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Germination and Seedling Growth of Pulse Crop (*Vigna* Spp.) as Affected by Soil Salt Stress

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ABSTRACT

Vigna is a genus of flowering plants in the legume family, Fabaceae with worldwide distribution. In the present study, effect of salt stress was investigated in four species of *Vigna viz. V. mungo* (urd), *V. angularis* (rais), *V. radiata* (moong) and *V. aconitifolia* (moth) at germination and seedling growth stages in a glasshouse experiment. Seeds of selected crops were surface sterilized and placed under three salt stress levels of 0 (control), 50 and 100 mM using NaCl solutions. Three replicates for each treatment was used for this experiment. The results indicated that increase in salt stress levels caused a reduction in germination percentage, germination rate, shoot length, root length and seedling dry weight of each *Vigna* species. The overall results indicated that among the four species, *V. aconitifolia* was the most sensitive, whereas *V. mungo* was the least sensitive species to the salinity in almost every aspect of growth.

Keywords: Germination percentage, Salinity, Seed vigour, Seedling growth, Sensitivity.

INTRODUCTION

By altering its metabolism, growth and development, abiotic stresses can directly or indirectly affect the physiological status of an organism [1, 2] and adversely affect agricultural productivity[3]. Salinity is the most destructive factor among the abiotic stresses, which considerably limits the productivity of crops. Salinity has affected a large area of land in the world which is increasing day by day and it is the more prominent problem in irrigated crop fields. Pitman and Lauchli [4] estimated that at least 20% of total irrigated lands in the world are salt-affected. The anthropogenic activities resulted in secondary salinity that disrupt the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops (transpiration) [5].

The ability of plants to utilize water is reduced due to salinity thus, resulted in reduced growth rate and also change metabolic processes in plants[6, 7]. In addition, it decreases plant growth and yield depending on the plant species, salinity levels and ionic composition of salt[8]. Seed germination, seedling growth, vigour, vegetative growth, flowering and fruit set are adversely affected by high salt stress, ultimately causing diminished economic yield and also quality of product. Major organic osmolytes such as protein and proline accumulated naturally in many plant species when subjected to different abiotic stresses[9]. These compounds play adaptive role in mediating osmotic adjustment and protecting sub cellular structures in stressed plants.

Salt stress is reported as a serious problem[10]. Salinity decreases crop productivity and threatens the global food balance[11]. It is present in soluble form from low level to high level in soil atmosphere and resulted in decreased water uptake both during imbibition and seedling establishment followed by uptake of ions[12, 13]. Throughout the world, salinity stress negatively impacts agricultural yield affecting production whether it is for subsistence or economic gain[14]. The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyper osmolarity and ion disequilibrium. In addition, crop plant must be capable of satisfactory biomass production in a saline environment[15, 16]

The genus *Vigna* L. includes many wild and cultivated species with pantropical distribution[17]. Among the pulse crops (*i.e.* annual leguminous food crops) *Vigna* is far the most important. For mankind *Vigna* species are important sources of high quality proteins and amino acids and like many other leguminous plants; they play a key role in crop rotation due to their ability to fix nitrogen. To support the awareness on this matter; the United Nations declared 2016 the International year of pulses. Salinity stress is one of the main factors limiting legume productivity in many parts of the world[18]. In this study, an attempt was made to examine the effect of salinization on four species of *Vigna viz*. *V. mungo* (urd), *V. angularis* (rais), *V. radiata* (moong) and *V. aconitifolia* (moth) through glasshouse experiment. The main aim of this study was to compare salt (NaCl) stress tolerance potential of selected *Vigna* spp.

MATERIAL AND METHODS

In this study two salt stress levels (50 mM (S1) and 100 mM (S2)) were prepared using NaCl. In addition one control (C) (0 mM (C)) was also maintained.

