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Salt tolerance of mung bean (*Vigna radiata* (L.) Wilczek) at two growth stages

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Abstract

The salt tolerance of two cultivars of mung bean (*Vigna radiata* (L.) Wilczek), AuMg 588 and Mg 6601, was assessed at germination and at the seedling stage. Increasing salt concentration significantly reduced the percentage germination, fresh and dry weights, protein and carbohydrate contents of all plant parts, leaf area, shoot and root lengths, shoot/ root ratio, chlorophylls a, and b, and total chlorophyll in both the cultivars. At high salinities, cv AuMg 588 had greater fresh and dry weights of all plant parts, than cv Mg 6601, but the latter had greater chlorophyll a, and b, and total chlorophyll contents than cv AuMg 588. Cultivars did not differ significantly in other characters measured. *Vigna radiata* is very sensitive to salt.

Introduction

The accumulation of salts in the soils of arid and semi-arid regions is a continuing threat to crop production, and in such areas has caused many social and economic problems. Attempts by soil scientists to adopt management practices to reduce soil salt concentrations which are expensive, cannot be contemplated in most of the countries affected.

A possible alternative, is the introduction of crop species/cultivars capable of tolerating the higher soil salinities and which can produce economic yields under such conditions. This biotic approach to overcome the salinity problem has received considerable attention from many workers (Ashraf *et al.*, 1986; Epstein, 1985; Noble, 1983; Shannon, 1978).

Most leguminous species are within the salt sensitive group of crops (Maas and Hoffman, 1977). *Vigna radiata* (L.) Wilczek (mung bean) is a leguminous crop on which very little work has been done, although it is one of the most important crops for food, feed and fodder, and green manure.

The present work was carried out to assess the salt tolerance of this crop at the two stages, germination and seedling.

Materials and methods

Seeds of *Vigna radiata* (L.) Wilczek cv AuMg 588, and cv Mg 6601 were supplied by Dr Abdullah, Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. Seed was surface sterilized with 0.1% $HgCl_2$ solution for five minutes before experimentation.

Germination experiment

This experiment was carried out in a small, controlled growth chamber at a constant temperature of 20°C, 30 W m^{-2} light intensity for 12 h daily, and a relative humidity of 65%. Seeds of each cultivar were sown in Petri dishes (10 cm diameter and 2 cm deep) at 20 seeds per Petri dish. The salt treatments were as follows:-

Salt treatments
Electrical Conductivity (EC) dSm^{-1} at 25°C

3.0 (control)	— Hoagland nutrient solution
6.0	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)

9.0	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)
12.0	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)
15.0	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)

A completely randomized design was used with three replicates. 5 ml of appropriate solution was applied to each Petri dish every day after rinsing out the previous solution from the Petri dish. Daily counts of the number of germinated seeds were recorded for 12 days, and the experiment was then terminated.

Experiment No. 2–Effect of salt on seedling growth

Silica sand was soaked in 2% (v/v) HCl and then thoroughly washed with tapwater and rinsed with distilled, deionised water. 15-cm diameter plastic pots were filled with 1.75 kg dry sand. The salt concentrations of the solutions used in the experiment were as follows:-

EC 3.0 dSm^{-1} control	— Hoagland nutrient solution
EC 6.0 dSm^{-1}	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)
EC 9.0 dSm^{-1}	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)
EC 12.0 dSm^{-1}	— Hoagland solution, + NaCl + $CaCl_2$ (1:1 by equiv. wt.)

One hundred seeds of each cultivar were germinated on moist filter paper in Petri dishes, and six randomly chosen, three days old seedlings of each cultivar were transplanted, equidistant from each other, into each pot.

The experiment was conducted in a glasshouse at 22°C under 12 h daylength. Seedlings were grown for a further two days irrigated with full strength nutrient solution (Arnon and Hoagland 1940), after which time salt treatments were begun by adding aliquots of EC 3.0 $dS.m^{-1}$ solution every other day, until the appropriate salt concentrations were reached. Treatment continued with addition of 800 ml of the appropriate solution every other day to each pot. This volume of treatment solution was such as to displace the solution already present in the sand, the completeness of replacement being monitored by the electrical conductivity of the effluent solution from the pots.

