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RESPONSE OF SWEET SORGHUM (SORGHUM BICOLOR) TO CALLUS INDUCTION AND SALT STRESSES

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ABSTRACT

Response of three varieties of sweet sorghum (*Sorghum bicolor*) namely: Hunny, Brandies and Prawel, to callus induction, embryogenic production, and i*n vitro* salt tolerance were studied. In addition electrophoretic patterns of proteins and peroxidase isozyme patterns were studied to evaluate the response the obtaining calli of sweet sorghum to salt treatments with different concentrations of NaCl**.** The results obtained showed differential effect among the tested varieties. Brandies variety showed high response for callus induction, callus weight and salt tolerance comparing with other varieties. The present results might be useful in evaluative purposes in breeding program.

INTRODUCTION

Sweet sorghum (*Sorghum bicolor*) is a promising new crop. It has the possibility to replace sugarcane for syrup production and this will spare a large area of sugarcane preserved for this purpose, (Maareg *et al.*1993).

Sweet sorghum growing period is about 4 months compared with sugarcane (12-16 months), also cost of cultivation of sweet sorghum is 3 times lower than that of sugarcane, and sweet sorghum water requirement is 4000 m^3 wich less than the sugarcane water requirement (36000 m^3/HA). The Ethanole production process from sweet sorghum is eco- friendly compared to that from molasses, ethanol burning quality is superior less sulphur than of sugarcane and high octane rating.

Sweet sorghum has been found as moderately salt tolerant with a threshold level of about 6.8 dSm-1 and a slope in 16.0 percent, (Ludlow *et al.,*1990).

Because of the classical methods of breeding are very slow to produce salt tolerant plants, mutations and cell selection employing tissue culture might be used. Possible contribution to agriculture was the spontaneous or induced mutations which could be selected through tissue culture methods mentioned by Nabors, (1985), Hanning and Nabors (1988); The techniques of plant cell culture facilitate the rapid production of variant cell lines via selection procedures. These variant cell lines are useful for research into the genetics and biochemistry of plant cells in biotechnology for the production of new plant varieties and secondary metabolites. Rapidly growing fine suspension cultures or friable calluses are generally the most suitable for

selection purpose, where it is possible to regenerate plant from variant cells, selection techniques have potential for the production of crop varieties with new characteristics such as herbicide resistance (Saunders *et al.,* 1992) and salt tolerance (Freytage *et al.* 1990). This work, however, aims at:

1- Testing three varieties of sweet sorghum for capability to calli induction using *in vitro* culturing techniques.
2- Testing the effect of salt stresses on selected varieties, using different concentrations of sodium chloride (NaCl) on calli as a primary attempt for in vitro selection for salt tolerance.
3- Detecting biochemical genetic markers by analyzing the banding patterns of proteins and isozymes data.

MATERIALS AND METHODS

The present study was carried out to investigate the induction of callus of three varieties of sweet sorghum on MS media and the effect of different levels of salinity on the obtaining calli. The electrophoretic patterns of proteins and isozymes were also studied.

Plant materials:

Three varieties of sweet sorghum (*Sorghum bicolor*) namely: Hunny, Brandies and Prawel. They were kindly obtained from Sugar Crops Research Institute, (SCRI) Agriculture Research Center, Cairo, Egypt.

Methods

1. Tissue Culture Technique

1.1. Culture media

Murashige and Skoog's (MS 1962) was used as culture media.

1.2. Establishment of Sweet sorghum calli

Seeds of the genotypes were surface sterilized by immersing in 0.1% mercuric chloride for 20 min and washed with sterilized water by 3-4 changes, after that they were soaked for 24h in sterilized water. The mature embryos separated away by means of sterilized forceps from soaked seeds and culture. Nineteen mature embryos were placed on surface of agar solidified medium in Petri dishes. Experiments were performed in Petri dishes 10 cm in diameter using 25-30 ml of medium in each dish. Separation and culturing of mature embryos were carried out under aseptic conditions. Thirteen mature embryos were cultured in each Petri dish. Each dish represented one replicate and 12 replicates for every genotype were performed.

2. Salinity tolerance

To estimate the degree of salt tolerance among the tested varieties which cultured on (MS) medium containing different concentrations of sodium chloride (NaCl), the embryogenic calli were subdivided into groups of 40 calli, each group was grown on MS media which containing three concentrations of salt NaCl; EC= 5, EC= 10 and EC= 20.