EXPERIMENT

Seeds of selected *Vigna* species *viz. Vigna aconitifolia*, *V. angularis*, *V. mungo* and *V. radiata* commonly grown in Kumaun Himalayan region were collected from the native market of Nainital. Healthy and uniform seeds of all species were surface sterilized and washed with distilled water. The seeds were placed in pots with sterile soil and about 10ml distilled water for control or the respective solution was poured in every pot. There were 3 seeds per species in one pot and a total of 15 seeds were shown for creating replicate of one treatment. Germination test were conducted under condition of 12 hours light/dark cycle with 11ºC minimum and 36°C maximum temperature in the glasshouse of Botany Department, Kumaun University Nainital. Different concentration of salt solution was added gradually to pots. Number of germinated seeds was recorded daily after sowing of seeds up to 50 days. A seed was considered germinated when visible protrusions of plumule observed. After 50 days seedlings were harvested. Root and shoot length, fresh and dry weight were recorded for each species and each treatment. The germination percentage was determined by counting the number of germinated seeds every day. Root and shoot dry weights were recorded after oven drying at 60°C for 48 hours.

After final count germination percentage (GP) and germination rate (GR) was calculated by the following formulae[19]-

Germination Percentage (GP):

$$GP = \frac{\text{Number of Total Germinated Seeds}}{\text{Total Number of Seeds Tested}} \times 100 \quad ...(1)$$

Germination Rate (GR):

$$GR = \frac{\text{Number of Germinated Seeds}}{\text{Day of First Count}} + \text{-----------------} + \frac{\text{Number of Germinated Seeds}}{\text{Day of Final Count}} \quad ...(2)$$

The shoots and roots were separated and the fresh weights were measured; after being oven dried at 60º C for 24 hours, the dry weights were taken immediately. According to each salt stress treatment, the fresh and dry weights in reference to control were calculated in percent, by using following equation:

3 (a): Fresh Weight Percentage reduction (FWPR):

$$FWPR\% = 100 \times [1-(\text{Fresh Weight}_{\text{Salt Stress}}/\text{Fresh Weight}_{\text{Control}})]$$

3 (b): Dry Weight (DW) Percentage Reduction:

$$DWPR\% = 100 \times [1-(\text{Dry Weight}_{\text{Salt Stress}}/\text{Dry Weight}_{\text{Control}})]$$

Relative Water Content (RWC)

The water content respective to the fresh weight was calculated as described by Sumithra[20]-

$$RWC\,\% = \frac{FW - DW}{FW} X\,100 \qquad ...(3)$$

Seed Vigor Index (SVI)

This index was determined[21]-

$$SVI = \frac{\text{Germination Percentage } X \text{ Means of Seedling Length (Root+Shoot)}}{100} \qquad ...(4)$$

Salt Tolerance Index (STI)

It is quantified by the ratio, respectively to the controlled, of the total dry weight in salt stress, (in percent), and calculated by the following equation:

$$STI = \frac{\text{Total } DW_{\text{Salt Stress}}}{\text{Total } DW_{\text{Control}}} X\,100 \qquad ...(5)$$

Response Breadth (RB)

It was determined using following formula:

$$B = \frac{1}{\left(\sum_{i=1}^{s} P_i^2\right)} S \qquad ...(6)$$

Where B is the niche breadth; P_i is the proportional response of species at i^{th} treatment (water/salt stress level) and S is the number of size class.

Response Index (RI)

The Response Index (RI) was calculated as per the formula for the magnitude of inhibition versus stimulation by imposed stress on seed germination and seedling growth using following formula[22]:

When germination of treatments (T) is lower than the control (C):

$$RI = (T/C) - 1$$

When germination of treatments (T) is higher than the control (C):

$$RI = 1 - (C/T).$$

If RI > 0 Treatment stimulated germination

If RI = 0 No effect

If RI < 0 Treatment inhibited germination

Root: Shoot Ratio (R: S)

$$R{:}\ S\ Ratio = \frac{\text{Root Dry Weight}}{\text{Shoot Dry Weight (Leaf+Stem)}} \qquad ...(7)$$

Leaf Weight Ratio (LWR):

$$LWR = \frac{\text{Leaf Dry Weight}}{\text{Total Seedling Dry Weight}} \qquad ...(8)$$

Statistical Analysis

Data were analysed statistically by using the software SPSS (version 16.0).

RESULTS AND DISCUSSION

Analysis of variance showed significant effect on germination and seedling growth due to species as well as salt stress levels (Table1).

