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MARKER- ASSISTED SELECTION FOR ABIOTIC STRESS TOLERANCE IN SUGARCANE (*Saccharum spp.*).

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Abstract

This investigation was carried out to develop a model for molecular marker-assisted selection (MAS) to identify the promising cultivars that posses tolerance to environmental stresses (i.e. drought and salt) at a very early stage of the breeding program. Four cultivars of sugarcane were used in this study, i.e., G86/20, G95/21, G54/9 and F160. The performance of the four cultivars in sand culture experiment revealed that G.86/20 and G95/2 were superior in their salt and drought tolerance according to their performance in some yield- related traits. Cultivar G54/9 was the third, followed by F160, which was very sensitive to the two stresses. Some molecular genetic markers (SDS protein, esterase and acid phosphatase, isozymes,) were developed for the four cultivars in relation to the two stresses.

INTRODUCTION

Sugarcane (*Saccharum spp.*) is the most important sugar crop in the world. In Egypt, sugarcane has been grown since 1850. It is mainly cultivated in Upper Egypt in Qena, Aswan, Asyout, Sohag and Naga Hamad. Modern sugarcane cultivars are complex polyploids, which may contain over 100 chromosomes and a little is known about their genome structure.

Biotic (insect and plant diseases) and abiotic stresses (drought, salinity and sow and high temperatures) are considered limiting factors for plant productivity (Boyer, 1982). A rating system for evaluating the extent of drought tolerance in sugarcane varieties was developed. Drought tolerance was classified into five grades according to the ability of sugarcane roots to survive water deficit by Sheu *et al.* (1997), Abdel-Tawab *et al.* (1999) recorded data on some vegetative traits on seedlings of four cultivars of sugarcane subjected to drought, salinity and combined effects. The analysis of

variance indicated significant differences between the four cultivars in respect to their relative tolerance to such stresses.

Molecular markers such as proteins and isozymes have recently shown excellent potentiality to assist selection of quantitative trait loci (QTLs) associated with these markers (Stuber, 1992 and Ramagopal and Carr (1991) investigated changes in gene expression induced by salinity in a suspension culture of sugarcane. The data suggested that a multitude of mechanisms at the transcriptional, post-transcription; and post-translational levels may contribute to the control of gene expression in the salt-adapted sugarcane cells. Zhang *et al.* (1996) found that water stress increased the production of oxygen radicals in sugarcane leaves and decreased scavenging activity for active oxygen. Water stress increased malondialdehyde (MDA) content, menbrane permeability and free radical production and decreased superoxide dismutase and catalase activities and GSH [glutathione] content as a molecular marker linked with salt tolerance. Abdel-Tawab *et al.* (1999) obtained isozyme and RAPD markers associated with salt and drought tolerance in some cultivars of sugarcane.

The present study aim is to evaluate three promising varieties for environmental stress tolerance (drought and salinity) in comparison with G54/9 as a standard commercial variety and to detect molecular markers associated with drought and (or) salinity tolerance using SDS-protein electrophoresis and isozyme patterns.

MATERIAL AND METHODS

This study was carried out in the greenhouse and the laboratories of the Department of Genetics, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt, and the laboratories of Sugar Crops Research Institute ARC. Sugarcane cultivars used in this study are presented in Table (1)

Table (1): The four cultivars of sugarcane tested in this study and their parentages.

Cultivar*	Parents
1-G86/20	Co 421 x Co 453
2-G95/21	Sp7040 x Sp77-302
3-G54/9	NCo310 x F37-925
4-F160	NCo310 x F141

1. Sand culture experiment

The same four cultivars were sown in a sand culture experiment, which was conducted according to Heakel *et al.* (1981). Modified-Hoagland solution suggested by

Johnson *et al.* (1957) was used as the base nutrient solution. The cultivars were sown in a completely randomized experiment. Drought and salinity treatments were initiated on day 21 after planting. Control was irrigated with the base nutrient solution every three days, while salinity treatments were irrigated with the base nutrient solution plus 6000 ppm NaCl every three days. Drought stress treatments were irrigated with the base nutrient solution every two weeks. Samples were taken for genetic, biochemical and molecular analyses at the end of the experiment.

Data were recorded for all plants after 72 days for the following traits: plant height (cm), number of leaves/plant, stem diameter (cm), plant fresh weight (g), plant dry weight (g), and leaf area (cm^2).

