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Full Length Research Paper

Protein electrophoretic profiles and physiochemical indicators of salinity tolerance in sorghum (*Sorghum bicolor* L.)

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The present study was conducted to quantify the response of chlorophyll, protein concentration and electrophoretic patterns of leaf and root soluble proteins to increasing salinity in the rooting medium of three varieties (Payam, Kimia and Jambo) of sorghum (*Sorghum bicolor* L.) and to evaluate the usefulness of these parameters in identifying salt-resistant varieties. This work was carried out with five concentrations of salinity (0, 50, 100, 150 and 200 mM NaCl) and two sampling time points (sampling after 15 and 30 days salt treatment) using a split-split plot design with randomized complete block layout. With increasing salinity, the K+ concentration was found to decrease and the amount of Na+ and Na+/K+ increase significantly (P≤0.05) in roots and shoots. Chlorophyll a, b, total chlorophyll concentration and leaf and root soluble protein contents decreased with increasing salinity. The electrophoretic pattern of soluble proteins of cv. Jambo showed that after 15 days NaCl (200 mM) and 30 days NaCl (100 and 150 mM) treatment, a new polypeptide of molecular weight 50 kDa was expressed.

The expression of this polypeptide might have been due to the plant adapting to NaCl via expression of a stress-resistant gene. This polypeptide was synthesized under salt stress and is suggested as a marker protein for salt adaptation.

Keywords: Chlorophyll, electrophoretic patterns, soluble proteins, salinity, sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is moderately tolerant to salinity (Maas et al., 1986) and is widely grown in the semi-arid areas of east Africa on soils prone to salinity. Problems related to the agricultural systems, deficiencies of agricultural research plans, traditional agriculture, lack of agricultural knowledge and information among Third World farmers have caused irreparable damage to plant productivity in economies of these countries. Besides, salinity of soils and waters has led to development of salt marshes and caused serious problems for crops and pasturage plants.

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Abbreviations: PMSF, Phenylmethanesulfonylfluoride; **PAGE,** polyacrylamide gel electrophoresis.

Classical methods of screening for salt tolerance were based on the plant yield and are very costly and time consuming. Environmental salinity resistance in plants is recognizable through some parameters. For example, measurements of major physiological and biochemical traits, including chlorophyll content and protein concentration, can be used to monitor plant responses to salt stress (Belkhodja et al., 1994; Misra et al., 2006). Paying attention to plant adaptation and salinity resistance mechanisms (e.g. Na^+ exclusion or sequestering) is one of the important steps in their selection and breeding for salt adaptation and salinity resistance (Sunseri et al.,1998).

Crops in saline soils are faced with reductions in water absorption, insufficient nutrient availability, accumulation of toxic ions (Na^+ and Cl^-), or K^+ and Ca^{++} depletion in plant tissues (Salisbury and Ross, 1992), disturbances in metabolic activity such as respiration, photosynthesis (Rowson and Munns, 1984), altered enzyme activity

(Greenway and Osmond, 1972) and imbalance in absorbed elements. Plant species reveal many differences in sensitivity and reaction to water potential reduction that result from drought, frost, or salinity. Molecular breeding of salt-tolerant plants using either molecular markers or genetic engineering enhances their resistance to both hyper-osmotic stress and ion toxicity (Arzani, 2008; Chinnusamy et al., 2005).

Salt-sensitive genotypes of wheat show poor capacity to regulate entry of Na^+ into cells, thus inhibiting down-stream cellular functions (Serrano, 1996; Amtmann and Sanders, 1999; Tester and Davenport, 2003). In addition, tissue tolerance to Na^+ via sequestration in the vacuolar compartment contributes to salt tolerance in phenotypes (Munns and James, 2003). No variation was found among wheat genotypes for unidirectional root uptake of Na^+. The major differences in Na^+ transport between the genotypes were in the rate of transfer to the shoot (net root xylem loading) and the preferential accumulation of Na^+ in the leaf sheath versus the leaf blade (Davenport et al., 2005).

Genetic analysis of a cross between durum wheat Line 149 and cv Tamaroi revealed two genes that exert a major effect on Na^+ exclusion (Munns et al., 2003). A quantitative trait locus for low Na^+ concentration in leaf blades was mapped to the distal region on the long arm of chromosome 2A and named *Nax1*. It was suggested that a second gene (*Nax2*), independent of Nax1, contributes to the full expression of the Na^+ exclusion trait (Lindsay et al., 2004). Both *Nax* genes restrict the transport of Na^+ from roots to shoots which results in enhanced K^+–Na^+ discrimination in the leaf blade. *Nax2* has a mechanism similar to that described for *Kna1* in bread wheat. The mechanism conferred by *Nax1*, which was characterized by the deposition of Na^+ in the leaf sheath, is not confined to the wheat germplasm containing the *Nax1* gene. Preferential deposition of Na^+ in the leaf base has been described for rice (*Oryza sativa*), common reed (*Phragmites communis*; Matsushita and Matoh, 1991) and sorghum (Lacerda et al., 2003). An equivalent of the *Nax1* gene may be present in these other species.

One significant effect of salinity on plant growth occurs through changes induced in the osmotic strength of the growth medium. Plants have several mechanisms for balancing osmotic pressure changes in the root medium. Crops under saline conditions decrease the cellular osmotic potential by increasing the concentrations of free amino acids, inorganic cations and insoluble particles, accumulation of which helps to maintain the osmotic balance (Salama et al., 1994).

Genes that are up-regulated by salt stress mainly belong to several groups, based on their possible functionality. These genes encode the *LEA* proteins, enzymes (involved in the biosynthesis of osmolytes, hormones, detoxification and general metabolism), transporters (ion transporters, ABC transporters and aquaporins) and regulatory molecules such as transcription factors, protein kinase and phosphatases. The most common and widely reported genes that are stress-regulated are perhaps the *LEA* or *LEA*-like genes. *LEA* genes encode late embryogenesis abundant proteins (Dure et al., 1989). They have been identified in many plant species. Despite their wide occurrence, the functions of this group of polypeptides are ill-defined except in a few cases where over-expression of individual *LEA* genes resulted in some degree of stress protection (Xu et al., 1996). The finding that enhanced expression of the transcription factors regulating the expression of these *LEA*-like genes can increase the tolerance of transgenic plants to cold, drought, or salt stress demonstrates that these proteins do have a protective effect against abiotic stresses (Jaglo-Ottesen et al., 1998; Kasuga et al., 1999).

The identification of specific characteristics related to salt resistance such as proteins, amino acids and specific carbohydrates will provide potential biological markers useful in the identification and genetic manipulation of salt-resistant plants and plant cells. One approach to study the molecular mechanism of the plants' reaction to salinity is to use polyacrylamide gel electrophoresis to identify differentially regulated proteins involved in the physiology of salt resistance (Zorb et al., 2004). The aim of this study is to use protein electrophoretic profiles and some physiochemical traits to compare salt-tolerant varieties of sorghum.

MATERIALS AND METHODS

Three varieties of sorghum (*S. bicolor* L.), namely Payam, Kimia and Jambo, were used to identify salt-tolerant cultivars. These sorghum varieties were chosen for their commercial importance. However, the salt tolerance mechanisms of these cultivars have not been studied. Seeds of these cultivars were provided by the Karaj Seed and Plant Research Institute in Iran. Experiments were conducted in the greenhouse at Shahrekord University, Sharekord, Iran. Five concentrations of salinity (control, 50, 100, 150 and 200 mM) and two sampling time points (15 days and 30 days salt treatment) were used. Replications and sampling were arranged according to a split-split plot design with randomized complete blocks layout. Seeds were planted in a greenhouse in pots containing a peat-sand-perlite mix. Eight days after planting, the seedlings were irrigated with Hoagland nutrient solution (Hoagland and Arnon, 1959). Application of nutrients was started at low concentrations (10%) and increased gradually to the desired level of concentration by the 10th day after planting, at which time the seedlings were treated with NaCl. Salt treatments were initiated by adding 50 mM of NaCl per liter of culture solution per day. The plants were harvested 15 and 30 days after the final salinity level was reached.

Chlorophyll measurement

Using three plant samples, chlorophyll concentration was determined from fully expanded leaves (leaf number 8). A leaf sample of 0.1 g was ground and extracted with 5 ml of 80% (v/v) acetone in the dark. The slurry was filtered and absorbencies were determined at 645 and 663 nm. Concentration of chlorophyll a (Chl.

Table 1. Analysis of variance of Chl a, b, total Chl, shoots and roots soluble protein recorded in 3 varieties of sorghum in 2 sampling of exposure to 5 salinity levels.

