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Molecular Profiling Using Protein Markers for Salt Tolerance in Sugarcane

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ABSTRACT

This study was carried out between 2006 and 2008 with the aim of evaluating five sugarcane varieties (G.84-47, G.98-28, G.95-21, G.95-19 and Phil8013) for their tolerance to salinity stress in comparison with the commercial cultivar G.T.54-9; SDS-protein analyses were conducted to identify protein markers associated with salinity tolerance for selection of promising lines tolerant to salt stress at an early stage of the sugarcane breeding program. The performance of varieties was assessed in sand culture using salinized (8000 ppm NaCl) nutrient solution under greenhouse conditions. G.95-21 and G95-19 had superior salt tolerance to salt for most yield-related traits, while commercial varieties G.T.54-9 and Phil8013 were more sensitive. Two SDS-protein markers (MW = 32 and 85 kDa) were positively associated with salt tolerance, since they were identified as being expressed exclusively in the resistant varieties (G.95-21 and G95-19) as a result of the stress.

Keywords: marker-assisted selection, *Saccharum* sp., salinity, SDS-protein, yield-related traits

INTRODUCTION

Sugarcane is the most important sugar crop in the world. Sugarcane (*Saccharum* spp.) has been grown in Egypt since 641 A. D. (Allam 1999) and is considered to be one of the most important industrial crops. Therefore, the improvement of sugar cane is one of the main objectives of the Egyptian agricultural policy. Modern sugarcane varieties are complex polyploids, which may contain over 100 chromosomes (Heinz 1987) and little is known about their genome structure (Roach and Daniels 1987; d'Hont *et al.* 2001).

Salinity can affect many processes in a plant's life cycle, and tolerance involves a complex interplay of characters (Neto *et al.* 2004; Sairam and Tyagi 2004). The physiology and biochemistry of salt tolerance were investigated by many researchers to screen overall plant performance for potential trait(s) to be used in breeding programs. Plants are generally relatively tolerant during germination, but become more sensitive following emergence and during early seedling stages of growth. Hence, it is imperative to keep salinity levels in the seedbed low at these times (Abdel-Tawab *et al.* 1998).

Electrophoretic techniques have been used to identify and characterize different crop varieties 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. Kasarada *et al.* (1998) reported that electrophoretic patterns of 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 protein markers are that most of the genome does not code for genes, and different biochemical procedures are required to visualize allelic differences for enzymes having similar 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).

The main objective of this investigation was to evaluate six sugarcane varieties for their tolerance to salinity stress and to detect putative markers associated with salinity tolerance using SDS-PAGE.

MATERIALS AND METHODS

Plant materials

Six sugarcane varieties (G.84-47, G.98-28, G.95-21, G.95-19, Phil8013 and G.T 54-9) were assessed in the 2007 and 2008 growing seasons for salt tolerance. These varieties were kindly provided by the Sugar Crops Research Institute (SCRI), Agricultural Research Center (ARC), Giza, Egypt (**Table 1**).

Sand culture experiment

The six varieties were sown in a sand culture experiment, which was conducted according to Heakel *et al.* (1981) in plastic dishes 45 cm in height, 50 cm in diameter and with a capacity to hold 50 kg of sandy soil. The plastic dishes were filled to 7 cm from the top with pre-washed fine sand. Five single-bud cuttings of a single variety were sown in each dish. Modified-Hoagland's solution, as suggested by Johnson *et al.* (1957), and recommended by Khaled (2005) was used as the base nutrient solution.

All sugarcane varieties were planted in a completely randomized experiment. Salinity treatments were initiated at 21 days after planting (DAP). Control plants were irrigated with the base nutrient solution every three days while salinity treatment was irrigated with the base nutrient solution plus 8 g/l NaCl every 3

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Table 1 Names, pedigrees and origins of the six sugarcane genotypes.

Variety name	Pedigree			Source of seed
	Female		Male	
Phil 8013	CAC 71-312	X	Phil 642227	Seed cutting from The Philippines
G 98-28	C 34-33	X	?	Local seed fuzz
G 95-21	Sp 79-2278	X	Sp 80-1043	Local seed fuzz
G 95-19	Sp 79-2278	X	Sp 80-1043	Local seed fuzz
G.T. 54-9	NCO 310	X	F 37-925	Seed fuzz from Taiwan
G 84-47	NCO 310	X	?	Local seed fuzz

? unknown parent

Table 2 Means of some yield-related traits of the six sugarcane varieties and reduction % (R %) at 72 days under control (C) and salinity (S) conditions with their overall means (M) in a sand culture experiment.