2. Statistical analyses:

All data were statistically analyzed using analysis of variance procedure proposed by Snedecor and Cochran (1969). The differences between means were compared using Duncan's multiple range test (Duncan, 1955).

3. Marker-assisted selection

3.1.SDS-protein electrophoresis

SDS-polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Laemmli (1970) as modified by Studier (1973) on each sample of the four sugarcane cultivars

3.2. Isozyme electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed on the same leaf samples using the method of Stegmann *et al.* (1985). Visualization of esterase and acid phosphatase bands was done according to Scandalios, (1964) and Shaw and Prasad (1970), respectively.

RESULTS AND DISCUSSION

The four sugarcane cultivars used in this study are: G54/9 a commercial cultivar and the three promising cultivars; G86/20, G95/21, and F160. Sand culture experiments were carried out on these four cultivars to study the effect of salinity (6000 ppm) or drought treatments comparing with the control to choose the tolerant cultivars on the basis of their performances for some yield-related traits.

*Thanks are due to Sugar Crops Research Institute, ARC for providing these cultivars.

1. Stress Experiments

1.1. Sand Culture Experiment

At 72 days from sowing in a sand culture experiment, data were recorded on the four cultivars for the following traits; plant height, number of leaves, leaf area stem diameter, plant fresh weight (g), and plant dry weight (g) under salinity, drought and control as shown in (Table 2)

Analysis of variance for these traits indicated significant differences between control concerning salinity or drought and among the four cultivars in their relative tolerance to such stresses. The interaction between cultivars and treatments was found to be significant for all the studied traits except leaf area.

As for plant height, means of the control, salt and drought treatments for the four cultivars showed significant differences among all of them. G86/20 exhibited the highest performance, followed by G95/21, G54/9 and finally F160. At the same time, each cultivar exhibited different responses under control, salt and drought treatments. The two cultivars; G54/9 and F160 showed no significant differences between the control and salinity treatment and between the control and drought treatment (except G95/21). However, cultivar G86/20 gave significant differences when the control treatment was compared with salinity or drought treatments (Table 2). However, cultivar overall means exhibited significant differences between the four cultivars where G86/20 showed the highest performance, followed by G95/21, G54/9 and finally by F160.

Regarding the number of leaves/plant, all cultivars did not exhibit significant differences between control and either of the two treatments (salt and drought) except G86/20 where the control recorded marked difference from both salinity and drought treatments, and G95/21 where the control differed only from drought treatment. The cultivar means confirmred the presence of a marked significant difference for F160 from all the other three cultivars.

As for stem diameter significant differences were observed between the control and the two treatments for the two cultivars; G86/20 and G95/21. While no significant differences were recorded between the control and each of the two treatments for the other two cultivars; G54/9 and F160. The cultivars overall means confirmed that the two cultivars G86/20 and G95/21 exhibited slight differences with lower performance than G54/9 and F160. As for plant fresh weigh (Table 2), significant differences be-

tween the control and the two stress treatment were observes for the three cultivars G86/20, G95/21 and G54/9. However, cultivar F160 recorded no significant differences between the control and the two treatments (salt and drought). The cultivars overall means indicated that G86/20 and G95/21 exhibited the highest performances with significant differences from G54/9 and F160.

Dry weight showed significant differences between the control and the two treatments for the two cultivars G86/20 and G95/21. While, no significant differences between control and the two treatments and between control and salt treatment were recorded for G54/9 and F160 cultivars, respectively. At the same time, cultivar overall means exhibited significant differences between the four cultivars, as G86/20 showed the highest performance, followed by, G95/21, G54/9 and F160, in that order.

Leaf area recorded significant differences between the control and the two stress treatments for the two cultivars G86/20 and G95/21. While it recorded no significant differences between the control and each of the two treatments for the other two cultivars; G54/9 and F160. Cultivar overall means of G86/20 and G95/21 were almost equal with no significant differences, while they recorded significant differences from the other two cultivars which were significantly different from each other (Table 2). Generally, G86/20 and G95/21 were the best performing cultivars and the most tolerant to salt and drought stresses followed by cultivar G54/9, while cultivar F160 was quite sensitive to each of the two stresses.

