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SALINITY INDUCED DISSMILARITY AND AFFECTED GENE EXPRESSION IN SOME GRAMINACEOUS TAXA

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ABSTRACT

The present study was conducted in order to gain a better understanding of the significance of genetic factors and gene expression which are related to the effect of salt stress on the polypeptide levels in plants. To achieve this, the current work tested six Graminaceous taxa of sorghum and forage crops under three levels of salinity (50, 100 and 150 mM NaCl). The varieties were examined morphologically, physiologically and at the level of molecular biology by SDS-PAGE analysis. Results revealed that growth inhibition was observed in the plants subjected to 100 mM NaCl for 9 weeks while those subjected to 50 mM were not severely affected and still grow with lower rate till the end of experiment. On the other hand, all plants can't continue to grow at the higher salinity level III. Only two of six varieties showed tolerant ability towards high salinity stress (level II 100 mM NaCl), one of these two varieties was not capable continuing alive under this level of salinity and the other could. Extractable sodium rose linearly with an increase in external NaCl concentration. The extractable sodium from plants treated with 50 and 100 mM NaCl were fold higher than in control plants. Dendrogram was used to identify changes that resulted when plants were grown in the presence of 50, 100 and 150 mM NaCl for 9 weeks. SDS-PAGE showed that protein patterns for control and salt stressed plant were changed as shown from R_f changing values and newly protein with lower molecular weight 16-30 kDa were found. This observation was noticeable markedly in variety Pearl millet at second level of salinity. Dendrogram revealed dissimilarity attributed to salt stress with similarity coefficient varied due to salt stress effect. This result markedly showed between level I of salinity in Pearl millet which give similarity coefficient value in level I close to control (0.86) and this Sc changed to 0.61 in level II of salt stress for the same plant. Salinity showed different sc index between the treated plants and its control. The study suggests that salt tolerance is not conferred by a single trait, but is the consequence of complex gene interaction.

Keywords: Salinity, Graminaceous taxa, Sorghum, Forage crops, SDS-PAGE, Similarity coefficient, Dendrogram,

INTRODUCTION

Salinity is a major abiotic stress in plant agriculture world wide (Zhu, 2001). About 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Rhoades and Loveday 1990). Thus, salinity limits the production of nearly 40% of agricultural lands over the world (Serrano and Gaxiola, 1994). Salinity affects plant water status, reducing the water potential and thus impairing many function (Ali *et al.*, 2000; Eisa & Ali 2005) and decreased external water potential which inhibits cell expansion and the build-up of Na^+ and Cl^- in the cytosol inhibits metabolism (Glenn *et al.*, 1999). Moreover, salinity is a complex environmental constraint that presents two main components: an osmotic components due to the decrease in the external osmotic potential (Ψ_s) of the soil solution, and an ionic component linked to the accumulation of ions which become toxic at high concentrations (mainly Na^+ and Cl^-) and to a stress-induced decrease in the

content of essential elements, such as potassium and calcium (Lefevre *et al.*, 2001). Therefore, salinity in soil or water is one of the major environmental stresses which significantly limiting agricultural crop production, because most crop plants are glycophytes and sensitive to high Na^+ ion concentration either in soil or/and water (Flowers & Yeo, 1992). Thus, salinity affects plants by multi numerous aspects, osmotic stress, ionic toxicity and nutrition imbalance (Gorham *et al.*, 1985). The degree to which salinity affects growth depends on the plant genotypes, environmental conditions and the extent of salinity (Dubey, 1997).

However, salt stress result in a wide variety of physiological and biochemical changes in plants like accumulation of low molecular weight solutes, such as proline (Yancy *et al.*,1982), absorption of inorganic ions both of which contribute to osmotic adjustment (Binzel *et al.*,1988 and Bohnert *et al.*, 1995). On the other hand, Ochiai and Matoh (2001) reported that plants (*Anneurolepidium chinense*) accumulate K^+ to maintain the osmotic pressure of the young leaf blades under 100 and 200 mmol NaCl salinity. Nevertheless, identification of intracellular solutes and the importance of the changes induced in their level under stress conditions could be relevant as metabolic traits of interest for breeders concerned with characterization of stress tolerant cultivars of crop plants. Salt tolerance of plants is a complex phenomenon that involves physiological, biochemical and molecular process. One approach to understanding the molecular basis of salinity tolerance is to identify stress-induced changes in the levels of proteins (Majout *et al.*, 2000).

The ability of plant to overcome the effect of salinity stress and to sustain its productivity may be the important goal to achieve the gab between production and consumption. Graminaceae is one of the solutions which could be used to fill this gab in two purposes, forage and grain. There is a need for salt-tolerant cultivars to bring the poorly utilized saline lands into proper cultivation (Holmberg and Bulow, 1998). Achieving this by plant breeding has been proved futile, as at the genetic level salinity tolerance is a quantitative trait, resistant to improvement by breeding (Fooland and Jones, 1993). Hence, there is a need to understand the biochemical and molecular mechanisms of salinity tolerance in plants so that the property can be introduced in the species of interest through genetic engineering (Rout and Shaw 2001). Sorting out the mechanisms by which plants might adapt to salinity, is still unclear because the lack of full knowledge on the molecular and genetic basis of such a complex trait.

The effects of salinity on plant growth have been the subject of intensive researches in crop plants in arid and semi arid areas (Safarnejad *et al.*, 1996). Saline soil and Water are two challenges target that affecting plant productivity (Rajagobal *et al.*, 2006). Therefore, many studies were needed to obtain salt-tolerant plants. Analyzed soluble proteins using electrophoresis or HPLC techniques were used to classify or identify species and cultivars like *Phaseolus vulgaris* L. cultivars. Also, they have been used to characterize species and individual inbred lines or varieties of many cultivated species including maize (Smith and Smith, 1992), and *Rhodiola* Sp. (Wang *et al.*, 2005).

The present study will deal with six varieties of sorghum and forage crops to evaluate its tolerant ability against salinity and the ionic balance. In addition, using SDS-PAGE to monitor the changing in protein pattern under salinity and the similarity between all these six cultivars to select and sorting its capability toward salinity. Also, it could be allow deeper insight into the molecular mechanisms of salt tolerance to achieve better understanding of the changes caused by salt stress. Thus, this study will have to be extended at the molecular level to indicate whether or not transcription or both are affected by salinity.

MATERIALS AND METHODS

Plants (Cultivars)

The present study was performed as pots experiment in an open area in Fac. Agric. Ain Shams Univ. Six graminaceous taxa (forage crops) were chosen as the model plants for the study. Seeds of 1) Sudan grass (*Sorghum bicolor* var. sudanensis cv. Giza 1; 2) hybrid forage sorghum (Local hybrid 102); 3) Pearl millet (*Pennisetum americanum* L., cv. Shandawel 1); 4) Teosinte (*Euchlaena mexicana*); and 5,6) *Sorghum bicolor* (hybrid Shandawel 1 and hybrid Shandawel 2). Grains used for this study were obtained from Forage Crop Research Institute, Agriculture Research Center, Minstry of Agriculture, Giza, Egypt.

