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EVALUATION OF SALT TOLERANCE IN SOME DURUM WHEAT BY USING IN VITRO TECHNIQUE

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ABSTRACT

The objective of this study was to increase salt tolerance in *Triticum durum* wheat varieties by selecting the high tolerant genotypes from the total genotypes under salt stress by using mature embryos. Mature embryos of four *Triticum durum* genotypes were grown on MS medium for germination under 4 concentrations of salt solutions (2000,4000,6000 and 8000 ppm). The germination percent at the high salt concentration (8000 ppm) was 77.7% S1, followed by77.1%D2, 66.2%ID12 and finally 64.5%D1, respectively.

Plantlets on these media were transferred to pots with sand and irrigated with the same salt solutions. The produced grains were recultured for get some grains for propagation. Proline accumulation was increased with increasing sodium chloride salt in the medium. SDS-PAGE profile revealed an increasing in some bands intensity in 8000ppm than in the control and presented two bands with molecular weight 32and 28kda related to proline accumulation in the cells. The four *Triticum durum* wheat genotypes had the ability to resist salt stress.

Key words: *In Vitro* culture, salt stress, *Triticum durum*, SDS-PAGE.

INTRODUCTION

Plant responds to abiotic stress actively to survive the stress by turning on some metabolic pathways or by modifying gene expression, the final aim being to survive the stress. The stress factors specially salinity negatively affects plant growth and productively of the crop under stress. *In vitro* selection schemes for the isolation of salt tolerant cell lines have been successful in various crop species, Kirti *et al.*(1991).

Three of the *Triticum durum* genetic materials used were introduced from ICARDA for the breeding program in the Breeding Unit in Genetic Resources Department. Sabry *et al.*(2006) regenerated plantlets of wheat on MS media under 3 different concentrations of sea salt . They concluded that somaclonal variation could be a successful tool for the production of salinity tolerant lines regardless of the tolerance degree for the original parent cultivar. Proline is considered as an important amino acid that serves as an osmoprotectant in many plant species. Abo-Doma (1997) and Qader *et al.* (1981) confirmed that free Proline accumulation in barley and wheat crops increased with salt stress. El-Farash *et al.* (1993) found that NaCl had an effect on gene expression of the soluble protein profiles in callus culture. Rashed *et al.* (1994)used SDS-PAGE in wheat at 8000 and 10000 ppm NaCl salt concentration. They found that, there were more bands below 43kDa and higher intensities with higher salt concentrations. Abo-Doma (1997) found that protein band (50KDa)and a newly 37.5KDa occurred in high intensity of NaCl . It is very important to increase salt tolerant in wheat in Egypt to increase wheat production regarding to area affected with salt tress. The aim of this study was to generate high salt tolerant varieties of *Triticum durum*

by selecting high salt tolerant genotypes under saline conditions,to determinat the effect of salt stress on osmolyte such as proline and also elucidating some biochemical genetic markers such as SDS-PAGE protein.

MATERIAL AND METHODS

This study was carried out through 2004 to 2007, in the tissue culture unit lab., Genetic Resources Department, Ecology and Dry Land Agriculture Division, DRC, Mataria, Cairo, Egypt. Four genotypes of *Triticum durum* namely D1,D2,ID12 and Sohag1 (salt tolerant up to 6000ppm) were used.

Their pedigrees are:

Name Origin Pedigree

D1: Hurani Syria ICD BMABL-223-ORP

D2: Omtel-1 Mex / Syria ICD85-6AP-TR-4AR-OTR

ID12:cross:Ru/3/ch21563/cr Mexico ICD81-9062-7TR-1AP-2AP-OAP

S1: Sohag I Egypt

Excised embryos of the four mentioned *Triticum durum* were cultured on MS media supplemented with 0.5mg 2,4-D+ 0,5mg BA +30.0g sucrose / l and different concentrations of NaCl as a stress agent in the media. The pH value was adjusted with drops of 0.1N of H Cl or NaOH at 5.7 . Media was solidified by adding 3.0 grams of phytagel /l and dispersed into baby food jars before autoclaving at 121C° for 15 min.

Grains of the four *Triticum durum* genotypes were soaked in 30% of commercial of Sodium hypochlorite solution for 20 min, then washed with sterilized water five times and then soaked in sterilized water over night until culture. Embryos were excised from wheat grains and cultured on MS media with NaCl treatments (2000,4000,6000 and 8000 ppm/L). The concentration 2000 ppm was considered as control. Cultures were incubated at 8h dark and 16h light.

Germination percent of the four *Triticum durum* genotypes was recorded after 25 days . The length of growing plantlets were measured on these media. Samples of the plantlets were kept for proline determination . Plantlets were cultured in culture pots filled with prewashed sand by HCl and distilled water . Plants were irrigated with salt stress solution and Hoagland solution suggested by Johnson *et al.*(1957) as a nutrient supplement, the control pots were irrigated with Hoagland only.