As a general conclusion, G86/20 and G95/21 proved to be relatively the most tolerant cultivars for salt and drought treatments based on their performance in sand culture experiment. These results are comparable with Plaut *et al.* (2000), who studied the effect of salinity on leaf growth of sugarcane cultivars and found that both leaf dry weight and area decreased with increasing salinity.

2. Molecular Markers for Salt and Drought Stresses:

2.1. SDS-protein Electrophoresis

SDS-electrophoretic patterns of water-soluble protein fractions in the leaves at 72 days of age for the sugarcane plants in sand culture experiment exhibited a maximum number of 15 bands, which were not necessarily present in all samples. The banding patterns of the four sugarcane cultivars under control, salinity and drought stresses are shown in Figure (1) and some SDS-protein markers for stress tolerance are shown in Table (3).

Regarding the tolerant cultivars G86/20 (lanes 1, 5, and 9, Fig.1) and G95/21 (lanes 2, 6, and 10, Fig. 1 and Table 3), band 2 (89.6 KDa) appeared in salt an drought treatments, while they were absent in the control, as well as in the two sensitive cultivars. This band could be considered as a positive molecular marker for salt and drought tolerance. At the same time, in the two tolerant cultivars, bands 4 and 10 (72 and 47 KDs) were present in drought treatment only which may be considered as positive molecular markers for drought tolerance since they were absent in the two sensitive cultivars.

These results are comparable with those of Ericson and Alfinito (1984) they found that some protein bands, were enhanced under drought stress. However, Hurkman and Tanaka (1988) reported that there were quantitative differences (intensity) between protein types in barley under salinity stress comparing with control, while in both treatments there were no differences in the appearance or disappearance of bands between the two treatments. In addition, Fahmy *et al.* (1992) in maize, Allam and Abdel-Tawab (2001) in sugarcane.

2.2. Isozyme Markers

The electrophoretic patterns for esterase and acid phosphatase isozymes are show in Figures 2 and 3, sand culture experiments and are summarized in Table (4).

The electrophoretic pattern of esterase isozymes of the four sugarcane cultivars in the sand culture experiment under control, salt and drought treatment are shown in Figure (2) and Table (4). They exhibited a total number of nine bands which did not necessarily appear in all leaf samples. Some adaptive bands appeared which were probably due to the effect of drought and salinity treatments compared with the control. Est-8 appeared in the two tolerant cultivars both under salinity and drought stresses. In salt treatment the activity (intensity) of bands varied also among tolerant and sensitive cultivars. While in drought treatment, this band was absent in the sensitive cultivar G54/9, so this band may be used as positive molecular marker for salt and drought tolerance in sugarcane.

Our results are in agreement with those of Abdel-Tawab *et al.* (2001) who reported molecular markers for esterase insozymes linked with salt tolerance in maize. In sugarcane, Abdel - Tawab *et.al.* (1999) and Allam and Abdel - Tawab (2001) reported specific esterase bands linked with stress tolerance which were considered as molecular markers in this respect.

Acid phosphatase isozymes of the four sugarcane cultivars in the sand culture experiment showed a maximum number of five bands, which were not necessarily present in all genotypes (Fig. 3) and Table (4). Data for the positive markers for salt and drought tolerance markers are present in Table (4). AcP-5 appeared in the two tolerant cultivars both under salinity and drought while it was absent in the two sensitive cultivars. This band may be used as positive molecular marker for salt and drought tolerance.

These results are in partial agreement with Pollak *et al.* (1984) who reported strong association of acid phosphatase with some quantitative traits in maize.

It is evident from the aforementioned discussion that cultivars G86/20 and G95/21 were distinguished by the relative abiotic stress tolerance (i.e. drought and sanlinity) compares with cultivars G54/9 and F160 which showed sensitivity to these stresses. In addition, SDS-protein, estrase and acid phosphatase markers associated with salt an/or drought tolerance were developed which could be used in screening a large number of sugarcane accessions in the early stages of breeding for stress tolerance to identify the most tolerant accessions and concentrate the time and efforts on a limited number of promising lines. This will enhance the breeding program in cost-effective way.

Table (2): Means of some yield-related traits of the four sugarcane cultivars at 72 days under control (C), salinity (S) and drought (D) conditions with their overall means (M) in a sand culture experiment.

