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BIOCHEMICAL GENETIC MARKERS AND MORPHOLOGICAL CHARACTERISATION OF EGYPTIAN COTTON GENOTYPES UNDER SALINITY STRESS

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ABSTRACT

Cotton plants are considered salt tolerant fiber plants, with adaptive limits between 7.5 to 27 mmhos/cm. This experiment was carried out in three successive growing seasons; 2002, 2003 and 2004 in pots (three salinity levels) using seven genotypes (Dendera,Giza 90, Giza 83,Giza 45, and the F_1 , SF_2 sensetive plants and TF2 tolerant plants of the hybrid Giza 83 x Giza 45) of the Egyptian cotton. SDS-PAGE of soluble protein analysis was employed to evaluate the biochemical genetic markers and genetic relationships among the tested genotypes. Giza 83 variety exhibited the highest values of No. of open bolls/plant, proline content and seed cotton yield/plant compared to the other genotypes under 8000ppm salinity level. The results indicated that physiological and yield characteristics were more sensitive to salinity than the technological characteristics.Asimilarity matrix was generated from the SDS-PAGE analysis; its values ranged from 0.250 to 1.000 , and a dendrogram was generated using UPGMA similarity coefficients. One band of molecular weight 37.23kDa can positively differentiate the salt sensitive parent (Giza 45) and the sensitive group of F2 plants. On the other hand, six of the other polymorphic bands with molecular weights (81.6, 40.95, 34.72, 28.91, 26.1 and 17.1 kDa) can negatively differentiate the same sensitive genotypes from all other genotypes tested. Such bands could also be used as indicators to distinguish the salt-sensitive cotton lines in breeding programs.

Key words :***Gossypium barbadense, Salt tolerance, SDS-PAGE, Similarity coefficients, Dendrogram, Biochemical genetic markers.***

INTRODUCTION

In Egypt, cotton (*Gossypium barbadense* L.) is considered one of the most important fibre and oil crops contributing to national income. Egyptian cotton has a good reputation as long and extra long fibre cotton .The whole production of Egypt is classed as Extra fine cotton. Approximately 15 % of Egypt's production is Extra long fibre staple (1 ⅜ inch and above) and the remainder is long fibre staple (1⅛ to less than 1 ⅜ inch).

Due to the semi-arid climatic conditions prevailing in Egypt, and the presence of restricted drainage conditions, a large portion of the soils in the northern part of the Nile Delta has become more or less affected by salinity. The presence of salinity in the soil solution can affect plant growth in two ways i.e., reducing water availability due to osmotic pressure, and causing a characteristic toxic effect on plant metabolism by a specific effect of the constituent ions in the saline media (El-Falaky 1985 and Zein *et al* 2003).

As a result of the limited soil in the Delta and the valley, the opinions suggested cultivating the cotton crop in the new reclaimed lands. Moreover, cotton is considered a salt tolerant fibre plant since its adaptive limits ranges between 7.5 to 27 mmhos/cm (Mass and Hoffman 1977).

The disturbance in cotton metabolism under highly saline conditions that differ greatly among plant genotypes mainly affects yield and its components. So, several studies focused on evaluating and selecting the available genotypes and determining the best growth enhancing treatments of such genotypes under salinity stress conditions.

Now, one of the most important methods of improving new Egyptian cotton varieties is hybridization to induce new genetic variability. For any crop, improvement program, the analysis of genetic diversity is the main step for chosing the proper program. Most of genetic diversity studies on Egyptian cotton varieties have been carried out on the basis of morphological characteristics.

Electrophoretic techniques for protein polymorphism have been used as identificatin method.which provide correlation between the altered expression of specific genes and changes in the environment.These changes in expression of genes would be involved in adaptation and could be used as biochemical genetic markers for salt stress (Abdel-Tawap *et al* 2003 ; Rashed et al 2004 : Abou Deif *et al* 2005 and Afiah *et al* 2007).

Nowadays, protein marker is used extensively to identify and study the genetic characters and relationship of many plants. Many authors recommended the use and applications of SDS-protein as a rapid method to identify and characterize cotton species and cultivars. SDS-PAGE of soluble protein serves as laboratory genotyping or fingerprinting of cotton cultivars and can also be used to determine cultivar uniqueness (Khalil *et al* 1998 and Lu and Myers 2002).

This investigation was carried out to study the effect of salt stress on some Egyptian cotton genotypes through yield charateis, physiological, technological and biochemical parameters (i.e., SDS protein) to detect markers for salt tolerance in cotton that can subsequently be used in further stress tolerance breeding programs.