Source of variation	df	Chl. a (mg/g leaf)	Chl. b (mg/g leaf)	Total chl. (mg/g leaf)	Shoot protein (mg/g leaf)	Root protein (mg/g root)
Salinity	4	1.113**	2.756**	7.348**	69.180**	5.909**
Genotype	2	0.001^{ns}	0.030*	0.044*	13.623**	1.126**
Sampling	1	2.548**	1.288**	7.459**	100.36**	4.871**

ns: Non-significant; *: significant at P = 0.05; **: significant at P = 0.01.

Table 2. Analysis of variance of Na^+, K^+ and Na^+/K^+ ratio of shoot and root recorded in 3 varieties of sorghum, in 2 sampling of exposure to 5 salinity levels.

Source of variation	df	Na^+ shoot (mg/g leaf)	K^+ shoot (mg/g leaf)	Na^+/K^+ shoot	Na^+ root (mg/g root)	K^+ root (mg/g root)	Na^+/K^+ root
Salinity	4	18209.9**	16445.0**	8.3**	9864.2**	3370.8**	10.8**
Genotype	2	2001.6**	44.0 ^{ns}	0.86**	1147.2**	1264.2**	2.5**
Sampling	1	12376.3**	4393.7**	5.5**	450.6**	4411.7**	4.6**

ns: Non-significant; *: significant at P = 0.05; **: significant at P = 0.01.

a), chlorophyll b (Chl. b) and total chlorophyll were estimated by the equations of Hendry and Price (1993). The total concentration of chlorophyll was obtained from the relation:

Total Chl = Chl. a + Chl. b

Measurement of Na^+ and K^+ concentration

For this measurement, 0.2 g of dried sample of leaf or root was placed in a porcelain crucible inside a furnace at 550°C for 2 h. After the samples were removed from the furnace and before they were cooled completely, 10 ml of 2 N HCl was added and the solution heated for several minutes till the ash dissolved completely. After filtering the solution with ash-less filter paper, the filtered solution volume was diluted to 100 ml with distilled water and the concentration of Na^+ and K^+ measured with a flame photometer (Genway flame photometer, model PFP-7).

Protein analysis

Soluble proteins were extracted from leaf and roots of seedlings. One gram of tissues was homogenized in 3 ml Tris-HCl buffer (0.1 M, pH 7.5) containing 1 mM phenylmethanesulfonylfluoride (PMSF), at 4°C. The homogenate was centrifuged at 18,000 x g for 30 min. Protein present in the supernatant was measured by a modification of the Bradford (1976) method using crystalline bovine albumin to establish a standard curve. This protein extract was concentrated before loading on the gel. Samples with equal protein content of 40 µg were subjected to discontinuous polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions as described by Leammli et al. (1970) with little change. Electrophoretic separation was performed at 4°C for 7 h, using 15% polyacrylamide gels. After electrophoretic separation, the gels were treated with fixing and staining solution for 3 h. The gels were then photographed and scanned using a 2202 ULTROSCAN Laser Densitometer (LKB). The leaf and root soluble protein contents under salinity stress were determined according to Bradford (1976), using bovine serum albumin (BSA) as standard protein.

Statistical analysis

Analysis of variance (ANOVA) was performed with the statistical program Minitab (Minitab Inc; College Park, PA). Means comparisons were conducted using the least significant difference (LSD) test at 5% of probability level.

RESULTS AND DISCUSSION

Results of analysis of variance showed that salinity had significantly ($P \leq 0.01$) reduced the amount of chlorophyll a, b, total chlorophyll, K^+ and leaf and root soluble protein concentrations and raised the Na^+ concentration in leaves and roots (Tables 1 and 2).

Chlorophyll a, b and total chlorophyll

In the present study, decreases in the contents of chlorophyll a, b and total chlorophyll were observed when the NaCl concentration in the media increased (Figure 1). Loss of chlorophyll and leaf bleaching are often responses to stress conditions that occur due to senescence, that is, degradation and recycling of resources. The total chlorophyll content was significantly lower in the leaves of salt-treated shoots than in those of control shoots. No significant difference was observed between varieties in the amount of Chl. a at the first and second sampling time points (Figure 2). In cv. Jambo, the content of Chl. a at 150 mM salinity concentration showed an increase compared to that in the 100 mM treatment, but further increase in salt concentration (200 mM) caused it to decrease (Figure 1). There were no significant differences in Chl. a among the tested genotypes. With

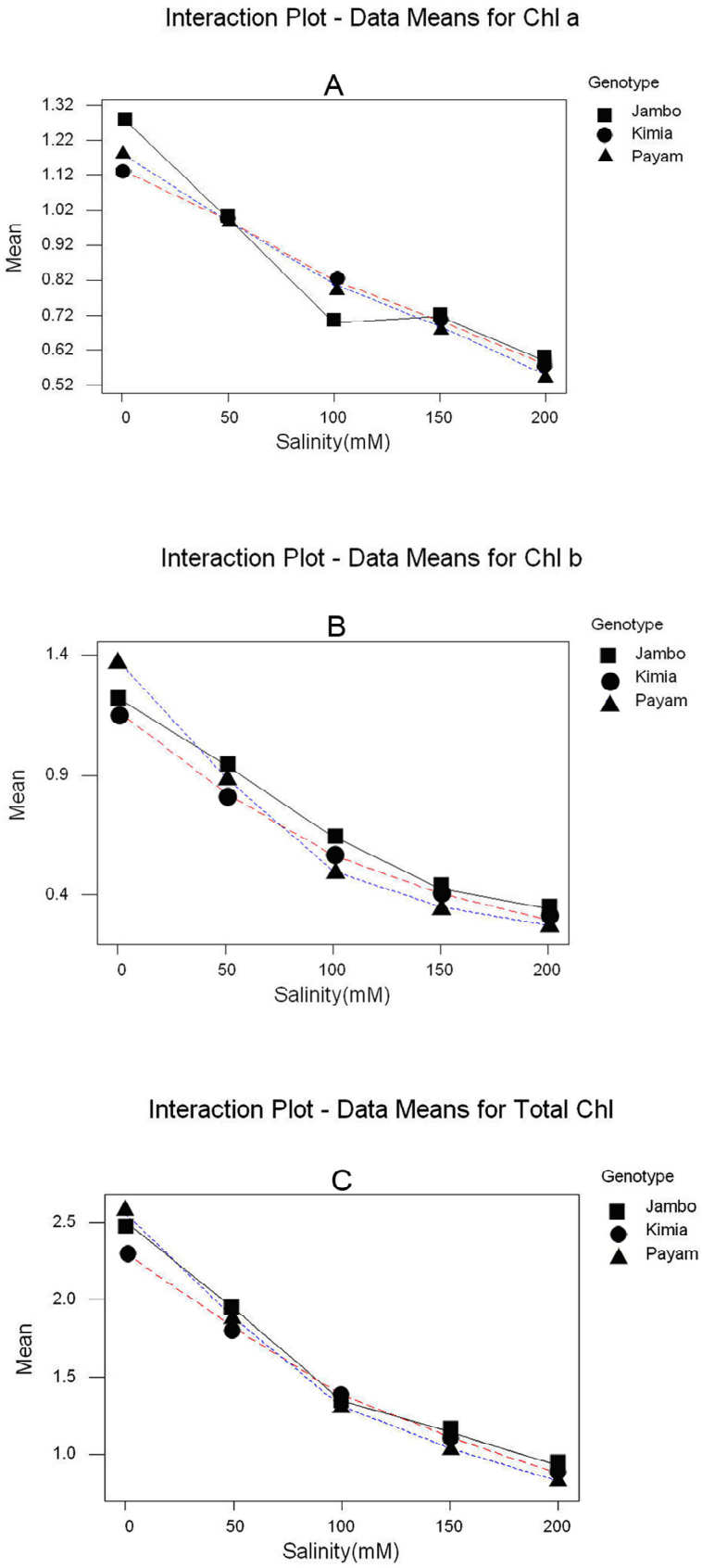


Figure 1. Effect of increasing salinity on the amounts of chlorophyll a (A), b (B) and total chlorophyll (C) of three sorghum varieties.