Table 1: Variance analysis (ANOVA) for traits investigated for the four species of *Vigna* in response to salinity stress

		Mean square			
Parameters	**Df**	**SL**	**RL**	**GP**	**GR**
Species	3	15.55NS	33.22*	6982.30*	0.011*
Salt Stress levels	2	42.722*	14.74*	3497.72*	0.009*

*Significant at 5% level; Df: degree of freedom, NS: not significant, SL: Shoot length, RL: Root length, GP: Germination percentage, GR: Germination rate.

Effect on Germination Percentage

In each species, salinity stress significantly affected germination percentage and germination decreased with increase in salinity level (fig. 1a). Maximum germination percentage (49.99%) was recorded in the control while the lowest germination (6.66%) was recorded for 100 mM salinity level. At control, maximum germination was observed in *V. angularis* (49.99%) and the minimum germination percentage was observed in *V. aconitifolia* (6.66%) while at high (100mM) salinity level, maximum germination (19.99%) was recorded for *V. mungo* and minimum (6.66%) for *V. aconitifolia*. The sensitivity index showed significant difference between the species in response to salt stress. The osmotic effect due to salinity was the main inhibitory factor that reduced germination[23]. Salinity is one of the most important factors limiting plant growth and delaying seed germination as well as final germination percentage[24]. The final germination percentage of the seeds treated with high salt concentration was much lowers than that of the control seeds, indicating that exposure to high concentration of NaCl strongly affected germination. Increasing concentration of NaCl probably caused the decrease in water potential gradient between the seeds and their

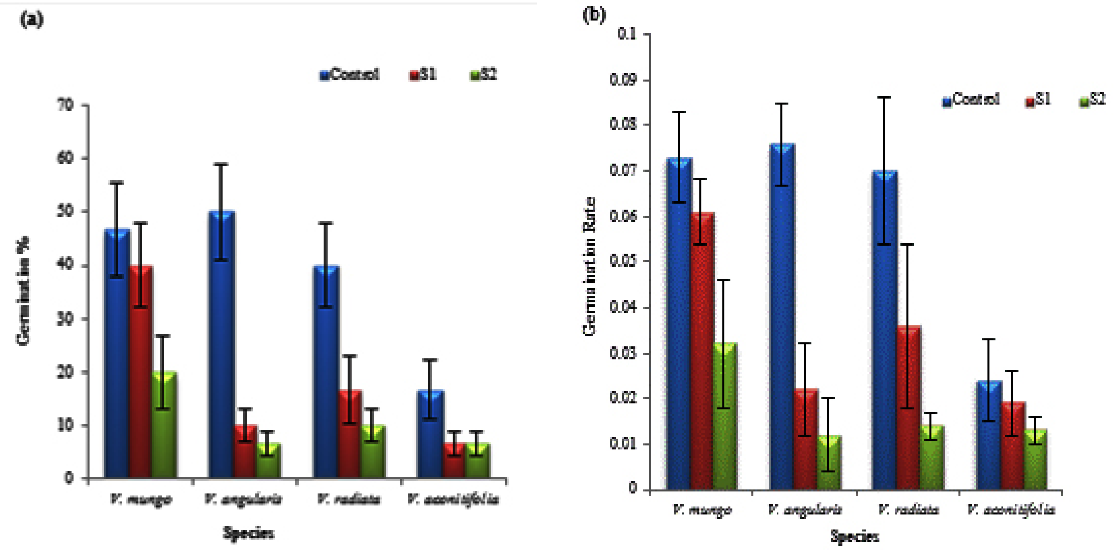

Fig. 1: Effect of salt stress on (a) germination percentage and (b) germination rate of selected *Vigna* species

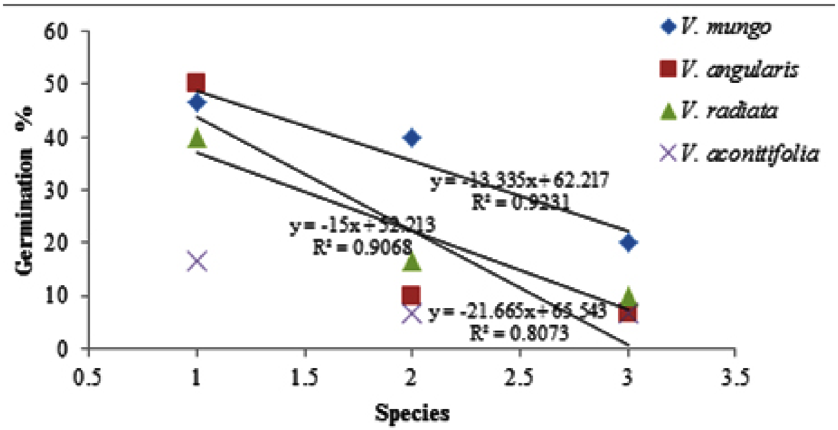

Fig 2: Regression analysis of the salt stress effect on germination percentage of *Vigna* species