Salinity treatment

Experimental bottom drainage pots (144) with 30 cm diameter were filled with 9.5 kg HCl-washed sand and divided into six groups. Ten grains were sown per pot, on June 2nd 2002 then, two weeks after sowing, plants were thinned to one plant per pot. Complete randomized design with six replicates was used in this study. Each replicate included four treatments which were, tap water (control), 50, 100 and 150 mM NaCl. Irrigation was done to field capacity. During the experiment every pot was fertilized with 4.5 L nutrient solution of modified Arnon and Hoagland (1940). Irrigation with saline water was started after 10 days from sowing, and continued till the end of experiment. The experiment was continued for 9 weeks.

Morphological parameters

After 7 weeks, plant height (cm), leaf area (cm^2), shoot & root fresh weight in gram (g), shoot/root ratio, stem diameter (cm), blade and sheath weight (g), No. of tillers/plant and morphological characters were estimated and considered as growth parameters indices. Three types of leaves (old, middle & young) were taken to estimate leaves area. The youngest leaf was considered the full expanded leaf from the top (youngest mature physiological leaf); the middle leaf was the following third leaf to the youngest and the oldest green healthy leaf from the bottom. Leaf area was estimated according to Stickler *et al.* (1961) & Palamisway and Gomez (1974).

Elements analysis:

Leaves at three ages young, middle and old from top to bottom of the plant, were cut after 7 weeks from sowing. Then, sodium and potassium salts from both the blade and Sheath were determined using flame photometer Petracourt PFP1 according to Ali *et al.* (2000).

SDS-PAGE (Protein analysis):

Extraction of leaf water soluble proteins.

Fully expanded leaves (0.5 g) were ground in a mortar with a pestle at 4 °C. Homogenate was transferred to Eppendorf tubes that contain 1 ml water soluble protein extraction Tris buffer pH 7.5 in ratio 2:1 (w/v), then vortexes thoroughly. After that samples were centrifuged for 15 min at 2500 x g at 4 °C. After that supernatants containing water–soluble proteins were transferred to fresh tubes. For sure extraction all water–soluble proteins, pellet in Eppendorf tubes was resuspended in the same buffer and recentrifuged again to remove residual water-soluble proteins. Supernatant containing water-soluble proteins were collected, pooled in fresh tubes and then kept in deep freezer until use for electrophoretic analysis.

Poly acrylamide gel electrophoresis.

Sodium dodocyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12.5% (w/v) slab gels containing SDS (Laemmli, 1970) as modified by Studier (1973). The gels were cross-linked with 0.3% (w/v) N.N-methylene bisacrylamide at pH 8.3, and stacking gels were made 5.0% (w/v) polyacrylamide at pH 6.8. Leaf extract of water soluble proteins samples 50 µl were added to the same volume of Lan buffer and denaturized by heating at 100 °C for10 min in 1% SDS containing 2-mercaptoethanol (10% v/v), then used for gel loading after adding 10 µl bromophenol blue (0.025%). Lan buffer consist of 6 ml (1 M Tris pH 8.8) & 0.8 ml (0.25 M EDTA) and 93.2 ml double distilled water. Molecular weight of the protein was estimated from a low molecular weight standard (M.W. range from 14.3 to 97.4 kDa Pharmacia Montreal). Sample No. 2 at the second level of salinity (100 mM) was not included in protein analysis due to that plant was not survived till the sample date. SDS-PAGE bands obtained were detected, analyzed and plot the phonogram among sorghum and forage crops lines on UV-Transilluminator and photographed by Gel Documentation System UVP 2000.

Statistical analysis.

Statistical analysis was estimated using student's t-test ($p<0.05$) according to Snedecor and Cochran (1989).

RESULTS AND DISSCUSION

Morphological and Molecular Characteristics

The results illustrated in Figures & Tables revealed significantly decreases in plant height, shoot/root fresh weight (Fig. 1); leaves area (Fig. 2) blade, sheath weight Table (1), stem weight, length, diameter and tillers number (Table, 2), per plant for all sorghum and forage varieties by adding the first NaCl salinity level. No further growing was observed by increasing NaCl level up to 100 mM for varieties 1,4,5 and 6. Meanwhile, varieties 2 and 3 had survived and grown under 100 mM, but they had failed to continue growing by raising NaCl level up to 150 mM. On the other hand, the response of salinity was differed among all tested varieties.

Results revealed that growth inhibition was observed in the plants subjected to 100 mM NaCl for 7 weeks, while those subjected to 50 mM were not severely affected and still grow with lower rate till the end of experiment. Despite variety 1 & 3 gave high shoot, root fresh weight and plant height at control, but in general data in Figure 1 showed that variety 3 revealed better growths in shoot, root fresh weight and plant height among all tested varieties in salinity levels I & II treatments. Also, it is obviously shown from data in Fig. (1) that, applying the second level of salinity resulted in drastically lowering in fresh weight about 70% in variety 2 and 45% in variety 3 compared to the first level of salinity for the same varieties respectively. This drastically lowering in fresh weight in variety 2 at second level of salinity means that plant was suffered severely from salt stress and that interpret why this variety could not continue survival under salt stress of level II of salinity till the end of experiment.

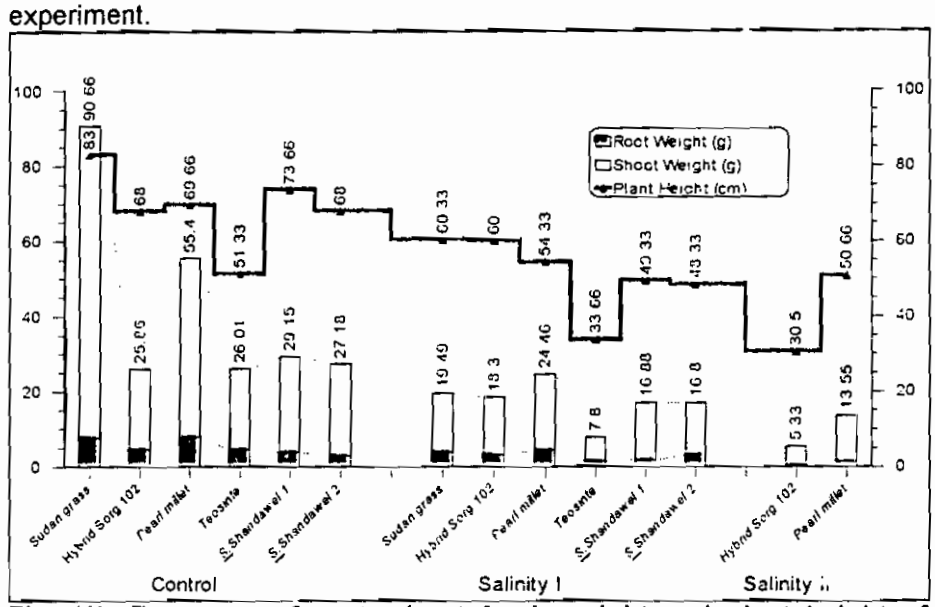

Fig. (1): Response of root, shoot fresh weight and plant height of different sorghum and forage varieties to effect of two levels of salinity (50 and 100 mM NaCl).