For biochemical analysis samples were taken after 25 days from culture on MS treated media. Plant leaf samples were used for proline determination using the method of Bates *et al.* (1973).

Biochemical genetic studies:

SDS- polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Studier (1973). Water soluble proteins were extracted from leaf samples of the four *Triticum durum* genotypes after 25 days on two contrast Salinity treatments, 2000ppm (control) and high concentration 8000ppm.Data were recorded as 1 the normal bands and +1(bands more dense than 1)

RESULTS AND DISCUSSIONS

Embryos of the four varieties *Triticum durum* genotypes (D1,D2,ID12 and Sohage1) were cultured for germination and subsequently plantlets on Murashige and Skoog salt medium (1962) supplemented with (0.5 mg BA+0.5 mg 2,4-D) in the presence of four sodium chloride concentrations (2000, 4000, 6000 and 8000 ppm). Plantlets of the four genotypes were grown well after 25days from culture date under intensive light for 16h and 8h dark .The plantlets were separated and transferred to the greenhouse in plastic pots (30cm dia.) filled with prewashed sand . Pots were irrigated with Hoagland solution (1950) weekly until maturity. Data in Table (1), show the germination percent and the length of the

plantlets of the four *Triticum durum* genotypes(D1,D2,ID12 and Sohag1). The germination percent was 100% with the four varieties at the treatment control (2000ppm) . The variety D1 exhibited high germination percent (81.7%) followed by 80.8 %, 80.3% and 74.2% with variety ID12,S1 and D2 respectively. The treatment 6000 ppm produced high germination percent (78.0%) with variety D1, 75.9% with D2, and 72.7% with S1,but ID12 was the lowest one (66.6%). In the treatment 8000 ppm. Sohage 1 was the highest (77.7%) followed by D2 (77.1%), while ID12 was 66.2% and D1 was 64.5% . Screening of genotypes of the varieties was occurred by using germination test, not all the genotypes have the capability to express themselves on salt stress and to complete live cycle. Some of these genotypes are high tolerant to the high salt stress . It is clear from Table (1) that, the main of plant length *In Vitro* was ranging from 20.01cm with Sohag1 to 18.5cm with ID12 in the control treatment. In the treatment 4000 ppm, the highest plant length was 19.2cm with Sohage1 followed by D1,D2 (18.24cm) and ID12 (17.7cm).With the treatment 6000ppm, D1 and D2 the main of plant length were 18.33cm, while with ID12 it was 15.9cm and Sohag1 was 15.83cm length. With the high salt concentration 8000pm, the plant lengths were 16.8, 16.45, 15.9 and 15.83cm with D1, D2, ID12 and Sohage1 respectively. The high concentration was the more effective in germination percent and plant length. These results are in agreement with that of Sabry *et al.* (2006) who regenerated plantlets of wheat on MS media under 3 different concentrations of sea salt (0.0,6000 and 9000 ppm) .The plantlets were transferred to pots 30cm diameter, which were filled with pre-washed sand and irrigated twice a week with Hoagland solution until they reach harvest time.

Some plants from each treatment of the four genotypes were chosen to measure root number and length . There was a negative relation between the mean of root number and salt concentration (Table 2). The control treatment showed the highest number of roots/ plant, which subsequently is decreased by increasing of salt concentration. This was observed in all genotypes tested. In the high salt concentrations (6000 and 8000 ppm), the mean of root number was ranging between 7.67 and 8.89 roots /plant, but was higher in the control and 4000 ppm treatments . Sohag1 was the best variety in root number. On the other hand, the mean of root length in was around 15.48 and 11.36 cm / root, this was noticed in the control and all salt concentrations .After the appearing of flag leaves, the sterile and the semi sterile plants were discarded from pots and spikes were cut off plants.

There were two or three grains in each spike of each genotype on the high concentrations. These grains were considered as high tolerant genotypes for high salt concentration (8000ppm) and were cultured at the next season to increase the yield of grains to generate high salt resistant *Triricum durum*.

These results are in agreement with those obtained by Sabry *et al.* (2006). They found that, there were reduction in the means of grain yield, plant high, and biochemical and physiological characteristics due to stress. while the aim of study (screening of the high salt tolerant genotypes) were disagreed with that obtained by Sabry *et al.* (2006) they concluded that somaclonal variation could be a successful tool for the production of salinity tolerant lines regardless of the tolerance degree of the original parent cultivar.

Proline content was measured from leaf extraction of the regenerated plantlets of the four genotypes and the 2000 and 8000ppm sodium chloride concentration (4000, 6000 and 8000ppm) Table (3).

