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The effect of NaCl on water relations, chlorophyll, and protein and proline contents of two cultivars of blackgram (*Vigna mungo* L.)

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Abstract

The physiological basis of salt tolerance of two cultivars of blackgram, cv Candhari Mash (relatively salt tolerant) and cv Mash 654 (salt sensitive), was assessed in salinized sand culture at the flowering stage. Increasing NaCl concentration in the rooting medium significantly reduced the chlorophyll a, chlorophyll b, and total chlorophyll, leaf water potential (Ψ_w), leaf solute potential (Ψ_s), and leaf turgor potential (Ψ_p) in both the cultivars. Leaf protein and proline content was increased as a result of increasing salt concentration in both cultivars. High salt concentrations had no significant effect on the seed protein content of both cultivars. At high salinities, cv Candhari Mash had significantly greater chlorophyll a, chlorophyll b and total chlorophyll, leaf water potential, solute potential, and turgor potential than cv Mash 654, but the latter had greater leaf proline content than cv Candhari Mash. Cultivars did not differ significantly for both leaf and seed protein contents.

The relatively salt tolerant cv Candhari Mash maintained high leaf water potential and turgor potential to resist salt injury. Leaf proline content had negative correlation with salt tolerance in blackgram.

Introduction

Although soil scientists have been successful in minimizing the extent and spread of salinity affected soils using many curative and preventative measures, the methods are highly expensive so that they cannot be contemplated particularly in developing countries.

A highly attractive biotic approach to overcome the salinity threat, *viz* 'Selection and breeding for salt tolerance' as suggested by Epstein and Norlyn (1977), seems to be efficient and very economic in the prevailing situations. Direct methods of selection and breeding for the improvement of salt tolerance in crops have been suggested, without reference to any appropriate mechanism of salt tolerance, by previous workers (Epstein, 1985; Shannon, 1985). However, the knowledge of physiological mechanisms of salt tolerance is crucial in seeking rapid and objective parameters to assist mass screening programmes (Yeo and Flowers, 1984).

Blackgram (*Vigna mungo* L) offers a great promise as one of the major pulse crops in many countries. In addition, it is also widely used as fodder and green manure. However, it is highly sensitive to salinity like many other leguminous crops (Maas and Hoffman, 1977).

A study to assess the relative salt tolerance of ten cultivars of blackgram (Ashraf *et al.*, unpublished data), using sand culture technique, showed cv Candhari Mash and cv Mash 654 to be relatively salt tolerant and salt sensitive respectively.

The present work was carried out to draw comparisons between the salt tolerance, water relations and some other physiological parameters of the two cultivars of blackgram.

Materials and methods

Seeds of two cultivars of blackgram (*Vigna mungo* L.), Candhari Mash and Mash 654, were obtained from Ayub Agricultural Research Institute Faisalabad, Pakistan. Seed samples of both the cultivars were surface sterilized in 5% sodium hypochlorite for 5 minutes before experimentation. Ordinary river sand was washed thoroughly with tap water, distilled water and finally with full strength Hoagland nutrient solution. Pots of 28 cm size were filled with 6.61 kg of dry sand. The experiment was conducted in a growth room at 28 ± 3°C with 12 h daylength, at light intensity of 36 W m^{-2} and a relative humidity of 64%.

The concentrations of NaCl used were, 0, 15, 30 and 45 mol m^{-3} in full strength nutrient solution. The experiment was placed in a randomized complete block design with four blocks. Each block had two cultivars and four salt treatments and was replicated twice. A total of 64 pots was used in the experiment. Two randomly chosen, five days old, pregerminated seeds of each cultivar were transplanted into each pot. All the pots were irrigated for 10 days with full strength nutrient solution.

NaCl treatments, in full strength nutrient solution, begun 15 days after the start of the experiment. The NaCl concentration was increased in steps of 15 mol m^{-3} on alternate days until the appropriate salt treatments were reached. Treatments continued with the addition of 2 L of the appropriate solution on alternate days to each pot.

When the plants started flowering, one fully expanded trifoliate leaf (4th leaf from the first trifoliate leaf) was taken from each plant for analysis of chlorophyll, water potential, solute potential, proline and total protein content. Seed was collected at maturity for N analysis.