Cultivar	Treat.	Plant Height (cm)	No leaves/ plant	Stem Diameter (cm)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Leaf area (cm^2)
G86/20	C	205.4^{a}	11^{a}	1.17^{a}	70.8^{a}	13.9^{a}	219.7^{a}
	S	180.5^{b}	8.3^{c}	0.97^{b}	33.1^{b}	11.2^{b}	144.3^{bc}
	D	163.3^{bc}	8.6^{bc}	0.82^{b-d}	16.0^{c-d}	6.2^{cd}	119.2^{b-d}
	M	183.1^{A}	9.3^{A}	0.99^{A}	39.9^{A}	10.5^{A}	161.0^{A}
G95/21	C	176.3^{b}	10.6^{a}	1.25^{a}	70.2^{a}	13.1^{ab}	254.0^{a}
	S	157.4^{bc}	9.9^{ab}	0.95^{bc}	33.2^{b}	8.5^{c}	150.1^{b}
	D	141.8^{cd}	9.1^{bc}	0.72^{de}	15.8^{c-d}	4.3^{d-f}	96.7^{d-f}
	M	158.5^{B}	9.8^{A}	0.97^{A}	39.4^{A}	8.6^{B}	166.9^{A}
G54/9	C	143.5^{cd}	10.1^{ab}	0.79^{b-d}	27.4^{bc}	4.8^{de}	111.4^{b-d}
	S	122.4^{d}	8.9^{bc}	0.77^{cd}	10.9^{d}	3.4^{d}	78.6^{d-g}
	D	131.0^{d}	9.2^{bc}	0.70^{de}	13.6^{d}	3.2^{ef}	106.2^{c-e}
	M	132.3^{C}	9.3^{A}	0.75^{B}	17.3^{B}	3.8^{C}	98.7^{B}
F160	C	76.3^{e}	8.8^{bc}	0.66^{de}	14.1^{cd}	1.5^{f}	56.7^{fg}
	S	80.2^{e}	7.7^{c}	0.56^{e}	5.3^{d}	2.4^{ef}	40.1^{g}
	D	83.4^{e}	7.9^{c}	0.69^{de}	10.8^{d}	2.6^{e}	65.7^{e-g}
	M	79.9^{D}	8.1^{B}	0.63^{C}	10.1^{C}	2.2^{D}	54.2^{C}

* Means within a given trait for each cultivar followed by the same small letter(s) are not significantly different by the Duncan,s Multiple R T ($p< 0.05$).

**Cultivar mean comparisons for each trait followed by the same capital letter(s) are not significantly different by the Duncan,s Multiple R T ($p<0.05$).

Table (3): SDS-Protein markers of four sugarcane cultivars in a sand culture experiment under control (C), salinity (S) and drought (D) treatments.

Band No.	M* (Kda)	Cultivars											
		G86/20			G95/21			G54/9			F160		
		C	S	D	C	S	D	C	S	D	C	S	D
2	89.6	-	+	+	-	+	+	-	-	-	-	-	-
4	72	-	-	+	+	-	+	-	-	-	-	-	-
10	47	-	-	+	-	-	+	-	-	-	-	-	-

* M = molecular weight marker + = present - = absent

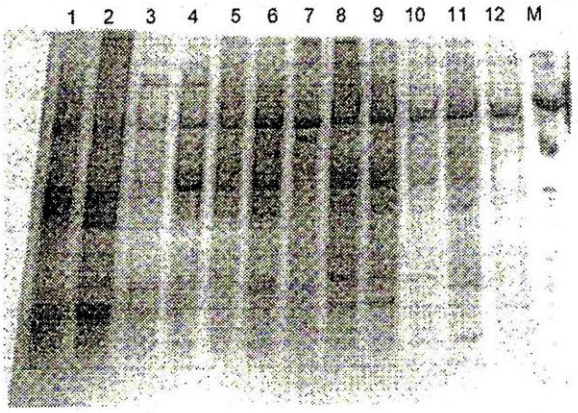

Fig. (1) SDS-PAGE profiles of sugarcane leaf protein (water soluble protein): lanes 1, 2, 3 and 4 are the four cultivars under control, 5, 6, 7 and 8 under salinity and 9, 10, 11 and 12 under drought treatment and M is a protein marker in a sand culture experiment, respectively.