MATERIALS AND METHODS

Experimental work of this study was carried out during the seasons 2002, 2003 and 2004. The seeds of Egyptian cotton cultivars were obtained from Cotton Research Dept, Cotton Research Institute, Agricultural Research Center, Giza, Egypt (Table 1). In the first season (2002). the hybridization between Giza 45 (as a salt sensitive parent) and Giza 83 (as a salt tolerant parent) according Afiah and Ghoneim (1999) was done at the experimental farm of the Faculty of Agriculture, Cairo University at Giza to

obtain the F_1 seeds. The F_1 plants were selfed to obtain F_2 seeds in 2003 season.

Table 1: Pedigree and classification of cotton varieties under investigation

Name	Pedigree	Classification
Giza 45	(G28x G7) x (G59A x G51B)	Extra-long staple
Giza 83	G67 x G72	Long staple
Giza 90	G83 x Dendera	Long staple
Dendera	G 31 selected from G 3	Long staple

. In both second and third seasons (2003 and 2004), the experiments were carried out in pots at the greenhouse of the Desert Research Center, El-Mataria, Cairo.The five genotypes Giza 45, Giza 83, their F_1 and the two checks (Giza 90 and Dendera) in 2003 season and the seven genotypes Giza 45, Giza 83, their F_1, SF_2, TF_2 and the two checks (Giza 90 and Dendera) in 2004 season were used.

The SF_2 and TF_2 genotypes are two different groups of plants; the SF_2 represent the salt-sensetive F_2 plants and the TF_2 represent the salt-tolerant F_2 plants .Determination of such groups of plants was carried out based on the following procedure:

Before flowering stage (50 days after sowing) one sample of bulked leaves was taken from seven plants of each studied genotype, except F_2. For F_2, 300 plants were measured for their leaf area and analysed for their proline content and accordingly they were divided into ten groups (each group included 30 individual plants); the first group included the highest estimates of these two parameters, while the tenth one included the lowest estimates.Leaf samples of individual plants of these two extreme groups were kept in the proper conditions until harvest. According to data recorded on seed yield /plant, leaves of the highest yielding plants of the 1st group and those of the lowest yielding plants of the 10 th group were only used and bulked to represent each of the two extreme groups i.e salt sensitive (SF_2)and salt tolerant (TF_2) groups to carry out the biochemical analysis.

Three salinity levels [tap water (210-260 ppm), 4000 and 8000 ppm] and sandy soil in the pots (plastic bags) were used. The diameter of each plastic bag (pot) was 45 cm and the height was 75 cm which was filled with pre -washed fine sand to about 7 cm from the bottom .The bags were irrigated every 4 days. Both salinity levels (4000 and 8000 ppm) were obtained by diluting sea water. Each pot contained two plants.

This experiment was conducted in a split plot design in three replicates. The main plots were assigned to salinity levels while, studied genotypes were applied in the sub-plots.

Nitrogen fertilizer at the rate of 60 kg N / fed was splitted after 30, 60 and 90 days from sowing in three equal doses as ammonium sulfate (20.6 % N). The K fertilizer at the rate of 30 kg K/fed (48% K_2O) as potassium sulfate was added before planting. Phosphate fertilizer at the rate of 50 kg P2O5 / fed (P2O5 15 %) was added in two equal doses before planting and before Nitrogen fertilizer (after 30 days from sowing).

At harvest, ten guarded plants were randomly collected from the two parents, their F_1 and check varities as well as ten plants of each of the two extremly F_2 bulked segregant groups from each replicate for studying the trais.

The data from experiment were subjected to the statistical analysis of variance and calculated means were separated by Duncan's (1955) multiple range test at 0.05 level, using MSTATC computer statistical software according to Russel (1991).

Data recorded

1- Leaf area (cm^2): [the mean leaf area of 4 th, 5th and 6th leaf on the main stem of five guarded plants].
2- Proline content (μ mol/g fresh weight) determined according to the method of Bates *et al* (1973).
3- No. of open bolls /plant
4- Boll weight (g)
5- Lint percentage (L %): calculated as the relative amount of lint in a seed cotton sample, expressed in percentage:

$$L\% = \frac{\text{weight of lint cotton in a sample}}{\text{weight of seed cotton in the same sample}} \times 100$$

6- Seed cotton yield /plant (g)
7- Fibre technology measurements

Some of the technological fibre properties of the studied genotypes were examined during the two growing seasons (2003 and 2004). The technological properties were measured using H V I according to ASTM D-4605-86 in the Laboratories of the Cotton Research Institute, Agricultural Research Center, Giza. Such properties were:

a- Fibre length (mm): Staple length (2.5% S.L. in mm) was measured using H .V. I according to ASTMD- 4605-86.
b- Fibre strength: Measured by H V I in gram/tex units.
c- Fineness (Micronair value): Fineness was expressed as micronair instrument reading measured by (H V I).