1. Chlorophyll content

Chlorophyll content was estimated following Witham *et al.* (1971). 0.5 g of fresh leaves were triturated in a porcelain mortar, with 80% acetone and filtered. When the extraction was complete, the filtrate was made up to 50 mL, thoroughly mixed and used to determine the chlorophylls a, b, and total spectrophotometrically at the appropriate wavelengths.

2. Leaf water potentials (Ψ_w)

Leaf water potential measurements were made with a water potential apparatus (Chas W. Cook and Sons, Birmingham, UK) soon after the leaves were excised from the plants.

3. Solute potential (Ψ_s)

The fresh leaf material was frozen in 2.0 cm^3 polypropylene tubes for two weeks, thawed and sap was extracted by crushing the material with a metal rod. After centrifugation at 8000 *g* for 4 min the sap was used directly for osmotic potential determination is an Osmometer TP 10B (Camlab Limited). Turgor potential was derived from the difference between solute and water potentials.

4. Total protein percentage

Nitrogen analysis of dry leaf and seed samples was carried out by the method described by Jackson (1958). Total protein = N × 6.25.

5. Proline estimation

0.5 g of fresh leaf tissue, from plants of each block, was homogenized in 10 mL of 3% sulfosalysalic acid and filtered through a Whatman No. 2 filter paper. Proline was estimated spectrophotometrically following the ninhydrin method described by Bates *et al.* (1973), using pure proline (Merck) as a standard.

Results

The data for mean chlorophyll content of the two blackgram cultivars, at varying salt concentrations, are given in Fig. 1, and analysis of variance of the data in Table 3. Increasing concentration of NaCl in the rooting medium significantly reduced the chlorophylls a, b, and total ($p \leqslant 0.001$), in both the cultivars. cultivars differed significantly in response to increasing salt concentration ($p \leqslant 0.001$), but the cultivars × treatments interaction was only significant for

chlorophyll b ($p \leqslant 0.05$). Cv Candhari Mash had significantly greater chlorophyll b than cv Mash 654 at 30 mol m^{-3} NaCl, but the chlorophyll b content of the two cultivars did not differ significantly for the rest of the salt concentrations.

Data for the three water relationship parameters, leaf water potential (Ψ_w), leaf solute potential (Ψ_s), and leaf turgor potential (Ψ_p) are presented in Table 1. Ψ_p was calculated as the difference between leaf water potential and solute potential. Increasing salt concentration had a significantly marked effect ($p \leqslant 0.001$) on the leaf water potential (Ψ_w) of both cultivars. Cultivars showed significantly different responses to increasing salt concentration ($p \leqslant 0.05$). Cv Candhari Mash had significantly higher leaf water potential than cv Mash 654 at 30 and 45 mol m^{-3} NaCl.

Analysis of variance of the data for leaf solute potential (Table 3) showed that cultivars had differing response to increasing NaCl concentration ($p \leqslant 0.05$). Cv Candhari Mash again had significantly higher solute potential (Ψ_s) than cv Mash 654 at high salt concentrations.

The leaf turgor potential responses of cultivars, to increasing NaCl concentrations, were significantly different ($p \leqslant 0.05$). Cv Candhari Mash maintained significantly higher turgor potential (Ψ_p) than cv Mash 654 at high salt concentrations.

Total protein percentage of dry leaves and seeds of the two cultivars are presented in Table 2 and leaf proline content in Fig. 2. With the increase in salt concentration the protein percentage of leaves was significantly increased in both the cultivars ($p \leqslant 0.05$), but the increase in protein content was not observed in seeds. Cultivars did not differ significantly in protein content of both leaves and seeds. Since cv Mash 654 did not produce seed at 45 mol m^{-3} NaCl the protein analysis was not carried out for the seed of Candhari Mash at this salt treatment because of possible effects on the statistical analysis of the data.

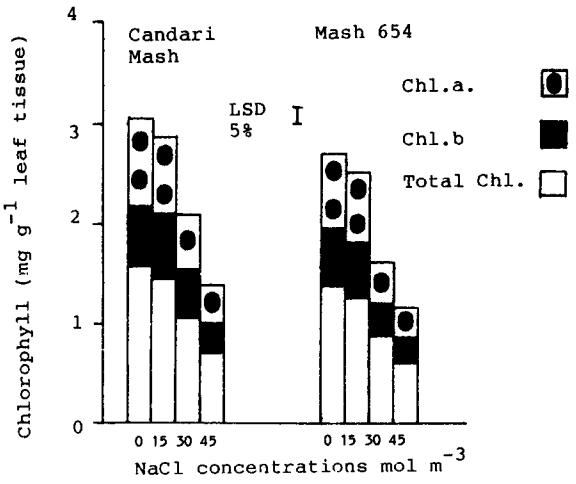

Fig. 1. Mean chlorophyll content (mg g^{-1} leaf tissue) of two blackgram cultivars grown in sand culture at different NaCl concentrations.