The plants were harvested 30 days after the start of the experiment. Plants roots were removed carefully from the sand, and washed with distilled deionised water. Shoots and roots were separated. After recording the fresh weights of all plant parts, and measuring leaf area using a simple graphic method, plant material was dried at 70°C for three days and dry weights were recorded.

Chlorophyll content

Chlorophyll content was estimated by the method described by Witham *et al.* (1971). After 20 days growth, one leaf of the same age, was taken from each plant of both cultivars for chlorophyll analysis. One gram of the fresh leaves were taken and triturated in a porcelain mortar with 40 ml of 80% acetone. The supernatent liquid was transferred to a buchner funnel fitted with a Whatman No. 40 filter paper and the filtrate was collected in a 1000 ml graduated flask. Trituration of the sample was repeated with successive 30 ml portions of 80% acetone until all the chlorophyll had been extracted. The pestle, mortar and filter were rinsed with the successive extracts, which were collected in the litre flask. When the extraction was complete, the filtrate was made up to 100 ml, thoroughly mixed and used to determine the chlorophylls spectrophotometrically at the appropriate wavelengths. The chlorophylls were calculated using the following formulae;

$$1.\ \text{mg Ch. ag}^{-1}\ \text{tissue} = [12.7\ (D\ 663) - 2.69\ (D\ 645)]\ X\ \frac{V}{1000\ X\ W}$$

$$2.\ \text{mg Ch. bg}^{-1}\ \text{tissue} = [22.9\ (D\ 645) - 4.68\ (D\ 663)]\ X\ \frac{V}{1000\ X\ W}$$

$$3.\ \text{mg total Ch./g tissue} = (D\ 652 \times 1000/34.5)\ X\ \frac{V}{1000\ X\ W}$$

Total protein percentage

Nitrogen analysis of samples of leaves, stem, and roots was carried out (Jackson, 1958). Total protein = N × 6.25.

Table 1. Mean germination percentage of two mung bean cultivars at varying salinities (NaCl + $CaCl_2$ 1:1 by equiv. wt)

Variety	Salinity as EC dS/m at 25°C					LSD at 5%
	3.0 (control)	6.0	9.0	12.0	15.0	
AuMg 588	96.0	83.3	66.7	56.7	46.7	0
Mg 6601	93.3	76.7	70.0	60.0	53.3	

Total carbohydrates

Total carbohydrates were estimated by the method of Ashwell (1957). 0.2 g of a dried and well ground sample was digested with 6 *N* HCl and total carbohydrates were determined using a spectrophotometer, and calculated using the following formula: Total carbohydrate % age = conc. of glucose solution/absorbance of glucose solution × absorbance of sample Anaylsis of variance was carried out on the data for each parameter.

Results

The data for the percentage germination of the two mung bean cultivars at varying salt concentrations are presented in Table 1. Increasing concentration of salt significantly reduced the germination percentage ($p \leqslant 0.001$) in both the cultivars. Cultivars did not differ significantly, and the interaction treatments X cultivars was also non-significant.

Table 2. Mean fresh and dry weights per plant (mg) of shoots and roots, and mean shoot/root ratio of two mung bean cultivars after 30 days growth at different salinities

Plant part	Variety	Salinity as EC dS/m at 25°C			LSD 5%
		3.0 (control)	6.0	9.0	
Fresh weight					
Shoots	AuMg 588	1550	780	321	230
	Mg 6601	1276	696	245	
Roots	AuMg 588	800	290	111	132
	Mg 6601	650	270	92	
Dry weight					
Shoot	AuMg 588	324	166	81	61
	Mg6601	258	115	59	
Roots	AuMg 588	90	31	31	27
	Mg 6601	60	26	9	
Shoot/root ratio					
	AuMg 588	3.6	5.35	6.53	0
	Mg 6601	4.3	4.42	6.92	

Table 3. Mean shoot and root length per plant (cm), and mean leaf area per plant (cm^2) of two mung bean cultivars after 30 days growth at different salinities