Cultivar	T[a]	PH[b] (cm)	No leaves/plant	Stem diameter (cm)	FW[c] (g)	DW[d] (g)	Mean
Ph 8013	C	95.9	9.1	0.8	12.4	3.0	24.2
	S	62.2	6.9	0.6	6.1	1.8	15.5
	M	79.1	8.0	0.7	9.3	2.4	19.9
	R %	35.1	24.2	25.0	50.8	40.0	35.0
G 98-28	C	165.0	11.6	0.9	31.5	5.5	42.9
	S	140.8	10.2	0.9	12.5	3.9	33.7
	M	152.9	10.9	0.9	22.0	4.7	38.3
	R %	14.7	12.1	0.0	60.3	29.1	23.2
G 95-21	C	236.2	12.7	1.3	81.4	16.0	69.5
	S	227.6	11.5	1.1	68.1	14.9	64.6
	M	231.9	12.1	1.2	74.8	15.5	67.1
	R %	3.6	9.4	15.4	16.3	6.9	10.3
G 95-19	C	202.7	12.2	1.4	80.7	15.1	62.4
	S	198.0	11.4	1.2	68.2	13.8	58.5
	M	200.4	11.8	1.3	74.5	14.5	60.5
	R %	2.3	6.6	14.3	15.5	8.6	9.5
G.T 54-9	C	150.7	10.6	0.8	16.2	3.7	36.4
	S	87.7	7.1	0.6	10.6	1.7	21.5
	M	119.2	8.9	0.7	13.4	2.7	29.0
	R %	41.8	33.0	25.0	34.6	54.1	37.7
G 84-47	C	187.8	10.5	0.9	18.4	7.1	44.9
	S	173.1	8.9	0.7	15.2	5.1	40.6
	M	180.5	9.7	0.8	16.8	6.1	42.8
	R %	7.8	15.2	22.2	17.4	28.2	18.2

[a] Treatment; [b] Plant height; [c] Fresh weight; [d] Dry weight

days. Samples were taken for biochemical and molecular analyses at the end of experiment. Data were recorded on all plants after 72 DAP for the following traits: plant height (cm), number of leaves/ plant, stem diameter (cm), fresh weight (FW; g), total biomass production (dry weight; DW), and a visual ranking for plant vigor (mean of four independent ranks).

Statistical analysis

The data were statistically analyzed using analysis of variance according to Bernardo (2002). Differences between means were compared after mean separation using analysis of variance (ANOVA) using Duncan's multiple range test (Duncan 1955) at $P \leq 0.05$.

Protein electrophoresis

SDS-PAGE was used to extract proteins from the studied varieties and determine their fingerprints in terms of water-soluble and -insoluble proteins. Protein fractionations were performed exclusively on a vertical slab gel (19.8 cm × 26.8 cm × 0.2 cm) using a BIO-RAD electrophoresis apparatus according to the method of Laemmli (1970) as modified by Studier (1973). All gels were scanned using the Gel Doc 2000 Bio-Rad system and analyzed with TotalLab software package v. 2009 supplied by NonLinear Co. TotalLab was used to calculate a matrix of pair-wise differences that was used to plot the relationships among the sugarcane varieties as a dendrogram based on UPGMA-based cluster analysis.

RESULTS AND DISCUSSION

Sand culture experiment

At 72 DAP in a sand culture experiment; data were recorded on the six varieties for the following traits: plant height, number of leaves and stem diameter under salinity and control treatments (**Table 2**).

ANOVA for these traits indicated significant differences between control and treatment among the six varieties in their relative tolerance to such stresses. However, the interaction between varieties and treatments was found to be significant for all the studied traits.

Table 2 indicates that the effects of salt stress varied across the studied traits in all varieties, the plant height, number of leaves/plant, stem diameter, FW, total biomass production (DW) were decreased by salt stress. G 95-21 and G 95-19 recorded the highest average plant height, number of leaves/plant, stem diameter, FW and DW, while the lowest was scored for Phil 80-13 and G.T.54-9 (**Table 2**). Varieties G 95-21 and G 95-19 showed the lowest percent reduction in plant height, number of leaves/plant, FW and DW, while Phil 80-13 and G.T.54-9 had of the highest percent reductions in plant height, number of leaves/plant, stem diameter and DW (**Table 2**). G 98-28 showed the lowest percent reduction in stem diameter. On the other G 98-28 scored the highest percent reduction in FW (**Table 2**).