3. Biochemical analysis

Protein fingerprinting and peroxidase isozyme patterns were examined for Sweet sorghum varieties to detect the degree of salinity tolerance.

3.1. Protein fingerprinting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using discontinuous buffer system as described by Laemmli (1970). The procedure of protein electrophoresis was carried out following the method described by (Gomathi and Vasantha, 2006).

Protein staining

Gels were stained with stain solution (0.1 % Coomassie blue R- 250, 40% methanol, 10% glacial acetic acid) for 2 hours, and distained with a solution of (1:3:6) glacial acetic acid; methanol; and water; respectively.

Past program (1999), was used to detect the presence or absence for protein bands, the zymograms of the varieties were photographed, the images were scanned by computer using Total Lab Software (2000),(virg. 1.1).

3.2. Isozymes electrophoresis

Agar- Starch- Polyvinyl pyrrolidine (PVP) gel was employed to study Peroxidase isozyme patterns for identification and comparison between varieties; Hunny, Brandies and Prawel. However, the procedure described by Ghonema, (2005) was used.

Peroxidase staining solution

100 ml of 0.01M sodium acetate – acetic acid buffer (pH 5.0) containing 0.1gm benzidine and 0.5 % hydrogen peroxide (H_2O_2) were used as a staining solution.

4. Statistical analysis

Data were statistically analyzed using Past program (1999).

RESULTS

Calli were obtained by tissue culture techniques. The in vitro selection technique was used to test the effect of different concentrations of NaCl (EC= 5, EC= 10 and EC= 20) stresses on calli to determine the tolerance of each genotype. On the other hand, peroxidase isozyme patterns and protein fingerprinting were tested, as genetic biomarkers, to determine the genetic variability among the tested varieties.

1. Tissue culture

1.1. Evaluation of embryogenic callus

Distinction between embryogenic and non.embryogenic callus was carried out on the basis of callus external aspect. Embryogenic calli were proven to be glossed aspect, compact, characterized by their yellow color and their globular structure, while nonembryogenic callus were found to be wet aspect, translucent and were more brownish in color. After 3 weeks of culturing, the number of embryogenic calli was recorded for each sweet sorghum varieties. These data were transformed into percentages expressed as percentage of embriogenic calli per total number of obtained calli.

1.2. Callus induction

Based on the obtaining results as shown in (Table, 1) and (Figure, 1), one can conclude that the varieties Hunny gave the lowest percentage of calli

(65%). While, the highest percentage of calli (with 72 %) was recorded for variety Prawel. Brandies. Variety gave intermediate percentage (with 71%).

Table (1): The percentags of Callus induction after 21 days on (MS) media

Sweet sorghum Varieties					
Hunny		Brandies		Prawel	
Total-explants Per-dish	%Response of callus induction	Total-explants Per-dish	%Response of callus induction	Total-explants Per-dish	%Response of callus induction
13	47	13	66	13	88
13	46	13	86	13	85
13	46	13	53	13	66
13	69	13	69	13	62
13	54	13	62	13	53
13	57	13	57	13	84
13	57	13	85	13	64
13	92	13	68	13	92
13	92	13	82	13	71
13	85	13	61	13	62
13	67	13	85	13	66
13	64	13	73	13	74
Calli induction (%)	65	71		72	

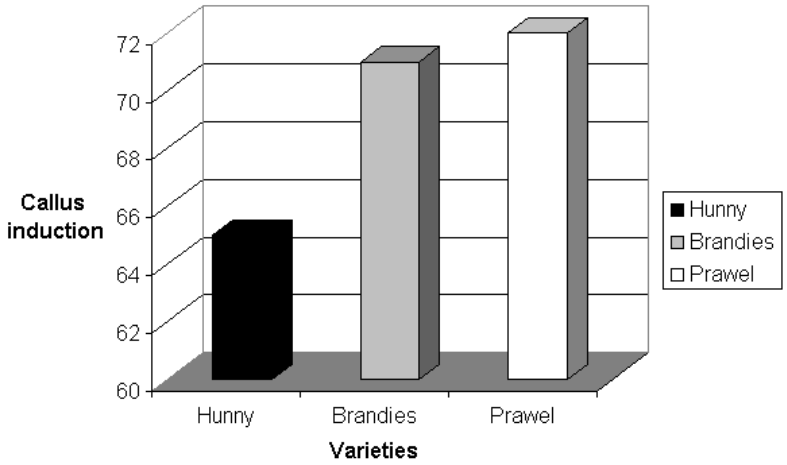

Figure (1): Callus induction percentage for three sorghum varieties.