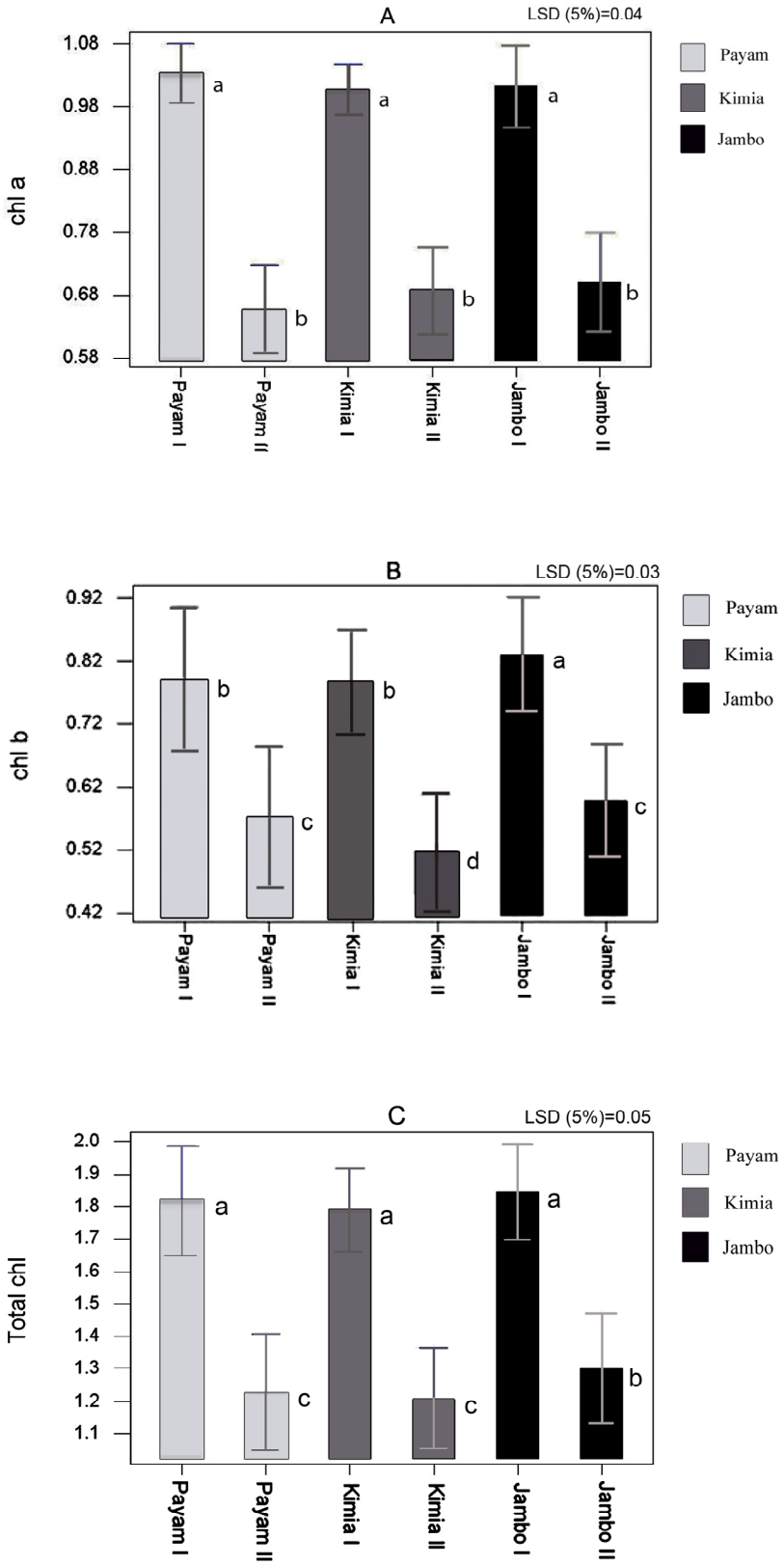


Figure 2. Chlorophyll a (A), b (B) and total chlorophyll (C) concentrations of the three sorghum varieties recorded at two sampling points (sampling after salt application for 15 (I) and 30 (II) days). Significant values at the 5% level are indicated with letters.

Table 3. Mean comparisons of Chlorophyll a, b, total chlorophyll, shoot and root soluble protein concentrations of 3 sorghum varieties under 5 salinity levels.

Salinity (mM)	Genotype	Chl. a (mg/g leaf)	Chl. b (mg/g leaf)	Total chl. (mg/g leaf)	Shoot protein (mg/g leaf)	Root protein (mg/g root)
Control	Payam	1.182 b	1.378 a	2.560 a	9.200 c	2.398 c
	Kimia	1.148 b	1.168 b	2.303 b	9.833 b	2.725 b
	Jambo	1.281 a	1.222 b	2.507 a	11.31 a	2.895 a
50	Payam	0.989 c	0.894 cd	1.883 cd	7.787 f	2.135 e
	Kimia	0.991 c	0.828 d	1.820 d	8.135 e	2.235 d
	Jambo	0.996 c	0.943 c	1.942 c	8.967 d	2.355 c
100	Payam	0.809 d	0.498 fg	1.308 e	6.693 h	1.610 h
	Kimia	0.819 d	0.563 f	1.383 e	6.790 g	1.810 g
	Jambo	0.697 e	0.644 e	1.345 e	7.770 f	1.922 f
150	Payam	0.688 e	0.349 hij	1.038 g	5.360 l	1.275 j
	Kimia	0.705 e	0.404 hi	1.110 fg	5.732 k	1.590 h
	Jambo	0.718 e	0.425 gh	1.145 f	6.213 i	1.885 f
200	Payam	0.549 f	0.272 j	0.821 i	4.710 n	1.050 k
	Kimia	0.578 f	0.294 j	0.873 hi	5.115 m	1.225 j
	Jambo	0.587 f	0.340 ij	0.928 h	6.027 j	1.365 i
LSD value (α = 5%)		0.04	0.07	0.09	0.07	0.06

Data represent means of 3 replications. Means followed by the same letter are not significantly different at the 5% probability level.

increase in salinity, the Chl. a content decreased uniformly in all varieties; however, an unusually high decrease was observed in Jambo at 100 mM salinity. In the investigation of two sensitive and tolerant varieties of sorghum, a considerable decrease of Chl. a was observed in a tolerant variety at 100 mM salinity (Netondo et al., 2004).

At the first sampling time point (15 days after treatment), Jambo contained the most Chl. b relative to other varieties but there were no difference between varieties in the content of total Chl. By contrast, at the time point of 30 days after treatment, Jambo and Payam contained the most Chl. b and Kimia the least; Jambo also contained the most total Chl. (Figure 2). Results showed that Jambo contained more Chl. b and the other two varieties contained less at the 50 mM and 100 mM salinity levels, but there was no significant difference between Jambo and Payam at 50 mM salinity and among all cultivars at 150 and 200 mM salinity (Table 3). Jambo had higher total Chl. content than other varieties at 50, 150 and 200 mM salinity but there was no significant difference among cultivars at 100 mM salinity (Table 3).

Large increases in sodium concentration in the shoot can cause premature leaf senescence leading to reduction in biomass, productivity and yield (Munns et al., 2000). An investigation of the effects of 50 mM NaCl on sensitive and tolerant varieties of rice reported that the total Chl concentration in both the resistant and sensitive varieties was reduced by salinity and a highly significant difference between varieties was recorded after 28 days salt treatment (Lutts et al., 1996). When plants are subjected to environmental salt stress, the balance between the production of reactive oxygen species, that is, superoxide and hydroxyl radicals and singlet oxygen and the quenching activity of antioxidants is disturbed (Singha and Choudhuri, 1990). Hydrogen peroxide and singlet oxygen may play an important role in the mechanism of NaCl injury in *Vigna catjary* and *O. sativa* leaves. Reactive oxygen species are known to be the main mediators of oxidative damage to various cellular components such as membrane fatty acids, proteins, nucleic acids and chlorophyll (Singha and Choudhuri, 1990; Seneratina et al., 1985).

Na^+ and K^+ content and the Na^+ /K^+ ratio

There were significant differences ($P \leq 0.05$) between varieties in root and shoot concentrations of Na^+ and Na^+/K^+ ratio (Table 4). At all five salinity levels, Payam contained the most Na^+ and Na^+/K^+ in both roots and shoots and Jambo the least. Increasing salinity levels caused Na^+ and Na^+/K^+ to increase in both roots and shoots. The increase of Na^+ content in sorghum due to increases in salinity had been observed previously (Igarta, 1995; Lacerda et al., 2003). High levels of Na^+ in the shoot led to physiologic and osmotic problems in plants. Therefore, lower accumulation of this ion in the shoot could play a role in salinity tolerance. Lower accumulation of Na^+ in shoots results from either lower Na^+ uptake by the root, or from a reduced amount of transmission from roots to shoots (Tester and Davenport, 2003; Flowers and Yeo, 1995). Increased Na^+ concentration in shoot tissues was reported to be the consequence of increasing salinity in the root medium of wheat (Santa–Maria and

Table 4. Mean comparisons of Na^+, K^+ and Na^+/K^+ ratio of shoot and root of 3 sorghum varieties under 5 salinity levels.

