improvement programs. Exploitation of natural genetic variations, either through direct selection in stressful environments or through the mapping of quantitative trait loci (QTLs – regions of a genome that are associated with the variation of a quantitative trait of interest) is one of the basic genetic approaches that are currently being used to improve stress tolerance (Foolad, 2004; Flowers, 2004; Lindsay et al., 2004). The twelve (12) *Vigna* genotypes expressing wide range of genotypic variance of STI value based on analysis of the heredity parameters will provide the practicable and theoretical values for breeding of salt tolerant cultivars in *Vigna* genotypes.

Salt stress, increased protein bands intensity and induced some new bands. It is believed that stress-induced proteins allow plants to make biochemical and structural adjustment that enable them to cope with the stress conditions (Ricard et al., 1996). However, proteins produced under salt stress are not always associated with salt tolerance. It is suggested that stress protein could be used as important molecular markers for the improvement of salt tolerance using genetic engineering techniques (Pareek et al., 1997). The salt induced protein bands detected varied among the crops, for example 26 kDa in tobacco (Singh et al., 1987), 22 kDa in radish (Lopez et al., 1994), 54 kDa and 23-24 kDa in finger millet (Uma et al.,1995), etc. In this investigation, salt induced bands were found between 22 and 45 kDa in salt tolerant and some moderate genotypes. Ashraf and Harris (2004) also stated that, the most prominent was the induction of a 25 kDa protein and an increase in the amount of a 33 kDa protein. The results indicated that increasing of protein band patterns exposed to salt stress was relatively genotype dependent. Salt stress-specific proteins cause either increases (Dubey, 1982) or decreases (Levitt, 1972) in the level of total and/ or soluble proteins. Furthermore, salt tolerance and salt sensitive genotypes have different patterns of protein profiles (Rani, 1988). Salt tolerant cultivars (V7, V4) and some moderate genotype (V12) showed higher band intensity and an increase in new bands when compared with the other cultivars. This led to the suggestion that, protein bands accumulation can be used as an indicator in the selection of salt tolerant cultivars. This result is in agreement with those of Abdel-Haleen, (2007), who reported an increase in protein band which might be involved in mungbean tolerance. Increase of protein profile under salinity stress, especially at 225 mM NaCl suggested that salinity promotes the fixation of inorganic nitrogen into protein, thus, favoring protein synthesis (Dorgham, 1991). However, opposite result was reported by Beltagi et al. (2008) who stated that reduction of protein bands was observed from untreated plants to NaCl stress plants. Evaluation of selected accessions at the three salt-stress levels was

clustered into 2 distinct groups based on their STI values. The accessions which make up group A in the cluster analysis correspond to the most salt-resistant species of V7 and V4. Concerning wild relatives, ancestor of mungbean (*V. radiata* var. *sublobata*), (V6), showed sensitivity to salt stress, whereas, that of black gram (*V. mungo* var. *silvestris*), (V12), was observed relatively tolerant salt stress up to a 150 mM NaCl level. However, the number of stains used for the evaluation of salt tolerance among the wild accessions was so few that further collection and evaluation are still necessary. Jeannette et al. (2002) reported that wild *Pheseolus* species were ranked as the most tolerance salinity stress.

In conclusion, this study investigated the tolerance of twelve (12) *Vigna* species to four concentrations of NaCl (0, 75, 150 and 225 mM NaCl). On the basis of the growth parameters measured, the result demonstrated genetic variation in early seedling growth responses to salinity among and within the *Vigna* species. New protein banding patterns were detected among the tolerant genotypes, while sensitive genotypes showed no variation between the control and the salt treatments. Among the twelve (12) *Vigna* genotypes studied, V4 and V7 showed better performance and are recommended for general cultivation in salt affected areas (Ayeyawadi and Bago Division) due to their tolerance to salt stress. For the future prospects, they can be utilized through appropriate selection and breeding for their improvement in salt tolerance. Genetic evaluation of genotypes based on salt tolerance indices could be exploited in the breeding of salt tolerant genotypes among the *Vigna* accessions.

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