surrounding media[25]. According to the correlation coefficient, germination percentage was found to have a positive correlation with other measured parameters (Table 2).

Effect on Germination Rate

The highest germination rate was observed in control (0.076) and rate decreased as the salinity level increased (fig. 1b) which is in conformity to Kaya[26]. Among species, *V. angularis* (0.012) showed lowest germination rate at all salinity levels as compared to other three species. Germination rate and germination percentage (fig. 2) both showed negative correlation to salt stress levels.

Effect on Initiation and Completion of Germination

The lowest emergence time taken by *V. radiata* was 9 days while rest of the three species took maximum 15 days for initiation (I) in controlled conditions. It has been reported that salinity delays germination[27] and at high salinity levels seed germination features were deteriorated. The lowest completion time (9 days) was taken by *V. angularis* while *V. mungo* (18 days) and *V. aconitifolia* (21 days) took more time to complete germination (Table 3).

Effect on seedling Length

In the present experiment, each species showed decline in root length with increasing salinity level. Among species, *V. mungo* (4.32 cm) showed maximum root length as compared to other three species (fig. 3a). The gradual decrease in root length with the increase in salinity as observed might be due to inhibitory effect of NaCl salt to root growth compared to that of shoot growth[28]. Similarly, shoot length was decreased with increasing salinity stress. *V. mungo* (3.95 cm) produced maximum shoot length as a compared to *V. angularis* (2.34 cm), and *V. radiata* (2.45 cm) (fig. 3b). Shoot length was more

Table 2: Correlation matrix for analysed variables

	SL	RL	GR	GP	SD	SVI	R : S
SL	1	0.9058	0.6827	0.8172	0.7859	0.9618	0.5251
RL		1	0.8118	0.9280	0.9030	0.8307	0.2552
GR			1	0.9682	0.9823	0.7402	0.0538
GP				1	0.9979	0.8296	0.1325
SD					1	0.8106	0.1093
SVI						1	0.4790
R : S							1

SL: Shoot length (cm), RL: Root length (cm), GR: Germination rate, GP: Germination percentage, SD: Seedling dry weight (g), SVI –Seed vigour Index, R: S: Root: Shoot ratio.

Table 3: Effect of salinity stress on initiation and completion of seed germination of four *Vigna* species

Salt stress levels	Species *V. mungo* I		*V. angularis* I		*V. radiata* I		*V. aconitifolia* I	
	I	C	I	C	I	C	I	C
Control(0mM)	09.0± 0.0	17.5± 3.5	09.0± 1.0	16.0± 2.0	08.0± 1.0	12.5± 0.5	15.0± 1.0	16.5± 1.5
50mM	09.5± 0.5	16.5± 1.5	08.5± 0.5	09.0± 1.0	08.0± 1.0	09.5± 0.5	10.0± 0.0	11.0± 0.0
100mM	14.5± 0.5	15.0± 0.0	15.0± 0.0	15.0± 0.0	12.0± 0.0	15.0± 0.0	21.0± 0.0	21.0± 0.0

suppressed than root by salinity at each salinity level (Table 4). Plant height decreased with increasing salinity stress and *V. radiata* showed maximum susceptibility to salinity stress (fig 4 & 5).

Effect on seedling Weight

V. angularis (0.21 g) has the maximum fresh weight (fig. 6a) as well as dry weight (0.03 g) (fig. 6b). The least affected species was *V. aconitifolia* with the continuous decrease in both fresh and dry weight with increasing stress levels (fig. 6a). Shoot length, root length and dry weight decreased with increasing salt stress in transgenic rice[29].

