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EFFECT OF SALINITY STRESS ON MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] DURING CONSECUTIVE SUMMER AND SPRING SEASONS

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Abstract: Mungbean is an ecologically important food grain legume crop. Susceptibility towards salinity stress has limited the productivity of mungbean. The effect of salt stress on two popular mungbean varieties ('Pusa vishal' and 'Pusa ratna') has been compared during summer and spring seasons. The experiment was carried out at two salinity stress levels (50 mM and 75 mM NaCl). Significant variations and adaptability among stressed and non-stressed plants were observed in both varieties. The plants in early vegetative stage were found more resistant to salinity as compared to plants in late vegetative and reproductive stage. Salt stress, high temperature and salinity induced osmotic stress severely limited the plant growth, morphology, physiology and yield characteristics during summer. Measured parameters were less affected during spring season. The tolerant variety 'Pusa vishal' exhibited less reduction in plant height, total chlorophyll and carotenoid contents, plant length, leaf area, rate of photosynthesis, number of pods per plant and grain yield at high salinity level. However, the susceptible variety 'Pusa ratna' showed higher reduction for the measured parameters under salinity stress. A delay in pod ripening during spring season resulted in less pod-shattering. The present study may help to execute further research on screening of large mungbean germplasm for salt tolerance during spring season. The germplasm screening may help to identify resistant genotypes for genetic improvement of mungbean for growing in saline soil.

Key words: mungbean, salt stress, photosynthesis, grain yield.

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Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is an important diploid crop with 2n = 2x = 22 chromosomes. It belongs to the genus *Vigna* that is composed of more than 150 species originating mainly from Africa and Asia where the Asian tropical regions have the greatest magnitude of genetic diversity (USDA-ARS GRIN, 2012). The *V. radiata* var. *sublobata,* the wild progenitor of mungbean, is widely dispersed from West Africa to Northern Australia and Papua New Guinea (Undal et al., 2011). India is the largest producer of pulses which contributes 35.7% to the global pulse production (FAOSTAT, 2013). Worldwide, a total of 43,027 mungbean accessions are held *ex situ* at different institutes (AVGRIS, 2012; WIEWS, 2012).

The symbiotic association of mungbean roots and Rhizobia reduces the cost for nitrogen fertilizers (Limpens and Bisseling, 2003). The short life span and nitrogen fixing ability make mungbean a valuable crop in cropping systems and sustainable agriculture production (Somta and Srinives, 2007).

Salinity stress is one of the most atrocious environmental factors restricting the productivity of mungbean in arid and semiarid regions (Abd-Alla et al., 1998).Due to natural salinity and human interferences, the arable land is continuously transforming into saline that is expected to have overwhelming global effects, resulting in up to 50% land loss by 2050 (Saha et al., 2010; Hasanuzzaman et al., 2013). Salt stress imposes substantial adverse effects on the performance and physiology of the crop plants, which eventually leads to plant death as a consequence of growth arrest and metabolic damage (Hasanuzzaman et al., 2012). However, the intensity of adverse and injurious effects of salinity stress depends upon the nature, plant species, duration, stage, concentration, and mode of salt application to the crop. An evaluation of the crop plants in saline environment will certainly provide suitable material as a resource of agronomic traits or genes that can be introduced in the salt sensitive legume crops by breeding (Nair et al., 2012). Recently, Sehrawat et al. (2013a) reviewed that mungbean also encounters the cumulative adverse effects of other environmental factors as insects, pests, high temperature, pod-shattering along with salinity causing high yield loss. Due to the complex nature of salinity stress and lack of suitable techniques for introgression of desirable agronomic traits or resistant genes, little progress has been made in developing salt tolerant mungbean varieties (Singh et al., 2011).

The present research has been performed to observe the performance of two popular varieties of mungbean under salt stress during summer and spring seasons to find out the suitable season for the screening of large mungbean germplasm for salt tolerance.