On the other hand, data illustrated in Fig.(2) showed that leaf area was gradually increased from old to middle and young leaves, and this gradient increase was still right under salinity treatments. Also, Fig. (2) showed the same shape for all crops in control and in the first level of salinity treatment. The accumulated leaf area for three leaves of plants under control and the first salinity level gave the same trend as shown from the imposable shape of these two treatments, but they were reduced in salinity treatments compared to control. It is cleared that the highest leaf area plant (Shandawel 1) could not grow under salinity II. This may be attributed to water restriction for growth, beside lowering in physiological process which reflected in lowering leaf area, stem (weight, length & diameter) and blade, sheath weight as shown in Table (1&2).

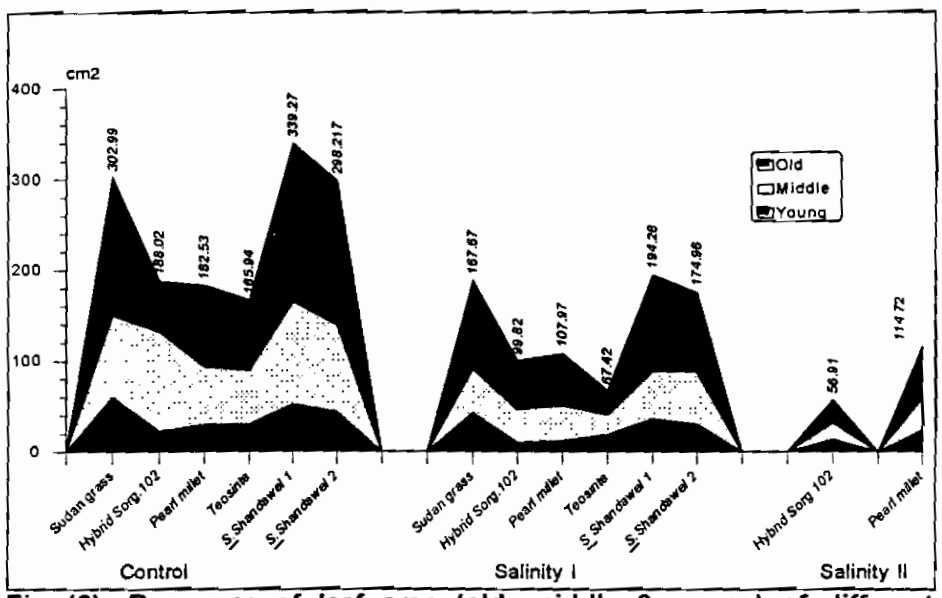

Fig (2): Response of leaf area (old, middle & young) of different sorghum and forage varieties to effect of two levels of salinity (50 and 100 mM NaCl).

Table (1) shows that stem weight, length and diameter were significantly affected with salinity stress especially Teosinte which drastically lowered in all morphological parameters as shown in Table (1) and Figures (1&2). It is shown from Figure (2), although No 5 & 6 (Shandawel 1& 2) gave the highest leaves area in control and in the first salinity level, they could not continue to be alive under a higher level of salinity II. This may be interpret on the fact these varieties have the highest surface leaves area among tested plant, so that they transpire much water and need more water, meanwhile water represents limited factor in salinity stress. Therefore, they could not adapted to salt stress at the high salinity level II corresponding to varieties 2 & 3 (hybrid sorghum & Pearl millet), which have less leaf area thus they could survival.

Respecting to shoot/root ratio Table (1), it is obviously cleared that ratio was decreased by adding the first level of salinity, except sample No.2 (hybrid sorghum) was increased in first and second level of salinity. However, this decrease of shoot/root ratio resulted in more decrease in shoot than root as affected by stress. Therefore, that reflected more sensitivity for shoot than root under salt stress. This behavior was revealed in excluder plants. That was agreeing with Marchenar, (1995) who cleared that the excluder plants prevent the translocation of Na from root to shoot to avoid the harmful effect of Na toxicity. On the other hand, the plant accumulates sodium in sheath higher than blade to protect photosynthesis active tissues. These afore-mentioned notes were in agreement with the results presented in Table (3).

Table (1): Morphological characteristics of different sorghum and forage varieties under two levels of salinity (50 and 100 mM NaCl).

Variety	Stem Weight			Stem Length			Stem Diameter			Shoot : Root ratio*			Tillers number / pot		
	Control	Salinity I	Salinity II	Control	Salinity I	Salinity II	Control	Salinity I	Salinity II	Control	Salinity I	Salinity II	Control	Salinity I	Salinity II
1	6.23	1.81		12.33	4.5		6.3	1.8		7.1	4.1		1	-	-
2	2.17	1.32	0.315	5.50	3.25	1.2	2.2	1.3	0.34	4.3	4.9	11.4	1	-	-
3	1.96	0.67	0.676	3.93	1.93	2.4	2.0	0.7	0.70	5.1	4.6	9.35	4	3	1
4	1.14	0.31		1.76	0.66		1.0	0.3		7.3	5.7		3	1	-
5	1.84	1.12		3.70	2.6		1.8	1.1		6.7	4.9		-	-	-
6	1.53	1.03		2.86	2.36		1.5	1.1		8.5	4.4		-	-	-

All data is expressed as mean. Shoot/Root Ratio could be calculated from data represented in Fig. (1).

Table (2): Blade and Sheath fresh weight in gram for three leaves (old, middle & young) of different sorghum and forage varieties under two levels of salinity (50 & 100 mM NaCl).

Variety	Control			Salinity I			Salinity II		
	Old	Middle	Young	Old	Middle	Young	Old	Middle	Young
Blade1	1.09	0.38	2.23	0.49	0.74	1.46			
Sheath	0.65	1.65	1.82	0.53	0.67	1.61			
Blade2	0.38	1.15	1.96	0.36	0.81	1.42	0.18	0.33	0.47
Sheath	0.40	1.34	1.82	0.32	0.87	1.33	0.18	0.28	0.45
Blade3	0.52	1.17	2.08	0.34	0.72	1.36	0.37	0.72	1.13
Sheath	0.76	1.20	1.45	0.25	0.62	0.86	0.34	0.69	0.86
Blade4	0.58	1.32	1.80	0.39	0.50	0.69			
Sheath	0.83	1.30	1.60	0.52	0.49	0.67			
Blade5	0.62	1.51	2.78	0.48	0.82	1.70			
Sheath	0.69	1.39	2.1	0.43	0.64	1.49			
Blade6	0.61	1.54	2.56	0.36	0.91	1.35			
Sheath	0.62	1.27	2.03	0.37	0.83	1.20			

All data is expressed as mean.

Regarding to Table (2), it has been found that blade & sheath weight for old, middle and young leaves significantly affected by salinity treatment. As shown in Figure (2), leaf weight gave a high value in control corresponding to treated plants with salinity. The blade weight was more affected than sheath weight after salinity treatments. However, plants were still growing under salt stress in lower rates and this was clearly in varieties 2, 3 (hybrid sorghum & Pearl millet) which were given lowest weight of blade and sheath at level II of salinity compared with control and level I of salinity. Generally, significant reduction in growth characters like plant height, root & shoot fresh weight, leaf area, leaf weight, was found in salinity treatment. These results are in agreement with Ritambhara *et al.* (2000); Dubey, (1997); Mittal and Dubey, (1991), who reported that genotypes of crop species differing in salt tolerance, when they had grown under increasing levels of NaCl salinity, show distinct morphological differences as well as alterations in behaviors of key enzymes of various metabolic pathways.