To compare, between embryogenic and nonembryogenic percentages as shown in (Table, 2 and Figure, 2) it was found that variety Brandies had the lowest percentage of embryogenic (with 62%). While the highest percentage of embryogenic calli was recorded for the varieties Hunny (with 68%). On the other hand, variety Prawel was found to be intermediate in calli percentages (67%).

Table (2): The percentages of embryogenic and nonembryogenic calli of the different varieties of sweet sorghum

Hunny		Brandies		Prawel	
% Empryo-genic	% Nonembryo-genic calli	% Empryo-genic	% Nonembryo-genic calli	% Empryogenic	% Nonembryo-genic calli
4	4	6	4	6	9
4	2	8	5	7	5
4	3	4	4	7	5
7	2	5	4	6	4
6	3	6	3	6	2
6	2	6	9	9	3
5	3	6	2	6	4
8	5	9	3	6	3
8	4	5	4	7	3
8	2	8	5	12	3
7	2	7	5	11	2
6	3	10	2	12	3
68%	32%	62%	38%	67%	33%

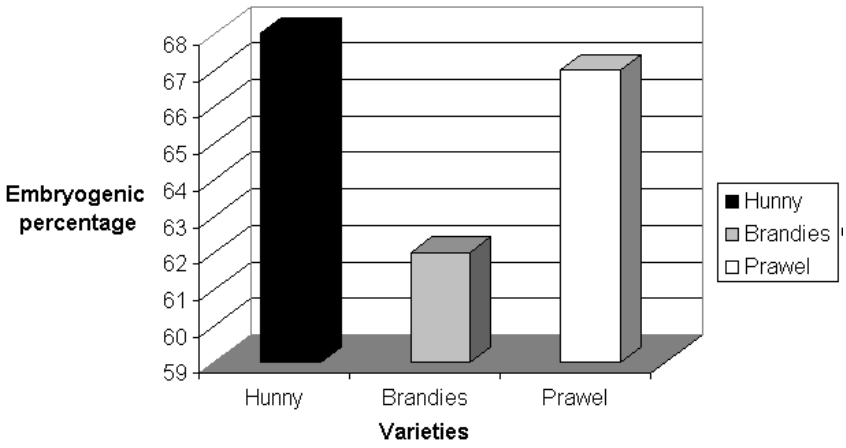

Figure (2): Embryogenic callus percentage for three sorghum varieties.

2. *In vitro* salt tolerance

Response of varieties of sweet sorghum (*Sorghum bicolor*) for *in vitro* salt tolerance was examined by exposed growing calli to different concentrations of NaCl (EC=5, EC=10 and EC=20) in culture growth media (MS) for 2 weeks. All comparisons for salt tolerance responses, necrosis percentage and relative fresh weight growth (RFWG) of calli were recorded.

2.1. *In vitro* salt treatments

In the absence of NaCl the Prawel variety showed the lowest necrosis (28%), while Brandies variety showed intermediate necrosis (30%) and the highest necrosis was found in Hunny variety (36%). After addition of the NaCl salt to culture medium caused an increase in calli necrosis for all varieties (Table, 3 and Figure, 3). Interestingly, the percentages of necrosis were

increased to be 55, 75 and 82 for Hunny after treatments with the three NaCl concentrations (EC=5, EC=10 and EC=20), respectively. While for Prawel it was increased from 40%, to 67% and 72%, respectively. The percentages of necrosis for Brandies variety were 45, 60 and 65; respectively.

Table (3): Percentages of necrosis in callus of sweet sorghum varieties at different concentrations of NaCl

NaCl concentrations	varieties		
	Hunny	Brandies	Prawel
0	36	30	28
EC=5	55	45	40
EC=10	75	60	67
EC=20	82	65	72