Material and Methods

The seeds of two popular mungbean varieties ‘Pusa vishal’ and ‘Pusa ratna’ were procured from the Division of Genetics, Indian Agricultural Research Institute, Pusa campus, New Delhi and used as plant material for this study. Three salinity levels of 0 mM NaCl (Control) , 50 mM NaCl (T_1), and 75 mM NaCl (T_2) were prepared by dissolving sodium chloride in the water used for irrigation to impose salt stress. The control treatment (0 mM NaCl) was without sodium chloride.

Rhizobium treated seeds of two mungbean genotypes (‘Pusa vishal’ and ‘Pusa ratna’) were sown in earthen pots containing 10 kg of soil, sand, and farmyard manure in 1:2:1 ratio. The pots were lined with 400 gauge polythene bags to avoid leaching of the salt during irrigation. The whole experiment was conducted in completely randomized block design (RBD) with 8 replications per treatment. The experiment was carried out in an open field during summer while in covered area during spring season. The covered area was made up of bamboos and transparent polythene. The covering protected the plants from various stress causing factors like rain, strong wind etc. which can interfere with the salinity treatment. The removal of the weeds was done by hand regularly and the scheduled irrigation practice was maintained manually at regular time intervals for the crop season. The plants were thinned to 5 plants per pot after one week of seed germination. The NaCl solutions of two concentrations, i.e. 50mM (T_1) and 75mM (T_2) were applied to the plants, i.e. 2.5 l/kg of soil. The salt (NaCl) solution was applied after the emergence and expansion of first trifoliate leaves in both varieties to impart salt stress. The plants provided with equal volume of water without NaCl were used as control (C).

The effect of salt stress on growth and physiological characteristics (total chlorophyll and carotenoid contents, photosynthetic rate) was measured at different stages of the crop, i.e. 1) vegetative, 2) flowering, and 3) pod filling growth stage. The data was collected and average values were calculated for all the stages (vegetative, flowering and pod filling growth stages). Total chlorophyll content and carotenoid content were estimated as described earlier (Hiscox and Israelstam, 1979; Sehrawat et al., 2013b). Briefly, chlorophyll content was estimated by extracting leaf material in dimethyl sulfoxide. Samples were heated in an incubator at 65ºC for 4 h followed by cooling to room temperature. The absorbance of extracts was recorded at 663nm and 645nm. Chlorophyll content was calculated using the earlier reported formula (Arnon, 1949) as described below:

$$\text{Chl a: } [12.7 \times A_{663} - 2.69 \times A_{545}] \quad (1)$$

$$\text{Chl b: } [22.9 \times A_{645} - 4.68 \times A_{663}] \quad (2)$$

$$\text{Total chlorophyll} = 20.2 \times A_{645} + 8.02\ A_{663} \times V/W \times 1000 \quad (3)$$

The values thus obtained were expressed in ug/ml of extract (solvent). Values in mg/g of fresh wt. were obtained by multiplying the above values with "v/w x 1000," where V is volume of extract; W is fresh wt. of sample. The value of total carotenoids (mg g^{-1}) was determined according to the formula described by Lichtenthaler and Wellburn (1983).

$$\text{Carotenoids} = [1000\ A_{470} - (3.27\ \text{chl a} + 104\ \text{chl b})]/229 \quad (4)$$

The plant growth, leaf size and leaf color, necrosis and chlorosis in leaves under saline environment were also observed visually and the photographs were taken at regular time intervals for comparison of control and salt treated plants. The yield related characteristics as number of pods per plant, weight per hundred seeds and grain yield per plant were recorded for salt treated and control plants. The recorded data was subjected to statistical analysis of variance (ANOVA) using OPSTAT program (HAU, Hisar, India).

Results and Discussion

Salinity caused a significant reduction in plant growth and associated developmental parameters. Considerable effect of salt treatment on plant height, number of trifoliates, leaf expansion area, and leaf color was observed in both varieties during summer and spring seasons (Tables 1 and 2; Figure 1). However, the variety 'Pusa vishal' showed less reduction in these traits, which indicates its considerable adaptability in stressed conditions up to the maturity as compared to the susceptible variety 'Pusa ratna'. Salt stress caused low intra-cellular water potential and water scarcity around the root zone due to which roots failed to absorb sufficient water and nutrients for adequate plant growth (Mohammed, 2007; Sunil et al., 2012). A decrease in root and shoot growth under saline environment caused reduced total plant growth (Sehrawat et al., 2013b; 2013c). Growth inhibition under salt stress may be due to the diversion of energy from growth to maintenance (Greenway and Gibbs, 2003).

