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ASSESSMENT OF GENETIC DIVERSITY AMONG ELEVEN SWEET SORGHUM CULTIVARS (*Sorghum bicolor* L.) UNDER SALT STRESS

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ABSTRACT

Sweet sorghum were assessed in seasons 2006 and 2007 for salt tolerance on basis of the traits; plant height, shoot fresh weight, shoot dry weight, root dry weight, root length, total chlorophyll and shoot/root ratio. The sweet sorghum cultivars were Atlas, Umbrella, Planter, MN 3556, Leoti, Smith, Honey, MN 4418, Sugar Drip, MN 4490 and Rex. Also molecular genetic fingerprints for these cultivars using SDS-PAGE protein markers was determined. Rex, MN 4490 and Umbrella were the highest cultivars in the studied traits under salt stress (the most salt tolerant cultivars) as they had the highest rank for all the traits while, Atlas, Leoti and MN 4418 showed the lowest rank in this respect. The banding patterns of the four protein assessments, i.e., total protein, water-soluble protein, water-nonsoluble protein and kafirin revealed wide variations of different bands (22, monomorphic and 65 polymorphic bands). The relationships among sorghum cultivars based on the overall polymorphisms were in partial agreement with the known lineage of these cultivars.

Key words: ***Sweet sorghum, Salt stress, Fingerprints, Genetic similarity.***

INTRODUCTION

Salinity can affect many processes in the plant's life cycle, so that tolerance will involve a complex interplay of characters. The physiology and biochemistry of salt tolerance were investigated by many researchers to screen overall plant performance that could be used in breeding programs. Plants are generally relatively tolerant during germination but become more sensitive following emergence and early seedling stages of growth. Hence, it is imperative to keep salinity in the seedbed low at these times (Azhar and McNeilly, 1989; Abdel-Tawab *et al* 1998).

Sweet sorghum is considered as one of the commercial sources of syrup and for sugar production besides sugarcane and sugar beet. The productivity of this crop could be improved, as it possesses a wide range of genetic diversity. The use of modem molecular techniques such as markers assisted selection is required to enhance sorghum breeding programs. Analyses of the extent and distribution of genetic variation in a crop are essential in understanding the relationships between accessions and to sample genetic resources in a more systematic fashion for breeding and conservation purposes.

Electrophoretic techniques have been used to identify and characterize different crop cultivars and to assess the uniformity, purity and agronomic traits (Teng *et al* 1988). Since its introduction by Beitz and Wall (1973), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used to separate proteins in the recent years (Kasarada *et al* 1998). They reported that electrophoretic patterns of the protein fractions are directly related to the genetic background of the protein and can be used to certify the genetic makeup of wild, cultivated, or newly derived plants. Protein markers, including seed storage proteins, structural proteins, and isozymes are among the first group of molecular markers. They are the basis for a newly emerging research area called proteomics. They also provide some of the most cost-effective tools for data point generation, especially, when iso-electric focusing equipment is used to precisely distinguish between very similar versions of proteins.

The major limitations of these markers are that most of the genome does not code for genes - different biochemical procedures are required to visualize allelic differences for enzymes having and different functions. Besides, many proteins are post-transcriptionally regulated, which might underlay DNA sequences polymorphism and thus can mask variation at that level (Hash and Bramel - Cox, 2000).

This study aimed to: (1) determination of molecular genetic fingerprints for 11 sorghum cultivars using SDS-PAGE for proteins markers related to salt tolerance and (2) elucidation of the genetic relationships among these cultivars.

MATERIALS AND METHODS

Plant materials

Eleven sweet sorghum cultivars were assessed in the growing seasons 2006, 2007 for salt tolerance on the basis of the traits; plant height, shoot fresh weight, shoot dry weight, root dry weight, root length, total chlorophyll and shoot/root ratio. The cultivars Atlas, Umbrella, Planter, MN 3556, Leoti, Smith, Honey, MN 4418, Sugar Drip, MN 4490 and Rex were kindly provided by Sugar Crops Research Institute (SCRI), Agricultural Research Center (ARC), Giza, Egypt. Their origin is shown in Table (1).

Table1. Code number, name, source and origin of the eleven sorghum cultivars under investigation.