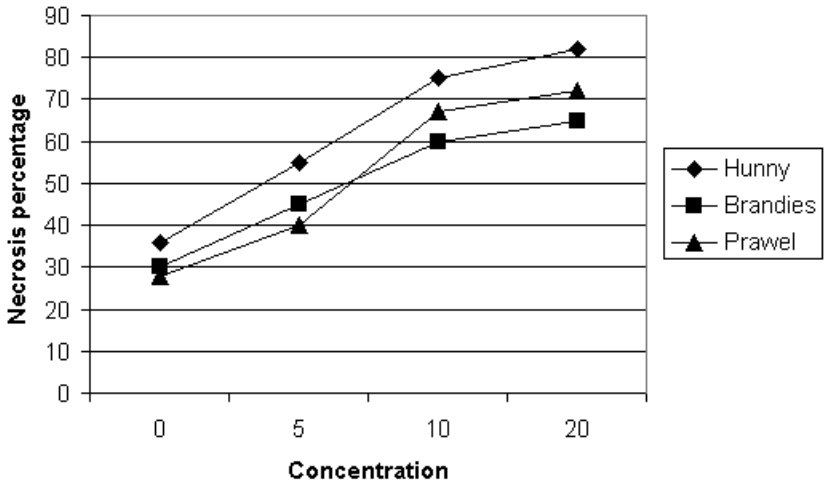

Figure (3): Necrosis percentage of the three sweet sorghum varieties before and after treatments with different concentrations of NaCl.

2.2. Callus weight (mg)

The control of the three varieties Prawel, Hunny and Brandies was significantly different. It was (240, 210 and 170 mg) for varieties Prawel, Hunny and Brandies; respectively. Callus weight was affected by salinity concentrations. It was found to be decreased with the increasing of the used concentrations (Figure, 4). Callus weight for variety Prawel was (200, 110 and 50 mg) for treatments with EC=5, EC=10 and EC=20; respectively. For variety Hunny it was (180, 115 and 40 mg). While it was (150, 90 and 80 mg) for variety Brandies after the same mentioned treatments; respectively.

3. Biochemical markers.

Protein profiles were studied after NaCl treatment. To detect differences among varieties, Sodium Dodydyl Sulphate Polyacrylamide Gel Electrophoresis (SDS- PAGE) was employed.

3.1. Protein studies

Table (4) shows the total number of protein bands for variety Brandies. It was (9, 13, 12 and 11) after treatment with EC= 0, 5, 10 and 20 dSm/l; respectively. The total number of bands detected for the variety Hunny was 8, 15, 10 and 9. While for variety Prawel showed total number of bands: 8, 12, 12 and 10 for the same mentioned treatments; respectively.

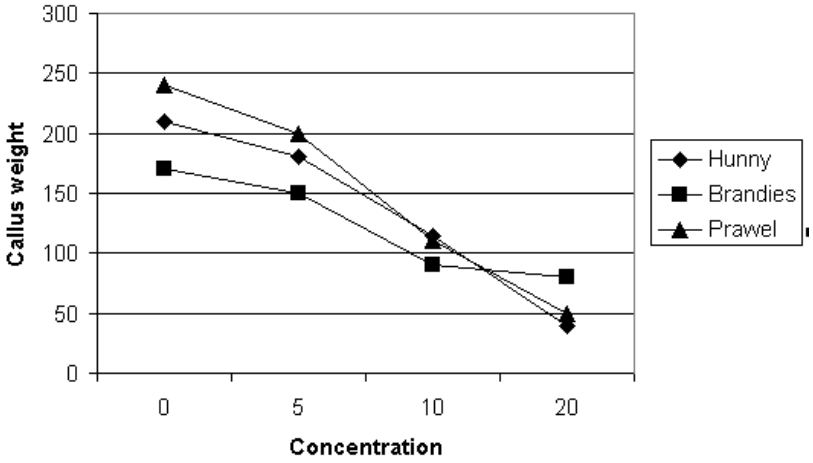

Figure (4): Percentage of callus weight for the three sweet sorghum varieties before and after treatments

Table (4): Total number of protein bands detected in sorghum varieties before and after treatments with different concentrations of NaCl.

Varieties	NaCl concentrations			
	C	EC=5	EC=10	EC=20
Brandeis	9	13	12	11
Hunny	8	15	10	9
Prawel	8	12	12	10

Figure (5), shows the SDS electrophoretic patterns in different Sorghum varieties. Electrophoretic profile would be described starting from the most anodal zone as follows:

Zone (1): It consists of arrange of several bands with high molecular weight from 115 to 225 kDa.

Zone (2): It consists of arrange of several bands with medium molecular weight that ranged from 55 to 87 kDa.

Zone (3): It consists of arrange of several bands with low molecular weight that ranged from 17 to 35 kDa.

Zone (4): It consists of arrange of several bands with very low molecular weight that ranged from 7 to 13 kDa.