Salinity caused reduction in chlorophyll and carotenoid contents which in turn resulted in pronounced chlorosis and necrosis in leaves (Figure 1). Decrease in photosynthetic pigments reduced the photosynthetic efficiency of the plants in 'Pusa vishal' and 'Pusa ratna'. Reduction in chlorophyll and carotenoid contents was higher during summer season as compared to control plants (Table 1 and Figure 1). Greater magnitude of these contents was obtained in 'Pusa vishal' in both seasons that may be responsible for its more resistance towards salt stress than the susceptible variety 'Pusa ratna'. Salinity stress caused swelling of membranes in chloroplasts of sensitive plants which affected their chlorophyll content, or it occurred due to excess ions (Na^+ and Cl^-) in leaves which induced loss of chlorophylls (Wahid et al., 2004; Arulbalachandran et al., 2009). Accumulation of toxic ions under salinity stress reduced the water and osmotic potential that further caused disturbances in photosynthetic processes (Khan et al., 2010). Loss of chlorophyll content caused chlorosis of leaves that later turned into necrosis. These adverse effects finally caused senescence and plant death. The results are in agreement with the earlier findings on mungbean (Sehrawat et al., 2013b; 2013c). The symptoms of yellow mosaic virus were also observed enormously in salt stressed plants with significant variations in both varieties during rainy season.

Table 1. Average values and standard errors of means obtained for various physiological and yield characteristics in two mungbean varieties under salt stress during summer and rainy seasons.

Traits	Salt stress level	'Pusa vishal'		'Pusa ratna'	
		Summer season	Rainy season	Summer season	Rainy season
Total chlorophyll content (mg g^{-1} of dry weight)	Control	16.72±0.410	18.06±0.434	14.21±0.286	16.56±0.258
	50mM	15.22±0.227	17.55±0.272	9.45±0.147	12.31±0.169
	75mM	12.61±0.309	13.66±0.319	4.45±0.161	8.65±0.144
Carotenoid content (mg g^{-1} of dry weight)	Control	3.63±0.044	3.87±0.059	3.27±0.052	4.51±0.084
	50mM	3.22±0.038	3.72±0.048	2.35 ±0.038	3.45±0.067
	75mM	2.43±0.031	2.84±0.034	1.68±0.026	3.06±0.048
Leaf area (cm^2)	Control	1248.55±0.098	1440.58±0.147	1203.47±0.166	1376.59±0.101
	50mM	1092.24±0.112	1234.14±0.168	758.77±0.241	1042.25±0.134
	75mM	879.69±0.124	989.79±0.194	289.36±0.274	473.49±0.214
Rate of photosynthesis (μmol m^{-2} s^{-1})	Control	15.24±0.067	18.01±0.141	14.77±0.089	16.77±0.109
	50mM	12.21±0.114	15.51±0.227	9.11±0.136	10.62±0.147
	75mM	9.33±0.123	12.34±0.189	4.23±0.192	6.14±0.186
Plant length (cm)	Control	70.14±0.822	77.41±0.901	68.84±0.704	73.12±0.799
	50mM	59.17±0.641	64.32±0.546	44.87±0.546	50.69±0.447
	75mM	48.79±0.437	57.34±0.389	19.67±0.223	28.88±0.335
Weight of hundred seeds (g)	Control	4.55±0.162	5.44±0.172	3.82±0.161	4.12±0.102
	50mM	3.91±0.067	4.40±0.093	1.09±0.183	2.02±0.071
	75mM	2.77±0.098	3.06±0.078	0.00±0.00	1.25±0.043
No. of pods per plant	Control	43±0.111	54±0.143	40±0.108	49±0.138
	50mM	37±0.098	45±0.126	12±0.058	16±0.069
	75mM	18±0.077	34±0.094	0±0.00	4±0.031
Grain yield per plant (g)	Control	5.49±0.122	7.02±0.184	5.11±0.183	6.59±0.172
	50mM	3.22±0.063	5.40±0.141	0.47±0.144	1.03±0.088
	75mM	1.78±0.082	3.89±0.118	0.00±0.00	0.38±0.092