Variety	Salinity as EC dS/m at 25°C			LSD 5%
	3.0 (control)	6.0	9.0	
Shoot length				
Mg 6601	10.10	6.11	3.7	0
	8.74	6.40	3.8	
Root length				
AuMg 588	13.87	9.93	4.6	0
Mg 6601	14.36	9.95	5.1	
Leaf area				
AuMg 588	27.0	15.02	9.6	4.02
Mg 6601	22.76	17.90	9.9	

Fresh and dry weights of different plant parts of the two cultivars are presented in Table 2. Cv. AuMg 588 produced significantly greater shoot and root fresh weights than cv Mg 6601 in the control treatment ($p \leqslant 0.05$). Although statistically non-significant, cv AuMg 588 had greater shoot and root fresh weights than cv Mg 6601 at higher salt concentrations. A similar trend was seen for shoot and root dry weights of both cultivars.

The shoot/root ratio (Table 2) of both cultivars increased at high salt levels, compared with the controls, but there was no significant difference between cultivars.

Mean shoot and root lengths of the two cultivars are given in Table 3. Cultivars did not differ in their response to salt with regard to shoot or root length; although there was a significant inhibitory effect of salt on both the shoot length and root length of both cultivars. The two cultivars had significantly different leaf areas ($p \leqslant 0.05$) in the control treatment (Table 3) but did not differ significantly at the high salt concentrations. Also at the higher salt concentrations, the leaf area of both the cultivars was reduced significantly ($p \leqslant 0.05$) compared with control treatment.

The mean chlorophyll content of the two cultivars is given in Table 4. Cv Mg 6601 had significantly ($p \leqslant 0.05$) greater Ch. a, b, and total chlorophyll than the cv AuMg 588. The chlorophyll contents of both the cultivars decreased significantly at EC 6.0 and 9.0 dS/m compared with the controls.

Table 4. Mean chlorophyll content ($mg.g^{-1}$ leaf tissue) of two mung bean cultivars after 30 days growth at different salinities

Chlorophyll	Salinity as EC dS/m at 25°C			LSD 5%
	3.0 (control)	6.0	9.0	
AuMg 588				
a	0.70	0.38	0.27	a 0.17
b	0.57	0.37	0.23	b 0.19
Total	1.17	0.74	0.58	Tot. 0.40
Mg 6601				
a	0.78	0.59	0.39	
b	0.65	0.58	0.31	
Total	1.63	1.19	0.79	

Different plant parts of the two cultivars did not differ significantly in protein or carbohydrate content (Table 5). At EC 6.0 and 9.0 $dS.m^{-1}$ the protein percentage of all plant parts increased in both the cultivars although this difference was not statistically significant. Carbohydrate content decreased significantly at EC 6.0 and 9.0 $dS.m^{-1}$ in all plant parts of both the cultivars ($p \leqslant 0.05$) compared with control treatment.

Table 5. Mean protein and total carbohydrate percentages of two mung bean cultivars after 30 days growth at different salinities

Plant part	Salinity as EC dS/m at 25°C			LSD 5%
	3.0 (control)	6.0	9.0	
Protein percentage				
AuMg 588				
Leaves	18.69	19.88	22.40	Lvs. 0
Stem	12.25	14.99	16.21	St. 0
Roots	10.13	13.24	14.15	Rts. 0
Mg 6601				
Leaves	19.22	20.32	23.10	
Stem	11.81	13.83	17.20	
Roots	11.09	12.71	14.41	
Total carbohydrate percentage				
AuMg 588				
Leaves	43.81	29.52	19.41	Lvs. 0
Stem	29.52	24.76	18.62	St. 0
Roots	12.38	11.43	9.70	Rts. 0
Mg 6601				
Leaves	40.48	27.62	20.10	
Stem	27.14	23.33	17.90	
Roots	13.81	12.86	10.30	

Discussion

The high sensitivity of the mung bean to salt at the germination stage is clear from the adverse effect of increasing salt concentrations on the percent germination of the two cultivars examined here (Table 1). This was due to reduced absorption of water by the germinating seeds and/or the toxic effects of certain ions on them (Bernstein and Hayward 1958).

At the seedling stage the shoot/root ratio of cultivars did not differ significantly which suggests that shoots and roots were affected by salt in a similar way. For dry matter AuMg 588 showed greater tolerance than Mg 6601 at high salt concentrations.