Effect on Relative Water Content

Relative water content was greatly influenced by salinity level. Among the species, *V. mungo* (17.86%) was most susceptible to salinity in terms of RWC and it showed lowest RWC at high salinity level (Table 5) while *V. radiata* showed better performance under the salinity stress. The water content was significantly reduced by the increase in NaCl concentration[30]. Reduced water contents with increased salt stress was reported in *Trigonella*[31], while enhanced root moisture contents with increasing salinity levels was reported in *Medicago polymorpha*[32].

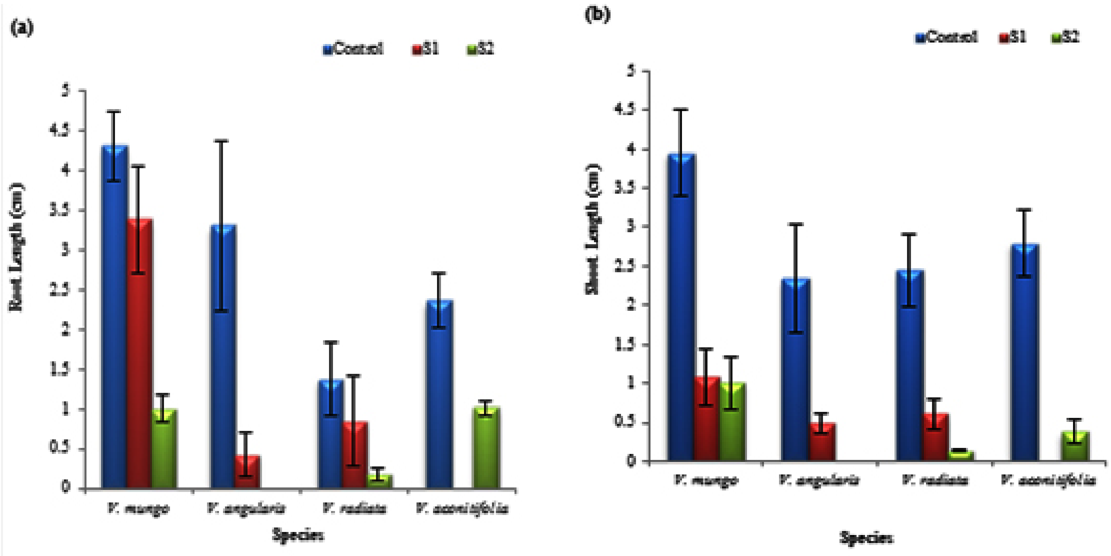

Fig. 3: Effect of salt stress on (a) root length (cm) and (b) shoot length (cm) of selected *Vigna* species

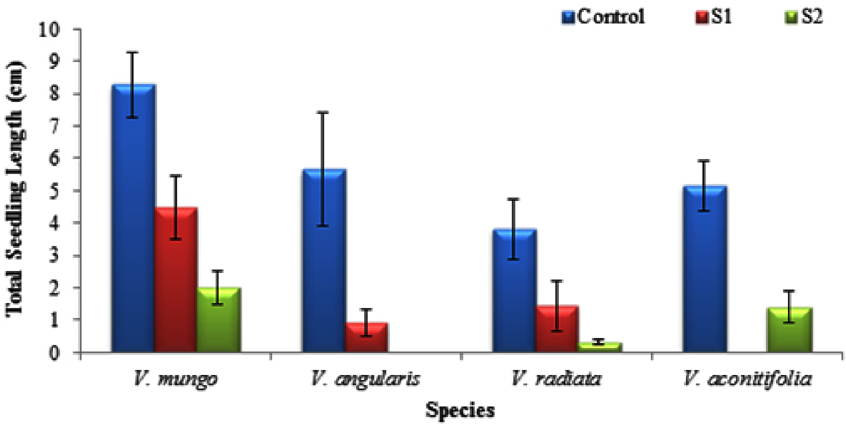

Fig. 4: Effect of salt stress on total seedling length (cm) of selected *Vigna* species

Effect on Salt Tolerance Index

Salt tolerance index decreased with increasing salinity stress. In the present experiment, *V. mungo* (46.45%) showed the highest STI at 50 mM salt stress level while *V. radiata* showed the lowest STI (20.8%) at 100 mM salt stress level (Table 5). It has also been reported that salinity suppresses the uptake of essential nutrients like P and K which could adversely affect seedlings growth and vigor[33].