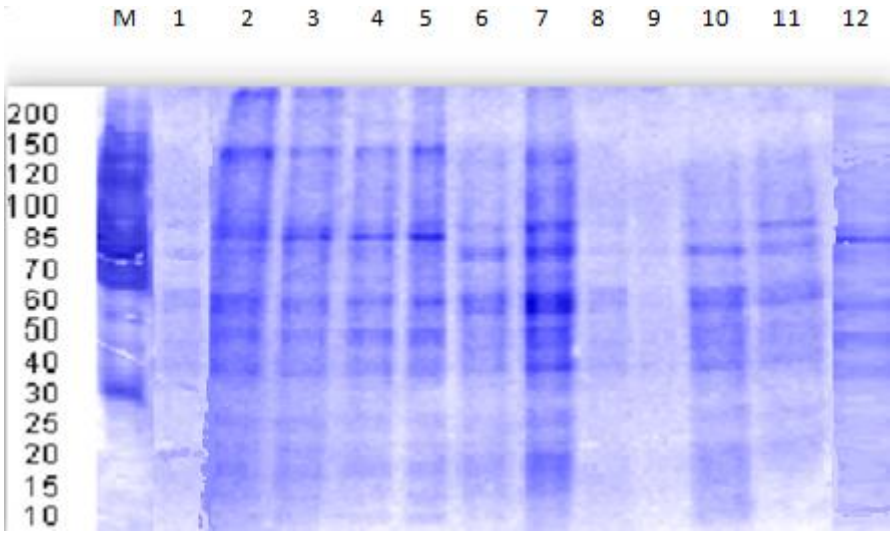

Figure (5): SDS electrophoretic protein patterns in the three Sorghum varieties

where

1- Brandies control	**5-Hunny control**	**9-Prawel control**
Brandies under EC=5	**2- 6- Hunny under EC=5**	**10-Prawel under EC=5**
3- Brandies under EC=10	**7- Hunny under EC=10**	**11-Prawel under EC=10**
Brandies under EC=20 4-	**8- Hunny under EC=20**	**12-Prawel under EC=20**

In untreated samples of Brandies variety nine electrophoretic bands with Mw of; 220, 120, 87, 85.5, 75, 55, 30, 17 and 10 kDa were observed. While, after treatment with 5 dSm/l of NaCl four new protein bands with 65, 54, 28 and 13.5 kDa were observed. Treatments with10 dSm/l of NaCl produced a new protein bands with 82, 23 and 7 kDa which absent in untreated sample. Moreover, new bands were obtained with 150 and 11 kDa which due to treatment with high NaCl concentration (20 dSm/l).

However, in untreated samples of Hunny variety eight protein bands with Mw of; 120, 88, 75, 30, 25, 17, 13 and 11 kDa were observed. Additionally, seven new bands with Mw of; 225, 117, 32, 22, 18, 13.5 and 7 kDa were found as a result of treatment with 5 dSm/l of NaCl. While with 10 dSm/l it was found two new bands with Mw of; 87 and 55 kDa. Finally, in treatment with 20 dSm/l of NaCl produced one new band with 115 kDa compared with that of the control.

The third examined variety (Prawel) showed eight protein bands with Mw; 87, 85, 35, 30, 20, 16.8, 11 and 7 kDa in untreated samples. Furthermore, four new bands were found with Mw; 190, 148, 33 and 21 kDa due to treatment with 5 dSm/l of NaCl. In addition, four new bands with Mw; 70, 25, 21 and 15.3 kDa were appeared after treatment with10 dSm/l of NaCl. Again, two new protein bands were produced with Mw; 148 and 9.8 kDa after treatment with high NaCl concentration (20 dSm/l).

3.2. Peroxidase Isozymes

The obtained data showed that there were differences in numbers, densities and position of bands in varieties between salt treatments within every variety of sweet sorghum after salt treatments. All differences in the peroxidase isozymes might be due to the activities and numbers of genes which controlling the peroxidase enzyme.

3.2.1. Peroxidase Isozyme patterns

The differences among the three varieties according to the photographs of peroxidase isozyme patterns are shown in (Figure, 6).

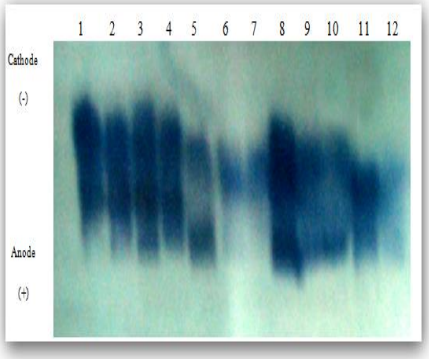

Figure (6): photograph of the Peroxidase Isozyme patterns for the three varieties of sweet sorghum.

