

# Proteins, Sugars and Ion Leakage as a Selection Criterion for the Salt Tolerance of Three Sorghum Cultivars at Seedling Stage Grown Under NaCl and Nicotinamide

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## ABSTRACT

The interactive effects of salinity stress (- 0.90 MPa NaCl) and seeds presoaking in 100 ppm. nicotinamide (vitamin PP) in three sorghum cultivars (Dorado, Hagen Shandawil and Giza 113) were studied to examine their response to salinity stress and the possible role played by vitamin PP in regulating salt- induced changes in seedling growth, protein patterns and some relevant metabolic activities of these cultivars. According to the dry mass production and tissue water content, the cv. Dorado seemed to be more or less unaffected by salinity, whereas the cv. Giza 113, was severely reduced, the cv. Hagen Shandawil was almost intermediated. Consequently, carbohydrate and protein contents were increased in cv. Dorado and Hagen Shandawil, while the opposite occurred in cv. Giza 113. On the other hand, while proline content decreased in cv. Hagen Shandawil and Giza 113, it markedly increased in cv. Dorado as a result of salinity stress.  $\text{Na}^+ / \text{K}^+$  ratio was higher in cv. Dorado than in cv. Giza 113. cv. Hagen Shandawil was intermediate. Treatment with vitamin PP was accompanied with higher values of saccharids and protein compared with the untreated seedlings, which partially alleviated the adverse effect of salinity on growth. This was linked with a marked variation in protein patterns among these cvs. Salinity stress led to the appearance of 67 and 26 kD (in cv. Dorado) and 45 kD (in cv. Hagen Shandawil). While in cv. Giza 113, no new band appeared. Seeds treated with vitamin PP lead to appearance of bands with 67 and 46 kD in cv. Dorado and 38, 26 and 18 kD in cv. Hagen Shandawil, while in cv. Giza 113, the 67 and 26 kD which had disappeared by salinity, they reappeared under the interactive effect of salinity and vitamin PP. This confirms the metabolic importance of the 67 and 26 kD proteins in salt tolerance.

**Key Words:** Amino acid; Carbohydrate; Potassium; Proline; Protein patterns; Sodium

## INTRODUCTION

Soil salinity, especially if caused by  $\text{Na}^+$  and  $\text{Cl}^+$  ions, adversely affects the growth of plants and their yield. There is much variability both between and within varieties; differences in vigour accounting for much of the variation in the survival of salinity (Yeo *et al.*, 1990; Shen & Yan, 2003). It is commonly hypothesised that seedling growth inhibition under salt – stress is associated with altered water relations (osmotic effects), specific ion effects (excess or deficiency) or carbohydrates availability (Munns, 1993; Lazof & Bernstein, 1998). Salinity stress was found to induce the accumulation of carbohydrates in various plants (Zhang & Archbol, 1993; Schubert *et al.*, 1995). Sundaresan and Sundhakaran (1995) reported that the response of free amino acids content to salt stress differed depending on cultivars and stage of development. Abdel-Samed (1991) showed that, the biosynthesis of amino acids was nearly opposite to that of proline accumulation. Differences were found in the accumulation patterns of  $\text{Na}^+$  and  $\text{K}^+$  under salinity stress. The salt tolerant plants maintained a high  $\text{K}^+$  content and higher  $\text{K}^+/\text{Na}^+$  ratio compared with the salt sensitive plants (Liu & van Staden, 2001; Azooz, 2002).

Salinity stress has been reported to disturb the integrity of wheat and barley cell membrane resulting in increased membrane permeability (Mansour *et al.*, 1993). Maintenance of membrane integrity and selective uptake of essential minerals are the parts that confer salt tolerance (Schröppel-Meier & Kaiser, 1988). Ismail (2003) reported that under salt stress, membrane permeability was higher in maize (less-salt tolerant) than that of sorghum (more- salt tolerant).

Stress tolerance is dependent upon the genetic and biochemical characteristics of the cultivars. Therefore, attempts have been made by certain investigators to differentiate stress-tolerant and stress - sensitive genotypes of crops, on the basis of profiles or levels of soluble proteins in germinating seeds and growing plant parts, which exist in the sets of genotypes differing in salt tolerance (Dubey & Rani, 1990; Barakat, 2003). It is well documented that, environmental stresses cause important modification in gene expression (Soussi *et al.*, 2001). Gene expression is manifested by the appearance of proteins, which are not present before the stimulation. Salinity promotes the synthesis of salt stress - specific proteins (Ben - Hayyim *et al.*, 1989), many of these proteins were suggested to protect

the cell against the adverse effect of salt stress. Accumulation of 26 kD protein (osmotin) is a common response to salt stress (Guerrier, 1998).

Vitamins are required in trace amounts to maintain normal growth and proper development of all organisms. These compounds act as coenzyme systems and thus take part in the regulation of metabolism. Presoaking of seeds with optimal concentration of vitamins has been shown to be beneficial to seedling growth under saline condition by increasing physiological availability of water and nutrient (Ashfaq *et al.*, 1983; Azooz *et al.*, 2002; Barakat, 2003).

Thus, the question arises, what could happen if the plants were treated with both salinity and vitamin PP? Could the exogenously added vitamin mitigate the adverse effects of salinity? Therefore, the aim of work was to determine the changes in seedling growth, protein patterns and some relevant metabolic activities associated with salt tolerance or salt sensitive sorghum cvs and the possible role played by vitamin PP in regulating salt-induced changes in these parameters, if they are subjected to salinity or salinity vitamin interaction.