Salinity (mM)	Genotype	Na^+ shoot (mg/g leaf)	K^+ shoot (mg/g leaf)	Na^+/K^+ shoot	Na^+ root (mg/g root)	K^+ root (mg/g root)	Na^+/K^+ root
Control	Payam	8.74 o	128.0 b	0.068 j	14.18 i	55.19 de	0.270 j
	Kimia	10.56m	144.0 a	0.073 j	10.54 j	66.48 c	0.157 k
	Jambo	9.66 n	102.6 d	0.094 j	13.87 j	78.11 a	0.177 k
50	Payam	24.66 j	88.43 e	0.303 h	27.60 g	51.16 f	0.651 h
	Kimia	21.61 k	104.9 cd	0.238 hi	27.77 g	54.91 e	0.606 h
	Jambo	17.55 l	112.7 c	0.183 i	21.63 h	67.11 b	0.326 i
100	Payam	52.64 g	90.54 e	0.633 f	58.01cd	45.43 h	1.35 e
	Kimia	45.29 h	69.95 f	0.681 f	53.72 e	48.50 g	1.15 f
	Jambo	41.07 i	84.0 e	0.517 g	44.58 f	55.51 d	0.80 g
150	Payam	97.25 a	59.15 gh	1.77 b	70.85 b	35.60 l	2.02 b
	Kimia	66.75 e	59.67 gh	1.18 d	67.74 b	36.64 k	1.90 c
	Jambo	56.72 b	66.07 fg	0.92 e	53.75 e	42.12 i	1.32 e
200	Payam	94.26 b	48.03 i	1.94 a	79.75 a	30.31 n	2.70 a
	Kimia	82.46 c	47.66 i	1.71 b	58.75 c	31.39 m	1.90 c
	Jambo	71.77 d	57.41 hi	1.32 c	54.71 de	38.40 j	1.45 d
LSD value (α = 5%)		0.47	8.05	0.14	3.48	0.47	0.10

Data represent means of 3 replications. Means followed by the same letter are not significantly different at the 5% probability level.

Epstein, 2001; Munns et al., 2003) and other cereals (Flowers and Hajibagheri, 2001). The absorption patterns of Na^+ vary in different genotypes; salt-sensitive genotypes accumulated more Na^+ in their shoot tissues. Under control conditions, Payam had the least Na^+ in the shoots and Kimia the most, but at all salinity levels Jambo accumulated the least Na^+ in shoots (Table 4). In a study of two sensitive and tolerant groups of sunflower, it was reported that the tolerant genotypes accumulated less Na^+ in their shoots than sensitive genotypes (Ashraf and Tufail, 1995).

At all salinity levels, Jambo also had less Na^+ content in its root tissues (Table 4) and was lower than that of shoots. This is because compared to shoot tissues, roots accumulate less Na^+. In halophytes, the Na^+ absorbed by roots is transferred to the shoot and consequently residual Na^+ in roots is lower than in other types of plants (Greenway and Munns, 1980). Little research has been carried out on roots with regard to either salt- or water stress. Roots might seem the part of the plant most vulnerable as they are directly exposed to salt or to drying soil, but they are surprisingly robust. As shown earlier, their growth rate is not affected as much as that of shoots. Ionic status of roots is better than that of shoots; their ion concentrations do not increase with time as much as in leaves and Na^+ concentration is often lower than that of the external environment; this rarely happens in leaves. For example, in the roots of wheat grown in 150 mM NaCl, content of Na^+ was only 20 - 40 mM, the variation being due to the different genotypes (Gorham et al., 1990).

In our study, K^+ was lower in roots than in shoots (Table 4) and did not make a significant contribution to root osmotic adjustment. This raises the interesting question of whether organic solutes could be used for osmotic adjustment, as K^+ is often lower in roots than in shoots (Gorham et al., 1990). However, there is no evidence that organic solutes are more likely to accumulate in roots than in shoots; on the contrary, they are often lower in roots than in shoots. For example, proline and glycine betaine concentrations on a fresh weight basis were five times lower in roots than in shoots of barley grown at levels of over 100 - 200 mM NaCl, even though the Na^+ concentration was much lower in roots than in shoots (Wyn and Storey, 1978). Because osmotic adjustment is presumably as important for roots as for shoots, it is likely that unknown solutes are involved. Surprisingly, little is known about the metabolic regulation of osmotic pressure in relation to organic solute synthesis and compartments in response to salinity, either in shoots or in roots.

The K^+ concentration of roots and shoots decreased significantly ($P \leq 0.05$) by increasing salinity (Table 4). The K^+ content in crops is important in salinity tolerance (Munns et al., 2000). At both time points, Jambo had the lowest content of Na^+ and the lowest Na^+/K^+ ratio and the highest content of K^+ in both roots and shoots (Figure 3).

Generally, tolerant genotypes maintain higher K+ levels under salinity stress (Ashraf and Tufail, 1995). Because of the negative effect of Na^+ on K^+ uptake and increasing salinity, rising Na^+ levels in plant tissues disrupt the K^+ status of the plant. High Na+ concentration

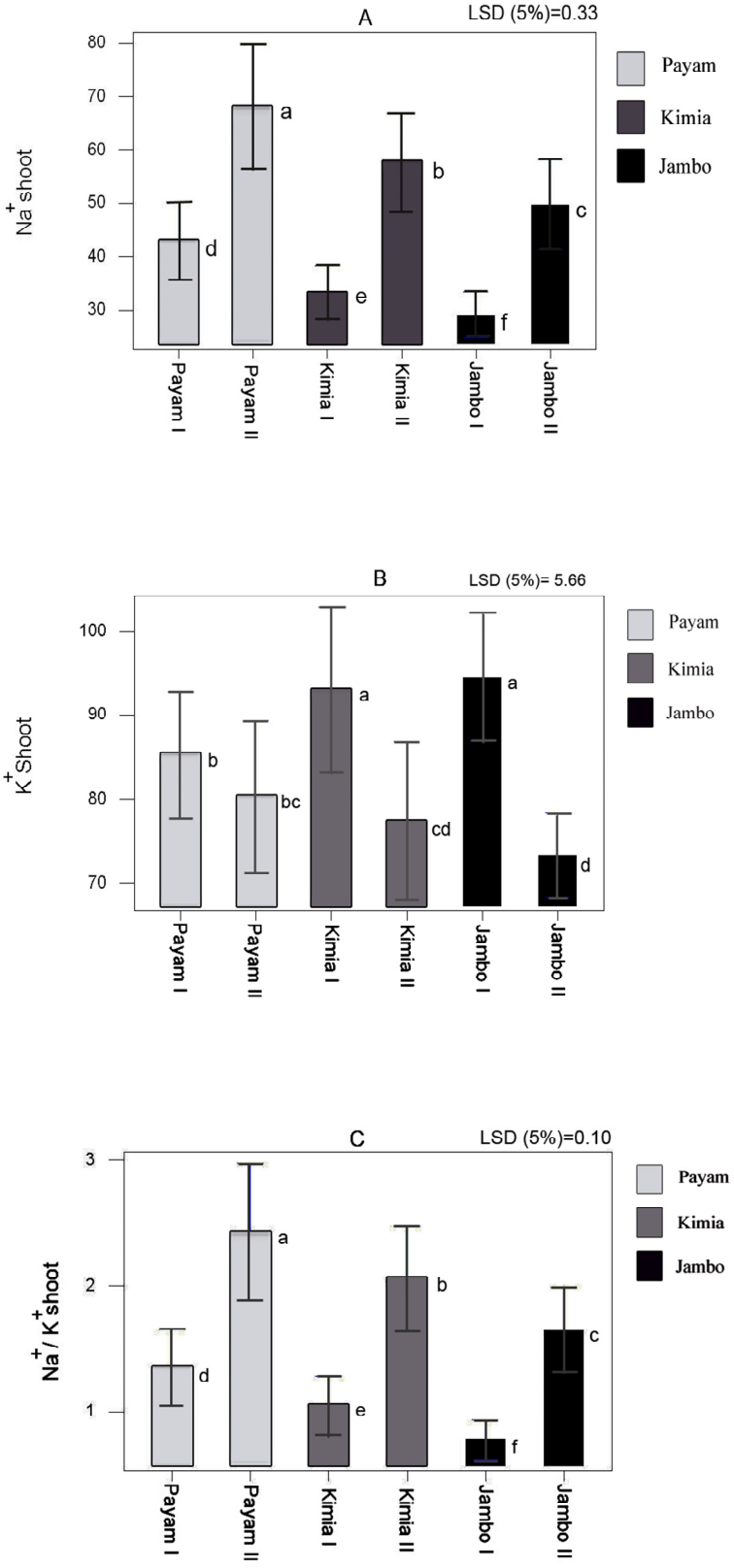
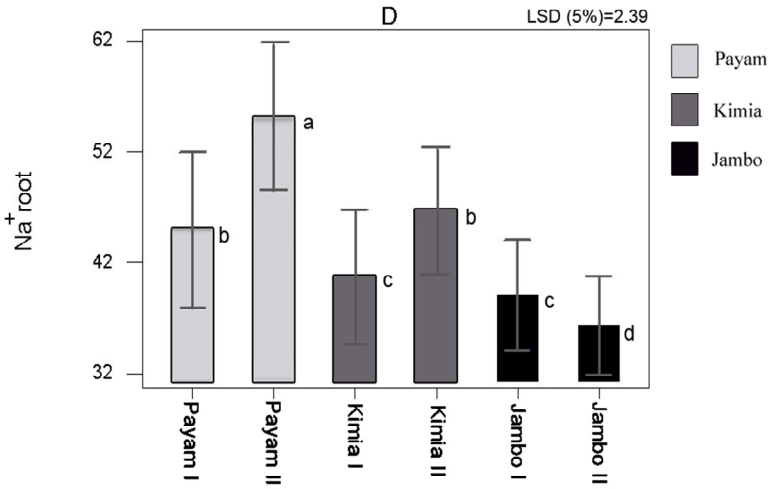
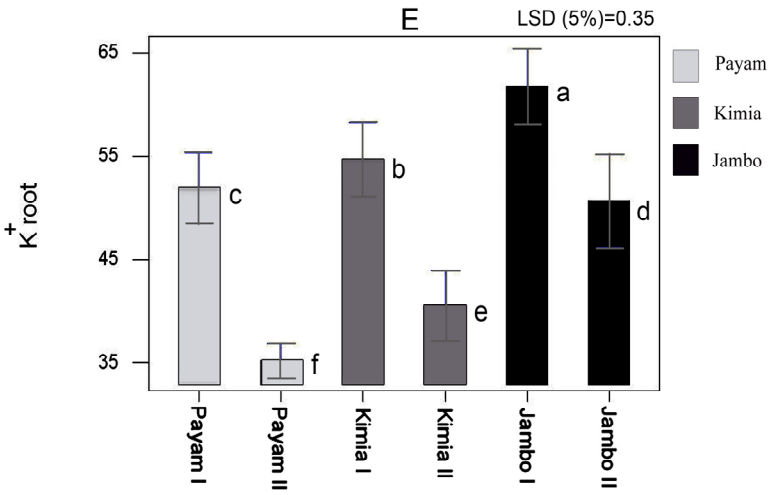