Fibre technology measurements were determined on composite lint yield samples of 2003 and 2004 seasons.

Biochemical genetic studies

Leaf samples were taken from all tested genotypes including varieties, F_1 plants of hybrid (Giza 83 x Giza 45) and leaf samples of F_2 groups, i.e SF_2 and TF_2.

SDS-protein electrophoresis

SDS-PAGE (SDS-polyacrylamide gel electrophoresis) was carried out according the method of Laemmli (1970). Young leaves were collected from 10 plants of each genotype and then one gram of leaves the 10 plants was treated with liquid nitrogen and ground with 2 ml Lan,s buffer (2X) using mortar and pestle. Samples were transferred to Eppendorf tubes, and kept at O °C over night, then centrifuged for 20 min at 12000 rpm at 4 °C. Supernatants containing water- soluble protein fraction were then kept under – 80 °C until used and subjected for further analysis by SDS electrophoreses.

Scoring and data analysis

Genetic similarity Dice coefficients among genotypes were estimated according to Sokal and Sneath (1973). The banding patterns of bulked sampels were compared among genotypes tested. Bands were scored as present (+) or absent (-). For constructing a dendrogram dealing with genetic relationships among genotypes tested, the data generated from SDS-PAGE was introduced to SPSS pakage program according to binary values (+,-). This matrix was subjected to unweighted pair-group method for arithmetic averages analysis (UPGMA) to generate a dendrogram using average linkage procedure. All-computing were carried out using NTSYS-pc software (Rohlf, 1993).

Results and Discussion

Leaf area

It could easily be noticed from Table (2) that constant saline conditions caused significant decrease in leaf area comparing with control (tap water) for all genotypes under pots experiments. By comparing cotton genotypes tested under salinity levels (tap water, 4000 and 8000 ppm) the data revealed that Dendera variety was the highest for such trait by using both tap water and also by using 4000 ppm level during the two growing seasons followed by F_1 plants > Giza 90 > Giza 83 > Giza 45 in the first growing season (2003) and followed by TF_2 plants group> Giza 90 > Giza 83 > SF_2 plants > Giza 45.

However, Dendera variety gave the lowest value of leaf area during the growing seasons (2003 and 2004) under high salinity level (8000 ppm).

Table 2. Leaf area and proline content in cotton varieties and hybrids during 2003 and 2004 growing seasons under three salinity levels.

Genotype		Leaf area (cm^2)		Proline content (μ mol/g)	
		2003	2004	2003	2004
Giza 45	Tap Water (control)	6.104e	6.333fg	2.225f	2.213h
Giza 83		6.667d	6.500f	2.890d	3.107ef
F_1		7.655c	-	2.560e	-
SF_2		-	6.261g	-	2.505g
TF_2		-	9.038b	-	2.529g
Check varieties:					
Giza 90		8.436b	8.056d	2.900d	3.233e
Dendera		10.569a	10.256a	2.380ef	2.400g
Giza 45	4000ppm	2.927h	2.970l	2.519e	2.513g
Giza 83		3.964g	4.613i	3.962c	3.937d
F_1		4.944f	-	4.001c	-
SF_2		-	4.043j	-	2.560g
TF_2		-	7.197e	-	4.067d
Check varieties:					
Giza 90		4.855f	4.827h	4.300b	4.467c
Dendera		8.210b	8.470c	3.012d	3.040f
Giza 45	8000ppm	1.798i	1.807n	3.120d	3.150ef
Giza 83		3.375h	3.398k	5.597a	5.600a
F_1		3.274h	-	5.490a	-
SF_2		-	2.867l	-	3.990d
TF_2		-	3.423k	-	5.583a
Check varieties:					
Giza 90		2.960h	2.934l	5.630a	5.643a
Dendera		1.761i	2.007m	5.387a	5.300b

Means followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan,s multiple range test.

The data showed that Giza 83 and F_1 plants in the first growing season (2003) and TF_2 plants during the second growing season (2004) had the highest leaf area and seemed to be the best genotypes under the high salinity level (8000 ppm).

Several authors reported that, leaf area was decreased by increasing the concentration of the salts (Hoffman *et al* 1971 and Kamal *et al* 1995) or might be due to stunted growth by salination because of fewer cells, judged by DNA content (Nieman 1965). However, Meiri and Poljakoff-Mayber (1967) reported that the reduction in leaf area was due to a reduction in cell size.