## MATERIALS AND METHODS

Three different cultivars of *Sorghum vulgare* L. (Dorado, Hagen Shandawil and Giza 113) were selected in the present work. These cultivars were supplied by Agriculture Research Center, Giza, Egypt. The seeds of each cultivar were divided into two groups: the first group was soaked in distilled water (control), the second group was soaked in 100 ppm aqueous solution of nicotinamide (vitamin PP) for 8 h and air - dried. The seeds were surface sterilized by pre treating with 1% sodium hypochlorite and rinsed three times with distilled water. Twenty seeds of first and second group from each cultivar were germinated in sterilized petri dishes on filter paper containing appropriate amount of water (control) or - 0.90 MPa NaCl (four replicates from each). The petri dishes were incubated at 28-30°C in dark conditions. Distilled water was added as needed to compensate for evaporation loss. After seven days, the seedlings were harvested.

Membrane permeability of the excised leaves was measured by EC according to Yan *et al.* (1996). Briefly, the washed leaves were cut into 1 cm pieces and placed in a glass beaker containing 10 mL deionised water. The beakers were kept at 30°C for 3 h and the conductivity of solution was measured by a conductivity meter. The same samples were boiled for 2 min and then their conductivity was measured again when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated as follows, EC (%) =  $(C1 / C2) \times 100$ . Where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

Dry mass was determined after drying the freshly harvested seedlings in an aerated oven at 70°C to constant weight. Carbohydrates were determined by the anthrone

sulphuric acid method described by Badour (1959). The dried tissue of seedling was extracted by distilled water (in case of soluble or HCl in case of total carbohydrates). 1mL of the carbohydrate extract was mixed with 9 mL of anthrone sulphuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbency was read spectrophotometrically (*Spectronic Genesys ZPC*, Rochester, NY, USA) at 620 nm against blank containing only distilled water and anthrone reagent. The insoluble carbohydrate was calculated by subtract soluble from total carbohydrate.

Proline content was estimated according to Bates *et al.* (1973) for free proline determination. A known weight of dried tissue was homogenized in 10 mL of 3% sulfosalicylic acid and filtered. Two mL of the filtrate was reacted with 2 mL glacial acetic acid and 2 mL of acid - ninhydrin reagent in a test tube and heated for 1 h at 100°C. The reaction mixture was extracted with 4 mL toluene. The chromophore was aspirated from the aqueous phase and the absorbency was read at 520 nm using toluene as a blank.

Free amino acids were extracted and estimated according to the method of Lee and Takahashi (1966). About 0.1 mL of the water extract containing free amino acids was mixed with 1.9 mL of ninhydrin-citrate- glycerol mixture in a test tube for 20 min at 100°C. The absorbency was read at 570 nm against blank (only distilled water and the same reagent).

Sodium and potassium were determined by flame photometer method (Williams & Twine, 1960).

**Electrophoresis.** The samples after harvest were collected and ground in liquid nitrogen. The proteins were then extracted and their concentrations were determined according to Bradford (1976). Protein samples were prepared by mixing clear supernatant (equal amounts) with 0.025% Bromophenol Blue before their loading onto gel wells for electrophoresis (*Bio Rad, Protean II XI Cell*). The electrophoresis was carried out using 13.5% acrylamide gels. Gels were run at constant current 20 mA per gel at 4°C in run buffer containing 0.025 M Tris, 0.192 M glycine and 0.1% SDS. Protein bands were visualized by a modified Comassie Brilliant Blue R-250 (CB) with Bismark Brown R (BBR) according to Choi *et al.* (1996). The protein bands were quantified using a video system and image analysis computer program, according to Mair and Ninnemann (1996), to measure the density of stained protein bands.

**Statistical analysis.** The data of all experiments were subjected to analysis by the least significant differences test (L.S.D.) using SPSS program.

## RESULTS AND DISCUSSION

NaCl salinity significantly affected dry mass, water content and membrane permeability of sorghum cultivars seedlings, but to a different degree (Table I). Dry mass and water content were significantly reduced, while membrane permeability (solute leakage) was markedly increased as

**Table I. Dry mass (DM) mg<sup>-1</sup> seedling, water content (%WC) and membrane permeability (%EC) of sorghum cultivars seedlings in response to the interactive effects of salinity (- 0.90 MPa NaCl) and vitamin PP (100 ppm)**

Treatments	Cv. Dorado			Cv. Hagen Shandawil			Cv. Giza 113		
	DM	%WC	% EC	DM	%WC	% EC	DM	%WC	% EC
Control	14.4	90.1	22.9	13.9	89.4	23.5	19.7	88.9	25.5
Vitamin PP	15.2	90.3	20.6	14.3	89.5	22.9	19.8	89.1	31.3B
NaCl	12.7	89.7	25.6A	10.1A	88.4B	33.9B	11.9B	87.2B	47.2B
NaCl + Vitamin PP	13.4	90.3	22.4	11.4	88.6A	28.6B	12.6B	88.6	39.7B
L.S.D. at 5%	2.1	1.8	2.4	3.1	0.64	0.75	3.5	0.23	0.98
L.S.D. at 1%	3.1	2.7	3.5	4.6	0.94	1.10	5.1	0.34	1.43

Means values in each column which are significantly different (P = 0.05) are followed by A letter and the highly significantly different (P = 0.01) are followed by B letter, as compared with control (0 .0 NaCl)

**Table II. Carbohydrates content (mg g<sup>-1</sup> dry mass) of sorghum cultivars seedlings in response to the interactive effects of salinity (- 0.90 MPa NaCl) and vitamin PP (100 ppm)**

Treatments	Cv. Dorado			Cv. Hagen Shandawil			Cv. Giza 113		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Control	38.4	63.7	102.1	36.7	61.4	98.1	35.1	70.0	105.1
Vitamin PP	38.4	70.1	108.5B	46.7B	63.5	110.2B	37.8	71.8	110.6B
NaCl	76.8B	116.9B	193.7B	45.5B	95.2B	130.7B	25.1B	65.8A	90.9B
NaCl + Vitamin PP	80.2B	128.6B	208.8B	66.8B	118.6B	185.4B	35.1	68.5	103.6
L.S.D. at 5%	3.7	8.4	3.8	2.8	4.9	4.7	4.6	3.3	3.7
L.S.D. at 1%	5.4	12.8	5.6	4.0	6.3	5.9	5.7	4.8	5.0