Sodium and Potassium Interaction

Data in Table (3) showed extractable sodium (µmol/g fw) from blade and sheath at various three age stages for different sorghum and forage crops varieties under control and salinity treatments. Extractable sodium rose linearly with an increase in external NaCl concentration. The extractable sodium from plants treated with 50 and 100 mM NaCl was fold higher than in control plants (Table, 3). Results revealed that extractable sodium was increased gradually from young to old parts either in blade or sheath and the level was higher in blade than in sheath. Although the same trend was observed under salinity condition, sodium was increased significantly in all parts (blade or sheath) compared to control plants. In contrary, the opposite trend was shown in young parts in some tested varieties, whereas most extractable sodium was found in sheath corresponding to blade and this trend was shown also under salinity treatment. This may be explained on the fact that the plant tends to protect the photosynthetic tissues from the harmful of a high level of salinity and avoid the sensitive parts from destroying. Also, high amounts of sodium in old part are the plant strategy to overcome onto salinity through transferring sodium to this less working part and failed it later. This was found clearly in variety 3 (Pearl millet) which could continue growing up to the end of the experiment under salinity level II, while the other varieties could not. This plant revealed high amount of extractable Na in blade in all treatments, control, level I & II of salinity compared to different tested varieties. The more sodium in photosynthetic tissues (blade and sheath) of plant and still alive, this mean tolerant capability for this plant against salt stress. It has been found that the extractable sodium considerably increased along with increasing salt stress compared with control. The results are in agreement with Vera-Estrella *et al.* (2005) who reported that, under salt stress, the major site of Na^+ accumulation occurred in old leaves, followed by young leaves and taproots, with the least accumulation occurring in lateral roots, as well as Blumwald (2000) who stated that the ability of plant cells to maintain low cytosolic sodium concentrations is an essential process associated with the ability of plants to grow in high salt concentrations.

Table (3): Sodium (µmol/g fw) extractable from Blade and Sheath leaf of three leaves for different sorghum and forage varieties under two levels of salinity (50 & 100 mM NaCl.

Variety	Control						Salinity I						Salinity II					
	Old		Middle		Young		Old		Middle		Young		Old		Middle		Young	
Blade1	8.61		5.61		2.73		17.03		12.10		4.43							
Sheath		12.04		2.53		1.81		13.04		11.29		4.91						
Blade2	10.29		3.10		2.00		18.62		6.57		4.47		37.68		23.97		5.00	
Sheath		3.47		1.38		1.86		8.69		6.79		7.19		48.31		18.32		8.69
Blade3	21.73		8.25		4.18		38.36		14.49		8.95		47.47		18.60		8.09	
Sheath		5.37		2.31		1.97		24.0		12.62		11.17		30.69		13.23		11.12
Blade4	11.24		4.87		7.25		43.14		25.04		9.04							
Sheath		4.71		3.74		2.71		15.05		14.72		5.84						
Blade5	15.65		6.62		3.23		21.55		6.25		3.32							
Sheath		4.02		3.62		2.49		18.35		13.05		10.85						
Blade6	11.97		5.67		2.54		14.49		14.42		7.27							
Sheath		3.78		2.60		1.28		28.90		20.63		12.90						

All data is expressed as mean. (µmol/g fw) = micromole/ gram fresh weight.

Table (4): Potassium (µmol/g fw) extractable from Blade and Sheath leaf of different sorghum and forage varieties under two levels of salinity (50 & 100 mM NaCl.

Variety	Control						Salinity I						Salinity II					
	Old		Middle		Young		Old		Middle		Young		Old		Middle		Young	
Blade1	6.19		5.71		2.06		11.74		4.16		2.62							
Sheath		6.49		1.09		1.22		4.92		4.00		2.24						
Blade2	10.09		3.00		1.72		2.98		2.27		1.56		11.08		6.15		2.77	
Sheath		4.02		2.59		2.53		3.59		1.58		1.67		4.68		2.74		1.53
Blade3	17.8		7.41		5.01		11.28		4.26		1.63		3.73		1.81		1.35	
Sheath		6.05		1.95		2.11		13.19		3.53		2.76		7.59		4.00		3.39
Blade4	6.08		3.66		2.68		7.08		3.68		1.55							
Sheath		3.23		2.19		1.96		2.36		1.25		0.80						
Blade5	18.30		6.55		2.78		7.19		2.52		1.71							
Sheath		3.78		3.31		2.77		4.28		2.75		2.31						
Blade6	11.32		3.63		1.43		13.64		3.45		2.78							
Sheath		3.21		1.81		1.47		7.25		2.95		1.99						

All data is expressed as mean. (µmol/g fw) = micromole/ gram fresh weight.

Concerning to Table (4), extractable potassium behaves the same trend like sodium, whereas the old part (blade or sheath) contains high level of K. Meanwhile salinity resulted in lowering K compared to control plant. The increasing of salinity led to lowering K in middle blade and also in old leaf for variety 2,3 & 5 beside the young leaf noticed lowering K level under salinity effect except No 1 and 6, whereas K increased in this tissues. The same trend was observed at the second level of salinity, but variety 2 showed increasing K in all leaves compared with control. These results were in agreement with Ochiai and Matoh (2001) who reported that plants *Anneurolepidium chinense* accumulated K^+ to maintain the osmotic pressure of the young leaf blades under 100 and 200 mmol NaCl salinity.

The data also showed that variety 3 had more extractable K in blade among all other tested plant. This may explain its survival and better growth. Besides, it did not suffer from K shortage under salinity stress. Moreover, this variety revealed better growth character at the second level of salinity (Figs. 1&2); this may be attributed to its capability to gain more K among all other variety as shown in Table (4). That means plant could translocate K from old to middle leaf and move K to compensate K shortage which resulted in from salinity stress effects. These results are in accordance with Blumwald (2000) who reported that the ability of plant cells to maintain low cytosolic sodium concentrations is an essential process associated with the ability of plants to grow in high salt concentrations.

Bio-Physiognomy Underlying Salinity.

(SDS-PAGE).

In the present study, SDS-PAGE criteria of leaf proteins were performed on 13 samples of sorghum and forage crops. Water soluble protein extracted from all tested samples (13 samples) 6 controls; 6 salinity level I and one sample No.3 level II of salinity subjected to SDS-PAGE analysis illustrated in Figure (3). Molecular characterization R_f , MW & peaks density of analyzed proteins are presented in Table (5).