It appeared that Proline content was increased in the genotypes under salt stress when compared with the control. The elevation in proline content varied between the four genotypes tested.

These data indicated that proline content increased in response to salt stress and its accumulation in the plant was relatively more with the high salt concentrations than in the control (2000ppm).

This means that tolerant genotypes accumulate Proline as osmoprotectant against salt stress in plant cell. This agrees with Abo-Doma (1997) who found that the tolerant clones showed higher accumulation levels of Proline than sensitive clones in barley and confirmed that the free proline accumulation in barley and wheat crops increased with salt stress. Qader *et al.* (1981) found that the extracted free proline from the leaves of wheat genotypes grown under sodic conditions increased in all varieties with an increase in sodicity stress .

Electrophoresis analyses were carried out on soluble SDS-protein fraction for the four wheat cultivars under salt free treatment and high salt concentration (8000ppm) as shown in Figure (1). The maximum number of bands was 11 as shown in Table (4) for the densitometer analysis of SDS-PAGE. There were two bands at the MW 32 and 28 kda presents a differential expression under salt stress in their profile and there were difference in the intensity of the two bands which were shown to be increasing with increasing of salt stress. This result revealed that there are two bands at MW32 and 28 kda related with the protein accumulation in plant leaves in response to salt stress. These results are in agreement with that of Abo-Doma (1997) who found that protein band (50KDa) and a newly 37.5 KDa occurred in a higher intensity in 200 mM NaCl treated plants in both the tolerant and sensitive cultivars as compared with the control plants.

Also, El-Farash *et al.*(1993) found that, sodium chloride had an effect on gene expression of the soluble protein profiles in callus culture. Rashed *et al.* (1994) used SDS-PAGE in wheat at 8000 and 10000 ppm NaCl salt concentration to find the protein bands associated with salt stress. They found that, there were more bands below 43kDa and higher intensities with higher salt concentrations. The four *Triticum durum* wheat genotypes (D1,D2,ID12 and Sohag1) had the ability to tolerate salt stress and to produce considerable yield under salt stress, but ID12was the best one followed by Sohag-1 and D1 while D2 was the lowest one.

Table (1): The mean shoot length of the four *Triricum durum* plantlets under salt concentrations (2000, 4000, 6000 and 8000ppm).

Treatments	Germination %				The length of regenerants (cm)			
	D1	D2	Id12	S 1	D1	D2	Id12	S 1
Control	100	100	100	100	19.5	19.2	18.53	20.01
4000ppm	81.7	74.2	80.8	80.3	18.5	18.24	17.7	19.2
6000ppm	78.0	75.9	66.6	72.7	18.33	18.33	16.58	16.92
8000ppm	64.5	77.1	66.2	77.7	16.8	16.45	15.9	15.83
Mean					18.28	18.05	17.17	17.99

Table (2): The mean root number and length of the four *Triricum durum* plants under salt concentrations (2000, 4000, 6000 and 8000ppm) *cultured in pots.*

Treatments	The mean number of root				The mean root length (cm)			
	D1	D2	Id12	S1	D1	D2	Id12	S1
Control	11.33	10.11	9.99	11.22	14.45	14.17	14.78	15.48
4000ppm	10.44	9.67	8.67	9.11	14.44	13.35	13.43	15.12
6000ppm	7.67	7.78	8.89	8.33	13.8	11.99	11.89	14.32
8000ppm	7.89	8.33	8.56	8.78	13.19	12.61	11.36	14.39

Table (3): Proline content ug of the four *Triticum durum* genotypes under salt stress (2000,4000,6000 and 8000ppm).

Variety	Proline content			
Salt concent.	Control	4000	6000	8000
D1	14.3	18,7221	37,40091	57,65148
D2	6,922551	14.25	16,85421	21,9795
Id12	11.07	13.64465	38.856	66,53531
S1	15.25	37,37813	42.762	59.14

Table (4): Densitometer analysis of SDS-PAGE. band number, Molecular weight of band and intensity as present band or absent.

MW kDa	Samples No.								
	Control (2000ppm)					8000ppm			
	Band no.	D1	D2	ID12	S1	D1	D2	ID12	S1
72KDa	1	1	1	1	1	1	1	1	1
66KDa	2	1	1	1	1	1	1	1	1
56Kda	3	1	1	1	1	1	1	1	1
Kda52	4	1	1	1	1	1	1	1	1
48KDa	5	1	1	1	1	1	1	1	1
KDa 42	6	1	1	1	1	1	1	1	1
32Kda	7	1	1	1	1	+1	+1	+1	+1
28Kda	8	1	1	1	1	+1	+1	+1	+1
14Kda	9	1	1	1	1	1	1	1	1
7Kda	10	1	1	1	1	1	1	1	1
KDa	11	1	1	1	1	1	1	1	1