In general, G95-21 and G95-19 were the most tolerant varieties to salt stress (**Table 2**). These results were comparable with those of Plaut *et al.* (2000), who studied the effect of salinity on leaf growth of sugarcane cultivars and found that leaf DW decreased with increasing salinity. Abdel-Bary *et al.* (2005) in maize and Rashed *et al.* (2006) in sorghum also reported that by increasing salinity levels,

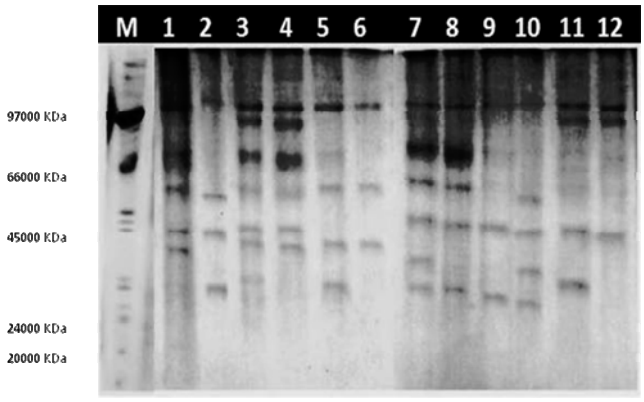

Fig. 1 SDS-PAGE profiles of sugarcane leaf protein (water soluble fraction). Lanes 1-6 = six varieties under salinity; lanes 7-12 = six varieties under control treatment.

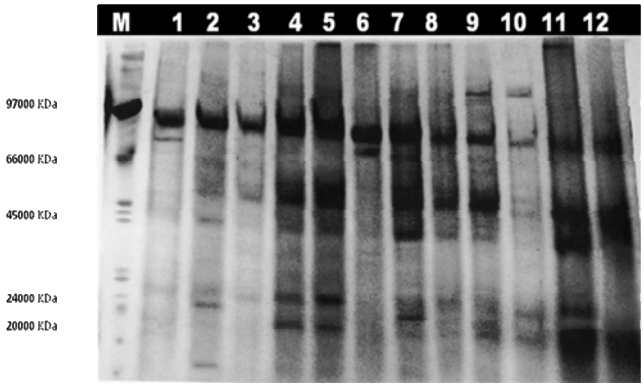

Fig. 2 SDS-PAGE profiles of sugarcane leaf protein (water-non soluble protein). Lanes 1-6 = six varieties under salinity; lanes 7-12 = six varieties under control treatment.

FW, DW and plant height decreased. They concluded that these traits could be used as indicators for the effect of salt stress on plant growth.

Molecular markers for salt stresses

The SDS-electrophoretic patterns of water-soluble protein fractions for the sugarcane cultivars under control and salt treatment in a sand experiment exhibited a maximum number of 18 bands, which were not necessarily present in all samples (**Fig. 1**). Tolerant varieties G95-21 and G95-19 exhibited band nos 4 (85 kDa) and 12 (32 kDa) which were present exclusively in the salinity treatments. These proteins may be considered as positive molecular markers, stimulated but not constitutive, for stress tolerance, as they were expressed after the salt stimulus and as they did not occur in the sensitive genotypes (**Table 3**). In addition, band no 14 (26 kDa) could be considered as an adaptive band, expressed in G 95-21 only after application of the salt treatment.

The SDS-electrophoretic patterns of water non-soluble protein fraction in the leaves at 72 DAP for the sugarcane varieties exhibited a maximum number of 15 bands, which were not necessarily present in all samples (**Fig. 2**). Genotypes G95-21 and G95-19, regarded as salt tolerant, exhibited band no. 3 (90 kDa), which appeared in the salt but not in the control treatment, nor in the sensitive varieties (**Table 4**). This band could be considered as a positive molecular marker for salt tolerance.

The present results were comparable with those of Ericson and Alfinito (1984) who found some protein bands that were enhanced in tobacco under drought stress. However, Hurkman and Tanaka (1988) reported that there were quantitative differences (intensity) between protein types in barley under salinity stress compared with control, and that

Table 3 SDS-protein marker (water-soluble protein fractions) of the six sugarcane varieties under control (C) and salinity (S) treatments.