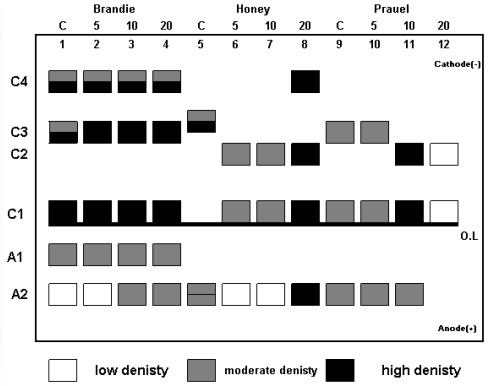

Figure (7): Zymogram of the peroxidiase Isozyme patterns for the three varieties of sweet sorghum.

where

1-Brandies control	**5-Hunny control**	**9-Prawel control**
2. Brandies under EC=5	**6- Hunny under EC=5**	**10-Prawel under EC=5**
3- Brandies under EC=10	**7- Hunny under EC=10**	**11-Prawel under EC=10**
4- Brandies under EC=20	**8- Hunny under EC=20**	**12-Prawel under EC=20**

Several bands were found in all varieties, some were migrating towards the cathode and the others migrating towards the anode. Cathodal bands were designated C1, C2, C3 and C4, while the two anodal bands were designated A1 and A2 according to their mobility from the origin line. Zymogram of the peroxidase isozymes is given in (Figure, 7).

No changes in total number of bands were detected between the control plants of Brandies variety and after treatments with different concentrations of NaCl. While total number of bands were different especially in cathodal bands in control of Hunny variety and after treatments with EC=20. On the other hand, the Prawel variety showed no variation in band numbers between untreated control and treated material, except after treatment with the highest concentration (EC=20) was noted one anodal band (Table, 5).

Table (5): Peroxidase bands for sweet sorghum (Sorghum bicolor) varieties

Varieties	Treatment	Number of Bands	Cathodal Bands	Anodal Bands
Brandies	(control)	5	3	2
	NaCl (EC= 5)	5	3	2
	NaCl (EC= 10)	5	3	2
	NaCl (EC= 20)	5	3	2
Hunny	(control)	2	1	1
	NaCl (EC= 5)	3	2	1
	NaCl(EC= 10)	3	2	1
	NaCl (EC= 20)	4	3	1
Prawel	(control)	3	2	1
	NaCl (EC= 5)	3	2	1
	NaCl(EC= 10)	3	2	1
	NaCl(EC= 20)	2	2	-

From Table (6) it was found that the first cathodal band (C1) was present in all varieties before and after treatments, except untreated samples of Hunny variety.

Table (6): Electrophoretic bands of peroxidase of sweet sorghum after treatments with NaCl

Sampels	Cathodal bands			Anodal bands		
	C1	C2	C3	C4	A1	A2
Brandies C	+	-	+/-	+/-	+	+
5	+	-	+/+	+/-	+	+
10	+	-	+/+	+/-	+	+
20	+	-	+/+	+/-	+	+
Hunny C	-	-	+/-	-	-	+
5	+	+	-	-	-	+
10	+	+	-	-	-	+
20	+	+	-	+/+	-	+
Prawel C	+	-	+	-	-	+
5	+	-	+	-	-	+
10	+	+	-	-	-	+
20	+	+	-	-	-	-

Moreover, the second cathodal band (C2) of peroxidase enzyme was expressed in samples of Hunny variety treated with 5, 10 and 20 concentration of NaCl and Prawel variety treated with 10 and 20 concentration of NaCl, while in the reminder samples of all varieties was absent. The third cathodal bands (C3) expressed with high activity in all samples of Brandies variety as homozygous locus except with control sample which was found to be heterozygous. Then, turned to be heterozygous locus in untreated samples of Hunny variety and disappeared in the rest of samples under treatments with NaCl. At final, (C3) cathodal band existed only in untreated Prawel variety and treated one with 5 dSm/l of NaCl. The fourth cathodal band (C4) expressed in all samples of Brandies variety in both treated and untreated as a heterozygous. Then, turned to become a homozygous band in Hunny variety which treated with high level of NaCl (20

dSm/l), while disappeared in reminder samples of Hunny and all samples of Prawel variety.

The first anodal band (A1) was expressed only in variety Brandies, and was absent in all samples of Hunny and Prawel varieties. The second anodal band (A2) was found in all samples of all varieties except Prawel variety which treated with 20 dSm/l of NaCl (Table, 6).