Figure 3. Amounts of Na^+ (A), K^+ (B), Na^+/K^+ (C) in shoots and Na^+ (D), K^+ (E), Na^+/K^+ (F) in roots of the three sorghum varieties recorded at two sampling points (sampling after salt application for 15 (I) and 30 (II) days). Significant values at the 5% level are indicated with letters.





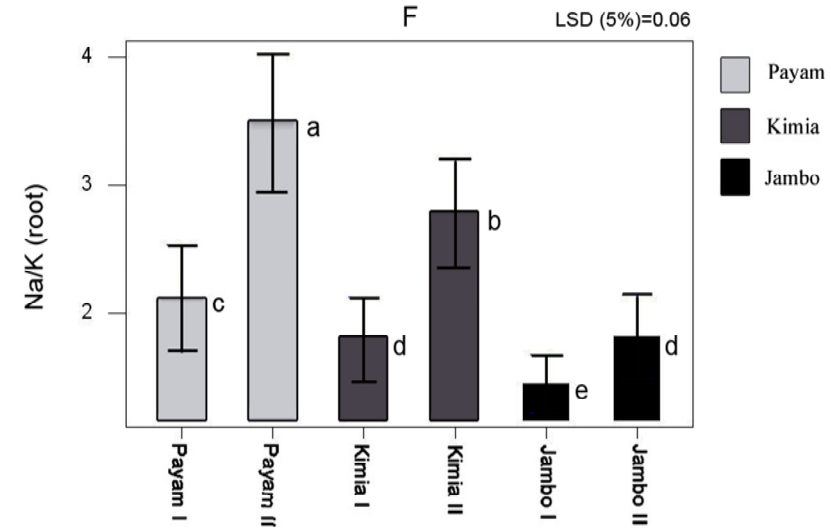


Figure 3. Contd.

strongly inhibits the uptake by roots and their accumulation of K^+. Some scientists have related decreasing K^+ content to the accumulation of toxic ions (Na^+ and Cl^-), carbohydrates, amino acids and proline. Because K^+ is a macronutrient involved in turgor control, inhibition of K^+ uptake should stunt growth (Lacerda et al., 2003; Renault et al., 2001).

Based on the various concentrations of Na^+ and K^+ observed in plant tissues in saline conditions, it was suggested that motion of K^+ is due to its substitution with Na^+ in vacuoles. The three genotypes reacted differently under different salinity concentrations, but generally, reaction of Jambo was better than that of the other two varieties and so K^+ concentration in its roots at all salinity levels was high (Table 4).

A survey of the genotypes at different salinity levels showed that the Na^+/K^+ ratio increased in shoots with increasing salinity. Jambo had a lower Na^+/K^+ ratio in shoot and root tissues at all salinity levels (Table 4). As in the tolerant genotypes, Na^+ transmission to the shoot is limited, they are more partial to uptake of K^+ than of Na^+. In an investigation of the effects of different salinity concentrations on Na^+/K^+ ratios of two tolerant and sensitive groups of wheat, it was observed that tolerant genotypes curtail the uptake of Na^+ and so their Na^+/K^+ ratio is lower than in the sensitive genotypes (Santa-Maria and Epstein, 2001; Munns et al., 2000).

Leaf and root soluble protein concentrations

In this study, it was observed that the three plant varieties showed significant differences ($P \leq 0.05$) in leaf and root soluble protein content. The cultivar Jambo had the highest soluble protein content in both shoots and roots and Kimia the least (Table 3).

Salt stress reduces protein synthesis, increases protein hydrolytic enzyme activity, decreases amino acid synthesis and interferes with tertiary and quaternary enzyme structures leading to decreases in soluble protein content (Dubey and Rani, 1989). Increasing the salinity level caused decreases in the soluble protein content of shoots and roots, with the highest and the lowest levels found at salinity levels of 0 (control) and 200 mM, respectively (Figure 4). Protein synthesis in plants growing in saline environments was affected negatively. In seedlings and in later stages of growth, salinity disrupts protein synthesis and could lead to proteolysis. Although salinity causes decreases in protein synthesis and increases in proteolysis in different plants, in many plants, the protein content increases under saline conditions, e.g. in budding seeds (Dubey and Rani, 1987), seedlings (Dubey and Rani, 1989) and different parts of plants (Elsamad and Shaddad, 1997).

At each time point and at all salinity levels, cv. Jambo had the most leaf and root soluble protein content and Payam the least (Figure 4 and 5). This could be due to greater resistance of Jambo to salt stress. A higher soluble protein content has been observed in salt-tolerant cultivars of barley, sunflower, finger millet and rice (Ashraf and Harris, 2004). This finding tallied with the observations of Dubey and Rani (1989) who found larger quantities of soluble proteins in seedlings of salt-tolerant rice varieties than in sensitive varieties. During the first 5 to 20 days of salt treatment, it was observed that the amount of total and soluble protein in resistant varieties of seedlings was higher than in the sensitive varieties.

Proteins that accumulate in plants under saline conditions could provide a storage form of nitrogen that is re-utilized later (Singh et al., 1987) and could play a role in osmotic adjustment. They may also be synthesized *de novo* in response to salt stress or may be constitutively expressed at low concentration. It was concluded that a number of proteins induced by salinity are cytoplasmic and can cause alternations in cytoplasmic viscosity of the cell (Hasegawa et al., 2000).