+1 means that bands are more dense, while the normal bands were recorded as 1

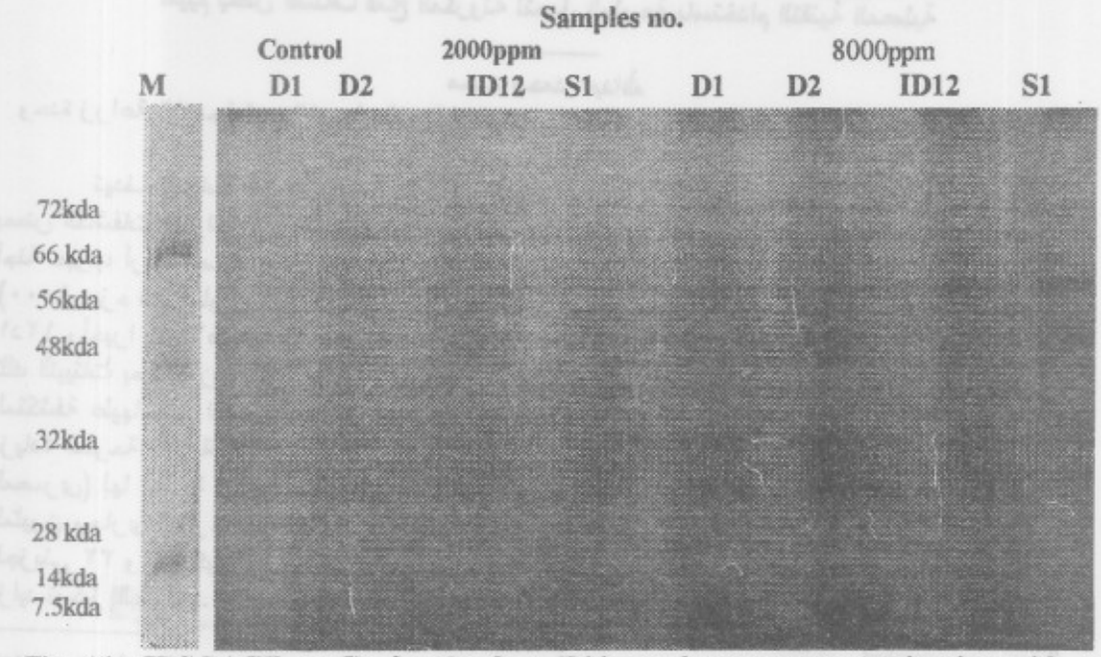

Fig. (1): SDS-PAGE profile for the four *Triticum durum* genotypes. *in vitro* with salt concentrations (control and 8000ppm) .

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تقييم بعض اصناف قمح المكرونة لتحمل الملوحة باستخدام التقنية المعملية

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تهدف الدراسة الى تقييم بعض اصناف قمح المكرونة وزيادة قدرتها على تحمل الملوحة وإيجاد بعض الكاشفات الوراثية البيوكيميائية المتعلقه بالإجهادات الملحية. كانت نسبة الإنبات الناتجة من زراعة أجنة حبوب أربعة أصناف من قمح المكرونة على بيئة موراشيج أند سكوج المحتوية على تركيزات ملوحة (٨٠٠٠ جزء فى المليون كلوريد صوديوم) ٧٧,٧% فى سوهاج ١ يليه ٧٧,١% فى د٢ ثم ٦٦,٢% فى ١د١٢ وأخيرا ٦٤,٥% فى د١. النباتات النامية على تلك البيئة وتكون منها نبيتات متحملة للملوحة نقلت تلك النبيتات بعد تكون مجموع جذرى إلى أكياس الأقلمة ورويت بمحلول هوجلاند مع تركيزات الملوحة المتكشفة عليها حتى المحصول. قدر البرولين فى عينات الأوراق ووجد هناك ازدياد فى تركيز البرولين بزيادة الملوحة مقارنة بالكنترول. كما وجد أن أصناف قمح المكرونة الأربعة (د١، د٢، ١د١٢ وسوهاج ١ المصرى) لها القابلية لزيادة تحمل الملوحة لإنتاجها بعض الحبوب تحت التركيز الملحى ٨٠٠٠ جزء للمليون وجارى إكثارها فى الحقل. وباستخدام الفصل الكهربي للبروتينات وجد ان هناك حزمتين عند الوزن الجزيئى ٣٢ و ٢٨ كيلو دالتون يتزايد فيهما تركيز الحزم البروتينيه مقارنة بالكنترول وان لهما ارتباط مع تزايد تركيز البرولين.

الكلمات الدالة –: زراعة الانسجة –اجهاد ملحى–قمح المكرونة – الفصل الكهربي للبروتينات– برولين