Band No	MW (KDa)	Varieties							
		G 95-21		G 95-19		G.T. 54-9		Phil 8013	
		C	S	C	S	C	S	C	S
4	85	-	+	-	+	-	-	-	-
5	78	+	-	+	+	+	-	-	+
9	47	-	-	-	-	-	-	+	-
12	32	-	+	-	+	-	-	-	-
14	26	-	+	-	-	-	-	-	-

+ = present; – = absent

Table 4 SDS-protein marker (water-non soluble protein fractions) of the six sugarcane varieties under control (C) and salinity (S) treatments.

Band No	MW (KDa)	Varieties							
		G 95-21		G 95-19		G.T. 54-9		Phil 8013	
		C	S	C	S	C	S	C	S
3	90	-	+	-	+	-	-	-	-
4	85	-	-	+	-	-	-	-	+
11	40	-	-	-	-	+	-	-	-

+ = present; – = absent

there were also similarities in the appearance or disappearance of bands between the two treatments. In addition, Fahmy *et al.* (1992) in maize, Abdel-Tawab *et al.* (1999) in sugarcane, and Rashed *et al.* (2001) and Khaled *et al.* (2008) in sorghum reported specific protein bands linked with tolerance to different stresses.

Genetic similarity and cluster analysis based on protein analysis

Genetic similarity indices among the six varieties ranged between 5% (G 84-47 and G 98-28) and 89% (G 95-21 and G 95-19). Similarity was 20% between G 84-47 and Phi1 8013, 67% between G.T. 54-9 and G 84-47 and 60% between G.T. 54-9 and G. 95-19. Also similarity was 18% (G.T. 54-9 and Phil 8013), and 25% (G.T. 54-9 and G. 98-28) (**Table 5**). These results suggested a relatively wide genetic diversity among these varieties, particularly between G 84-47 and G 98-28, (recently released as promising varieties) and also between G.T.54-9 and Phi1 8013 (currently grown commercially).

A dendrogram, representing the relationships among the six varieties, indicated that varieties Phil 8013 and G. 98-28 were the most diverse among the studied sugarcane varieties (**Fig. 3**). The dendrogram separated the six sugarcane

Table 5 Similarity matrix among the six sugarcane varieties based on protein profile.

	Phil 8013	G. 98-28	G.95-21	G. 95-19	G.T. 54-9
Phil 8013					
G. 98-28	0.22				
G.95-21	0.40	0.29			
G. 95-19	0.36	0.25	**0.89**		
G.T. 54-9	0.18	0.25	0.44	0.60	
G. 84-47	0.20	**0.05**	0.25	0.22	0.67

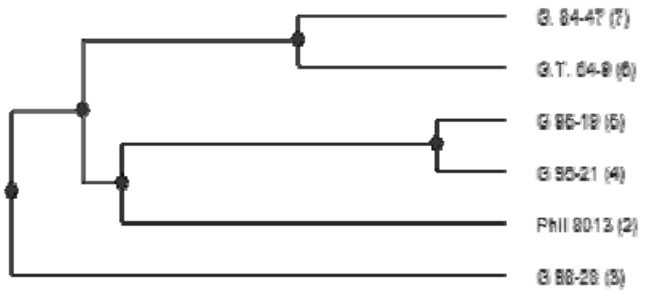

Fig. 3 Dendrogram representing the relationships among six sugarcane varieties based on similarity indices derived from protein profile.

varieties into two main clusters, with Ph8013 placed in a separate cluster by itself and with the remaining varieties making up the second cluster. The second cluster was subdivided further into two sub-clusters. The first sub-cluster was subdivided into two sub-sub-clusters; one of them included varieties G 95-21 and G 95-19 and the other contained G 98-28 only. The dendrogram matched the six varieties closely to the known pedigree information available for these varieties (**Table 1**). Varieties G.T 54-9 and G 84-47 occurred in the second sub-cluster.

This investigation revealed that protein markers could be useful tools to assist breeders in the selection and breeding of sugarcane varieties tolerant to salinity stress.

CONCLUSIONS

The performance of 6 studied varieties under greenhouse conditions in sand culture and experiments revealed that G.95-21 and G95-19 were superior in their tolerance to salt in most of their yield-related traits, while the commercial varieties G.T.54-9 and Phil8013 were more sensitive on the basis of their performance under these experimental conditions. The potential of these protein markers in screening for salt tolerance in sugarcane may need to be assessed further in inheritance studies involving a population developed between resistant and susceptible genotypes.

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