Influence of salinity on the soluble protein profile of root and shoot

To understand the molecular basis for mechanisms of salt tolerance in plants, it is important to identify constitutive and induced differences among genotypes, especially in those which show differential responses. Study of the leaf protein profiles of cv. Jambo showed that polypeptides with a molecular weight of 52 kDa in the 200 mM salinity solution at the first time point (sampling after 15 days salt treatment) and in the 100 to 200 mM salinity levels at the second time point (sampling after 30 days salt treatment) had disappeared. Conversely, protein bands of 50 kDa mw were observed in the 200 mM salinity solution in the first sampling and in the 100 and 150 mM salinity solution in the second sampling (Figure 6). There is a difference between short exposure to salt (quick changes in gene expression that put in place an adaptation response) and longer term gene expression changes, which are due to an adaptation response. The proteins induced later are likely to be related to the adaptation response to salt. Those induced earlier are often signal transduction components (hormones, kinases, transcription factors) that are required to establish the adaptation response (Arzani, 2008). After rice seedlings were suddenly exposed to 150 mM NaCl, the genes expressed in the roots at 15 min were different from those expressed after 1 week (Kawasaki et al., 2001). It is likely that many genes induced soon after salt is applied are related to water stress rather than specifically to salt stress. In wheat roots, a gene expressed in the early phase of the stress response, 6 h after sudden exposure to 250 mM NaCl, was identified as a protein kinase and was induced by abscisic acid as well as by salinity (Shen et al., 2001). Whether these changes in gene expression relate to the acclimation to long-term tolerance of salinity stress is yet

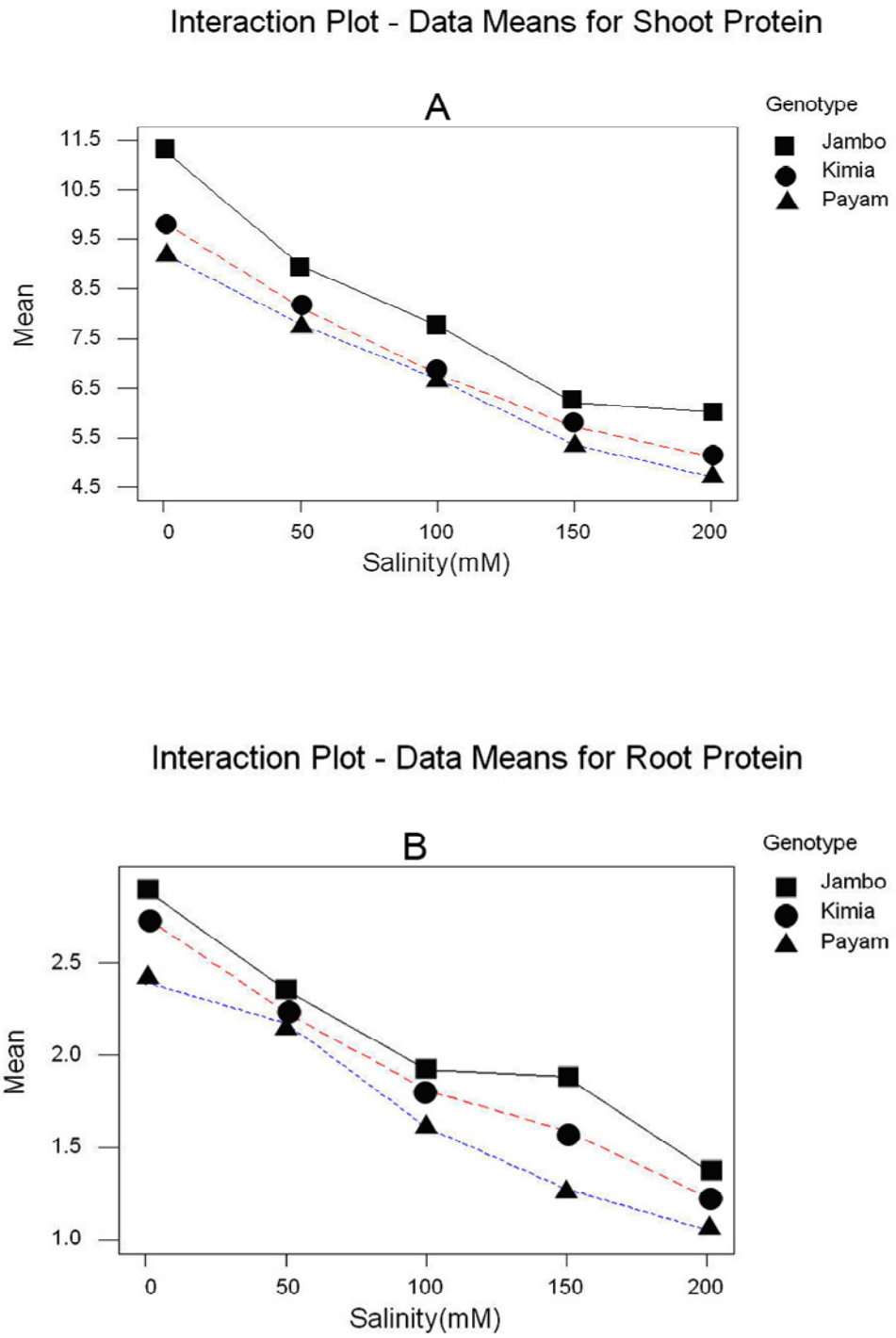


Figure 4. Effect of increasing salinity on soluble protein content in shoots (A) and roots (B) of the three sorghum varieties (Payam, Kimia and Jambo).

to be investigated. In a sensitive line, contrary and long-term salt stresses induce senescence and cell death.

Protein bands of 50 kDa mw were observed in Kimia at salinity level of 200 mM at the first collection time point and at a salinity level of 100 mM in the second time point; intensities of the bands were similar to those of Jambo (Figure 6). In Payam, this protein was not observed at any of the salinity levels. It is possible that this 50 kDa polypeptide that was expressed in Jambo at high salinity levels is a result of the expression of stress-tolerant genes and we could use it as a marker to map populations and check linkage with salt tolerance. In

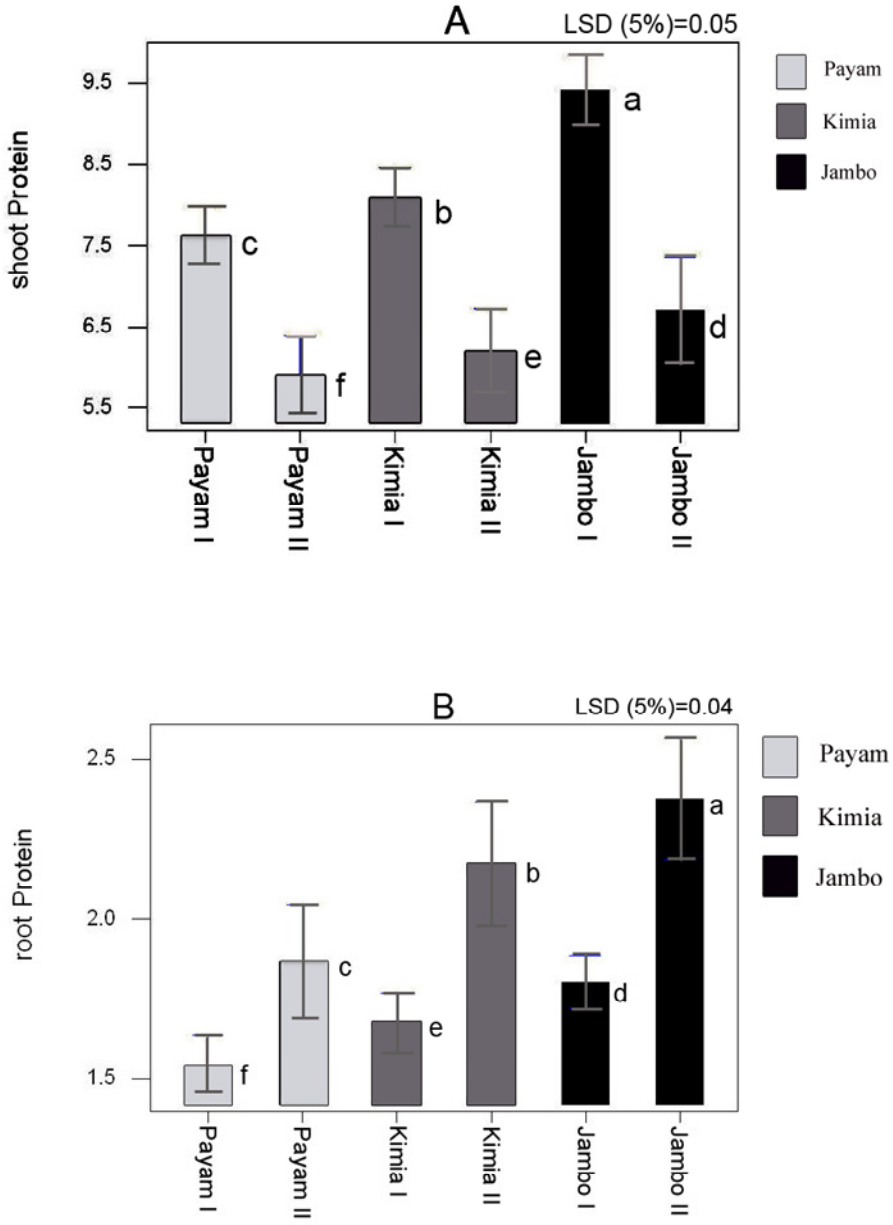


Figure 5. Soluble protein content in shoots (A) and roots (B), of the three sorghum varieties recorded at two sampling points (sampling after salt application for 15 (I) and 30 (II) days). Significant values at the 5% level are indicated with letters.