Code number	Cultivars	Source	Origin
1	Atlas	USA	West and South Africa
2	Umbrella	Egypt	Mississippi
3	Planter	Egypt	East and South Africa
4	MN 3556	USA	Mississippi
5	Leoti	Egypt	Mississippi
6	Smith	Egypt	Mississippi
7	Honey	USA	Mississippi
8	MN 4418	USA	Mississippi
9	Sugar Drip	USA	Mississippi
10	MN 4490	USA	West and South Africa
11	Rex	USA	Mississippi

Methods

1. Morphological traits

The eleven sorghum cultivars were grown in a sand culture in plastic pots, which were filled with pre-washed fine sand. Three grains were sown in each pot. The grains were sown in a complete randomized experiment with three replications. Hoagland solution was used twice a week for two weeks after germination as a base nutrient medium. Then plants were subjected to salt stress at four concentrations (0, 4000, 6000 and 8000 ppm NaCl) and the experiment was terminated after 60 days. The collected data were statistically analyzed according to Steel and Torrie (1980). The differences among means were compared using Duncan's new multiple range test (Duncan 1955).

2. Proteins electrophoresis

Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the genetic background for the studied cultivars involved the more highly tolerant and sensitive cultivars for salt stress by their protein fingerprints such as water-soluble and water-nonsoluble proteins, total protein and kafirin (alcohol-soluble protein). Protein fractionations were performed exclusively on vertical slab (19.8 cm x 26.8 cm x 0.2 cm) gel using the electrophoresis apparatus manufactured by BIO-RAD according to the method of Laemmli (1970) as modified by Studier (1973).

3. Densitometric scanning

All gels were scanned using the Gel Doc 2000 Bio-Rad system and analyzed with software package supplied by the manufacturer. SPSS computer program was used to calculate the pair wise differences matrix and plot the dendrogram among sorghum cultivars.

RESULTS AND DISCUSSION

Morphological traits

Table (2) show the means of means for the seven studied traits under three salt concentrations. The results indicated that the effects of salt stress varied across the studied traits in all cultivars, the total root length, root dry weight, shoot fresh weight, shoot dry weight, shoot\root ratio, total chlorophyll and plant height were decreased by salt stress. The highest average shoot fresh weight was recorded for cultivars Rex (4.92 g), Umbrella (3.16 g) and MN 4490 (2.53 g), while the lowest was obtained from Atlas (1.76 g), MN4418 (1.86 g) and Leoti (1.89 g). For shoot dry weight the means ranged from (3.02 g) in cultivar Rex, to (0.97 g) in Atlas. The results also indicated that the highest plant height was scored for cultivars Rex (43.045 cm), MN 4490 (40.365 cm) and Umbrella (39.105 cm), while the lowest was scored for cultivars Atlas (31.265 cm), Leoti (31.905 cm) and MN 4418 (32.045 cm). The results of root dry weight indicated that, the highest mean was scored for cultivar Rex (1.615 g), while the lowest was scored for cultivar Atlas (0.395 g). On the other hand, the results of root length indicated that the highest mean was scored in cultivar Rex (275.951 cm), while the lowest was scored in cultivar Atlas (110.151 cm). In addition, mean total chlorophyll content was highest in cultivar Rex (30.39 %), while the lowest content was scored for cultivar MN 3556 (18.75 %). The results of shoot / root ratio indicated that, the highest ratio was exhibited by cultivar Rex (2.35), while the lowest was scored for cultivar MN 4490 (0.58). As shown in Table (3), it could be concluded that Rex, MN 4490 and Umbrella were the highest cultivars in the studied traits under salt stress, in a descending order the most salt tolerant cultivars as they showed the highest rank overall the studied traits while, Atlas, Leoti and MN 4418 were the lowest in this respect (the most salt sensitive cultivars) as they exhibited the lowest rank overall the seven investigated traits. The results were in agreement with the findings of Hassanein *et al* (1993), who reported that increasing salinity levels decreased shoot dry weight. This trait can also be taken as an indicator for the effect of salt stress on plant growth. Similar findings were reported by Nasir *et al* (2000) who concluded that the decrease in shoot fresh weight under salinity conditions can be considered as indicator for the effects of salt stress on plant growth.