Means values in each column which are significantly different (P = 0.05) are followed by A letter and the highly significantly different (P = 0.01) are followed by B letter, as compared with control (0 .0 NaCl)

**Table III. Proteins content (mg g<sup>-1</sup> dry mass) of sorghum cultivars seedlings in response to the interactive effects of salinity (- 0.90 MPa NaCl) and vitamin PP (100 ppm)**

Treatments	Cv. Dorado			Cv. Hagen Shandawil			Cv. Giza 113		
	Soluble	Insoluble	Total	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Control	18.9	136.3	155.2	16.5	145.1	161.6	20.6	137.0	157.6
Vitamin PP	20.0	141.6B	161.6A	17.4	148.1	165.5A	22.4	148.0B	160.4
NaCl	42.2B	131.5B	173.7B	39.4B	120.4B	159.8	23.0	123.9B	146.9B
NaCl + Vitamin P	46.2B	132.7A	178.9B	41.5B	119.8B	161.3	25.1A	125.1B	150.2B
L.S.D. at 5%	4.3	3.1	5.2	3.2	4.2	2.9	3.2	5.0	3.8
L.S.D. at 1%	6.2	4.5	7.6	4.7	6.1	4.3	4.1	7.3	5.7

Means values in each column which are significantly different (P = 0.05) are followed by A letter and the highly significantly different (P = 0.01) are followed by B letter, as compared with control (0 .0 NaCl)

compared with control. These effects of salinity were much lower in cv. Dorado and Hagen Shandawil than cv. Giza 113, where the reduction in dry mass of cv. Dorado, cv. Hagen Shandawil and cv. Giza 113 was about 11, 27 and 40%, increase in membrane permeability was about 12, 44 and 85%, respectively, as compared with control. The contents of carbohydrate (Table II) were considerably increased in response to salinity in cv. Dorado and Hagen Shandawil, while in cv. Giza 113, they were decreased. In addition, the contents of carbohydrate were much higher in cv. Dorado (about 2-fold) than that of cv. Giza 113, while cv. Hagen Shandawil was intermediate. Seeds presoaking in vitamin PP, increased dry mass, production of carbohydrates and water content, while on the other hand decreased the membrane permeability of sorghum cvs. as compared with untreated seedlings.

Dry mass production and tissue water content seemed to be more or less unaffected in cv. Dorado by salinity, whereas the cv. Giza 113 was severely reduced, the cv. Hagen Shandawil was almost intermediate. Concomitantly,

membrane permeability was positively correlated with dry mass reduction (it severely increased in the salt sensitive cv. Giza 113). Increase electrolyte leakage is generally considered as an index of membrane damage (Yan *et al.*, 1996). Consequently, tolerance index was lower in cv. Giza 113 than the other two cultivars.

The best tolerance was judged by major differences in carbohydrate contents, which are frequently associated with active osmotic adjustment (Zhang & Archbold, 1993). The amount of carbohydrate in cv. Dorado was more than 2-fold comparing with Giza 113. Moreover, the soluble carbohydrate in cv. Dorado was nearly 2-fold under NaCl salinity than of control, while in cv. Giza 113, it was significantly reduced. Thus, the superiority of cv. Dorado was closely associated with its ability to accumulate carbohydrates, whereas the sensitivity of cv. Giza 113 was linked with the marked depletion of carbohydrates. This consequently increased the tissue water content of cv. Dorado as compared with cv. Giza 113. Thus, cv. Dorado is considered to be more "water saving" compared to the other

**Table IV. Total free amino acids and proline contents (mg g<sup>-1</sup> dry mass) of sorghum cultivars seedlings in response to the interactive effects of salinity (- 0.90 MPa NaCl) and vitamin PP (100 ppm)**

Treatments	Cv. Dorado		Cv. Hagen Shandawil		Cv. Giza 113	
	Amino acids	Proline	Amino acids	Proline	Amino acids	Proline
Control	8.12	1.218	7.70	1.522	16.24	1.414
Vitamin PP	7.06	1.155	6.52	1.015B	15.74	1.136B
NaCl	14.72B	1.915B	12.71B	0.994B	21.66A	0.751B
NaCl + Vitamin PP	13.35B	1.458	14.16B	0.877B	23.69B	0.669B
L.S.D. at 5%	2.92	0.277	2.72	0.178	4.30	0.159
L.S.D. at 1%	4.25	0.403	3.96	0.260	6.26	0.232

Means values in each column which are significantly different (P = 0.05) are followed by A letter and the highly significantly different (P = 0.01) are followed by B letter, as compared with control (0 .0 NaCl)

**Table V. Contents of Na<sup>+</sup>, K<sup>+</sup> and Na<sup>+</sup>/K<sup>+</sup> ratios (mg g<sup>-1</sup> dry mass) of sorghum cultivars seedlings in response to the interactive effects of salinity (- 0.90 Mpa NaCl) and vitamin PP (100 ppm)**

Treatments	Cv. Dorado			Cv. Hagen Shandawil			Cv. Giza 113		
	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup> /K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup> /K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup> /K <sup>+</sup>
Control	20.3	52.3	0.388	23.2	53.9	0.430	35.5	49.4	0.718
Vitamin PP	18.5	65.4B	0.282A	20.0	54.9	0.364	34.5	50.8	0.679
NaCl	30.0B	83.0B	0.321	38.0B	59.9A	0.634B	74.0B	40.5B	1.827B
NaCl + Vitamin PP	23.5	90.3B	0.260A	27.4A	65.9B	0.415	63.0B	48.1	1.131B
L.S.D. at 5%	4.6	3.8	0.103	3.4	5.4	0.110	4.7	4.3	0.197
L.S.D. at 1%	6.7	5.6	0.160	5.0	7.8	0.173	6.8	6.3	0.287

Means values in each column which are significantly different (P = 0.05) are followed by A letter and the highly significantly different (P = 0.01) are followed by B letter, as compared with control (0 .0 NaCl)

sorghum cvs. investigated. From a physiological hand point, the synthesis and distribution of carbohydrates could be used a suitable selection criterion for the salt tolerance of sorghum. In this respect, Balibrea *et al.* (1997) stated that the sugar accumulation and its distribution in different parts of the plants could be a valid trait to discriminate genotypes of different tolerance to saline and osmotic stress. Handa *et al.* (1983) found that, the concentration of reducing sugars in the tomato increased with the degree of adaptation to the salinity stress conditions.