Mung Bean (*Phaseolus aureus* L.), salinity stress leads to changes in electrophoretic patterns of proteins of both shoot and root, but no protein specifically related to salinity stress was observed (Misra et al., 2006). The present data indicate that protein expression was identical in the sorghum genotypes when stress was imposed. However, as demonstrated in this study, salinity stress elicited genotype-specific protein changes in shoots but did not detect any changes in roots (Figure 6). Perhaps this is because of the low total protein content of roots and consequent lack of strong and observable bands in the gel profile. In halophytes, the root is more subject to stress than the shoot and the effect of salinity on the physiological and morphological characteristics of the root in halophytes is more complex than in the shoot. This could be the reason for the sharp decrease in soluble protein concentration of the root in the investigated sorghum varieties under salinity stress. Ramagopal (1987) reported that in barley, salinity stress induced genotype-specific protein changes in shoots but not in roots.

In all three varieties, the intensity of bands in the electrophoretic pattern of roots showed decrease at the

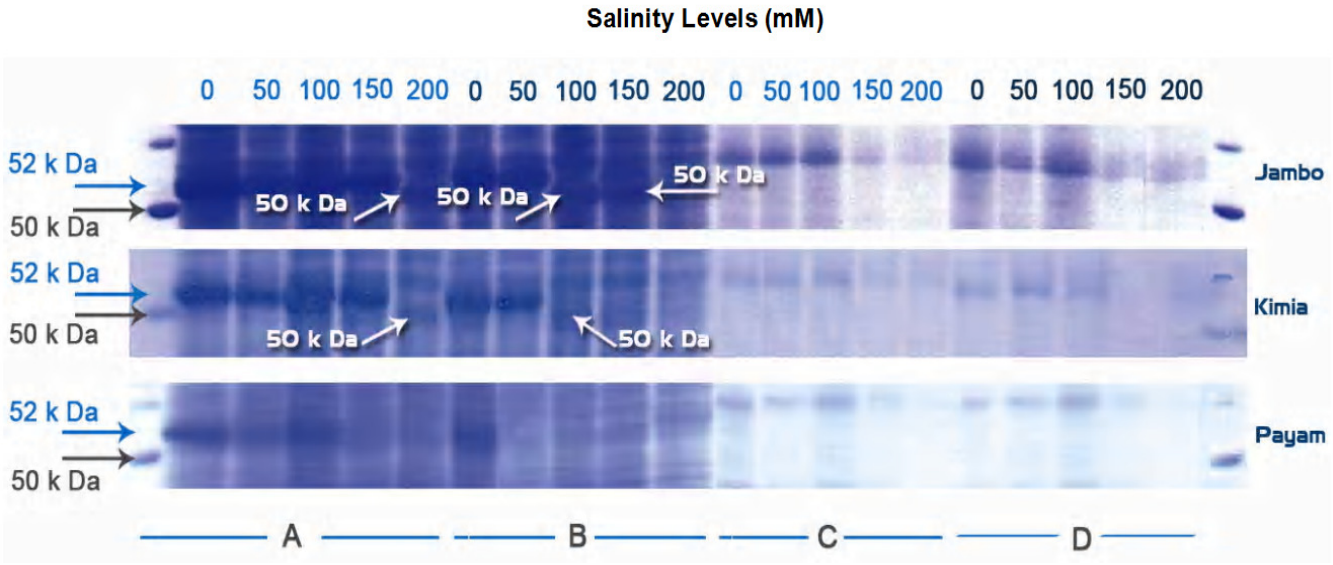


Figure 6. SDS-PAGE of soluble proteins of shoots (A and B) and roots (C and D) of the three sorghum varieties. A and C are electrophoretic patterns of proteins related to sampling after 15 days, B and D are related to sampling after 30 days salt treatment.

first sampling point at salinity levels of 150 and 200 mM and at the second time point at salinity levels of 100 to 200 mM; intensity of this decrease was higher at the 150 and 200 mM salinity levels (Figure 6). In a study of the effects of salinity on electrophoretic patterns of root proteins in Mung Bean, it was observed that protein bands with higher molecular weights (about 100 kDa) generally disappeared or reduced in intensity and instead protein bands with low molecular weights (about 16 to 23 kDa) were induced (Misra et al., 2006).

Under salinity stress, some of the major proteins that existed before stress disappear and new protein bands, usually of low molecular weight, are synthesized in tolerant genotypes. In this study, protein bands in Jambo with molecular weight of 52 kDa disappeared in high salt concentration and a new protein with molecular weight of 50 kDa was detected. Such changes also occurred in Kimia at lower intensity, but were not observed in Payam. In Jambo, this protein could be produced because of the expression of resistance by its genes to the salinity stress. Similar reports of specific synthesis of proteins under salt stress have been reported. A 25-kDa protein was discovered synthesized in salt-adapted cells of *Citrus sinensis* and was found to be associated with salt tolerance (Ben-Hayyim et al., 1993). It has been reported that in tobacco plants, cells that had adapted to salinity increased synthesis and produced some 10 polypeptides. One of these polypeptides called osmotin was unique in tobacco cells (Singh et al., 1987), because it was synthesized and accumulated by cells undergoing gradual osmotic adjustment to either salt or desiccation stress. Chretein et al. (1992) reported that polypeptides with molecular weights of 18, 27 and 49 kDa were synthesized in salt-adapted calli of Jojoba and were suggested as marker proteins for salt adaptation. The most obvious change concerned a protein of 22 kDa molecular weight, which manifested after exposure of radish (*Raphanus sativus)* to NaCl. A cDNA clone corresponding to this polypeptide was obtained and sequenced (Lopez et al., 1994).

Polypeptides synthesized specifically under salt stress in shoot tissues of a salt-tolerant Mung Bean cultivar (T-44) were found to be missing from those of the salt-sensitive cultivar SML-32 under salinity stress (Misra et al., 2006). Similar results have been reported from barley roots (Hukman et al., 1989) in which polypeptides of 26 and 27 kDa synthesized in a salt-tolerant cultivar under salinity stress was missing from a salt-sensitive cultivar in the presence of salinity. Since the protein which was specifically synthesized under salt stress only in cultivar Jambo was absent in the cultivar Payam in the presence of salinity, this protein may be considered a marker for salt tolerance in salt-tolerant cultivar Jambo. It has been suggested that these proteins that are synthesized either specifically or at a higher rate under salt stress play an adaptive role in plants during osmotic adjustment (Singh et al., 1987; Shirata and Takagishi, 1990), protecting the key cytoplasmic enzymes and protein synthesizing apparatus against adverse effects of high salt concentrations.

Conclusion

The chlorophyll, protein and K^+ contents of roots and shoots were less affected by salinity in Jambo than in other cultivars. Based on the higher amount of chlorophyll found in this variety under conditions of stress, we can relate the preferred photosynthesis and higher tissue

elasticity in the shoot to the higher resistance of this variety in conditions of stress. The protein of 50 kDa mw that was expressed in Jambo at high salinity levels could be a useful marker in the studies of genetic diversity and classification of adapted cultivars. Generally, Jambo was found superior to the other two varieties in all physiological traits tested here, which influence plant growth. It seems therefore that this variety is more tolerant to salinity than the other two.

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REFERENCES

Amtmann A, Sanders D (1999). Mechanisms of Na^+ uptake by plant cells. Adv. Bot. Res. 29: 76-104.

Arzani A (2008). Improving salinity tolerance in crop plants: a biotechnological view. *In Vitro* Cell Dev. Biol. Plant. 44: 373-383

Ashraf M, Harris PJC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Ashraf M, Tufail M (1995). Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). J. Agron Crop Sci. 174(5): 351-362.

Belkhodja R, Morales F, Abadia A, Gomez-Aparisi J, Ab-adia J (1994). Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). Plant Physiol. 104: 667-673.