Proline content

The increases of proline concentration in salt-stressed leaves play a protective function for enzymes in the cytoplasm by binding water to the proteins and thus maintained their hydration (Stewart and Lee 1974). Also these results are in agreement with those obtained by Chu *et al* (1976) on barley, Abou-El-Kher (1985) on maize and Ahmed (1988) on granium.

Under the pots experiment in Table (2) the levels of proline in salt stressed plants were higher than the control plants of all tested genotypes.In both growing seasons (2003 and 2004), Giza 90 followed by Giza 83 variety recorded the highest values of proline concentration in cotton leaves after 50 days from sowing.

Regarding to high salinity level (8000 ppm) Giza 45 recorded the lowest proline content followed by SF_2.

Also, under the high salinity level (8000 ppm) the data showed that, Giza 90, Giza 83 and TF_2 plants gave the highest values of proline content (Table 2).

Similarly Bar-Nun and Poljakoff-Mayber (1977) pointed out that, proline which increased in both halophyte (*Temarix tetragynal*) and glycophytes (*Pisum sativium*) plants when grown at various levels of NaCl, was considered as evidence that it may act as a cytoplasmic osmoticum.

Also, our results are in agrement with the findings of Kamel et *al* (1995). They reported an increase in proline concentration of Egyptian cotton plants exposed to salinity stress comparing to control. Furthermore, they found that, after shifting of plants to normal conditions proline decreased markedly than those of control.

Number of open bolls per plant

Mean number of open bolls was nearly the same for F_1 plants, Giza 83, Giza 90 and Dendera in the first growing season (2003) and for among Giza 83, TF_2 plants, Giza 90 and Dendera in the second growing season (2004). While there was a significant difference between the previous genotypes and Giza 45 in both growing seasons (2003 and 2004) and SF_2 plants in the second growing season (2004) under salinity levels (Table 3).

Table 3. Number of open bolls and boll weight in different varieties and hybrids during 2003 and 2004 growing seasons under three salinity levels.

Genotype		No of open bolls /plant		Boll weight (g)	
		2003	2004	2003	2004
Giza 45	Tap Water (Control)	8.667b	8.000bc	2.514c	2.510d
Giza 83		9.333b	8.333b	2.783b	2.811ab
F_1		9.333b	-	2.748b	-
SF_2		-	7.667c	-	2.609c
TF_2		-	8.333b	-	2.771b
Check varieties:					
Giza 90		10.667a	9.500a	2.760b	2.784b
Dendera		10.667a	9.500a	2.949a	2.871a
Giza 45	4000 ppm	4.000d	4.333h	1.993h	1.896j
Giza 83		6.667c	6.400de	2.274d	2.255ef
F_1		6.167c	-	2.213d-f	-
SF_2		-	6.000e	-	2.100h
TF_2		-	6.533d	-	2.220f
Check varieties:					
Giza 90		5.833c	6.167de	2.310d	2.329e
Dendera		5.833c	6.267de	2.246de	2.187fg
Giza 45	8000 ppm	2.933e	3.067j	1.754i	1.780k
Giza 83		4.667d	5.000f	2.102g	2.103h
F_1		4.667d	-	1.980h	-
SF_2		-	4.500gh	-	1.760k
TF_2		-	4.900fg	-	1.993i
Check varieties:					
Giza 90		4.000d	3.633i	2.171e-g	2.127gh
Dendera		4.000d	3.533i	2.117fg	2.114gh

Means followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan,s multiple range test.

Table 3. Cont.

Genotype		Lint percentage (%) (g)		Seed yield /plant	
		2003	2004	2003	2004
Giza 45	Tap Water (Control)	38.267de	38.600d-f	20.024e	21.574c
Giza 83		41.153ab	41.433b	24.463c	24.757b
F_1		41.067ab	-	23.740d	-
SF_2		-	39.233cd	-	20.388d
TF_2		-	42.067ab	-	24.833b
Check varieties:					
Giza 90		40.833ab	41.533b	27.073b	25.947a
Dendera		42.100a	42.267a	28.200a	26.133a
Giza 45	4000 ppm	35.867f-h	37.000hi	8.836h	9.643j
Giza 83		40.533b	38.800de	15.563f	14.620e
F_1		38.633cd	-	14.043g	-
SF_2		-	37.500gh	-	12.487h
TF_2		-	38.800de	-	14.200f
Check varieties:					
Giza 90		37.033ef	39.533c	14.001g	14.468e
Dendera		36.000f-h	36.333ij	13.712g	13.661g
Giza 45	8000 ppm	35.000h	35.900jk	5.341i	6.783n
Giza 83		39.717bc	38.000fg	9.168h	10.123i
F_1		38.267de	-	8.908h	-
SF_2		-	36.800hi	-	7.643m
TF_2		-	38.267ef	-	9.247k
Check varieties:					
Giza 90		36.567fg	37.900fg	8.775h	9.073k
Dendera		35.233gh	35.567k	8.763h	8.103l

Means followed by the same letter(s) are not significantly different at the 5% level, of probability according to Duncan,s multiple range test.