Table 2. Means of the investigated traits in the eleven sorghum cultivars

Cultivar	Shoot fresh wt. (g)	Shoot dry wt. (g)	Plant height (cm)	Root dry wt. (g)	Total root length (cm)	Total chlorophyll %	Shoot /root ratio
Atlas	1.76^{I}	0.97^{G}	31.265^{K}	0.395^{H}	110.151^{K}	20.87^{H}	2.17^{AB}
Umbrella	3.16^{B}	1.85^{B}	39.105^{C}	0.895^{D}	111.771^{J}	28.2^{C}	1.5^{C}
Planter	2.3^{E}	1.32^{D}	37.925^{E}	0.465^{G}	124.381^{J}	25.22^{D}	2.11^{B}
MN 3556	2.04^{FG}	1.23^{EF}	35.125^{G}	0.725^{E}	205.451^{H}	18.75^{K}	1.41^{C}
Leoti	1.89^{H}	1.06^{EFG}	31.905^{B}	0.395^{H}	225.051^{G}	19.93^{J}	1.48^{C}
Smith	2.08^{F}	1.18^{E}	38.395^{D}	0.465^{G}	259.371^{D}	21.94^{G}	1.29^{C}
Honey	2.39^{D}	1.38^{CD}	35.295^{F}	0.985^{C}	258.151^{E}	23.48^{F}	0.59^{E}
MN 4418	1.86^{H}	1.03^{EFG}	32.045^{I}	0.685^{F}	239.631^{F}	30.25^{I}	0.6^{E}
Sugar Drip	1.98^{G}	0.99^{FG}	34.055^{H}	0.875^{D}	261.001^{B}	24.37^{E}	0.91^{D}
Rex	4.92^{A}	3.02^{A}	43.045^{A}	1.615^{A}	275.951^{A}	30.39^{A}	2.35^{A}
MN 4490	2.53^{C}	1.47^{C}	40.365^{B}	1.345^{B}	260.431^{C}	28.92^{B}	0.58^{E}

Table 3. Ranking of different sorghum cultivars means for studied attributes across salt concentration treatments.

Cultivar	Shoot fresh wt. (g)	Shoot dry wt. (g)	Plant height (cm)	Root dry wt. (g)	Total root length (cm)	Total chlorophyll %	Shoot /root ratio	Total
Atlas	11	1	11	10	11	9	11	74
Umbrella	2	2	3	4	10	4	4	29
Planter	5	5	5	8	9	5	3	40
MN 3556	7	7	7	6	8	11	6	52
Leoti	9	8	10	11	7	10	5	60
Smith	6	6	4	9	3	8	7	43
Honey	4	4	6	3	5	7	10	39
MN 4418	10	9	9	7	6	2	9	52
Sugar Drip	8	10	8	5	4	6	8	49
Rex	1	1	1	1	1	1	1	7
MN 4490	3	3	2	2	2	3	2	17

Rank 1= highest and rank 11= lowest

These findings also were in agreement with Maiti *et al* (1996), who reported that increasing salinity levels decreased total chlorophyll contents. Moreover, the results were in agreement with the findings of Abdel Bary *et al* (2005) and Rashed *et al* (2006), who reported that increasing salinity levels resulted in decreasing shoot fresh weight, shoot dry weight, plant height, root dry weight, root length and shoot/root ratio. They concluded that these traits could also be taken as indicators for the effect of salt stress on plant growth.

Molecular genetic fingerprints of sorghum cultivars by protein analyses

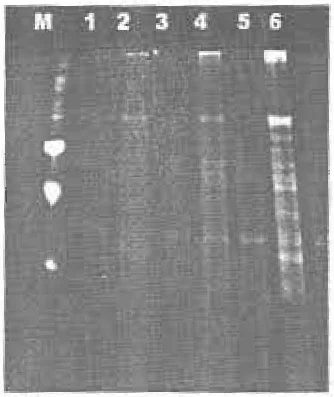
The banding patterns of the four protein fractions, i.e., total protein, water-soluble protein, water-nonsoluble protein and kafirin revealed wide variations of different bands (22, monomorphic and 65 polymorphic bands) as shown in Tables (4 and 5) and Figure (1). Total proteins showed higher polymorphic protein bands (87%), while water-soluble, water-nonsoluble and kafirin protein showed less polymorphic protein bands (71%, 70% and 69% respectively). The overall protein systems recorded 75% polymorphism, which was enough to discriminate among the 6 sorghum cultivars. These results were in agreement with those of Shadi *et al* (1999), who discriminated among 20 maize inbred lines based on SDS-PAGE of albumin and zein fractions and Rashed *et al* (2004) who discriminated among 17 sorghum cultivars based on SDS-PAGE of total protein, water-soluble protein, water-nonsoluble protein and kafirin. On the other hand, Aly *et al* (2000) used SDS-PAGE of water-soluble and water-nonsoluble proteins to differentiate among nine Egyptian rice cultivars and concluded that such differences were not enough to differentiate among the studied rice cultivars.