DISCUSSION

Mature embryos of the three varieties were used as an explants for calli induction. To evaluate *in vitro* salt tolerance, the selected embryogenic calli were transferred to Murashige and Skoog (MS) medium, supplemented with different concentrations of NaCl (EC= 5, 10 and 20) for two weeks.

Protein profiles of the three varieties treated with different NaCl concentrations were studied to detect the differences among the untreated and treated calli, Sodium Dodydyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used, peroxidase isozyme activities were examined also.

Tissue culture

It was known that the type and concentration of growth regulators are the most significant factors affecting successfully the culture media. Arzani, (2008) suggested that cell and tissue culture techniques have been used to obtain salt tolerant plants employing two *in vitro* culture approaches, the first approach is selection of mutant cell lines from cultured cells and plant regeneration from such cells (somaclones), *in vitro* screening of plant germplasm for salt tolerance is the second approach.

On the light of the present results the three varieties showed an average of callus induction (69.3) on MS media supplemented with (1.5 ml 2,4-D + 30gm sucrose+ 6 mg/l BAP). This result however, is in agreement with that reported by Ali *et al.,* (2008) who observed that among the auxins, 2, 4-D at 3.0 mg/l was more potent for callus induction and its subsequent growth, while the effect of auxin-cytokinin interactions was not significant with respect to callus formation. Miccah *et al,.* (2012) studied four Tanzanian open pollinated maize varieties namely; Kito, Situka M-1, Staha and TMV-1 were regenerated *in vitro* using immature zygotic embryos as ex-plants. Callus induction was achieved using Murashige and Skoog (MS) basal medium supplemented with 1, 1.5, 2 or 2.5 mg/l of 2, 4-D. Callus induction was significantly affected by the genotype of the varieties.

Embryogenic calli production

Distinction between embryogenic and nonembryogenic calli have been demonstrated by many scientists (Elkonin *et al.* 1995, Sharaf and Ouf., 1995 and Jogeswar *et al.* 2007). The average percentage of embryogenic callus was 65.66%. The highest percentage of embryogenic calli was 68% for Hunny variety. Lursard and Lupotto, (1990) represented evidence that embryogenic callus is genotype dependent as found in other crops (e.g. sorghum). Xuan WeiYan *et al.*, (2011) illustrated that Combinations of different concentrations of 2,4-D with other different hormones showed

significant and positive effects on induction of callus in two different genotypes of sweet sorghum. Miccah Songelael Seth *et al,.* (2012) reported that embryogenic callus induction percentage was significantly influenced by the genotype, 2, 4-D concentrations and their interaction effect implying differential response of the genotypes to 2, 4-D concentrations.

***In vitro* salt tolerance**

Callus necrosis

It was clear from the results that the addition of NaCl to culture media caused an increases in calli necrosis for all sweet sorghum varieties. In the absence of NaCl the average percentage for necrosis in sweet sorghum was 31.3% and at the highest concentration of NaCl used (EC=20) the lowest percentage of necrosis was 65% in sweet sorghum for variety Brandies. The addition of NaCl caused significant differences among all varieties of sweet sorghum, the average percentage of necrosis was (46.6, 67.3 and 73) at (EC= 5, 10 and 20) respectively. This results are in agreement with the data reported by (Hannging and Narobs., 1988 and Sharaf and Ouf., 1998) who observed a sudden decrease in calli number and that totopotincy of calli decreased by the increased of NaCl concentration in the media and was nearly lost when NaCl concentration were up to 11.6 gm/l. Vikas *et al.,* (2008) who reported a significant decrease in callus growth and cell viability occurred with ≥85.6 mM NaCl**.**

Callus weight

Calli were weighted before they transferred to Ms Media supplemented with different concentrations of NaCl. To tolerate higher degree of salinity in the medium, the calli's cell might possess more than dominant gene. This approach could be proved by the useful results reported by (Hurkman and Tanaka, 1987) who observed significant differences within and between different varieties for salt tolerance.

It was clear from these the present results that callus weight decreased with the increasing of NaCl in the culture media. This result is in agreement with that reported by Hefny and Abdel-Kader, (2009) who observed that salt stress resulted in significant reduction of dry weight of both tolerant and sensitive genotypes. Furthermore, These results are confirmed by the results obtained in sorghum by (Yang *et al.* 1990), in rice by (Lutts *et al.* 1996, Basa *et al.,* 2002) in sun flour by (Alvarez *et al.,* 2003), and in sweet orange by (Helaly and Hanan El-Hosieny 2011) who reported that NaCl reduced callus growth and this genotype responds differently to this stress. In the present study Brandies variety appeared to be more tolerant to NaCl *in vitro* than the other varieties of sweet sorghum, this result is fully agree with those obtained by Gandnu *et al.* 2004 they cleared that growing calli derived from sugar cane varieties CP70-321 and CO310 showed less necrosis and less relative fresh weight growth reduction under salt stress.

