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DIFFERENCE IN SALT TOLERANCE OF FOUR VARIETIES OF GREENGRAM

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ABSTRACT

Four high yielding *Vigna radiata* (L.) Wilczek genotypes were tested for their salt tolerance at germination, 35, 55 days after emergence of seedlings, and at maturity (81DAE). The genotypes displayed a substantial variability for salinity tolerance at all growth stages. The salt tolerance limits (EC_{50} values) of genotypes varying from 2.7 dS m^{-1} to 4.1 dS m^{-1}. Enhanced salt tolerance was expressed by high germination percentage, reduced tip burning, low chlorosis and necrosis of young leaves, greater number and area of green leaves, high chlorophyll contents, low leaf senescence and high seed yield per plant. A genotype 245/7 exhibited tolerance at germination and 35 DAE while genotype NM-54 was the most tolerant at later growth stages.

Key word: Chlorosis, economic yield, necrosis, salt tolerance, *Vigna radiata*.

INTRODUCTION

Salt tolerance changes considerably during the development of plants (Maas and Hoffman, 1977), most plant species being particularly salt sensitive at early vegetative growth stage. Corn (Maas *et al.*, 1983) and sorghum (Maas *et al.,* 1986) were more sensitive to salinity during the vegetative and reproductive stages and less sensitive during grain filling stage where as the seeding stage of rice proved to be the most sensitive to salinity (Akita, 1986).

Salinity also induces numerous physiological and biochemical changes in cells including reduced growth in sunflower (Wahid *et al.,* 1999b). Visible signs of leaf chlorosis in sugarcane (Wahid *et al.,* 1997), and delayed leaf expansion in bean due to salinity stress (Neumann *et al.,* 1988). Sodium chloride treatment produced greater effect on leaf senescence in *Oryza sativa* (Lutts *et al.,* 1996). Similarly, decrease in barley seed yield was observed due to salt toxicity (Isla *et al.,* 1998). The present study was undertaken to detect any phenotypic flexibility of salt tolerance in locally available genotypes of greengram [*Vigna radiata* (L.) Wilczek].

MATERIALS AND METHODS

The seeds of two *V. radiata* genotypes (245/7 and 241/11) obtained from the Department of Botany, University of Agriculture, Faisalabad, while two advanced *V. radiata* genotypes NM-89 and NM-54 were obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The genotypes were selected for tests and trails based upon contrasting seed characteristics. The experiments were carried out in the laboratory and Botanical Garden, University of Agriculture, Faisalabad. For germination studies, seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 minutes, followed by repeated washing with water, and sown in 180 petridishes lined with double layer of Whatman No.2 filter paper. The experimental design was completely randomized with three replications. Salinity (NaCl) levels, i.e., control, 4, 8 and 12 dS m^{-1} were added to petridishes before placing 10 seeds in each dish. The seeds were germinated at 30^{o}C under a 12h L / D cycle. The germination of seeds was recorded in terms of germination percentage (total number of seeds germinated / total number of seeds sown x 100).

Salt tolerance was determined at 35 and 55 days after emergence (DAE) of seedlings and at maturity (81DAE). The plants were raised in pots lined with polythene bags, containing 10 kg of sun dried, homogeneously sandy loam soil and three plants of equal size were maintained per pot. The pots (three replicates per treatment) were placed in a wire house in complete randomization, where the ambient temperature ranged between $30^{o}C\pm 3$ to $35^{o}C \pm 4$, relative humidity between $25\% \pm 7$ to $42\% \pm 5$ and annual rainfall was 68-88 mm. The physico-chemical characteristics of the soil were: Organic matter 1.51%; pH 7.6; ECe 0.4 dS m^{-1}; Cation exchange capacity (CEC) 13.2 meq per 100 g soil; Sodium absorption ratio (SAR) 0.07 meq L^{-1}; Na^{+} 3.99 meq L^{-1}; Cl^{-} 6.91 meq L^{-1}; $SO_4^{\ 2-}$ 1.53 meq L^{-1} and Ca+Mg 16.2 meq L^{-1}. The original ECe of the soil was accounted while developing salt levels. The plants were harvested 16 days after the onset of salinity treatments at each growth stage. The data collected for various parameters were analyzed statistically by ANOVA to find differences among various parameters and their interactions. Chlorophylls were extracted in 100 percent acetone and optical densities were determined at 663 and 645 nm.