Effect on Seed Vigor Index

Seed vigor index decreased with increasing salinity level indicating that salt concentration caused harmful effects on seeds[34]. In the present experiment (Table 5), the maximum seed vigor index (4.40) was recorded at control for *V. mungo* and the lowest at 50 mM for *V. angularis* (0.41). Seedling vigor index of maize was also significantly affected under different salt stresses[35]. It was reported that

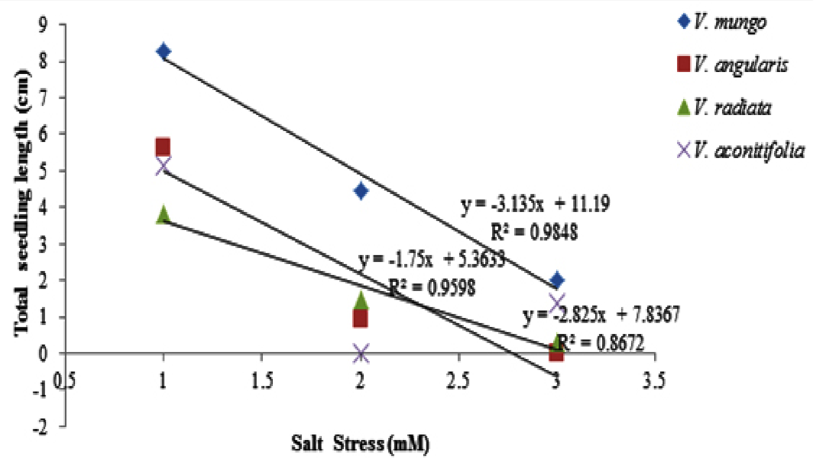

Fig. 5: Regression analysis of the salinity effect on total seedling growth (cm) of *Vigna* species

Table 4: Means (± standard error) comparison of species, salt stress level and their interaction on the studied traits

Species	SL	RL	GR	GP	SD	SVI	R : S
V. mungo	2.48± 0.34	2.87± 0.39	0.061± 0.01	45.55± 6.08	0.70± 0.11	2.26± 1.03	0.23± 0.12
V. angularis	1.02± 0.34	1.28± 0.46	0.036± 0.01	22.98± 5.51	0.39± 0.22	0.21± 0.12	0.05± 0.01
V. radiata	1.04± 0.42	0.75± 0.26	0.038± 0.01	21.50± 5.68	0.38± 0.22	0.65± 0.26	0.09± 0.03
V. aconitifolia	1.05± 0.52	0.56± 0.26	0.015± 0.01	8.88± 3.16	0.18± 0.04	0.30± 0.12	0.19± 0.05
Treatment (Salt stress)							
0 mM	2.95± 0.61	2.37± 0.56	0.06± 0.007	38.33± 6.65	0.021± 0.001	3.15± 0.79	0.12± 0.04
50mM	0.83± 0.31	1.18± 0.44	0.05± 0.007	25.83± 7.84	0.005± 0.002	1.36± 1.02	0.33± 0.21
100mM	0.41± 0.20	0.54± 0.27	0.015± 0.006	10.04± 5.54	0.004± 0.001	0.21± 0.08	0.17± 0.09

SL: Shoot length (cm), RL: Root length (cm), GR: Germination rate, GP: Germination percentage, SD: Seedling dry weight (g), SVI –Seed vigour Index (%), R: S: Root: Shoot ratio.

under stress conditions there is a decrease in water uptake both during imbibitions and seedling establishment and in the case of salt stress, this can be followed by uptake of ions[36]. Seed vigour index had positive and significant correlation with salt stress levels.