Biochemical markers

Protein fingerprint

Protein banding patterns had been studied in sweet sorghum by many authors (Wang *et al..,* 2002, Mohammadi and Hajieghrari, 2009 , Chai *et al.,* 2010 and Ngara *et al.,* 2012). Electrophoresis of soluble protein revealed some differences concerning band numbers and intensity for all treatments of

sweet sorghum. The result shows the association of marker protein bands. At the highest concentration of NaCl (EC=20) varieties showed new marker protein bands at kDa of (150 & 11), (115) and (148 & 9.8) for Brandies, Hunny and Prawel; respectively. These results are in agreement with that reported by Schmidit *et al.,* (2001) who indicated that 3 portion marker bands at kDa of 88, 65 and 50, these bands were suppressed in untreated samples. Chai *et al.,* (2010) reported that NaCl treatment resulted in the inhibition of growth and increased the content of free proline, soluble protein and malondialdehyde in sweet sorghum.

However, the most tolerant sweet sorghum variety (Brandies) could identified by two new bands at kDa (150 and 11). The present results would lead to conclusion that, the network of genetic material, which control soluble proteins associated with salinity tolerant is probably polygenic. This means that more than one genetic locus are responsible for the biosynthesis of proteins associated with salinity tolerant.

Peroxidase isozyme

Genetic differences among individuals might be identified by the use of isozyme analysis. The study of isozyme electrophoresis could give a rapid identification of different varieties. Isozyme polymorphism in sweet sorghum have been evaluated by many authors (Brewebaker and Nagia, 1992, Castaeda and Mata 2000, Guirllemo, 2000, Dong Huang and Backhouse 2006, Marambe and Ando, 2008 and Chai *et al.,* 2010). In the present study a maximum of 5 bands of peroxidase activity was found in sweet sorghum calli (control and treated with different concentration of NaCl). Hunny and Prawel varieties displayed fluctuation in band number and activity after salt treatment, while the band number and activity for Brandies variety not affected after NaCl treatment. The first anodal band (A1) is found in all investigated samples of Brandies variety treated with NaCl (EC= 5, 10 and 20) and control, this means that this band might be due to the activity of a genetic locus common only in this variety, which might serve the highest ability of this variety to tolerate the increased level of NaCl and this could represent a locus that serve a general metabolic function for salt tolerant in this variety. This result is in agreement with the data reported by El-Sayed *et al.,* (2007) who found that appearance of addition isozyme bands may be involved for improving salinity tolerance and could be used as biochemical genetic markers for salinity tolerance in breeding programs. Generally, sweet sorghum peroxidase isozyme patterns indicated that the salinity induced remarkable fluctuation in the number and activity of peroxidase isozyme pattern. These results supported the results mentioned by (Mohamed., 2002, and Sandra *et al..,* 2006) who showed that peroxidase activity markedly increased response to lower saline treatments. Chai *et al.,* (2010) observed NaCl treatment enhanced the activity of catalase, peroxidase in both shoots and roots, while decreased that of glutathione reductase in sweet sorghum.

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استجابة الذره السكرية لاستحداث الكالس وتحمل الملوحة

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تـم دراسـة اسـتجابة ثلاثـة اصـناف مـن الـذرة السـكرية وهـي Hunny و Brandies و Prawel وذلك للكالس المستحدث وانتاج الكالس الجنيني كما تمت دراسة مدى تحمل هذه الاصناف للملوحة.

وكذلك تم عمل التفريد الكهربي لنماذج البروتين والمشابهات الانزيميه لانزيم البيروكسيداز وذلك لتقييم حساسية الكلس الناتج للتركيزات الملحية المختلفة من الصوديوم كلوريد.

ولقد أظهرت النتائج المتحصل عليها تأثيرات مختلقة ما بين الاصناف المختبرة. وأظهر الكالس المستحدث للصنف Brandies استجابة عالية من حيث تكوين وزن عالى والتحمل للملوحة وذلك مقارنة بالاصناف الاخرى تحت الدراسة.

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