These results are in harmony with those obtained by EL-Garib and Kadry (1983) Jafri and Ahmed (1994) and Badran (2001). They concluded that there was a great variation between some cotton varieties in number of open bolls per plant under salinity stress conditions.

Boll weight

The highest boll weight was obtained by Giza 90 (2.31 and 2.329 g) followed by Giza 83 (2.274 and 2.255 g) under the salinity level (4000 ppm) during the two growing seasons 2003 and 2004 respectively (Table 3).

However, under the salinity level (8000 ppm) the differences among Dendera, Giza 90, Giza 45 were limited followed by F_1 plants in the first season (2003) and TF_2 plants in the second season (2004).

These results are in harmony with those obtained by El-Saidi and Hegazy (1980), Jafry and Ahmed (1994) and Abd El-Aziz *et al* (1998) who reached the similar conclusion that boll weight of cotton plant decreased significantly with increasing salinity level.

Lint percentage

With regard to Table (3) salinity greatly affected the lint percentage for tested cotton genotypes. There were wide differences among cotton genotypes in lint percentage. Giza 83 and F_1 hybrid in the first growing season (2003) and TF_2 plants followed by Giza 90 and Giza 83 in the second growing season (2004) were superior in lint % as compared with the other genotypes as SF_2 plants, Giza 45 and Dendera which were almost equal under high salinity level (8000 ppm). The TF_2 plants, Giza 83 variety and F_1 plants seemed to be the best genotypes for such trait.

These results are in agreement with those of El-Razaz *et al* (1997), Abd El-Rahim (1998), Afiah and Ghoneim (2000) and Badran (2001), who reported that high lint percentage was obtained from long stable varieties while low lint percentage was obtained from extra long stable varieties.

Seed cotton yield/plant

Under the salinity level 4000 ppm the highest mean seed cotton yield was obtained by Giza 83 (tolerant variety) while, Giza 45 variety recorded the lowest mean during the two growing seasons 2003 and 2004 as shown in Table (3).

Also, under the high salinity level (8000 ppm) Giza 83 variety (long staple) scored the high values during 2003 and 2004 seasons followed by the other genotypes except Giza 45 variety (extra long staple) and SF_2 plants group which recorded the lowest values (6.783 g and 7.643 g), respectively in the second season (2004). Similar results were obtained by some workers such as Ghoneim *et al* (1993) and Badran. (2001).

Fibre length

It appeared from the results of fibre length trait (Table 4) that the difference among genotypes was more pronounced than the difference among salinity levels. Also, the results indicated that fibre length of all tested genotypes during the two growing seasons (2003 and 2004) was greatly affected under 8000 ppm than under 4000 ppm salinity level.

On the other hand, Giza 45 variety recorded the highest mean fibre length under all salinity treatments followed by F_1 plants in the first season (2003) and TF_2 plants in the second season (2004) while the differences were less among the other genotypes.

This finding is in agreement with earlier reports of Bouzaidi and Amami (1980) and El-Saidi and Hegazy (1980), who found that fibre length of cotton, was reduced with 3-4 mm under saline condition from 0 to 6000 ppm (NaCl + Ca Cl2) as compared with the control. Also these results are in harmony with those obtained by Radwan *et al* (2002) and Zein (2003).

Micronaire reading

With regard to miconaire value as an indicator to fibre fineness (Table 4), it seemed to be relatively increased by increasing salinity level. At the same time, it was noticed that no significant differences were recorded between Dendera and Giza 90 variety for such trait under different salinity levels where they recorded the highest mean values (lowest fineness). While, Giza 45 variety recorded the lowest micronaire values (3.744) in both growing seasons (highest fineness) followed by F_1 plants which recorded 3.967 in the first season (2003) and TF_2 plants (3.778) in the second season (2004) followed by SF_2 plants (4.011) and Giza 83 variety (4.033 and 4.022 in 2003 and 2004 seasons, respectively).

Our results are in agreement with those of Afiah and Ghoneim (1999) and Badran (2001) who found that micronaire reading increased by increasing salinity level. Also, our results are in agreement with those obtained by Zeina *et al* (2001) who stated that micronaire value tended to increase as the soil salinity level increased.