Response Breadth (RB)

In the present study, *V. mungo* exhibited wider (0.88) response breadth as compared to other three species. In terms of response breadth species can be ranked as *V. mungo>V. radiata>V. aconitifolia> V. angularis* (fig. 7). Since *V. angularis* tend to show

Table 5: Effect of Salt Stress on different growth parameters of selected *Vigna* species

Species / Salt stress levels	FWPR **(%)**	DWPR **(%)**	RWC **(%)**	SVI	STI **(%)**
			50 mM		
V. mungo	92.17	53.53	17.86	04.40	46.45
V. *angularis*	97.73	77.94	47.31	00.41	23.54
V. radiata	-	-	-	-	
V. conitifolia	-	-	-	-	-
			100 mM		
V. mungo	91.04	96.87	54.12	00.84	25.50
V. angularis	-	-	-	-	-
V. radiata	98.24	79.19	29.47	00.64	20.80
V. conitifolia	07.55	70.76	78.04	01.23	29.23

FWPR: Fresh weight percent reduction, DWPR – Dry weight percent reduction, RWC: Relative water content, SVI: Seed vigour index, STI: Stress tolerance index.

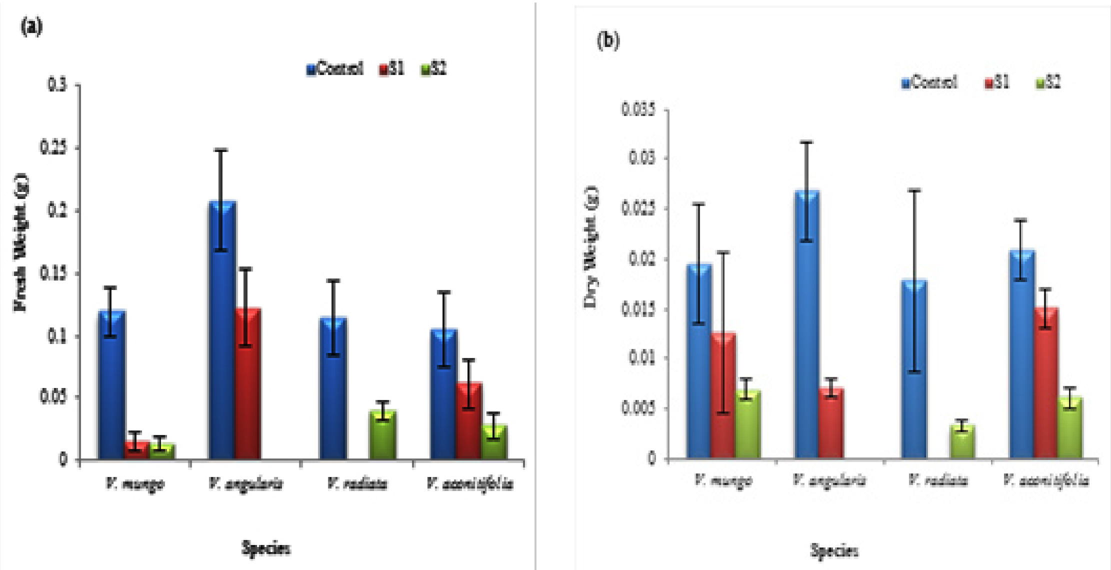

Fig. 6: Effect of salt stress on (a) fresh weight (g) and (b) dry weight (g) of seedling of selected *Vigna* species