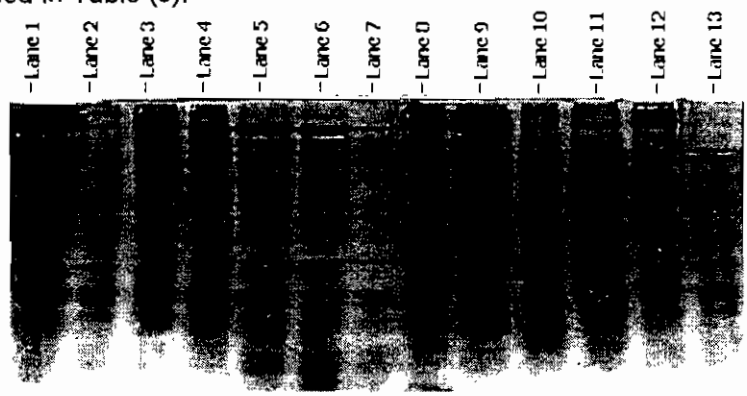

Fig.(3). **Proteins patterns of different plants subjected to salt stress and separated using one dimensional SDS-PAGE. Electrophoretic pattern of taxa under studies were Lan-1,2 Sudan grass (*Sorghum sudanensis*, Giza 1), Lan-3,4 hybrid forage sorghum (102), Lan-5,6,7 Pearl millet (*Pennisetum americanum* L.., Shandawel 1), Lan-8,9 *Euchlaena mexicana* (Teosinte); and Lan-10,11 Sorghum bicolor (hybrid Shandawel 1) and Lan-12,13 Sorghum bicolor (hybrid Shandawel 2), First No. control and second is salinity level I; Lan-7 Salinity level II for Pearl millet.**

Table (5): Molecular weight and peak density of protein pattern of six graminaceous taxa under two levels of salinity treatment.

Band			Lane 1 cont	Lane 2 sal	Lane 3 cont	Lane 4 sal	Lane 5 cont	Lane 6 sal	Lane 7 Sal. II	Lane 8 cont	Lane 9 sal	Lane 10 cont	Lane 11 Sal	Lane 12 cont	Lane 13 sal
	Rf	MW	Peak	Peak	Peak	Peak	Peak	Peak	Peak	Peak	Peak	Peak	Peak	Peak	Peak
1	0.033	176.58	190.80	------											
2	0.082	133.12	211.39	115.45	149.70	141.89	223.91	217.39	116.75	200.05	169.82	110.98	130.00	147.00	88.32
3	0.118	108.36			122.45	102.70	147.82	151.55	83.00				108.16*	103.48	------
4	0.135	97.40					142.09	114.80	------	152.11	------	95.43	------		
5	0.162	84.92	174.98	115.86	144.64	151.68	169.14	141.52	73.21	152.91	110.91	163.82	153.77	160.98	109.39
6	0.180	77.17											136.5*	148.66	118.64
7	0.189	73.55		129.59*		151.36*		130.95*			132.77*				
8	0.207	67.26	192.48	------	149.41	------	159.95	134.73	------	172.84	------	186.80	------		
9	0.216	64.37					149.02	------	------						
10	0.233	59.42										112.50	------		
11	0.241	57.30	154.50	104.39		117.45*									
12	0.256	53.67					137.57	103.75	------	150.66	------				
13	0.272	50.24		109.8*	136.77	------	146.34	110.41	------	143.30	------				
14	0.299	45.36	170.36	125.75	149.14	151.50				145.41	------	135.59	146.91	143.89	113.52
15	0.311	43.51		134.23*			133.00	111.55	------						
16	0.330	40.92	184.20	150.50	161.43	167.36	182.11	170.30	76.46	181.64	159.59	161.82	179.07	160.27	133.93
17	0.352	38.36		135.8*	141.14	------							149.0*		
18	0.373	36.28	171.39	133.86	138.5	147.66	149.32	140.52	100.14	139.66	143.16	117.89	153.55	131.09	130.43
19	0.407	33.47	161.82	------	125.64	137.93	115.20	------	------				140.0*	125.34	------
20	0.417	32.75		133.45*			115.14	------	------	114.66	------	102.02	138.05		119.82*
21	0.433	31.65		124.52*	141.36	132.91				127.18	123.41	105.80	------	114.57	------
22	0.443	31.00	162.98	------	116.25	126.39	116.98	102.82	------	121.18	125.00	112.59	129.11		103.43*
23	0.457	30.13					107.32	87.55	44.89		90.32*		100.05*		94.25*
24	0.478	28.86	133.64	90.02	93.89	93.02						77.98	------		
25	0.505	27.27								90.48	84.59				
26	0.523	26.21					117.95	93.66	70.75						
27	0.538	25.33	118.57	95.89	105.05	107.39				76.25	70.64	88.39	103.55	98.18	107.27
28	0.560	24.01				92.80*	72.82	------	------						
29	0.578	22.91					70.70	55.93	------						
30	0.645	18.79	106.84	87.95	94.50	96.43	88.93	84.16	69.93	87.23	75.41	70.52	98.45	96.02	88.07
31	0.665	17.63		89.52*	101.05	------	95.64	84.39	54.93		79.32*				
32	0.701	15.78	124.91	------	96.68	96.52			47.93*	88.70	85.89	86.61	103.25	95.41	96.14
33	0.751	14.16	122.80	------			102.11	99.30	65.39	86.05	------				
34	0.783	14.07	107.02	------	88.63	------									
35	0.861	12.79					87.70	94.84	61.32						
36	0.912	12.08					89.73	101.41	------						

* = means new protein or peptide. cont = control; sal = salinity level I; — = means peak was not found in salinity treatments. Lan-1,2 *Sorghum sudanensis* (Giza 1), Lan-3,4 hybrid forage sorghum (102), Lan-5,6,7 Pearl millet, Lan-8,9 Teosinte; Lan-10,11,12,13 hybrid Shandawel 1& 2 and Lan-7 Salinity level II for Pearl millet.

As shown from R_f values for patterns data in Table (5) revealed a presence of newly lowered molecular weight peptides either synthesized or small fraction of damaged proteins with Mw range 15 - 30 kDa in the Lane 2, 4, 7, 9, 11 and 13 as shown and determined by SDS-PAGE. Accordingly, Table (5) shows the maximum No of protein patterns found were 23 patterns in Lan-5 (control Pearl millet), while the minimum number was 12 patterns in Lan-7,13 (Pear millet & Shandawel 2) after salinity treatment. Therefore, as shown in Fig (3) that confirmed by the results in Table (5) peak density of protein patterns analyzed were decreased and appeared lowered density as found in control peak No. 2, 2 in Lan 1,5 and compared with peak No. 2,2 in Lan 2,7 in treated sample with salinity for the same varieties. Not only that, but also, some protein patterns were disappeared completely as observed in samples 5, 6 & 7 for Pearl millet due to the salinity treatments. Nevertheless, looking at Lan-5,6,7 it has been found that number of peaks was decreased from 23 to 21 and 12 at control, salinity I & II for Pearl millet respectively. In addition, new protein pattern which appeared may be from synthesis or protein degradation due to salinity effects. However, Table (5) revealed that Pearl millet is the only variety among all tested plants that have lowered Mw proteins as shown in control and salinity levels I & II with R_f values 0.86 & 0.91 in control & salinity I, while R_f was 0.86 in salinity II with molecular weight range 10-13 kDa. The presence of lower molecular weight protein reflects that plant could reduce the osmotic potential to be able to absorb water in high osmotic potential in soil due to salinity stress. This explains the survival of Pearl millet and its give tillers (Table, 1) despite salt stress. Also, it is observed that some peptides with Mw range 31-65 kDa in control plants were disappeared as the causal effect of salinity. This may be due to that plant turnover proteins to lower MW proteins and degraded it to lower Mw which is necessary to adjust osmotic potential or gene depression under hard stress state. Consequently, it is legal to showing some peaks in control samples were disappeared completely after salinity treatment as cleared in sample with R_f values 0.13, 0.20, 0.21, 0.25, 0.27, 0.31, 0.40, 0.41, 0.44, 0.56, 0.57 & 0.91 in control Pearl millet were not found in treated samples. Thus, salinity resulted in peaks reduction as found in sample Pearl millet control Lan-5; salinity I Lan-6 and Salinity II Lan-7, whereas peaks number was reduced from 23 to 21 and finally 12 protein patterns after treated with high salt stress. The study analyzed expression patterns of salt-responsive proteins about (23), and it found different expression groups. These proteins banding could be considered as unique biochemical markers as well as genetic markers for the respective salt stress. Qualitative and quantitative changes of protein patterns suggested a modulation of gene expression. The results were interpreted in the light of recent arguments on salinity stress and proteins. These results indicate that salt stress accelerate gene expression of these varieties and furthermore induces the encoding gene to produce this salt inducible proteins. Although, on one side peaks density were decreased after salinity treatment, but on the other side some patterns have been found more density such as patterns 35, 36 in Lan-5,6; bands 22, 27, 30 & 32 in Lan-10, 11and band 27 in Lan-12,13. This increase in peak density for lower molecular weight bands confirm tendency of plants to increase these lowered