Fibre strength

Under each salinity level, there was a wide difference among cotton genotypes in fibre strength (Table 4). Giza 45 variety and F_1 were superior in this trait as compared with other genotypes in the first season (2003). Also, in the second season (2004) TF_2 plants group scored the higest fibre strength (35.622 g/tex) followed by Giza 45 (35.311 g/tex). While, Dendera variety followed by Giza 90 were the least ones for this trait under high salinity level (8000 ppm) in both growing seasons.

On the other hand, SF_2 plants followed by Giza 83 variety scored moderate values of fibre strength under both 4000 and 8000 ppm salinity levels (Table 4).

These results are in agreement with those obtained by Abd El-Aziz *et al* (1998) who found that irrigation with saline water, under calcareous sandy soil had a highly significant effect on fibre strength. At the same manner, Badran (2001) stated that, fibre strength was more influenced by each of the two salinity levels (3750 ppm and 6624 ppm artesian irrigation water) than fibre length

Table 4. Mean performance for technological characters during 2003 and 2004 growing seasons under three salinity levels.

Genotype		Fibre length (mm)		Fibre strength (g/tex)		Micronair value	
		2003	2004	2003	2004	2003	2004
Giza 45	Tap Water (Control)	35.533a	34.600a	39.500a	38.333a	3.533h	3.500g
Giza 83		31.300de	31.533e	35.000d	35.067d	3.900f	3.900de
F_1		33.483b	-	38.167b	-	3.600h	-
SF_2		-	31.600e	-	35.667c	-	3.833ef
TF_2		-	33.500b	-	38.333a	-	3.567g
Check varieties:							
Giza 90		31.683d	31.583e	34.500d	35.200cd	3.933ef	3.933de
Dendera		31.333ef	31.433e	33.000e	31.900e	4.000ef	3.933de
Giza 45	4000 ppm	33.400b	32.800c	36.667c	35.733c	3.700g	3.733f
Giza 83		29.933h	29.833j	30.017f	30.933f	4.033e	4.000d
F_1		32.233c	-	36.400c	-	4.133d	-
SF_2		-	31.000f	-	31.733e	-	3.933de
TF_2		-	32.300d	-	36.600b	-	3.767f
Check varieties:							
Giza 90		30.733fg	30.600gh	30.333f	32.033e	4.333c	4.133c
Dendera		30.333h	29.967ij	29.333g	29.467g	4.167d	4.167bc
Giza 45	8000 ppm	31.767cd	30.733fg	33.567e	31.867e	4.000ef	4.000d
Giza 83		28.833i	28.600lm	29.433g	29.133g	4.167d	4.167bc
F_1		30.36gh	-	33.333e	-	4.167d	-
SF_2		-	29.333k	-	29.500g	-	4.267ab
TF_2		-	30.233hi	-	31.933e	-	4.000d
Check varieties:							
Giza 90		29.233i	28.967kl	28.000h	28.567h	4.467b	4.267ab
Dendera		28.733i	28.500m	27.833h	27.400i	4.567a	4.333a

Means followed by the same letter(s) are not significantly different at the 5% level of probability according to Duncan,s multiple range test.

Biochemical genetic markers for salt tolerance

The SDS-PAGE for protein in leaves carried out for seven Egyptian cotton genotypes (two contrasting parents, their F_1, two groups of their F_2, i.e SF_2 and TF_2 and two check varieties) grown under 8000 ppm salinity is illustrated in Fig (1) and Table (5).

Bands with different molecular weights were detected under 8000 ppm salinity level ranging from 12.2 kDa to 81.6 kDa. The total number of bands differs among genotypes from 7 for Giza 45 variety to 14 for the extreme TF_2 plants, which is classified as salt tolerant segregant group as well as Giza 83 variety.

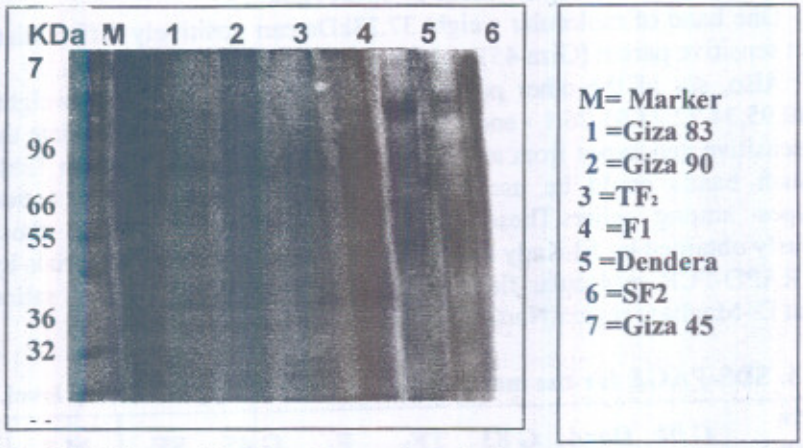

Fig 1. SDS- protein banding pattern in leaves of seven Egyptian cotton genotypes under salinity stress conditions (8000ppm).