Promotive effects of vitamin on growth and carbohydrate production indicated that, vitamin PP can play a role in mitigating the adverse effect of NaCl on metabolic activities relevant to growth, through enhancing cell division (Barakat, 2003) and /or cell enlargement. Kodandaramaiah (1983) has reported that, vitamins might act as activators of carbohydrates synthesis.

Soluble proteins in response to salinity (Table III) were highly significantly increased in cv. Dorado and Hagen Shandawil (more- salt tolerant), while in cv. Giza 113 (less- salt tolerant) they were slightly increased. On the other hand, insoluble and total proteins were significantly decreased as compared with non-salinized seedlings. These results support the hypothesis that the salt tolerance of plants correlated to the high protein contents (Azooz, 2002). Therefore, it may be suggested that, the accumulation of soluble protein could be regard as an indicator of the salt tolerance of these cultivars. Interestingly, as in the case of soluble carbohydrates; the soluble proteins in salt affected cv. Dorado approached 2-fold in comparing with cv. Giza 113. Thus, it can be concluded that cv. Dorado (more- salt tolerant) accumulated protein fractions under salinity stress

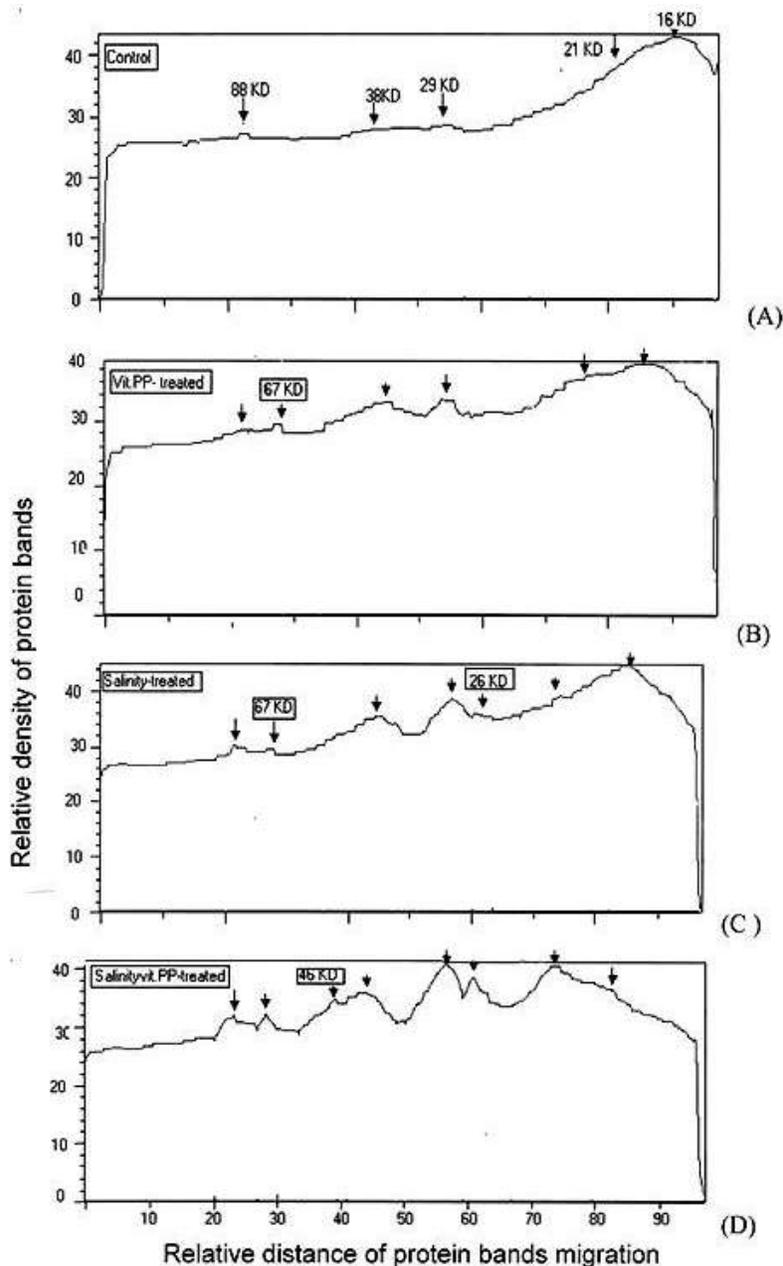
for two interrelated processes (survival and osmotic adjustment), while cv. Giza 113 (less - salt tolerant) failed to acquire these interrelations.

Free amino acids and proline exhibited variation among the tested sorghum cultivars (Table IV). In comparing with the control, salinity resulted in a marked accumulation of total free amino acids of the three sorghum cvs. Opposite trend was observed in free proline content except in cv. Dorado, where free proline content was slightly increased. Seeds presoaking in vitamin PP stimulated the biosynthesis of protein and decreased the biosynthesis of free amino acids and proline, except in salinized seedlings of cv. Hagen Shandawil and Giza 113 the biosynthesis of free amino acids was slightly increased as compared with seedlings subjected only to salinity stress.

There is surprising situation in the criteria of free proline among the three sorghum cvs., where salinity stress accumulated proline in cv. Dorado (the most tolerant cultivars), the opposite occurred in in cv. Giza 113, where the per cent reduction approached 50% as compared with control. Divitt *et al.* (1987) revealed that, free proline decreased with increasing salinity. Ashraf (1994) reported that, the salt - tolerant population accumulated greater amount of proline compared with non-tolerant.