The significant reduction in the leaf area of both cultivars at high salt concentrations may have been due to the suppression of both the cell enlargement and cell division by salinity (Meiri and Poljakoff-Mayber 1970).

In general, all the chlorophyll contents were reduced significantly at high salt concentrations in both the cultivars, although variation between cultivars was evident. It has been suggested by Strogonov *et al.* (1970) that the specific enzyme, which is responsible for the synthesis of green pigments was suppressed by the effect of salt. It has also been suggested by the same workers that total chlorophyll and the proportion of its components depend on the biology and the development stages of the plant and on the nature and concentration of the salts.

The data for protein percentage indicated that increase in salt concentration generally caused an increase in protein content in different plant parts of both the cultivars. These results were in close agreement with the findings of Iwake *et al.* (1958) and Sherazi *et al.* (1971) who also reported that high salt concentrations caused an increase in the N content in rice and high protein contents in maize respectively. It was possible that the crop had produced either a high amount of some amino acids or other quaternary ammonium compounds as a result of salinity stress (Wyn Jones 1981).

The reduction in the carbohydrate content of the two cultivars at high salt concentrations may have been due to the adverse effect of salinity on the carbohydrate metabolism through photosynthetic activity due to interaction of Cl ions (Strogonov *et al.*, 1970).

The results presented here showed that the mung bean was very sensitive to salinity as suggested by Maas and Hoffman (1977). In view of he great sensitivity of the crop to salt, the screening of a large number of native/exotic cultivars would be of great value. The identification of tolerant germplasm may provide material which could be exploited in specific breeding programmes to enhance the salt tolerance of the crop or the knowledge may be used in its own right when cultivating on saline soils.

References

Arnon D I and Hoagland D R 1940 Crop production in artificial solutions. Soil Sci. 50, 463–485.

Ashraf M, McNeilly T and Bradshaw A D 1986 The response of selected salt tolerant and normal lines of four grass species to NaCl in sand culture. New Phytol. 104, 453–462.

Ashwell G 1957 Colorimetric analysis of sugars. *In.* Methods in Enzymology 3, 84–85, Academic Press, New York.

Bernstein L and Hayward H E 1958 Physiology of salt tolerance. Ann. Rev. Plant Physiol. 9, 25–96.

Epstein E 1985 Salt-tolerant crops: Origins, development, and prospects of the concept. Plant and Soil 89, 187–198.

Iwake S, Kaawai M and Ikemoto S 1958 Studies on salt injury in rice plant. XVI. Proc. Sci. Soc. Japan. 27 (1), 77–79.

Jackson M L 1958 Soil Chemical Analysis. Constable and Co. Ltd. London.

Maas E V and Hoffman G J 1977 Crop salt tolerance – Current assessment. J. Irrig. Drainage Div. ASCE 103, 115–134.

Meiri A and Poljakoff-Mayber A 1970 Effect of various salinity regimes on growth, leaf expansion and transpiration rate of bean plants. Soil Sci. 109, 26–34.

Noble C L 1983 The potential for breeding salt tolerant plants. Proc. Royal Soc. Victoria 95 (3), 133–138.

Shannon M C 1978 The testing of salt tolerance variability among tall wheatgrass lines. Agron. J. 70, 719–722.

Sherazi S A U, Ahmed N and Khan M F A 1971 Effect of saline irrigation water on the yield and chemical composition of maize variety J-I. J. Agr. Res. 9, 165–169.

Strogonov B P, Kabanov V V Shevajakova N I Lapine L P Kamizerko E I Popov B A Dostonova R Kh and Prykhod'ko L S 1970 Structure and Function of Plant Cells in Saline Habitats. Nauka Koscow (Trans. Eng.) John Wiley and Sons, New York.

Witham F H, Blaydes D F and Devlin R M 1971 Experiments in plant physiology. pp. 55–58. Van Nostand Reinhold Co., New York.

Wyn Jones R G 1981 Salt tolerance. *In* Physiological processes limiting plant productivity. Ed. C B Johnson, pp. 271–292. Butterworths, London.