Table 5. The presence (+) and absence (-) of leaves protein profile of seven Egyptian cotton genotypes under 8000ppm salinity level.

Band No.	G 83	G 90	TF_2	F_1	Dend	SF_2	G 45	Mw
1	+	+	+	+	+	-	-	86.6
2	+	-	+	-	-	+	-	81.6
3	+	+	+	+	+	+	+	76.6
4	+	-	+	-	+	+	-	70.2
5	+	+	+	+	+	+	+	56.26
6	+	+	+	+	+	+	+	49.2
7	+	+	+	+	+	-	-	40.95
8	-	-	-	-	-	+	+	37.23
9	+	+	+	+	+	-	-	34.72
10	+	+	+	+	+	+	+	30.6
11	+	+	+	+	+	-	-	28.91
12	+	+	+	+	+	-	-	26.1
13	+	+	+	+	+	+	+	20.64
14	+	+	+	+	+	-	-	17.1
15	+	+	+	+	+	+	+	12.2
Total	14	12	14	12	13	9	7	

The results showed that six monomorphic bands with molecular weights ranging from 12.2 kDa to 86.6 kDa and the remainders were polymorphic bands with a percentage of 60% (Table 7).

One band of molecular weight 37.23kDa can positively differentiate the salt sensitive parent (Giza 45) and the sensitive group of F_2 plants.

Also, six of the other polymorphic bands with molecular weights (81.640.95,34.72,28.91,26.1 ‹ and 17.1 kDa) can negatively differentiate the same sensitive genotypes from all other tested genotypes as shown in Table (6). Such bands could be used to distinguish the salt tolerant cotton genotypes among others.These findings are in harmony with those previously obtained by El-Kady *et al* 2006 for the same genetic materials by using RAPD-PCR molecular fingerprint under irrigatin by artesian saline water at El-Maghara region,North Sinai Egypt.

Table 6. SDS-PAGE for the marker type under 8000 ppm salinity level.

Mw (kDa)	G 90	Dend.	G 83	TF_2	F_1	G 45	SF_2	M.T
81.6	+	+	+	+	+	-	-	Neg.
40.95	+	+	+	+	+	-	-	Neg.
37.23	-	-	-	-	-	+	+	Pos.
34.72	+	+	+	+	+	-	-	Neg.
28.91	+	+	+	+	+	-	-	Neg.
26.1	+	+	+	+	+	-	-	Neg.
17.1	+	+	+	+	+	-	-	Neg

Table 7. Number, types and percentage of polymorphism

Monomorphic bands	Polymorphic bands	Total	Polymorphic %
6	9	15	60

In the same concern, similarity matrix was developed by SPSS program based on the obtained data in Table (5).

Under 8000 ppm salinity level the highest similarity value (1.000) was scored between Giza 83 and the tolerant group of F_2 plants (TF_2) and also between Giza 90 and F_1 plants. While the lowest similarity value (0.250) was scored between Giza 83 and Giza 45 and also between Giza 90 and the sensitive group of F_2 plants (Table 8).

Table 8. Similarity matrix among the seven cotton genotypes based on SDS- Protein analysis under salinity level 8000 ppm.

Similarity	Giza 83	Giza 90	TF_2	F_1	Dendera	SF_2
Giza 83	-					
Giza 90	0.750	-				
TF_2	1.000	0.750	-			
F_1	0.750	1.000	0.750	-		
Dendera	0.867	0.857	0.867	0.857	-	
SF_2	0.364	0.250	0.364	0.250	0.304	-
Giza 45	0.250	0.300	0.250	0.300	0.273	0.636

The dendrogram of biochemical analysis (water soluble proteins) Fig (2) has categorized the seven studied cotton genotypes into two groups; the first contains five genotypes (Giza 90, Dendera, Giza 83, F_1 plants and the tolerant group of F_2 plants) and this cluster was further separated into two sub-clusters, within the first sub-clusters comes Giza 83 and Giza 90, while the second sub-clusters contains two groups, the first contain the tolerant group of F_2 plants and F_1 together and the second contains Dendera variety. On the other hand, the second group contained two genotypes, i.e. the sensitive group of F_2 plants (SF_2) and Giza 45.

