Pak. J. Biotechnol. Vol. 6 (1-2) 31-36 (2009) ISSN. 1812-1837

EVALUATION OF *TRITICUM DURUM* FOR SALT TOLERANT

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ABSTRACT

This work was conducted to evaluate 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* were grown on MS medium for germination under 4 concentrations of salt solutions (zero, 4000, 6000 and 8000ppm). The germination percent at the high salt concentration (8000ppm) 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 to obtain some grains for propagation. Among the saline tolerance cultures proline accumulation was increased. SDS-page profile revealed an increasing in band 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) varieties had the ability to resist against salt stress.

INTRODUCTION

Plant responds to abiotic stresses actively to survive under stress by turning on some metabolic pathways or by modifying gene expression, the final aim being to survive under stress. The stress factors especially 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).

The *Triticum durum* varieties have some genes, which are expressed at abiotic stress, make it possible to increase their response to stress. Mean while, Sabry *et al.*, (2006) have 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 osmo- protectant in many plant species. El-Farash *et al.*, (1993) found that NaCl had an effect on gene expression of the soluble protein profiles in callus culture. 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 genotype under saline conditions, to determine the effect of salt stress on osmolyte such as proline and also elucidating some biochemical genetic markers such as SDS-PAGE protein.

MATERIALS 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 cultivars 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-1Mex/Syria ICD85-6AP-TR-4AR - OTR

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

S1: Sohag 1 Egypt

Excised embryos of the four mentioned *Triticum durum* were cultured on Murashige and Skoog, 1962 media supplemented with 0.5mg/l 2,4-D+ 0.5mg/l BA +30.0g/l sucrose and different concentrations of NaCl as a stress agent in the media. The pH value was adjusted with 0.1N HCl /NaOH at 5.7, Medium was solidified by adding 3.0 grams of phytagel/l and dispersed into baby food jars before autoclaving at 121°C for 15 min.

Grains of the four *Triticum durum* varieties were soaked in 30% of commercial 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 (control, 4000, 6000 and 8000ppm/l). Cultures were incubated at 8h dark and 16h light.

Germination percent of the four *Triticum durum* varieties was recorded after 25 days. The length of growing plantlets was 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 Amon, 1950. 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-poly acrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as modified by Studier (1973). Water soluble proteins were extracted from leaf samples of the four *Triticum durum* varieties after 25 days on two contrast salinity treatments, salt free (control) and high concentration (8000ppm) of NaCl.

RESULTS AND DISCUSSION

Embryos of the four varieties *Triticum durum* (D1, D2, ID12 and sohage1) were cultured for germination and subsequently plantlets on Murashige and Skoog medium (1962) supplemented with 0.5 mg BA+0.5 mg 2,4-D in presence of four sodium chloride concentrations (0.0, 4000, 6000 and 8000ppm). Plantlets of the four varieties *Triticum durum* (D1, D2, ID12 and sohage1) were grown well after 25days from culture date under intensive light for 16h and 8h dark. The plantlets were separated and transferred to the green house in plastic pots (30cm dia) filled with prewashed sand. Pots were irrigated with Hoagland (1950) solution weekly until maturity. Data in Table-1 show the germination percent and the length of the plantlets of the four varieties *Triticum durum* (D1, D2, ID12 and sohag1).

Table-1: The mean shoot length of the four *Triricum durum* plantlets under salt concentrations (0.0, 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

The germination was 100% in all four varieties and control. 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 of 6000ppm 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 of 8000ppm Sohage 1 was the highest (77.7%) followed by D2 (77.1%), ID12 66.2% and D1 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 life cycle. Some of these genotypes are high tolerant to the high salt stress. It is clear from Table-1 that, the mean of plant length (In Vitro*)* was ranging from 20.01cm with Sohag1 to 18.5cm with ID12 in the control treatment. In the treatment 4000ppm, the highest plant length was 19.2cm with Souhage1 followed by D1, D2 (18.24 cm) and ID12 (17.7cm). With the treatment 6000ppm, D1 and D2 the mean 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 9000ppm). 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 varieties were chosen to measure root number and length. There was a negative relation between the main of root number and salt concentration as shown in Table-2. The control treatment showed the highest number of root/ plant, which subsequently decreased by increasing of salt concentration. This was observed with the four varieties. In the high salt concentrations (6000 and 8000ppm), the mean of root number was ranging between 7.67 and 8.89 root / plant, but was higher in the control and 4000ppm treatments. Sohag1 was the best variety in root number, and there were subroots with the roots of the four varieties. On the other hand, the mean of root length in each one of the four varieties 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 the four varieties in 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 height, 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 contents are measured by using leaf extract of regenerated plantlets on different culture media from varieties treated with sodium chloride concentration (4000, 6000 and 8000 ppm) and control as shown in Table-3. It appeared that Proline content was increased in the cultivars under salt stress when compared with the control. The elevation in Proline content varied between the four cultivars. Data has 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.

This means that tolerant varieties 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 based techniques 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. Table-4 shows densitometer analysis of SDS-PAGE. There were two bands at the MW 32 and 28kda 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.

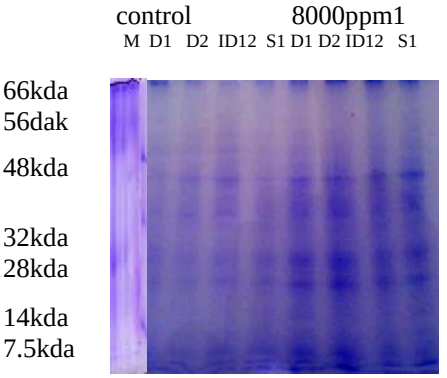

Figure-1: SDS-PAGE profile for the four *Triticum durum* vr. *in vitro* with salt concentrations (control and 8000ppm).

This result revealed that there are two bands at MW32and 28kda related with the protein accumulation in plant leaves in response to salt stress. These results are in agreement with that of Abo-Doma (1997) found that protein band (50KDa) and a newly 37.5KDa occurred in a higher intensity in 200 mM NaCl treated plants in both the tolerant and sensitive cultivars as compared with the control plants.

These results are in agreement with those of El-Farash *et al.*, (1993) who 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 10000ppm NaCl salt

concentration to relate the proteins with salt concentrations and 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 (D1, D2, ID12 and Sohag1) had the ability to tolerate salt stress and to produce considerable yield under stress, but ID12 was the best followed by Sohage and D1 while D2 was the lowest one.

Table-2: The mean root number and length of the four *Triricum durum* plants under salt concentrations (0.0, 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 contents (ug) in four *Triticum durum* varieties under salt stress (0.0, 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.								
	Band no.	1	2	3	4	5	6	7	8
72KDa	1	1	1	1	1	1	1	1	1
66KDa	2	1	1	1	1	1	1	1	1
Kda56	3	1	1	1	1	1	1	1	1
Kda	4	1	1	1	1	1	1	1	1
KDa48	5	1	1	1	1	1	1	1	1
KDa	6	1	1	1	1	1	1	1	1
Kda32	7	**1**	**1**	**1**	**1**	**+1**	**+1**	**+1**	**+1**
Kda28	8	**1**	**1**	**1**	**1**	**+1**	**+1**	**+1**	**+1**
Kda14	9	1	1	1	1	1	1	1	1
Kda7	10	1	1	1	1	1	1	1	1
KDa	11	1	1	1	1	1	1	1	1

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