Addition of NaCl to the rooting medium significantly increased the leaf proline content in both the cultivars ($p \leqslant 0.001$). Cultivars differed significantly in leaf proline content ($p \leqslant 0.01$). Cv Mash 654 accumulated significantly more proline ($p \leqslant 0.05$) in leaves at all salt concentrations as compared to cv Candhari Mash.

Discussion

Reference was made earlier to the fact that cv Candhari Mash was shown to be relatively salt tolerant in all growth parameters as compared to cv

Table 1. Mean leaf water potential (Ψ_w), solute potential (Ψ_s), and turgor potential (Ψ_p) of two blackgram cultivars at different salinities

Potentials	Salinity as mol m^{-3}				LSD 5%
	O (Control)	15	30	45	
Candhari Mash					
Ψ_w (−MPa)	1.56 (0.11)	1.62 (0.09)	1.86 (0.12)	2.11 (0.16)	Ψ_w = 0.21
Ψ_s (−MPa)	1.87 (0.13)	1.92 (0.15)	2.12 (0.17)	2.32 (0.14)	Ψ_s = 0.26
Ψ_p (MPa)	0.31 (0.03)	0.30 (0.03)	0.26 (0.02)	0.21 (0.02)	Ψ_p = 0.08
Mash 654					
Ψ_w (−MPa)	1.42 (0.08)	1.65 (0.09)	2.25 (0.04)	2.68 (0.15)	
Ψ_s (−MPa)	1.76 (0.11)	1.96 (0.11)	2.41 (0.13)	2.80 (0.20)	
Ψ_p (MPa)	0.34 (0.04)	0.31 (0.03)	0.16 (0.01)	0.12 (0.01)	

Figures in brackets represent standard errors.

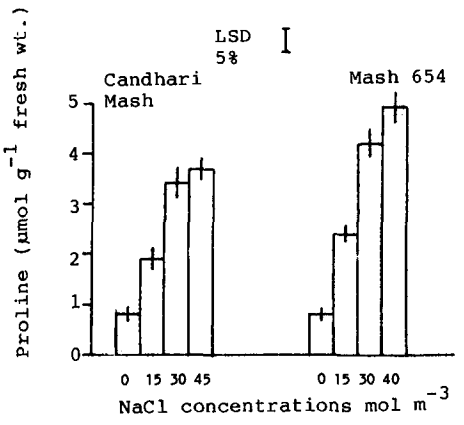

Fig. 2. Mean leaf proline content (μmol g^{-1} fresh wt) of two blackgram cultivars grown in sand culture at different NaCl concentrations.

Mash 654 (Ashraf *et al.*, unpublished data). The following discussion seeks to reveal the physiological parameters responsible for such tolerance with explanations of possible mechanisms involved.

From the results for chlorophyll content it is clear that all the chlorophyll contents were reduced significantly in both the cultivars as a result of increasing salinity. But variation between cultivars was apparent only for chlorophyll b. The more inhibitory effect of NaCl on the chlorophyll contents of cv Mash 654 could be due to the suppression of the specific enzyme which is responsible for the synthesis of green pigments (Strogonov *et al.*, 1970). It may also be due to the reason, suggested by the same workers, that the total chlorophyll and the proportion of its components depend on the biological processes and development stages of the plant and also on the type and concentration of the salts.