Additionally, there is another interesting situation in the criteria of free amino acids, where the amounts of amino acids in cv. Giza 113, were much more higher than that of cv. Dorado, even at the level of control (about 2-fold than that of cv. Dorado control). Thus, the accumulation of amino acids was in parallel with proline in cv. Dorado and in contrast in the other two cultivars. Since the accumulation of free amino acids in cv. Hagen Shandawil and Giza 113

**Fig. 1. Quantification of digitized video camera images of protein bands of cv. Dorado with densitometry computer program showing the changes in those bands in response to the effect of -0.90MPa NaCl (C) and 100 ppm vitamin PP (B) or both (D) as compared with untreated control seedlings (A). Newly appeared bands are indicated by (  $\square$  ), and the disappeared bands are indicated by (  $\bigcirc$  )**

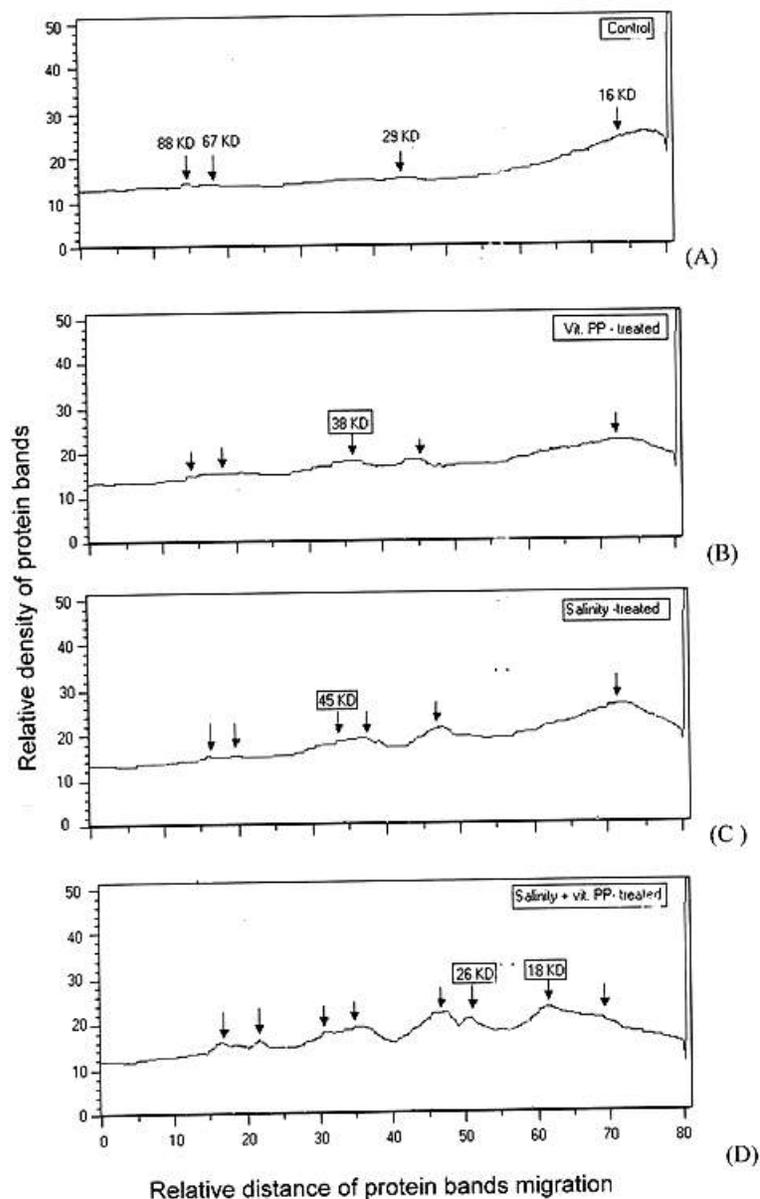


might be at the expense of proline, while in cv. Dorado is not. This means that the strategy of osmoregulation was different among the tested sorghum cvs.

The criteria in the accumulation of proteins, amino acids, proline and even carbohydrates was nearly the same in cv. Dorado (most of them accumulated by salt stress), while Giza 113 accumulated amino acids only. This means

that, the salt tolerance of cv. Dorado was associated with a marked equilibrium and interconversion between carbohydrate and nitrogen metabolism for osmoregulation and growth, while the sensitivity of cv. Giza 113 was accompanied with obvious disturbance in the synthesis and interconversion of both components, these metabolic disturbances lead to the depletion in the accumulation of

**Fig. 2. Quantification of digitized video camera images of protein bands of cv. Hagen Shandawil with densitometry computer program showing the changes in those bands in response to the effect of -0.90MPa NaCl (C) and 100 ppm vitamin PP (B) or both (D) as compared with untreated control seedlings (A). Newly appeared bands are indicated by (  $\square$  ), and the disappeared bands are indicated by (  $\circ$  )**