molecular weight peptides to face salt stress and adjust osmotic potential under salinity stress. These results are in accordance with Askari *et al.* (2006) mentioned that 27 protein spots were identified including proteins involved in oxidative stress tolerance, glycinebetain synthesis, cytoskeleton remodeling, photosynthesis, ATP production, protein degradation, cyanide detoxification, and chaperone activities and reported the expression pattern of these proteins and their possible roles in the adaptation of *Suaeda aegyptiaca* to salinity. Also, the results are in agreement with Vera-Estrella *et al.* (2005) who reported that salt stress increased both the H^+ transport and hydrolytic activity of salt cross tonoplast (TP) and plasma membrane (PM) H(*)-ATPases from leaves and roots.

Similarity Coefficients for control and treated samples.

Table (6) showed similarity coefficient values between varieties under study in normal and salinity condition. Data showed that varieties irrigated with tap water and do not suffer from salinity stress give genetic similarity coefficient ranged between 0.39-0.78 for control Pearl millet x Shandawel 2, and Teosinte x Shandawel 1 respectively. Meanwhile, this range was changed to 0.42-0.73, after treated varieties with salinity, except the Sc between hybrid Shandawel 1 & 2 in treated experiment recorded 0.64 in control and 0.86 in saline treatment. Table (6) showed that Sc values were decreased from 0.67 to 0.55 for Sudan grass (Giza 1) and hybrid Shandawel 2; from 0.75 to 0.62 between Hybrid sorghum and hybrid Shandawel 2; and from 0.79 to 0.67 between Teosinte and hybrid Shandawel 1 in control and saline treatment respectively. Hybrid Shandawel 1 & 2, which in normal condition varied with Sc 0.64 gave Sc 0.86 under salt stress which reflects that while these varieties differed in normal condition and gave different morphological characters, but under highly stress effects backed to original characters or induce the stable properties as the original (ancestor) plant.

Table (6). Similarity Coefficients as computed for all treatments under study.

Lane	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1	0.59	0.78	0.76	0.45	0.42	0.53	0.69	0.58	0.69	0.67	0.67	0.62
2		1	0.72	0.71	0.45	0.47	0.47	0.63	0.65	0.63	0.61	0.6	0.55
3			1	0.78	0.52	0.5	0.56	0.7	0.67	0.71	0.74	0.75	0.58
4				1	0.45	0.42	0.53	0.63	0.71	0.69	0.73	0.8	0.62
5					1	0.86	0.61	0.59	0.43	0.47	0.51	0.39	0.46
6						1	0.65	0.56	0.51	0.44	0.43	0.35	0.42
7							1	0.52	0.67	0.43	0.62	0.62	0.56
8								1	0.69	0.79	0.65	0.65	0.67
9									1	0.62	0.67	0.67	0.69
10										1	0.65	0.64	0.74
11											1	0.83	0.86
12												1	0.72
13													1

Similarity coefficient between control Pearl millet and its treated sample with the first salinity level recorded high value (0.86), this value was reduced to 0.61 in second salinity level for the same variety, which mean about 29% lowering happened in similarity due to the applied of higher salinity level.

As shown in Figure (4), Pearl millet and hybrid sorghum (102) have high Sc values 0.86 & 0.78 among all tested plants. These plants could still survival till the second level of salinity. On the other hand, all varieties that recorded Sc lower than 0.75 could not still survival at the second level of salinity, while those recorded Sc more than 0.75 could survive at the second level of salinity, i.e. Pearl millet which could still survival during the second level of salinity gave Sc 0.86. Thus, it could be concluded that the high Sc for Pearl millet compared with hybrid sorghum (102) interprets that the Pearl millet could still survival till the end of experiment. That means Pearl millet has a high potential to express as a tolerant plant.

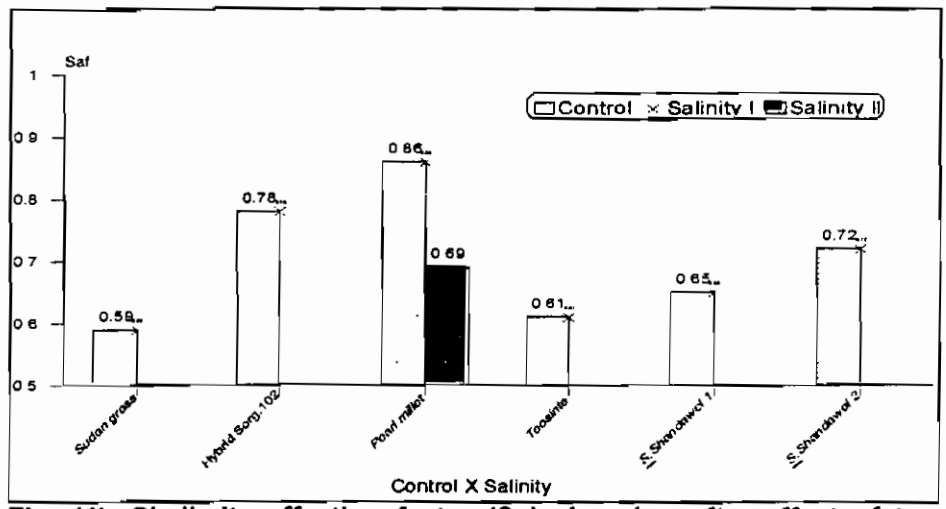

Fig. (4): Similarity affecting factor (S_{af}) changing after effect of two levels of salinity treatments on different taxa varieties.

Dendrograms of six taxa by SDS-PAGE analysis.