RESULTS

All the genotypes indicated significant ($P<0.05$) differences with respect to germination (Table 1). Under salinity, 245/7 had the highest germination percentage over the control in lab conditions, and was thus regarded as highly salt tolerant at this stage, followed by NM-54, NM-89 and 241/11.The accessions / genotypes exhibited increased symptoms of salt injury in the form of tip burning and chlorosis of leaves, stem chlorosis, necrosis of leaves and stem with a concomitant rise in salinity (Table 2). The degree of salt damage was greatly variable in the young and old leaves along with stem. Symptomatic determination of salt damage at seedlings stage (35DAE) revealed that NM-54 genotype at highest salinity level indicated a low degree of salt damage in the form of tip burning, chlorosis and necrosis on young leaves but these injuries were severe on older leaves and stem (Table 2). In contrast, 241/11 at 35DAE indicated the highest effect of salt damage on young leaves while it was lowest on the older leaves and stem. However, rest of the genotypes did not indicate any definite pattern of these injuries.

Table 1. Effect of NaCl salinity on seed germination % of greengram.

Genotype	Expt. Site	Germination %			
		Salt levels (ds/m)			
		control	4	8	12
245/7	Lab	100	99	83	80
	Field	94	88	67	64
241/11	Lab	95	90	78	68
	Field	82	70	65	54
NM-89	Lab	98	95	80	72
	Field	93	83	66	58
NM-54	Lab	100	95	82	75
	Field	93	85	68	60

Table 2. Symptoms and degree of salt injury in leaves and stem of four genotypes of greengram under 12 dS/m NaCl salinity at seedlings stage.

Sign of salt injury	Degree	Plant tissue		
		Young leaves	Old leaves	Stem
Tip burning	Low	NM-54	241/11	--
	↓	245/7	245/7	--
		NM-89	NM-89	--
	High	241/11	NM-54	--
Chlorosis	Low	NM-54	241/11	245/7
	↓	NM-89	245/7	241/11
		245/7	NM-89	NM-89
	High	241/11	NM-54	NM-54
Necrosis	Low	NM-54	241/11	241/11
	↓	NM-89	NM-89	NM-89
		241/11	245/7	245/7
	High	245/7	NM-54	NM-54

Qualitative symptoms of salt injury held relevance to the loss of chlorophyll. It was, therefore, imperative to determine chlorophyll content of the material under investigation. The statistical analysis for chlorophyll contents (Chl a, Chl b, total Chl and Chl a:b ratio) indicated highly significant ($P<0.01$) difference among the genotypes and growth stages with increased salinity levels (Table 3). The total chlorophyll contents were greater in NM-54 at higher level of salinity, while it was lowest in 245/7. In contrast to other chlorophyll parameters, the chl a:b ratio was greatly increased or variable in all the genotypes at all the growth stage with increasing salinity.

Table 3. Changes in some chlorophyll contents of four greengram genotypes at 55 days after emergence of seedling under NaCl salinity.

Genotype	Salt levels (dS/m)	Chlorophyll contents (mg/g of fresh green tissue)			
		Chl. a	Chl. b	Total Chl.	Chl.a:b ratio
245/7	Control	0.538	0.448	0.994	1.202
	4	0.413	0.387	0.794	1.079
	8	0.052	0.042	0.125	1.244
	12	0.038	0.032	0.016	0.865
241/11	Control	0.600	0.592	1.195	1.015
	4	0.486	0.413	0.934	1.133
	8	0.072	0.034	0.165	2.151
	12	0.060	0.019	0.069	3.157
NM-89	Control	0.631	0.581	1.208	1.086
	4	0.428	0.399	0.827	1.073
	8	0.101	0.071	0.171	1.423
	12	0.083	0.037	0.096	2.243
NM-54	Control	0.828	0.441	1.369	1.870
	4	0.759	0.419	1.005	1.812
	8	0.144	0.079	0.251	1.819
	12	0.107	0.058	0.139	1.840
Summary of significance of variance sources					
Genotypes(G)	3	**	**	**	**
Salinity(S)	3	**	**	**	**
G x S	9	**	**	**	**

**Significant at P=0.01

The number of green and senescent leaves and leaf area per plant were markedly reduced with increasing levels of applied salinity in all the genotypes at all growth stages (Table 4). Although under control condition, all the genotypes displayed higher number and area of green leaves and low senescent leaves per plant at 35, 55 and 81 DAE. The number of senescent leaves per plant increased with rising of salinity levels and with the passage of time i.e., at 35, 55 and 81 DAE while the number and area of green leaves decreased with high salt treatments, but increased at 35 and 55 DAE and markedly decreased at 81 DAE. The genotype NM-54, however, indicated differential behaviour for number of green and senescent leaves and leaf area per plant were much better at highest level of salinity (12 dS m^{-1}) while these parameters were lowest in 241/11.