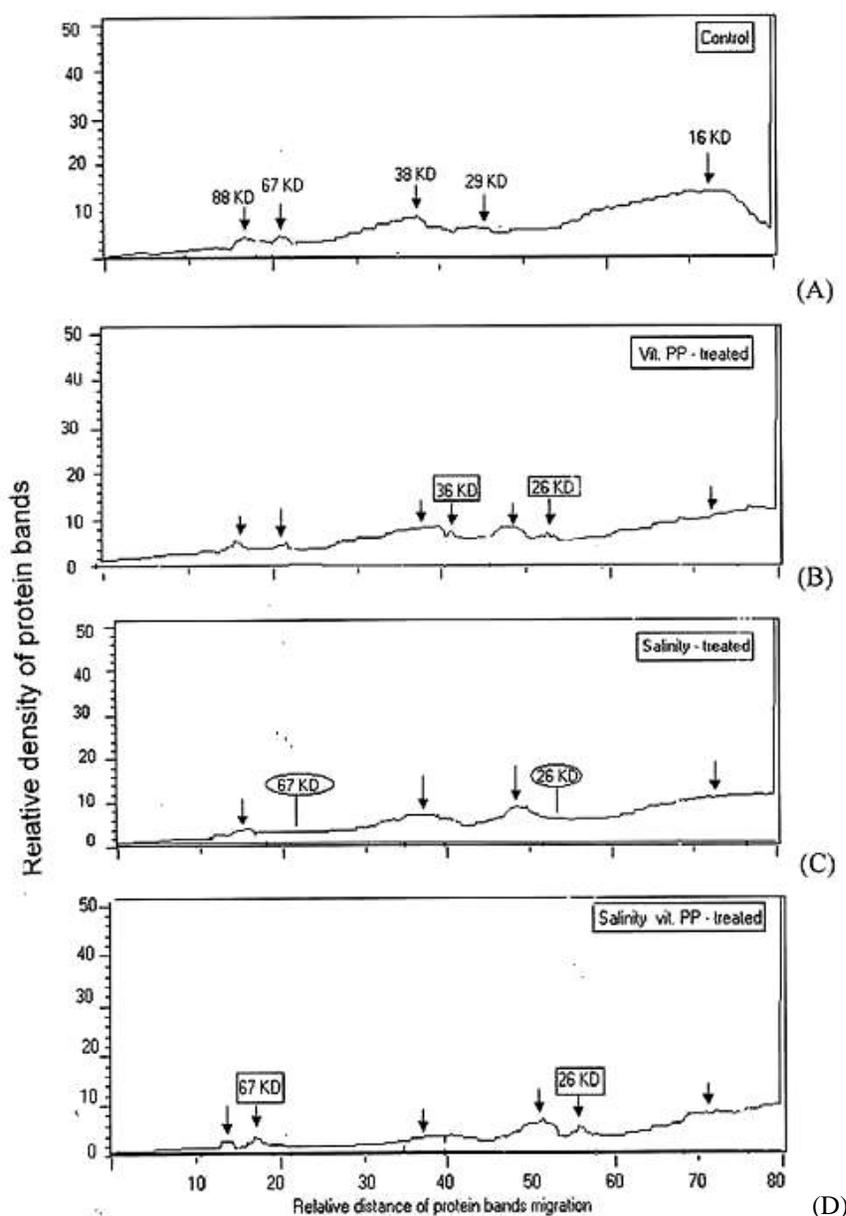


carbohydrates, proteins and even proline at the expense of free amino acids for survival only.

The content of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Na}^+/\text{K}^+$  ratio (Table V) as responses to salinity stress reveal that, there is a marked increase in the concentration of  $\text{Na}^+$  and  $\text{K}^+$ , of all sorghum cultivars, except in case of cv. Giza 113,  $\text{K}^+$  content was markedly decreased. Considerable variations in  $\text{Na}^+$  and  $\text{K}^+$  content within the three sorghum cvs. showed that,  $\text{K}^+$  content was higher, while  $\text{Na}^+$  was lower in cv. Dorado and

Hagen Shandawil than that of cv. Giza 113. Consequently, under salinity stress  $\text{Na}^+/\text{K}^+$  ratio was decreased to less than 1 in cv. Dorado (about 0.32) and Hagen Shandawil (about 0.63) and increased to more than 1 in cv. Giza 113 (about 1.83). Thus, in our results,  $\text{Na}^+/\text{K}^+$  ratio is a good index for salt tolerance of the three sorghum cvs. Application of vitamin PP, markedly retarded the accumulation of  $\text{Na}^+$  and stimulated the content of  $\text{K}^+$  in sorghum cultivars as compared with the values of untreated seedlings. This

**Fig. 3. Quantification of digitized video camera images of protein bands of cv. Giza 113 with densitometry computer program showing the changes in those bands in response to the effect of -0.90MPa NaCl (C) and 100 ppm vitamin PP (B) or both (D) as compared with untreated control seedlings (A). Newly appeared bands are indicated by (  $\square$  ), and the disappeared bands are indicated by (  $\circ$  )**



antagonistic relation between  $\text{Na}^+$  and  $\text{K}^+$  indicates that, vitamin PP could play a role in modifying  $\text{Na}^+ / \text{K}^+$  selectivity under salt stress (Apsalan & Gunes, 2001)

The protein patterns of sorghum cvs. seedlings in response to salinity stress or salinity and vitamin PP are quantified and represented in Figs. 1, 2 and 3 (A, B, C and D). Some variations can be noticed in the polypeptides profiles (represented by peaks) of SDS-treated proteins, among these cvs. The intensity of peaks was generally much

higher in cv. Dorado (the more salt tolerance) as compared with cv. Giza 113 (the least salt tolerance). Cv. Hagen Shandawil was intermediate. These changes in the intensity of protein patterns might be correlated with a specific adaptation of these cultivars to survive the adverse effect of salinity.

Seedlings treatment with NaCl Figs. (1C, 2C and 3C) showed that, an increase in the level of most polypeptide compared to those appeared originally in the control.

Moreover, a new bands with molecular weight of 67 and 26 kD in cv. Dorado (Fig. 1C) and unique 45 kD in cv. Hagen Shandawil (Fig. 2C)) were appeared. On the other hand, in cv. Giza 113, salinity stress led to the loss of polypeptides with 67 and 26 kD (Fig. 3C). These new proteins, which appeared in cv. Dorado and Hagen Shandawil (the more salt tolerant) and did not appear in cv. Giza 113 (salt sensitive) seedlings, may play an important role in triggering a special system helping the salt stress tolerance of these cvs. (Vierling, 1991).