A dendrogram of identity is presented in Fig. (5), which based on the results of SDS-PAGE analysis to find the phylogenic relationship among six Graminacea taxa (13 samples of six sorghum and forage crops). The relationship depicted in dendrogram were sorted into 7 clusters at similarity affecting coefficient 0.75 level designated I to VII (Fig. 5). In one side, cluster II, III, IV and VII each included salinity treated plants, respectively. On the other side, cluster V was composed of two control subgroups and cluster VI is mixed consists of three control subgroups & one salinity treatment. Meanwhile, control Pearl millet and its first saline treatment were grouped in cluster I. The greatest Sc (0.86) was found between plant 5 and 6 in cluster I. The lowest Sc (0.35) was observed in different clusters, for example 6 vs 12. The dendrogram clearly indicates that accession Pearl millet in control and two levels of salinity is isolated from the other accessions with Sc = 0.61, while Sc value was 0.86 between the same plant and its control along with first level of salinity. The relation between accessions was visualized by dendrogram.

On the other hand, regarding to dendrogram depicted in Fig. (5) two groups of variety can clearly be distinguished at 0.61 sc one group consists of Pearl millet control and its treatment sample at first level of salinity. This group gave the better performance growth despite the stress condition of salinity. The treated plant of this group revealed most similar protein patterns compared to control. This means that plant in this level of salinity does not severely suffer from stress and still related to the control plant which grows in normal condition. The other group contains subgroups of plants capable to grow in 50 & 100 mM NaCl and this group suffers severely from salt stress as confirmed from high number of protein patterns disappeared after salinity treatment as obviously shown in Table (5).

The produced dendrogram from SDS-PAGE analysis showed a close affinity and monophyllay among the species of genera i.e. Shandawel 1& 2 under salinity sc = 0.86. The dendrogram revealed that Pearl millet was moved from group I to II due to salinity effect at second level as a result of change Sc from 0.86 to 0.61. The same phenomenon was shown in the genera of studied taxa i.e. Teosinte and hybrid sorghum (102) as shown in Fig. (4), which mean salinity induced similarity changing or induce dissimilarity between the control plant and the plant itself treated or grown in saline condition. SDS-PAGE showed changing phylogenic relationship due to salinity stress which obviously cleared in dissimilarity found in alteration in similarity coefficient in dendrogram analysis. These results are in agreement with Brink *et al* (1989), Dinelli & Bonetti (1992), who used soluble protein analyzed by electrophoresis (IEF) and capillary electrophoresis or HPLC to classify or identify species and cultivars and as identification tool for *Phaseolus vulgaris* L. cultivars. Also, *Rhodiola* species (Wang *et al.*,2005).

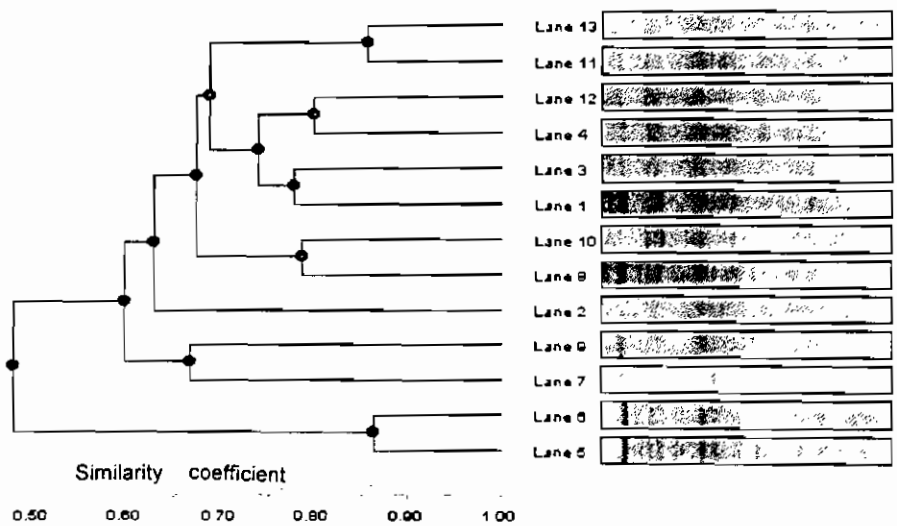

Fig. 5 : Dendrograms based on the converted data of water soluble protein peaks in SDS gel for six variety of sorghum and forage crops showing the clustering pattern between the varieties under two levels of salinity.

Dendrogram revealed that salinity resulted in change in similarity in protein patterns which may be due to that stress effect led to induce the gene expression to yield new protein and led to operate some genes which were not effective in normal conditions. So, it could be getting some resistant variety which able to grow under the salinity condition and give best performance in yielding. These results are in agreement with Singh *et al.*, (1987) who reported that salinity altered the pattern of only the synthesized proteins and these altered proteins could be classified into those accumulation was repressed, enhanced and which only induced in roots during germination and in both roots and shoots during the seedling stages beside both transcriptional and post-transcriptional mechanisms could involved in the regulation of gene expression. Moreover, El-Frash *et al.* (1993) reported that expression of some proteins (induced in salt stress tomato plants) was regulated depending on the salt level as well as the genetic background. Also, these results confirm that, salt tolerance is not conferred by a single trait but is the consequence of complex gene interaction (Bartels and Nelson, 1994). In addition, Youssef (1997) who found that electrophoretic patterns of water soluble proteins were more effective in identification of hybrid and cultivar and is relating heterotic electrophoretic protein. Also, Winicov and Bastola (1997) reported that abiotic stress lead to multigenic responses. Moreover, Winicov (1998) stated that diverse genes that are induced and repressed by dehydration such as oxidative stress defenses, also respond to salt stress.

The study recommended to increase cultivated lands with Pearl millet because is a highly cross-pollinated crop. It demonstrates the highest levels of tolerance to salinity as confirmed in the present work, moreover tolerant to drought and heat found in domesticated cereals and, consequently, is grown on >26 million ha in the arid and semiarid regions of Africa and India (Food and Agriculture Organization, 2000). Also, pearl millet is the only cereal that can be grown under dry land conditions and so plays a critical role in food security (Senthilvel, 2004). In addition to drought tolerance, millet has a relatively short growing season (60–90 d) that allows double cropping after wheat (*Triticum aestivum* L.) has been harvested and is an excellent forage and, because of its low hydrocyanic acid content, is the best annual grazing crop in the southern USA (Burton, 1995). The energy density of pearl millet is relatively high, arising from its higher oil content relative to maize, wheat or sorghum (Hill and Hanna, 1990). Pearl millet contains 27 to 32% more protein than maize, higher concentrations of essential amino acids, twice the ether extract, and higher gross energy than maize (Ejeta *et al.*, 1987).