At maturity (81DAE), applied salinity highly significantly reduced number of pod per plant, pod length, number of seed per pod and per plant, and seed yield per plant. With a significant interactions of genotypes and salt levels (Table 5). The effect of sodium chloride on the pattern of dry matter accumulation in seed of these beans were generally similar to the pod formation patterns, although pod formation stage was more sensitive to NaCl concentrations in all genotypes investigated here. The genotypes NM-54, however, indicated high and distinct behaviour for seed yield per plant while there was low in 241/11.

The salt tolerance limits (EC_{50}) based on germination percentage, chlorophyll contents, number and area of green leaves and seed yield per plant, revealed that the genotypes had relatively greater EC_{50} at germination and 35DAE, but it was reduced and variable at the later growth stages. On average, NM-54 with EC_{50} at 12 dS m^{-1} NaCl salinity was ranked as highly salt tolerant followed by NM-89, 245/7 and 241/11 (Table 6).

Table 4. Changes in number of green & senescent leaves and leaf area of four greengram genotypes at 35, 55 and 81 DAE under NaCl salinity.

Genotype	Salt levels (dS/m)	No.of Green leaves / plant			No.of Senescent leaves/plant			Leaf area / plant (cm^2)		
		35DAE	55DAE	81DAE	35DAE	55DAE	81DAE	35DAE	55DAE	81DAE
245/7	Control	11	16	9	2	3	4	9.93	15.92	16.31
	4	7	9	5	2	5	7	8.07	12.60	13.13
	8	4	5	2	3	6	9	2.98	5.41	5.82
	12	2	2	1	4	8	11	2.19	3.64	3.97
241/11	Control	10	14	7	2	3	4	9.27	15.78	16.22
	4	6	8	4	3	6	8	7.82	12.16	13.02
	8	3	5	2	4	7	10	2.75	5.32	5.67
	12	2	2	1	4	9	12	2.11	3.41	3.89
NM-89	Control	14	21	13	1	2	3	10.99	17.75	18.11
	4	10	13	7	1	3	6	9.51	14.91	15.12
	8	6	8	4	2	5	8	3.98	6.44	6.99
	12	3	5	2	3	6	9	2.99	4.82	5.35
NM-54	Control	17	24	18	1	2	3	12.53	18.89	19.21
	4	13	16	9	1	3	5	10.65	15.14	15.59
	8	7	10	5	2	4	6	5.11	7.13	7.91
	12	4	6	2	2	5	7	3.19	5.51	6.11
Summary of significance of variance sources										
Genotypes (G)	3	**	**	**	*	*	**	**	**	**
Salinity (S)	3	**	**	**	n.s	*	*	**	**	**
G x S	9	**	**	*	*	**	**	**	**	**

DAE: Days After Emergence of Seedling **Significant at P=0.01 *Significant at P=0.05 n.s Non-significant

Table5. Changes in some yield characteristics of greengram genotypes at maturity.

Genotype	Salt Levels (dS/m)	Yield Characteristics				
		No. of pod/plant	Av. Pod length (cm)	Av. No. of seed/pod	No. of seed/plant	Seed yield/plant
245/7	Control	6	5.2	7	41	3.12
	4	5	4.1	6	29	2.45
	8	3	3.1	4	11	1.25
	12	2	2.0	2	5	0.98
241/11	Control	5	4.2	6	34	2.61
	4	4	3.7	5	22	2.11
	8	2	2.9	3	8	0.99
	12	1	1.2	2	2	0.38
NM-89	Control	8	5.8	9	73	4.87
	4	6	4.7	7	45	3.34
	8	3	3.6	4	16	1.98
	12	2	2.7	3	8	1.12
NM-54	Control	9	6.1	10	88	5.37
	4	7	4.9	8	59	4.25
	8	4	3.9	5	23	2.81
	12	2	3.2	4	11	1.79
Summary of significance of variance sources						
Genotypes (G)	3	**	**	**	**	**
Salinity (S)	3	**	**	*	**	**
G x S	9	**	**	**	**	**

**Significant at P=0.01 *Significant at P=0.05

Table 6. Salt tolerance limits (EC_{50} values) of greengram genotypes at 12 dS/m NaCl salinity.