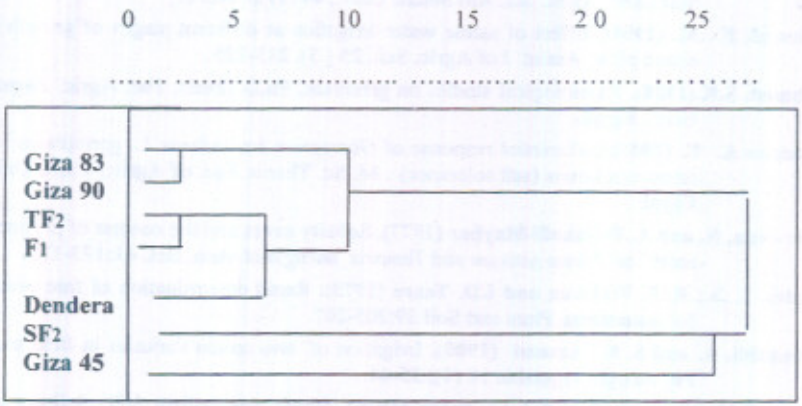

Fig.2. Dendrogram of seven Egyptian cotton genotypes based on the data derived from SDS - protein under salinity stress conditions (8000 ppm).

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الكاشفات الوراثية البيوكيميائية والتوصيف المورفولوجى لتراكيب وراثية من القطن المصري تحت الظروف الملحية المعاكسة

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يهدف هذا البحث الى محاولة الربط بين الكاشفات الجزيئية وصفة تحمل الملوحة فى بعض التراكيب الوراثية للقطن المصرى. وقد استخدم فى هذه الدراسة كل من الصنف جيزة 83 (متحمل للملوحة) والصنف جيزة 45 (حساس للملوحة)وكذلك الأصل الوراثى القديم دندرة والصنف المعتمد حديثا جيزة (90) كاصناف للمقارنة. وفى موسم 2003 تم زراعة الأصناف المختارة وكذلك نباتات الجيل الأول الهجينة (جيزة 83 x جيزة 45) فى صوبة قصارى فى مركز بحوث الصحراء وذلك تحت مستويات ملوحة مختلفة (الماء العادى، 4000 ، 8000جزء فى المليون) . وقد تم أخذ بعض القياسات الفسيولوجية مثل مساحة الورقة وكذلك محتوى الأوراق من البرولين بعد 50 يوما من الزراعة. كما تم أخذ بعض القياسات المحصولية مثل عدد اللوز المتفتح على النبات، وزن اللوزة ونسبة تصافى الحليج ومحصول النبات من القطن الزهر وكذلك تم عمل بعض القياسات التكنولوجية مثل طول التيلة والمتانة والنعومة .

وفى موسم 2004 تم زراعة الأباء المختارة وبذور الجيل الاول و الجيل الثاني الهجين الذى تم تقسيمه الى مجموعات عديدة حسب المحصول والصفات المساهمة فيه كمعيار لدرجة تحمل الملوحة واختيار مجموعة النباتات الفردية المتحملة ومجموعة النباتات الفردية الحساسة للملوحة لاستخلاص البروتين وتفريده كهربيا بطريقة SDS-PAGE .

* بالنسبة للصفات الفسيولوجية و المحصولية أشارت النتائج إلي وجود تأثير للعامل البيني بشكل واضح على أغلب الصفات المدروسة كما أظهرت النتائج أن مجموعة النباتات الفردية الشديدة التحمل للملوحة فى الجيل الثانى سجلت أعلى القيم بينما سجلت مجموعة النباتات الفردية الشديدة الحساسية للملوحة والصنف جيزة 45 أقل القيم بينما بالنسبة للصفات التكنولوجية فقد كان تأثرها بالعامل البيني محدود مقارنة بتأثر بالصفات

الفسيولوجية و المحصولية للتراكيب الوراثية المستخدمة.

- أظهرت نتائج التفريد الكهربى للبروتين وجود كاشف جزيئى موجب (37.23 كيلو دالتون) للصنف الأبوي جيزة 45 ومجموعة نباتات الجيل الثاني الشديدة الحساسية للرى بالماء المالح .
- كما يمكن تمييز هذه النباتات الحساسة للملوحة بستة كاشفات سالبة بأوزان جزيئية 181.6. 40.95 ، 34.72 ، 28.91 ، 26.1 ، 17.1 كيلو دالتون.
- ويربط النتائج السابقة يمكن للمربى اختصار الوقت والتكاليف المستخدمة فى عمليات التقييم والانتخاب لتحمل تلك الظروف البيئية المعاكسة باستخدام الكاشفات الجزيئية. .