Table 4 . List of proteins bands in sorghum cultivars

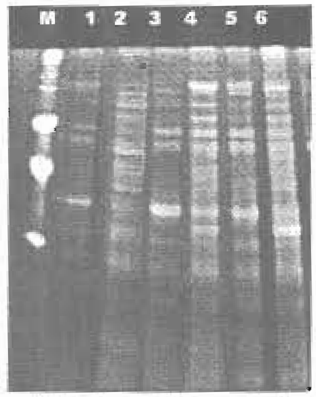
Marker number	Marker name	Range of MW	Number of bands/ cultivars					
			Atlas	Leoti	Rex	MN 4490	MN 4418	Umbrella
1	Total protein	148.22-39.36	5	7	8	11	7	6
2	Water-soluble protein	119.35-42.93	4	4	3	8	6	8
3	Water-nonsoluble protein	115.16-30.93	4	7	4	10	3	9
4	Kafirin	122.31-49.8	5	4	4	2	4	4

Table 5. Polymorphism detected for protein marker in the sorghum cultivars

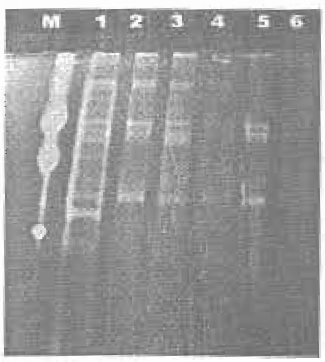
Marker number	Marker name	Mono-morphic	Poly-morphic	Total	% poly-morphism
1	Total protein	3	20	23	87
2	Water-soluble protein	7	17	24	71
3	Water-nonsoluble protein	8	19	27	70
4	Kafirin	4	9	13	29
	Total	22	65	87	75



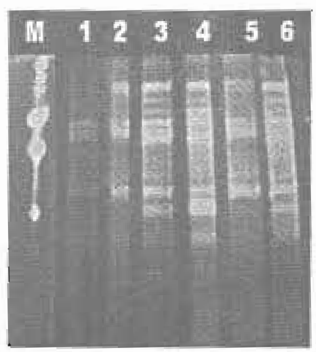
Water-nonsoluble protein



Water-soluble protein



Kifrin protein



Total protein

Figure 1. Protein banding patterns of the eleven sorghum cultivars 1-Atlas 2- Leoti 3- Rex 4- MN 4490 5- MN 4418 6- Umbrella

Genetic similarity analysis

The dendrogram tree among sorghum cultivars on the proteins level was detected by SPPS computer package. The analysis was based on the number of markers that were similar between any given pair of cultivars. Based on the protein polymorphisms, dendrogram tree was described in Figure (2).

Similarity matrix and dendrogram tree based on the polymorphisms of the overall markers were detected in Figure (2). The least average similarity was scored between cultivars MN 4490 and Atlas, while the highest average similarity was scored between cultivars Leoti and Rex.

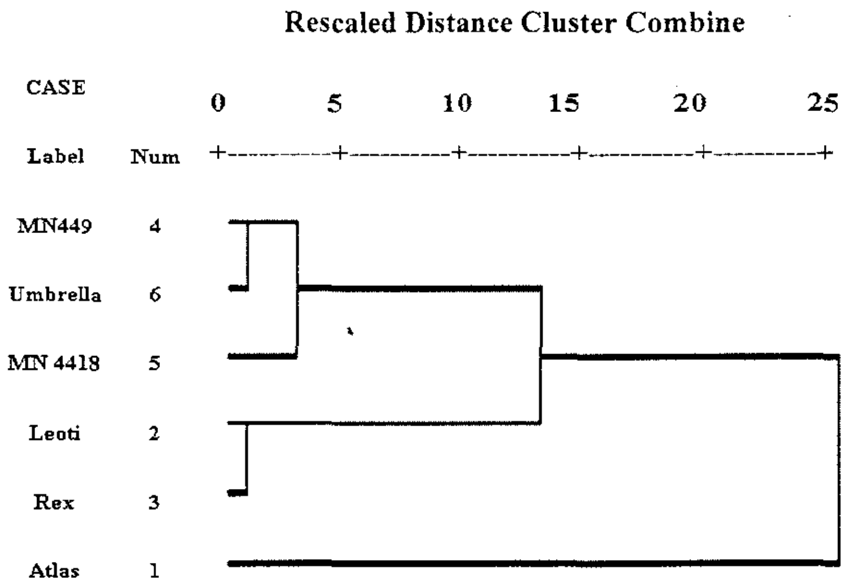


Fig 2. Dendrogram using Average Linkage (Between Groups)