Table (4): Isozymes marker bands for salinity and drought tolerance in the four sugarcane cultivars

Isozymes	Treat.	Band No.	Tolerant cultivars		Sensitive cultivars	
			G86/20	G95/21	G54/9	F160
Esterase	Drou.	8	+	+	-	+
Acid p.	Sal.	5	+	+	-	-
	Drou.	5	+	+	-	-

+ = present - = absent

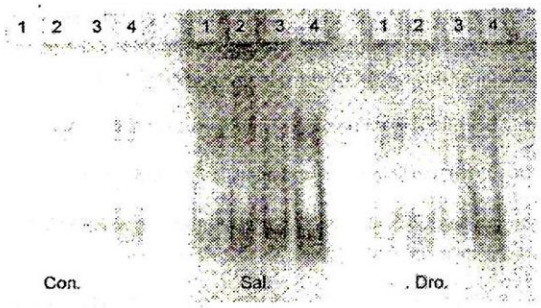

Fig. (3):Electrophoretic patterns of esterase isozymes lanes 1, 2, 3 and 4 are the four cultivars under control, respectively, 5, 6, 7 and 8 under salinity and 9, 10, 11 and 12 under drought treatment in sand culture experiment.

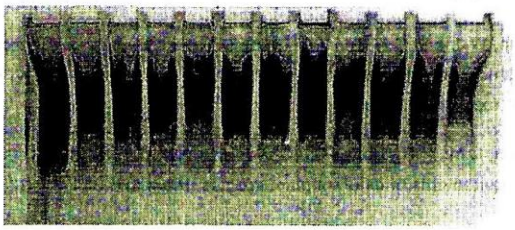

Fig. (4): Electrophoretic patterns of acid phosphatase isozymes; lanes 1, 2, 3 and 4 are the four cultivars under control respectively, 5, 6, 7 and 8 under salinity and 9, 10, 11 and 12 under drought treatment in sand culture experiment.

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أدلة إنتخابية مساعدة لتحمل الإجهاد البيئى فى قصب السكر

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الملخص العربى

تمت هذه الدراسة فى مزارع وصوب ومعامل قسم الوراثة كلية الزراعة جامعة عين شمس – شبرا الخيمة فى الفترة من ١٩٩٩ الى ٢٠٠٢ وكانت أهداف هذه الدراسة : – أولاً : تقييم ثلاثة أصناف مبشرة من قصب السكر G86/20 - G 95/21 - F160 لتحمل الملوحة و الجفاف (تم إنتخاب هذه الأصناف منذ عدة أجيال بمعهد بحوث المحاصيل السكرية بالجيزة لتحسين صفاتها الإنتاجية مقارنة بالصنف التجارى G54/9) ثانياً : الحصول على واسمات جزئية لتحمل الملوحة أو الجفاف للأصناف تحت الدراسة وذلك من خلال تقنيات التفريد الكهربائى للبروتينات ومشابهات الإنزيمات.

١ – أجريت تجربة المزارع الرملية لتقييم الأصناف الأربعة محل الدراسة وآختبار قدرتها لتحمل الملوحة (6000 جزء فى المليون) والجفاف (رية واحدة كل 15 يوم) وتم التوصل إلى أن الصنفين G86/20 &G95/21 متحملتين للملوحة والجفاف بدرجة أكبر من الصنف F 160 والصنف التجارى G54/9 .

٢ – تم عمل التحاليل البيوكيماوية على مستوى البروتين وذلك بإستخلاصه وعمل التفريد الكهربى له بإستخدام طريقة ال SDS - PAGE حيث تم الحصول على واسمات وراثية جزيئية مرتبطة بتحمل الملوحة والجفاف حيث ظهرت الحزم رقم ٢ و ٤ و ٨٦,٦) ١٠ و ٧٢ و ٤٧ كيلو دالتون فى الصنفين المتحملين G86/20 & G 95/21 كواسمات موجبة مميزة لتحمل الملوحة أو الجفاف .

٣ – تم إستخلاص المشابهات الإنزيمية estrase & acidphosphtase وعمل التفريد الكهربائى لها كذالك تم الحصول على واسمات وراثية جزيئية مرتبطة بتحمل الملوحة أو الجفاف وقد ظهرت الحزمة رقم 8 كواسمة موجبة مميزة لتحمل الملوحة أو الجفاف للمشابه الإنزيمى estrase. أما المشابهة الإزيمى acidphosphatase فإنه أظهر الحزمة رقم 5 كواسمة موجبة مميزة لتحمل الملوحة أو الجفاف فى السلالتين المتحملتين G86/20 G95/21.