Genotype	Growth Stages					
	Germination	35DAE	55DAE	81DAE	Mean	Rank
245/7	3.4	3.6	2.4	2.2	2.9	s
241/11	2.1	2.4	3.1	2.2	2.7	hs
NM-89	2.5	2.7	4.4	4.0	3.4	t
NM-54	3.1	3.3	5.3	4.7	4.1	ht

ht: highly tolerant; t: tolerant; s: sensitive; hs: highly sensitive; DAE: Days After Emergence of Seedlings

DISCUSSION

This study revealed that *V. radiata* L. genotypes, used here, have a high variability in phenotypic flexibility for salt tolerance in term of (a) germination percentage, (b) signs of slat damage, (c) chlorophyll contents, (d) number and area of green leaves, and (e) seed yield per plant.

Among the genotypes, 245/7 at 35 DAE while NM-54 at 55 & 81 DAE were rated as high salt-tolerant species ones in present work. A critical examination of the data revealed that salinity tolerance was based on the qualitative and quantitative characteristics such as high germination percentage, reduced chlorosis, necrosis and tip burning of young leaves, but increased stem chlorosis and necrosis, greater chlorosis and necrosis of older leaves, greater number and area of green leaves, higher chlorophyll contents, less number of senescent leaves, and high seed yield of tolerant genotypes found in all growth stages. Although the plants were not analyzed for ionic contents, a decreased chlorosis, necrosis and tip burning of young emerging leaves, enhanced chlorosis and necrosis of stem, greater number of senescent leaves allude to the exclusion of toxic ions from aerial parts of the tolerant genotype, so avoiding toxicity to the growing tissues. A similar mechanism has been suggested in sunflower genotypes (Ashraf and O'Leary, 1995; Francois, 1996; Wahid *et al.*, 1999b) and in barley and maize crops (Rawson *et al.*, 1988; Shannon, 1997; Shabala *et al.*, 1998).

The physiological potential of genotypes expressed in terms of photosynthetic efficiency of green foliage i.e., chlorophyll contents, revealed that a declining trend of these parameters with increasing levels of applied NaCl salinity in all the genotypes was observed at all the growth stages. The results of chlorophyll analyses (Table 3) showed that all the genotypes were rich in chlorophyll a, but relatively low in chlorophyll b. It was further founded that, NaCl salinity had no appreciable effect on chlorophyll a, chlorophyll b and total chlorophyll contents of green foliage of tolerant genotypes but a significant decrease was observed in case of sensitive genotypes in these chlorophyll parameters (Strogonov *et al.,* 1970). Furthermore, chlorophyll a:b ratio was decreased or greatly variable in all the genotypes at all the growth stages with increasing salinity levels. But, it is noteworthy that in present study the decrease in chlorophyll a:b ratio coincided with a decrease in chlorophyll b concentration, although, only chlorophyll a is involved in fluorescence (Lutts *et al.,* 1996).

The economic yield of crops is adversely affected under salt stress (Sarin *et al*., 1975). One of the attributes for reduced yield may be a reduction in pod and seed production by the plant. Gill (1979) in barley and Wahid *et al.* (1999b) in sunflower reported similar result of effect of soil salinity on seed yield. Furthermore, genotypes of greengram under present investigation differed widely in yield attributes under normal as well as under saline conditions. This may ultimately lead to reduced seed yield per plant under salinity and consequent lower the economic yield. Thus, it may be concluded that decline in yield under salt stress may be due to reduced number, area and efficiency in term of chlorophyll content of green foliage to fill the developing seeds during reproductive phase of crop.

In conclusion, although greengram elicited sensitive response to salinity, but there existed a significant phenotypic flexibility for salt tolerance, as reflected by EC_{50} values (Table 6). The author proposed that sustained photosynthetic area, greater number and efficiency of green foliage, and high seed yield per plant are useful criteria of selection for salinity tolerance in greengram. The genotype NM-54 can be successfully grown in moderately saline areas. The problem of relatively reduced germination can be overcome by achieving optimum plant density with the use of high seed rate.

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