The appearance of 26 kD in cv. Dorado (the more salt tolerance), while it was disappeared in cv. Giza 113 (the least salt tolerance), in response to salt stress may be linked to the differences in salt stress tolerance of these cvs. It is believed that the 26 kD plays important role in osmotic adjustment to the cells either by facilitating the accumulation of solutes or by providing certain metabolic alterations in the cell, which may be helpful in osmotic adjustment (Singh *et al.*, 1987). To confirm, the importance of 26 kD in salt stress tolerance, when cv. Giza 113 treated with vitamin PP, the 26 kD reappeared, which accompanied with improvement of the dry mass production and the salt stress tolerance of cv. Giza 113.

Seed presoaking in vitamin PP. generally, resulted in an increased the intensity of the most polypeptide bands, which were already apparent in vitamin PP untreated salinized and non- salinized seedlings. Moreover, In non-salinized seedlings, vitamin PP treatments led to the net synthesis of 67 kD in cv. Dorado (Fig. 1B) and 38 kD polypeptide in cv. Hagen Shandawil (Fig. 2B), while in cv Giza 113 (Fig 3B) two polypeptides with 36 and 26 kD were appeared as compared with seedlings untreated with vitamin PP.

In salinized seedlings, it is worthy to notice that, vitamin PP treatment showed that, (i) in cv. Dorado (Fig. 1D), the decrease in the intensity of 16 kD polypeptide was accompanied by increase in the intensity of 29 kD and enhanced the net synthesis of 46 kD. (ii) in cv. Hagen Shandawil (Fig. 2D), the high significantly decrease in 16 kD was accompanied by increase in the intensity of the most other polypeptides, in addition to two bands with molecular weight of 26 and 18 kD were appeared (iii) in cv.Giza 113 (Fig. 3D), the 67 and 26 kD polypeptides, which had disappeared in salt- stressed seedlings, reappeared when those seedlings treated with vitamin PP. The new bands and the significant increase in the intensity of the original bands appeared in the control indicate that, vitamin PP has profound effect on the qualitative and quantitative changes in the protein component of the plants, which might linked with the improvement of growth and the synthesis of saccharids and nitrogen components.

The new bands of protein patterns in the vitamin - treated sorghum cvs. may be due to *de novo* synthesis of new proteins or formation of peptides acting on higher molecular proteins (Gopala Roa *et al.*, 1987). Kodandaramaiah (1983) has shown, vitamin treatments

induced a significant alterations in the enzymes related to protein metabolism; which indicate that vitamins might act as activators of protein synthesis. The positive correlation between the gene expression induced by vitamin treatment and the differences in the ability of sorghum cvs. to overcome and cope with salinity stress indicate that, expression of the genes for these proteins may be involved in the ability of sorghum cvs. to tolerate salt stress. This correlation could be due to the existence of a general plant stress response mechanism that transduces salinity stress signals into physiological events (Hanson & Hitz, 1982; Chapin, 1991). Conceivably, such a transduction pathway could be also triggered by vitamin treatments (vitamins signal) and it could initiate a defined set of molecular responses after receiving an environmental cue (salt stress).

## CONCLUSION

From the above results, it can be concluded that, there were significant differences in salt tolerance between the three tested sorghum cvs. Based on the ability of experimental sorghum cultivars to cope with salinity stress, cv. Dorado was the most salt tolerant, cv.Hagen Shandawil is moderately salt tolerant, while cv. Giza 113 was salt sensitive. The salt tolerance of sorghum cultivars was associated with variation in protein patterns and other relevant physiological activities. The inhibitory effects of salt stress on seedling growth and other relevant physiological activities can be alleviated and /or modified to some extent, by presoaking the seeds in appropriate (100 ppm) concentration of vitamin PP.

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## REFERENCES

- Abdel-Samed, H.M., 1991. Physiological studies of some plants in relation to salinity injury. *Ph.D. Thesis*, pp: 1–242. Menia Univ. Menia, Egypt
- Alpaslan, A. and H. Gune, 2001. Interactive effects of boron and salinity on the growth, membrane permeability and mineral composition of tomato and cucumber plants. *Plant and Soil*, 236: 123–6
- Ashfaq, N., M.M.R.K. Afridi and S.A. Ansari, 1983. Effect of pyridoxine treatment of grain on yield of triticale. *Ann. Conf. Soc. Adv. Bot. Hisar, May*: 28–9
- Ashraf, M., 1994. Organic substances responsible for salt tolerance in *Eruca sativa*. *Biol. Plant.*, 36: 255–9
- Azooz, M.M., 2002. Physiological responses of seedlings of two wheat cultivars (cv. Seds-1 and cv. Banyswif-3) to salt stress tolerance. *J. Union Arab Biol. Cairo, Physiol. Algae*, 10: 39–55
- Azooz, M.M., A. M. Hassanein and F.A Faheed, 2002. Riboflavine (vitamin B2) treatments counteract the adverse effects of salinity on growth and some relevant physiological responses of *Hibiscus sabdariffa* L. seedlings. *Bull. Fac. Sci. Assiut Univ.*, 31: 295–303