The relationships among sorghum cultivars based on the overall polymorphisms were in partial agreement with the known lineage of these cultivars. Rashed *et al* (2004) detected a total of 85 polymorphic bands for protein markers, which gave adequate distinctions 17 among studied sorghum cultivars. However, the disparity among the protein analyses may be related to the amount of genome coverage characteristic of a particular marker system in the sorghum species and its efficiency in sampling variation in a population.

The previous studies and our observations are evident that protein analyses, which revealed high degree of polymorphism, are good tools in determining sorghum cultivars under study. They are generally much simpler to apply and more sensitive than traditional morphological and biochemical analyses because they are more polymorphic.

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تقديرالتباين الوراثى لأحدى عشر صنفاً من الذرة الرفيعة السكرية تحت ظروف الإجهاد الملحى

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أجريت تجربتان خلال موسمى ٢٠٠٦و٢٠٠٧ تم فيها دراسة التباين الوراثى بين إحدى عشر صنفاً من أصناف الذرة الرفيعة السكرية تحت ظروف الإجهاد الملحى. وهذه الأصناف هى Atlas, Umbrella, Planter, MN ٣٥٥٦, MN ٤٤١٨, MN ٤٤٩٠, Leoti, Simth, Honey, Sugar Drip and Rex وهذه الأصناف تم الحصول عليها من معهد بحوث المحاصيل السكرية – مركز البحوث الزراعية. حيث زرعت الأصناف تحت الدراسة فى أصص وتم معاملتها بمحلول هوجلاند كمحلول مغذى رئيسى والمعاملة بالتركيزات الملحية تحت الدراسة. تم تقييم الأصناف من خلال قياس سبعة صفات هى طول النبات ووزن المجموع الخضرى الغض والوزن الجاف للمجموع الخضرى والوزن الجاف للمجموع الجذرى والطول الكلى للمجموع الجذرى والمحتوى الكلى للكلوروفيل بالأوراق ونسبة المجموع الخضرى إلى المجموع الجذرى. وبناءاً على قياس هذه الصفات تم تحديد الأصناف المتحملة للإجهاد الملحى والأصناف الحساسة له.

وفى المعمل أجرى على الأصناف المتحملة والأصناف الحساسة للإجهاد الملحى أختبار تفريد البروتين من خلال استخلاص البروتينات الكلية والبروتينات التى تذوب فى الماء و والبروتينات التى لا تذوب فى الماء و والبروتينات التى تذوب فى الكحول وكانت أهم النتائج كالتالى:

١- أظهرت تجربة الأصص وجود أكثر من صنف متحمل للإجهاد الملحى حيث كان الصنف Rex أكثر الأصناف تحولاً للإجهاد الملحى يليه الصنف MN ٤٤٩٠ ثم الصنف Umbrella حيث أظهرت هذه الأصناف أعلى قيم متوسطات فى الصفات المدروسة, بينما أظهرت الأصناف Atlas, Leoti, MN ٤٤١٨ أقل القيم فى الصفات السابق ذكرها مما يجعلها من أكثر الأصناف حساسية للإجهاد الملحى.

٢- أظهرت نتائج تفريد البروتين وجود إختلافات واسعة بين حزم البروتينات الكلية والبروتينات التى تذوب فى الماء و والبروتينات التى لا تذوب فى الماء و والبروتينات التى تذوب فى الكحول المستخلصة من بذور الأصناف المتحملة و الحساسة للإجهاد الملحى, حيث أعطت ٨٧ حزمة منها ٦٥ حزمة متجمعة و٢٢ حزمة أحادية وعند دراسة صلات القرابة بين هذه الأصناف بناءاً على النتائج السابقة نجد أنها تتفق بدرجة كبيرة مع ما هو معروف عن تطور هذه الصناف والعلاقة بينها مما يؤكد أهمية هذه الحزم فى التعرف على الأصناف والتفرقة بينها. لذا يوصى البحث باستخدام هذه التقنية الجزيئية بجانب الطرق التقليدية للتفرقة بين الصناف وبعضها فى درجة تحملها للإجهاد الملحى.