Both varieties showed variable reduction in characteristics as plant length, number of pods per plant, weight per hundred seeds, and grain yield per plant in both seasons. However, the plants of ‘Pusa ratna’ failed to reach reproductive stage under high salinity due to death of all the plants (0% survival) during summer season and therefore, showed 100% yield loss (Table 1). Nevertheless, the plants of ‘Pusa vishal’ produced significant yield and good quality of seeds (bold seeds) under high salinity in both seasons. Salinity induced desiccation resulted in more flower shedding and pod-shattering during summer which further adversely affected the texture and quality of seeds. Reduced yield in mungbean under salt stress may be due to more flower shedding, reduced photosynthetic efficiency per day of plant to fill the developing seeds, and shattering of the pods (Wahid et al., 2004; Ahmed, 2009; Sunil et al., 2012). It was also observed that salt stress along with other pests (stem and pod borer) and yellow mosaic disease caused 80% to 100% yield loss in mungbean particularly during rainy season. Severe reduction in yield attributes due to salt stress showed similarities with earlier findings in mungbean and other related *Vigna* species (Sehrawat et al., 2013c; 2013d).

Table 2. Statistical analysis values (Critical difference; CD) for observed parameters during summer and spring seasons.

Traits	Summer season			Spring season		
	G[a]	T[b]	(G x T)[c]	G[a]	T[b]	(G x T)[c]
Total chlorophyll content (mg g^{-1} of dry weight)	0.71	0.39	1.21	0.69	0.41	1.27
Carotenoid content (mg g^{-1} of dry weight)	0.14	0.09	0.32	0.12	0.10	0.43
Leaf area (cm^2)	43.27	21.82	94.10	49.54	29.97	112.05
Rate of photosynthesis (µmol m^{-2} s^{-1})	0.58	0.27	1.12	0.62	0.36	1.15
Plant length (cm)	0.99	1.20	1.75	1.12	1.35	1.89
Weight of hundred seeds (g)	0.11	0.10	0.39	0.09	0.13	0.41
No. of pods per plant	0.60	0.42	1.00	0.58	0.46	1.24
Grain yield per plant (g)	0.18	0.11	0.52	0.21	0.11	0.62

[a]Genotype, [b]Treatment (salt), [c]Genotype × Treatment.

The investigated mungbean varieties showed considerable differences for all the measured traits in response to salinity stress. The effect of salinity was less during early vegetative stage but the percent decrease was increased significantly from flowering to pod-filling stage. Salt stress along with high temperature stress and salinity induced osmotic stress severely limited the plant growth, morphology, physiology and yield characteristics during summer but the average reduction in the measured and observed features was less during rainy crop season. The variety ‘Pusa vishal’ showed less reduction in observed traits. This variety produced significant yield under salt stress during both seasons. Results depicted its better resistance towards salt stress as compared to ‘Pusa ratna’ which was found susceptible towards salinity.