The decrease in the solute potential of plants grown under salt stress may result from water loss or an increase in dissolved solutes or a combination of both. A major proportion of the increase in dissolved solutes may be from uptake of salt (Slatyer, 1961). However, if comparisons are drawn between the salt tolerance and water relations of the two cultivars, it is quite clear that salt tolerant cv Candhari Mash had higher Ψ_w and Ψ_p than cv Mash 654 in order to avoid physiological drought. In fact, higher turgor potentials have been considered principal factors for maintaining growth at high salinities (Greenway and Munns, 1980). The significantly lower leaf-osmotic-potential in cv Mash 654, as opposed to that of cv Candhari Mash, may be related to the fact that halophytic, as well as glycophytic, plant species adjust to high salt concentration by lowering tissue osmotic potentials with increased uptake of solutes (Flowers *et al.*, 1977; Wyn Jones *et al.*, 1977). However, growth inhibition of cv Mash 654 may have been due to toxic effects of accumulated solutes despite osmotic adjustment in tissues (Flowers *et al.*, 1977; Wyn Jones *et al.*, 1977).

Although cultivars did not differ in leaf and seed protein content, increasing concentration of NaCl significantly increased the protein content in leaves of both cultivars. This may be due to the fact that salinity enhances protein synthesis in cereals (Langdale *et al.*, 1973) and promotes conversion of N into protein (Helal *et al.*, 1975). Therefore it can be inferred that salinity activates the metabolism of plants and hence the accumulation by the cells of more immediate metabolites. The results for protein percentage agree with those found in stargrass (Langdale *et al.*, 1973), in tomato (Besford, 1978), and in maize (Sherazi *et al.*, 1971) as these

Table 2. Mean protein percentage of leaves and seeds (dry weight basis) or two cultivars of blackgram when grown at different NaCl salinities in sand culture

Cultivar	Salinity as mol m^{-3}				LDS 5% Cv × Tmt = NS
	0 (Control)	15	30	45	
Leaves					
Candhari Mash	15.2 (0.51)	15.8 (0.46)	17.3 (0.23)	19.2 (0.36)	0
Mash 654	14.6 (0.32)	15.3 (0.22)	18.1 (0.27)	19.9 (0.31)	
Seeds					
Candhari Mash	22.8 (0.18)	22.6 (0.33)	23.9 (0.25)	ND	0
Mash 654	22.0 (0.21)	22.2 (0.26)	22.7 (0.36)	—	

ND, not determined.

Table 3. Analysis of variance summaries (mean squares) of different parameters of two cultivars of blackgram grown at different NaCl salinities in sand culture

Source of variation	Degrees of freedom (df)	Chl.a	Chl.b	Total Chl.	Leaf Ψ_w	Leaf Ψ_s	Leaf Ψ_p	Leaf protein	Leaf proline	df	Seed protein
Blocks	3	0.01NS	0.011NS	0.046NS	0.03NS	0.052NS	0.005NS	0.96NS	0.02NS	3	1.08NS
Cultivars (CV)	1	0.063***	0.071***	0.261***	0.09*	0.158**	0.013*	3.92NS	0.582**	1	2.01NS
Treatments (T)	3	0.098***	0.102***	0.456***	0.21***	0.440***	0.017**	8.22*	3.14***	2	2.86NS
CvXT	3	0.019NS	0.030*	0.059NS	0.07*	0.099*	0.012*	3.11NS	0.410*	2	1.96NS
Residual	21	0.01	0.009	0.031	0.02	0.031	0.003	2.04	0.098	15	1.5

*, **, ***, significant at 0.05, 0.01, and 0.001 levels of probability respectively; NS, non significant.

crops at high salinities produced an increase in protein content. However it is rather difficult to explain the tolerance of cv Candhari Mash on the basis of results for protein content.

The accumulation of proline in a wide variety of both halophytes and non-halophytes when subjected to various stresses and the role of proline in adaptive responses has been reviewed (Aspinall and Paleg, 1981). In this investigation, however, both the cultivars accumulated significantly greater proline in the leaves at high salinities than the control treatment, but this accumulation was significantly higher in cv Mash 654 than in Candhari Mash.

However, the results clearly show that proline levels in salt stressed plants were inversely correlated with ability to withstand salinity stress. Therefore, it may be concluded that proline cannot be used as an indicator of salt tolerance in blackgram as was suggested for soybean (Moftah and Michel, 1987). In general, proline levels in the leaves of the two cultivars were too low to have any significant osmoregulatory role in these cultivars. Proline may, however, be complimentary to other organic compounds such as glycinebetaine and glycerol which have been found to accumulate in numerous salt stressed plants (Flowers *et al.*, 1977; Levitt, 1980). Further research is necessary to investigate the osmoregulatory role of other organic compounds in salt stressed blackgram.

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