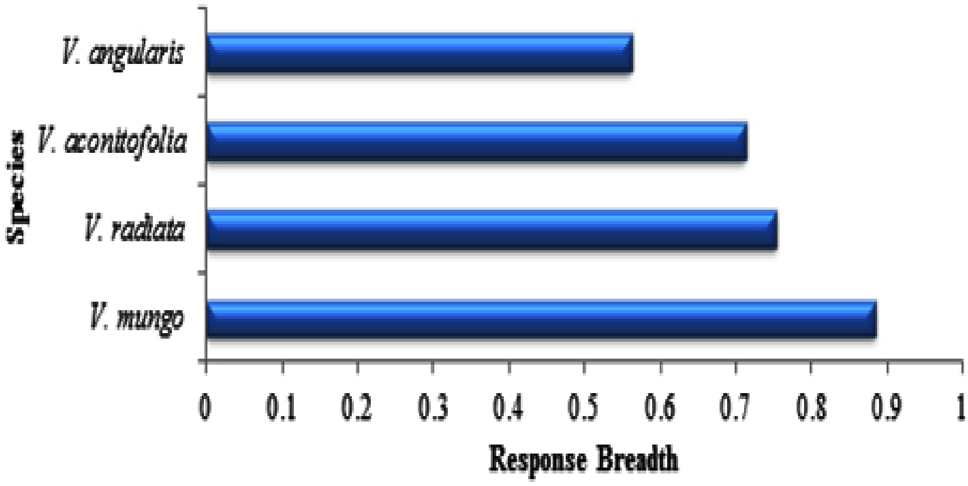

Fig. 7: Response Breadth for different *Vigna* spp. under salt stress

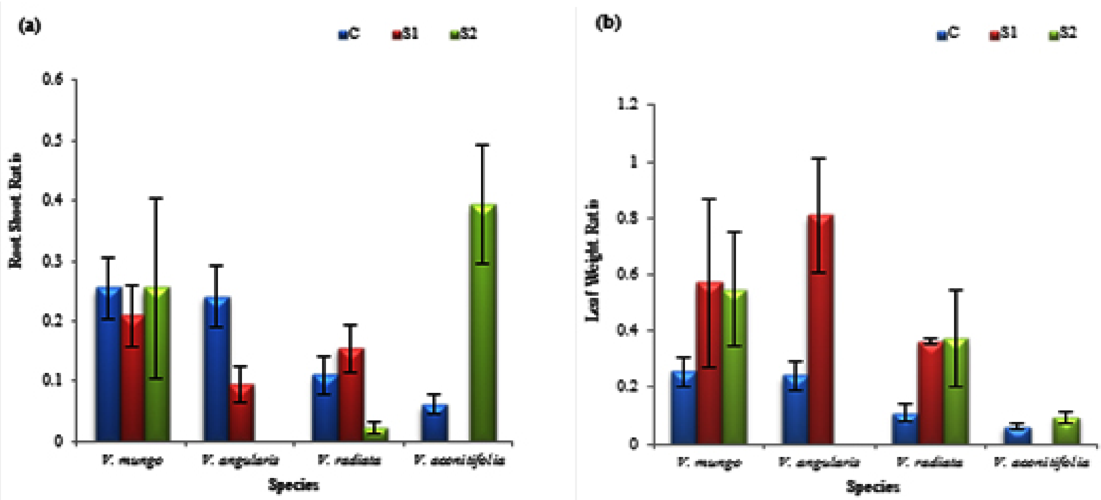

Fig. 8: Effect of salt stress on (a) root: shoot ratio and (b) leaf weight ratio of selected *Vigna* species

Table 6: Response Index (RI) for seed germination, root length, shoot length and total seedling dry weight in different species of *Vigna* due to salt stress

Species	Seed germination		Root length		Shoot length		Total seedling dry weight	
	50 mM	100mM	50 mM	100mM	50 mM	100mM	50 mM	100mM
V. angularis	-0.78	-0.36	-0.69	-	-0.35	-	-0.83	-
V. mungo	-0.56	-0.50	-0.16	-0.54	-0.54	-0.57	-0.28	-0.71
V. radiata	-0.50	-0.20	-0.23	-0.04	-0.33	-0.38	-0.61	-0.91
V. aconitifolia	-0.50	-0.25	-	-0.30	-	-0.40	-	-0.79

poor germination towards higher salt stress levels, it showed a narrower response breadth.

Response Index (RI)

The Response Index (RI) was mostly negative with different level of salinity stress in all species for every variables (Table 6) indicating that germination and growth was negatively affected by salinity stress in each species.

Root : Shoot Ratio

Salt stress levels did not have any linear relationship with root: shoot ratio. Maximum root: shoot ratio was measured in *V. aconitifolia* (0.39) at 100mM salt stress level, while minimum root: shoot ratio was measured in *V. radiata* (0.02) (fig. 8a).

Leaf Weight Ratio

In the present study, leaf weight ratio showed variability with respect to salt stress levels. Maximum ratio was observed in *V. angularis* (0.81) at S_1 (50 mM) salt stress level, while minimum was observed in *V. aconitifolia* (0.06) at S_2 salt stress level (fig. 8b).

CONCLUSIONS

In the present study, *Vigna* species were treated against salt stress at germination and early seedling growth stage and consequences unfolded vital information about their tolerance ability. Salinity exerts significant impact on every branch of growth parameters. Outcomes from the above study could be helpful in understanding the plant's nature against different levels of salt stress and that could be economically exploited by various able agencies.

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