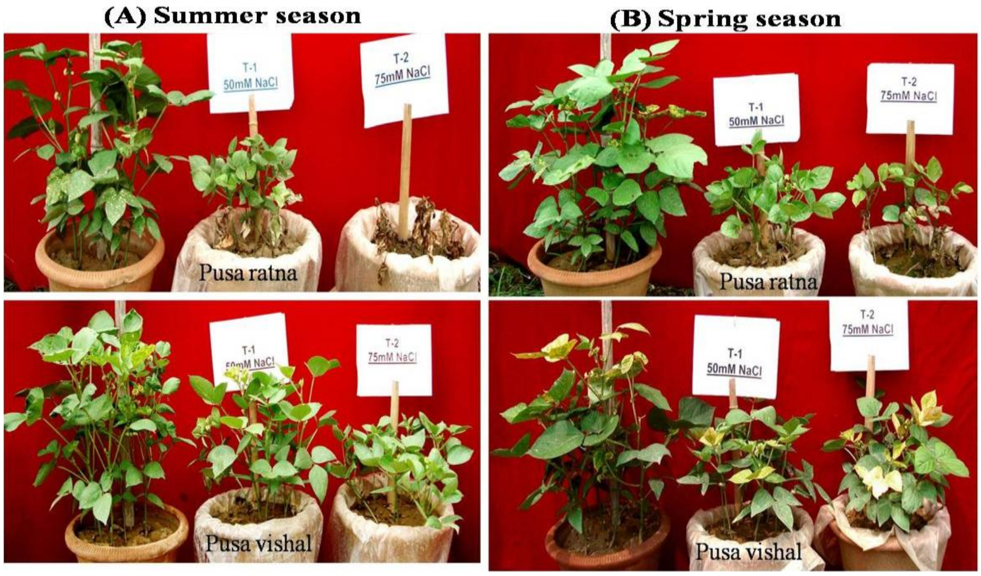

Figure 1. Effect of different levels of salt stress on selected varieties of mungbean in respective seasons.

Conclusion

The mungbean varieties, 'Pusa vishal' and 'Pusa ratna' exhibited significant and high variations for adaptation to salt stress. However, the mungbean plants were drastically affected during summer (due to cumulative effect of salinity and high temperature) as compared to spring season. The control treatment showed clear differences for all the measured features. Observations suggest that screening of the mungbean germplasm for salt tolerance during spring season is more efficient. The genetically diverse accessions resistant to salt stress may help to study the salt tolerance mechanism. The resistant accessions can be used as genetic resources in salinity breeding program to improve the genetic pool of mungbean and other related crops.

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UTICAJ STRESA SOLI NA ZELENU VIGNU [*VIGNA RADIATA* (L.) WILCZEK] TOKOM UZASTOPNE LETNJE I PROLEĆNE SEZONE

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R e z i m e

Zelena vigna je ekološki važna mahunasta kultura. Osetljivost na stres soli ograničava produktivnost vigne. Efekti stresa soli na dve popularne sorte vigne (’Pusa vishal’ i ’Pusa ratna’) su upoređivani tokom letnje i prolećne sezone. Ogled je izveden sa dva nivoa stresa soli (50 mM i 75 mM NaCl). Kod obe sorte su bile uočene značajne varijacije i prilagodljivost među biljkama koje su bile izložene stresu i onima koje nisu bile izložene stresu. Uočeno je da su biljke u ranoj vegetativnoj fazi bile otpornije na salinitet u poređenju sa biljkama u kasnoj vegetativnoj i reproduktivnoj fazi. Stres soli, visoka temperatura i osmotski stres uzrokovan salinitetom su jako ograničili rastenje biljaka, morfologiju, fiziologiju i karakteristike prinosa tokom leta. Mereni parametri su bili pod manjim uticajem stresa tokom prolećne sezone. Tolerantna sorta ’Pusa vishal’ je pokazala manju redukciju visine biljke, ukupnog sadržaja hlorofila i sadržaja karotenoida, visine biljke, lisne površine, intenziteta fotosinteze, broja mahuna po biljci i prinosa zrna pri visokom nivou saliniteta. Međutim, osetljiva sorta ’Pusa ratna’ je pokazala veću redukciju merenih parametara pri stresu soli. Kašnjenje sazrevanja mahune tokom prolećne sezone je rezultiralo manjim pucanjem mahuna. Ovo ispitivanje može pomoći za dalje istraživanje velike germplazme zelene vigne za toleranciju na stres soli u toku prolećne sezone. Ispitivanje germplazme može pomoći da se identifikuju otporni genotipovi za genetsko poboljšanje zelene vigne radi gajenja na slanom zemljištu.

Ključne reči: zelena vigna, stres soli, fotosinteza, prinos zrna.

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