Ben-Heyyim G, Faltin Z, Gepestein S, Camoin AL, Strosberg D, Eshdat Y (1993). Isolation and characterization of salt associated protein in citrus. Plant Sci. 88: 129-140.

Bradford MM (1976). A rapid sensitive method for the quantitation of microgram quantities of protein. Utilizing the principle of protein dye-binding. Anal. Biochem. 38: 248-252.

Chinnusamy V, Jagendorf A, Zhu JK (2005). Understanding and Improving salt tolerance in plants. Crop Sci. 45:437-448.

Chretein D, Guillot-Salamon T, Bahl J, Cantrel C, Jeanpaui D (1992). Lipid and protein changes in Jojoba callus under salt stress. Plant Physiol. 86: 372-380.

Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R (2005). Control of sodium transport in durum wheat. Plant Physiol. 137: 807-818.

Dubey RS, Rani M (1987). Proteases and proteins in germinating rice seeds relation to salt tolerance. Plant Physiol. Biochem. 14: 174-182.

Dubey RS, Rani M (1989). Influence of NaCl salinity on growth and metabolic status of proteins and amino acids in rice seedlings. J. Agron. Crop Sci. 162: 97-106.

Dure L, Crouch M, Harada J, Ho THD, Mundy J, Quatrano R, Thomas T, Sung ZR (1989). Common amino acid sequence domains among the LEA proteins of higher plants. Plant Mol. Biol. 12: 475-486.

Elsamad HAM,Shadad MAK (1997). Salt tolerance of soybean cultivars. Biologia Planarum, 39: 263-269.

Flowers TJ, Hajibagheri MA (2001). Salinity tolerance in *Hordeum vulgare*: Ion concentration in root cells of cultivars differing in salt tolerance. Plant. Soil. 231: 1-9.

Flowers TJ, Yeo AR (1995). Breeding for salinity resistance in crop plants, where next? Aust. J. Plant Physiol. 22: 875-884.

Gorham J, Wyn Jones RG, Bristol A (1990). Partial characterization of the trait for enhanced K^+-Na^+ discrimination in the D genome of wheat. Planta 180: 590-597.

Greenway H, Munns R (1980). Mechanisms of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol. 31: 149-190.

Greenway H, Osmond CB (1972). Salt responses of enzymes from species differing in salt tolerance. Plant Physiol. 49: 256-259.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 463-499.

Hendry GAF, Price AH (1993). Stress indications: chlorophylls and carotenoids. In: Hendry GAF, Grime JP (eds) Methods in comparative plant ecology. 1ed. Chapman and Hall, pp. 148-152.

Hoagland DR, Arnon DI (1959). The water culture method for growing plants without soil. Calif Agric Exp. Stn. Circ. 32: 307.

Hurkman WJ, Fornari CS, Tanaka CKA (1989). Comparison of the effect of salt on polypeptides and translatable mRNA in roots of a salt tolerant and salt sensitive cultivar of barely. Plant Physiol. 90: 1444-1465.

Igarta AE, Gracia AM, Lasa JM (1995). Field response of grain sorghum to salinity gradient. Field Crops Res. 42: 15-25.

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998). Arabidopsis CBF1 overxpression induces COR genes and enhances freezing tolerance. Science 280:104-106.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999). Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcriptional factor. Nat. Biotechol. 17: 287-291.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ (2001). Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13:889–905.

Lacerda CF, Cambraria J, Oliva MA, Ruiz HA, Prisco JT (2003). Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Rev. Bras. Physiol. Veg. 49: 107-120.

Leammli UK (1970). Cleavage of structural proteins during assembly of the head bacteriophage T4. Nature, 227: 680-685.

Lindsay MP, Lagudah ES, Hare RA, Munns R (2004). A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat. Funct. Plant Biol. 31: 1105–1114.

Lopez F, Vansuyt G, Fourcroy P, Casse-Delbart F (1994). Accumulation of 22 kDa protein and its mRNA in the leaves of *Raphanus sativus* in response to salt stress or water deficit. Physiol Plant. 91:605-614.

Lutts EV, Poss JA, Hoffman GJ (1986). Salinity sensitivity on sorghum at three growth stages. Irrig. Sci. 7:1-11.

Lutts S, Kinet JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78: 389–398.

Matsushita N, Matoh T (1991). Characterization of Na^+ exclusion mechanisms of salt-tolerant reed plants in comparison with salt-sensitive rice plants. Physiol Plant. 83: 170-176.

Misra N, Gupta AK, Dwivedi UN (2006). Changes in free amino acids and stress protein synthesis in two genotypes of green gram under salt stress. J. Plant Sci. 1:56-66.

Munns R, Hare RA, James RA, Rebetzke GJ (2000). Genetic variation for improving the salt tolerance of durum wheat. Aus. J. Agri. Res. 51: 69-74.

Munns R, James RA (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant and Soil 253: 201-218.

Munns R, Rebetzke GJ, Husain S, James RA, Hare RA (2003). Genetic control of sodium exclusion in durum wheat. Aust. J. Agric. Res. 54: 627-635.

Netondo GW, Onyango JC, Beck E (2004). Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci. 44: 806-811.

Ramagopal S (1987). Salinity stress induced tissue-specific proteins in barley seedlings. Plant Physiol. 84: 324-331.

Renault S, Croser C, Franklin JA, Zwiazek JJ (2001). Effect of NaCl and Na2SO4 on red-osier dogwood (*Cornus stolonifera* Michx). Plant Soil 233:261–268.

Rowson HM, Munns R (1984). Leaf expansion in sunflower as influenced by salinity and short term changes in carbon fixation. Plant Cell Environ. 7: 207-213

Salama S, Trivedi S, Busheva M, Arafa AA, Garaba G, Erdi L (1994). Effect of NaCl salinity on growth, cation accumulation, chloroplast structure and function in wheat cultivars differing in salt tolerance.

Plant Physiol. 144: 241-247.
Salisbury FB, Ross C (1992). Plant Physiology. 4th[ed]. Wadsworth Publishing Company, Belmont. California.
Santa-Maria GE, Epstein E (2001). Potassium/sodium selectivity in wheat and amphiploid cross wheat×*Lophopym elongation*. Plant Sci. 160: 523-534.
Senratina T, McKersie BD, Sinson AN (1985). Simulation of dehydration injury to membrane from soybean axes by free radicals. Plant Physiol. 77: 472-474.
Serrano R (1996). Salt tolerance in plants and micro-organisms: toxicity targets and defence responses. Academic New York, In: Jeon KW (eds) International review of Cytology: a survey of cell Biology. 165: 1-52.
Shen W, Gmez-Cadenas A, Routly EL, Ho THD, Simmonds JA, Gulick PJ (2001). The salt stress-inducible protein kinase gene, Esi47, from the salt-tolerant wheatgrass *Lophopyrum elongatum* is involved in plant hormone signaling. Plant Physiol. 125: 1429-1441.
Shirata K, Takagishi H (1990). Salt induced accumulation of 27 kDa proteins in cultured cells of rice plants. Soil Sci. Plant Sutr. 36: 153-157.
Singh NK, Braker CA, Hasegawa PM, Handa AK, Buckels MA, Hermodsm P, Franckoch E Regnier FE, Bressan RA (1987). Characterization of osmotin a thoumatin like protein associated with osmotic adaptation in plant cell. Plant Physiol. 85: 529-536.
Singha S, Choudhuri MA (1990). Effect of salinity (NaCl) stress on H_2O_2 metabolism in *Vigna* and *Oryza* seedlings. Biochem. Physiol. Pflanz. 186:69-74.
Sunseri F, Palazzo D, Montemurro N, Montemurro F (1998). Salinity tolerance in sweet sorghum (*Sorghum bicolor* L. Moench): field performance under salt stress. Ital. J Agron. 2: 111-116.
Tester M, Davenport R (2003). Na^+ Tolerance and Na^+ transport in high plants. Ann Bot. 91: 503-527.
Wyn Jones RG, Storey R (1978). Salt stress and comparative physiology in the Gramineae. IV. Comparison of salt stress in *Spartina* x *townsendii* and three barley cultivars. Aus. J. Plant Physiol. 5: 839-850.
Xu D, Duan X, Wang B, Hong B, Ho THD, Wu R (1996). Expression of a late embryogenesis abundant (LEA) protein gene, HvA1, from barley confers tolerance to drought and salinity in transgenic rice. Plant Physiol. 110: 249-257.
Zorb C, Schmitt S, Neeb A, Karl S, Linder M. Schubert S (2004). The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by mitigation of symptoms and not by a specific adaptation. Plant Sci. 167: 91-100.