Conclusion

In the present study growth characters and concomitant changes of protein banding pattern were studied on six varieties selected sorghum and forage crops. Polyacrylamide gel electrophoresis (SDS-PAGE) was employed to characterize those 13 samples (Graminaceous taxa). The mechanism by which salinity exerts its effect still entirely needs more investigation. The present study suggests some possibilities: The first, accumulate sodium into blade than sheath in old leaf and the inversely trend happened in young leaf to protect photosynthesis tissues. The second, potassium behaves the

opposite trend to sodium where it accumulates in young leaf compared to old and in blade than sheath. The third, may be plant induce transcription mRNA to synthesis new protein with lower molecular weight as regulatory protein for osmotic potential like 15-30 kDa, This regulatory proteins were not synthesis in the same plant in normal conditions. Therefore, the plant consider tolerant in this case if it could be able to synthesis these osmotic regulatory proteins. These proteins and their possible roles in the adaptation of forage crop to salinity should be considered to identify the mechanisms of salt responsiveness in leaves of pearl millet under different salt concentrations. The forth, salinity reduces and change similarity between varieties each other and the plant with it self due to salinity stress and so it could be concluded that salinity increase dissimilarity. Final but not finally, these results confirm that salt tolerance is not conferred by a single trait, but it is the consequence of complex gene interaction.

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الإستحثاث الملحى المحدث لتغير التشابه فى بعض النجيليات وتأثر التعبير الجينى

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تهدف هذه الدراسة لمعرفة الدور الذى تلعبه الملوحة على المستوى الجزيئى الحيوى والتعبير الجينى وانعكاس ذلك على التغيرات المورفولوجية والفسيولوجية والكيماوية الحيوية لستة أصناف نباتية تنتمى لمحاصيل العلف غير التقليدية وغير الشائع زراعتها فى مصر لاختيار أكثرها تحملا للملوحة وظروف الإجهاد الملحى لهذه الأصناف الستة تحت الدراسة ، وهى كالتالى : حشيشة السودان (جيزة ١) (Sudan grass) (*Sorghum bicolor* var. *sudanensis*, cv. Giza 1) ، وهجين سورجم العلف (١٠٢) hybrid forage sorghum ، والدخن Pearl millet (*Pennisetum americanum* L.) ، ذرة ريانة Teosinte (*Euchlaena mexicana*) ، ذرة رفيعة هجين hybrid sorghum bicolor (شندويل ١ و ٢) Shandawel 1 & 2 .

أظهرت الدراسة حدوث بعض التغيرات على المستوى الجزيئى والناتجة من التأثير الملحى الحاث للتعبير الجينى وتم تحديد تلك المتغيرات باستخدام تكنيك الهجرة الكهربية SDS-PAGE ، كما وجدت استجابة لبعض الأصناف للتأثير الملحى . وقد حددت درجات القرابة بين تلك الأصناف النباتية على أساس جزيئى حيوى . ولقد أظهرت الأصناف المقاومة صفات ظاهرية ونوعية نتيجة الإجهاد الملحى ، حيث أوضحت النتائج تأثر الصفات الخضرية (ارتفاع النبات وقطر الساق وعدد الأشطاء للنبات ومساحة النصل والوزن الغض) لمعاملات الملوحة ، كما أظهر التفريد الكهربى بالـ SDS-PAGE وجود تباين فى نوع وعدد المناطق البروتينية المفصولة وظهر وجود اختلافات فى التعبير الجينى بين الأنواع تحت الدراسة أولا على أساس وراثى جينى ثم ظهرت أيضا اختلافات نتيجة التأثير الإجهادى للملوحة ، و أظهرت الدراسة اختفاء كثير من المناطق البروتينية فى معاملات الملوحة وفى نفس الوقت لوحظ وجود وظيور بروتينات جديدة ذات وزن جزيئ منخفض يمكن تعليل ظهورها لمقاومة الضغط الإسموزى المرتفع نتيجة التأثير الملحى الإجهادى للنبات حيث تعمل على خفض الجهد الإسموزى للنبات لزيادة المقاومة للتأثير الملحى المتزايد ، كما تبين أن الدخن (الصنف رقم ٣) عند المستوى الأول من الملوحة أظهر قيم مرتفعة لمعامل التشابه Sc (Similarity coefficient) = ٠,٨٦ مع عينة الكنترول الخاصة بنفس النبات إلا أنه عند المستوى الثانى للملوحة المرتفع اختلفت قيمة معامل التشابه له فأعطى قيمة مختلفة مع العينة الكنترول Sc = ٠,٦١ أى أن العينتان ابتعدتا فى التماثل ، وظهرت تلك النتيجة بين كل المعاملات حيث كانت الملوحة تعطى قيم منخفضة مختلفة لمعامل التشابه مقارنة بالعينات الكنترول لنفس النبات. وكانت أعلا قيمة لمعامل التشابه Sc = ٠,٨٦ ، ٠,٧٨ لصنف الدخن وحشيشة السورجم الهجين عند المستوى الأول للملوحة ٥٠ مليمولر ، وارتفاع قيمة معامل التشابه للدخن وحشيشة السورجم يظهر تفسير لإمكانية تحملهم للمستوى الثانى للملوحة ١٠٠ مليمولر . كما أن قيمة Sc المرتفعة توضح استمرارية بقاءه حتى نهاية فترة التجربة ، فى حين لم يستطع السورجم الهجين (١٠٢) الاستمرار لنهاية التجربة ، أى أن الدخن يملك قدرة عالية على التعبير الجينى المقاوم للظروف الإجهادية الناتجة من الملوحة حتى ١٠٠ مليمولر.

وتخلص الدراسة إلى أنه ولابد عند حساب معامل التشابه الجينى GSC (genetic similarity coefficient) من خلال المخطط التفرعى دندروجرام (dendrogram) من الأخذ فى الاعتبار معامل التشابه نتيجة الإجهاد stress factor أو معامل التشابه المؤثر الفعال similarity affecting factor S_{af} لأى نوع من انواع المعاملة حيث أن الإجهاد stress يعتبر مؤثرا هاما على معامل التشابه الجينى حيث أوضحت الدراسة حدوث تغير لمعامل التشابه فى بعض الأصناف النباتية المدروسة تحت تأثير الإجهاد الناتج من التأثير الملحى المحدث لتغير معامل التشابه والذى ظهر من انتقال الدخن من مجموعة لأخرى عند زيادة مستوى الملوحة من ٥٠ إلى ١٠٠ مليمولر بقيم من ٠,٨٦ إلى ٠,٦١ عند زيادة مستوى الملوحة. كما تطرح الدراسة مفهوما جديدا لمعامل التشابه المؤثر (S_{af}) Similarity affecting factor) وهو عبارة عن معامل التشابه الناتج من التأثير الإجهادى المحدث على العينة تحت الدراسة وهى التأثير الملحى فى الدراسة الحالية. وتوصى الدراسة بالتوسع فى زراعة الدخن فى الأراضى ذات الملوحة المرتفعة كما يمكن استخدام الآبار التى زادت نسبة الملوحة بها فى الرى للنبات وذلك لما للدخن من قيمة بروتينية مرتفعة أعلا من الذرة وزيتية تقارب الذرة وقصر فترة زراعته (٦٠-٩٠ يوم) مما يجعله من المحاصيل المزدوجة العلفية التى يمكن توفرها صيفا كما أنه يتحمل الجفاف ، وكذلك يحتوى على الأحماض الأمينية الأساسية (Ejeta *et al.*,1987) لذلك يمكن الاستفادة من حبوبها فى التغذية وتقليص الفجوة الغذائية .