- Badour, S.S.A., 1959. Analytisch-chemische Untersuchung des Kaliummangels bei Chlorella im Vergleich mit anderen Mangelzuständen. *Ph.D. Dissertation* Göttingen
- Balibrea, M.E., A.M. Rus-Alvarez, M.C. Bolarin and F. Perez-Alfocea, 1997. Fast changes in soluble carbohydrates and proline contents in tomato seedlings in responses to ionic and non-ionic iso osmotic stresses. *J. Plant Physiol.*, 151: 221–6
- Barakat, H., 2003. Interactive effects of salinity and certain vitamins on gene expression and cell division. *Int. J. Agric. Biol.*, 3: 219–25
- Bates, L.S., R.P. Waldren and I.D. Tear, 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205–7
- Ben-Hayyim, G., Y. Vaadia and G.B. Williams, 1989. Protein associated with salt adaptation in citrus and tomato cells: Involvement of 26–kDa polypeptides. *Physiol. Plant.*, 77: 332–40
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. *Anal. Biochem.*, 72: 248–54
- Chapin, F.S., 1991: Integrated responses of plants to stress. *Biosci.*, 41: 29–36
- Choi, J., S. Yoon, H. Hong, D. Choi and G. Yoo, 1996. A modified Coomassie Blue staining of proteins in polyacrylamide gels with Bismarck Brown R. *Anal. Biochem.*, 236: 82–4
- Devitt, D.A., L.H. Stolzy, and C.K. Labanausks, 1987. Impact of potassium, sodium and salinity on the protein and free amino acid content of wheat grain. *Plant and Soil*, 103: 101–9
- Dubey, R.S. and M. Rani, 1990. Influence of NaCl salinity on the behavior of protease, aminopeptidase and carboxypeptidase in rice seedlings in relation to salt tolerance. *Australian J. Plant Physiol.*, 17: 215–22
- Gopala R.P., C.D. Reddy, and J.K. Ramaiah, 1987. Effect of B-vitamins on the protein component of clusterbeans *Cyamopsis tetragonoloba* L. *Taub. Ann. Bot.*, 59: 281–4
- Guerrier, G., 1998. Proline accumulation in salt-treated tomato: different proline precursors in *Lycopersicon esculentum* and *Lycopersicon pennellii*. *J. Plant Nutr.*, 21: 505–13
- Handa, S., R.A. Bressan, A.K. Handa, N.C. Carpita and P.M. Hasegawa, 1983. Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiol.*, 73: 834–43
- Hanson, A.D. and W.D. Hitz, 1982. Metabolic responses of mesophytes to plant water deficits. *Ann. Rev. Plant Physiol.*, 33: 163–203
- Ismail, A.M., 2003. Response of maize and sorghum to excess boron and salinity. *Biol. Plant.*, 47: 313–6
- Kodandaramaiah, J., 1983. Physiological studies on the influence of B – vitamins on leaf and fruit metabolism in clusterbeans *Cyamopsis tetragonoloba* L. *Taub. Ph.D. Thesis* submitted to Sri Venkateswara Univ. Tirupati, India
- Lazof, D.B. and N. Bernstein, 1998. Physiological studies on the influence of B-vitamins on leaf and fruit metabolism in clusterbeans *Cyamopsis tetragonoloba* L. *Taub. Ph.D. Thesis* submitted to Sri Venkateswara Univ. Tirupati, India
- Lee, Y.P. and T. Takanashi, 1966. An improved coloremeteric determination of amino acids with the use of ninhydrin. *Anal. Biochem.*, 14: 71–7
- Liu, T and J. van Staden, 2001. Growth rate, water relations and ion accumulation of soybean callus lines differing in salinity tolerance under salinity stress and its subsequent relief. *Plant Growth Regul.*, 34: 277–85
- Maier, J. and H. Ninnemann, 1996. Quantification of light-promoted condition of *Neurospora crassa* bd al–2 using a video system and densitometry software. *Fung. Genet. Newsletter*, 43: 68–70
- Mansour, M., O. Lee-Stadelmann and E. Stadelmann, 1993. Salinity stress and cytoplasmic factors. A comparison of cell permeability and lipid partiality in salt sensitive and salt resistant cultivars of *Triticum aestivum* and *Hordeum vulgare*. *Physiol. Plant.*, 88: 141–8
- Munns, R., 1993. Physiological process limiting plant growth in saline soils: Some damage and hypotheses. *Plant Cell Environ.*, 16: 15–24
- Schröppel-Meier, G. and W.M. Kaiser, 1988. Ion homeostasis in chloroplasts under salinity and mineral deficiency. I–Solute concentrations in leaves and chloroplasts from spinach plant under NaCl or NaNO<sub>3</sub> salinity. *Plant Physiol.*, 87: 822–7
- Schubert, S., R. Serraj, E. Plies-Balzer and K. Mengel, 1995. Effect of drought stress on growth, sugar concentrations and amino acid accumulation in N<sub>2</sub>-fixing alfalfa *Medicago sativa*. *J. Plant Physiol.*, 146: 541–6
- Shen, Y.Y. and S.G. Yan, 2003. Effects of salinity on germination of six salt-tolerant forage species and their recovery from saline conditions. *New Zealand J. Agric. Res.*, 46: 263–9
- Singh, N.K., C.A. Bracker, P.M. Hasegawa, A.K. Handa, M.A. Buckel, S. Hermodson, E. Pfankoch, F.E. Regnier and R.A. Bressan, 1987. Characterization of osmotin, a thaumatin-like protein associated with osmotic adaptation in plant cells. *Plant Physiol.*, 85: 529–36
- Soussi, M., M. Santamaria, A. Ocana and C. Liuch, 2001. Effect of salinity on protein and lipopolysaccharide pattern in salt – tolerant strain of *Mesorhizobium ciceri*. *J. Appl. Microbiol.*, 90: 476–81
- Sundaresan, S. and P.R. Sudhakaran, 1995. Water stress induced alterations in the proline metabolism of drought-susceptible and tolerant cassava *Manihot esculenta* cultivars. *Physiol. Plant.*, 94: 635–42
- Vierling, E., 1991. The role of heat shock proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 42: 579–620
- Williams and Twine, 1960. Flame photometric method for sodium, potassium and calcium. In: Peach, K. and M.V. Tracey, (ed.), *Modern Methods of Plant Analysis*, Vol. V., pp: 3–5. Springer-Verlag, Berlin
- Yan, B., Q. Dai, X. Liu, S. Huang and Z. Wang, 1996. Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant and Soil*, 179: 261–8
- Yeo, A.R., M.E. Yeo, S.A. Flowers and T.J. Flowers, 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical and Appl. Genet.*, 79: 377–84
- Zhang, B. and D.D. Archbold, 1993. Solute accumulation in leaves of a *Fragaria chiloensis* and a *F. virginiana* selection responds to water deficit stress. *J. American Soc. Hort. Sci.*, 118: 280–5

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