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In Vitro Prediction for Salt Tolerance in Wheat

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Abstract

Callus of three salt tolerant wheat genotypes and their hybrids was obtained from mature embryos on MS medium supplemented with different NaCl concentrations. Growth rate of callus decreased as the salinity levels were increased in the culture medium. There were highly significant differences among the tested genotypes and their hybrids in response to salt stress in callus growth rate. The results showed that callus growth rate and plant regeneration of hybrids were less decreased as salt concentration was increased compared with that of parents. Sakha-69 and hybrid (Sakha-69x Giza–157) were the most tolerant and scored high percentage in plant regeneration. Free proline content increased parallel to the increase of salinity. The results prove that the genetic differences in wheat salt tolerance could be tested early *in vitro* and the more the genotype is able to produce proline under the stress conditions, the more it will tolerate salinity.
Keywords: *Wheat, prediction, tissue culture, salt-tolerance, plant regeneration, proline.*

Introduction

Wheat production in the arid areas is confronted with several kinds of stresses. Salinity of soil and /or irrigation water is among them. Salinity is a major factor limiting crop productivity in arid and semi-arid areas of the world.

For many years, *in vitro* culture of plant tissues has been a useful tool to study salt tolerance mechanisms at the plant cell level. The salt tolerance mechanisms involved at the whole plant level could however be quite different from those involved at the plant cell level and an ontogenic evolution of salt tolerance was clearly demonstrated (Adams et al., 1992).

Plant cell culture technology has potential application for selecting cell tolerant to salts in the culture medium and from these tolerant cells regenerate plants which are more resistant to the salts than parental materials. Many laboratories have reported on the selection of NaCl-tolerant callus lines (Yang *et al.*, 1990). Plant regeneration trait is a good parameter to evaluate the effect of salinity on genotypes rather than grown calli

(Abdel- Hady *et al.*, 2001). However, Belkhodja (2000) stated that proline is admitted since a long time by various searchers as a marker of the stress tolerant plant.

The present investigation was conducted to predict salt tolerant calli and plants in wheat using tissue culture techniques.

Materials and Methods

This study was carried out in Botany Departments, National Research Centre, Dokki, Cairo, Egypt.

Plant material

Three wheat genotypes "Sakha-69, Giza-157, Gemmiza-3 and their crosses "Sakha-69 x Giza –157", " Sakha-69 X Gemmiza-3", and "Giza-157 x Gemmiza-3" were used.

Tissue culture conditions

Callus cultures for the three genotypes and their hybrids (F_1) were induced from mature embryos following procedures outlined by Ozias-Aktins and Vasil (1983). Mature embryos were rinsed in 70% ethanol for one minute, sterilized in clorox (2.25% sodium hypochlorite) for 5 minutes and washed with sterile distilled water for several times. The grains then were soaked in sterile water for 16 hours, after which mature embryos were excised and cultured with the scutellum in contact with medium. The culture medium contained the inorganic components of Murashige and Skoog medium (1962), plus 2 mg/l 2,4-dichlorophenoxyacetic acid (2, 4-D), 3% sucrose, 150 mg/l L-asparagine, 0.5 mg/l thiamine- HCl, 250 mg/l myo-inositol, 1g/l casein hydrolysate and 0.8% agar were used in ten replicates (Jars). The medium was adjusted to pH 5.8 and autoclaved for 15 minutes at 121°C. and the cultures were incubated at 23°C. with photoperiod of light/dark (16/8 h.). Callus was subcultured at 4 weeks intervals until enough callus weight was obtained to start growing on the stress media. The percentage of callus induction defined as the embryos forming over the total number of embryos and callus fresh weight (mg) was recorded.

Salt stress

Salt stress studied were carried out on calli of the three genotypes and their hybrids (F_1) using the same medium supplemented with different concentrations of NaCl (4000, 8000 and 12000 ppm). Ten jars of each genotype and their hybrids were used for each NaCl level.

The salt tolerant calli were transferred to regeneration medium (MS) containing 0.1 mg/l 2,4-D and free-hormone for root formation. Percentage of plantlets formation was calculated. Free proline content was determined colorimetrically in fresh plantlets as described by Bates *et al.* (1973).

The fresh weight of callus grown on NaCl containing media was determined by weighing callus before and after four weeks. The rate of increase in weight to the original fresh weight, before and after four weeks was calculated according to Bhaskaran et al. (1983).

Statistical analysis

The experimental design was complete randomized blocks. Analysis of variance and L.S.D. values were estimated according to Wynne *et al*. (1970).

Results and Discussion

Effect of genotypes

Data presented in Table (1) show the percentage of callus induction and callus fresh weight (mg) of the three tested genotypes and their hybrids of wheat from mature embryos. No significant difference was detected in callus induction between Sakha-69 and Giza-157, but there was significant difference between Sakha-69 and Gemmiza –3. Hybrids showed that the highest callus induction was 95% in (SK-69 x G-157), while the lowest was 70% in (G157 x Gem-3). A significant difference in callus induction was detected between (SK-69x G-157) and (G-157 x Gem-3). It could be concluded that the tested genotypes of wheat and their hybrids differed in their ability to callus induction. Similar results were obtained by Castillo *et al*. (1998) in barley.

Table(1): Effect of wheat genotypes on callus induction(%)and callus fresh weight (mg).

Genotypes	Callus induction %	Callus fresh weight (mg)
Sakha- 69	90.0	437.6
Giza-157	85.0	391.3
Gemmiza –3	65.0	339.6
Sak-69 x G –157	95.0	623.9
Sak-69 x Gem-3	85.0	563.4
G157 x Gem-3	70.0	431.1
L.S.D. 5%	22.22	61.23
L.S.D. 1%	29.92	88.19

On the other hand, there were no significant differences among the three tested genotypes for callus fresh weight except that between Sakha-69 and Gemmiza-3 which was highly significant. Sakha –69 gave the highest value (437.6 mg), while Gemmiza-3 had the lowest one (339.6 mg). Hybrid (Sakha-69 x G-157) scored the highest callus fresh weight over the other hybrids and highly significantly differed from (Giza-157 x Gemmiza-3). Our results agreed with those of Barakat and Abdel-Latif (1995).

Effect of salinity on callus growth rate

Table (2) and Figure (1) show the effect of salinity treatment on callus growth rate of the three genotypes and their hybrids (F_1). All salinity treatments highly significantly decreased the growth rate of the tested genotypes and their hybrids.

Table (2) : Effect of salinity on callus growth rate (%) of three wheat genotypes and their hybrids

Genotypes	Salinity level (ppm) NaCl				Genotypes means
	0	4000	8000	12000	
Sakha –69	204.7	182.0	113.2**	37.3**	134.3
Giza-157	192.3	151.0**	107.2**	23.2**	118.4
Gemmiza –3	173.0	105.6**	81.8**	16.5**	94.2
Sak-69 x G-157	326.3	301.3	148.9**	69.7**	211.6
Sak-69 x Gem-3	288.3	213.0**	135.7**	51.7**	172.2
G-157 x Gem-3	253.1	198.1**	123.2**	33.1**	151.9
Treatment means	239.6	191.8**	118.3**	38.6**	

L.S.D.	5%	1%
Genotypes (G)	10.9	13.3
Salinity (S)	15.1	18.6
G x S	27.3	33.8

The average of growth rate on untreated MS medium (0.0 ppm NaCl) gave the highest growth rate (239.6%), while the lowest one was recorded for the level of 12000 ppm NaCl (38.6%). The percentages of increase were 239.6, 191.8%, 118.3% and 38.6% at 0.0, 4000, 8000 and 12000 ppm NaCl, respectively.

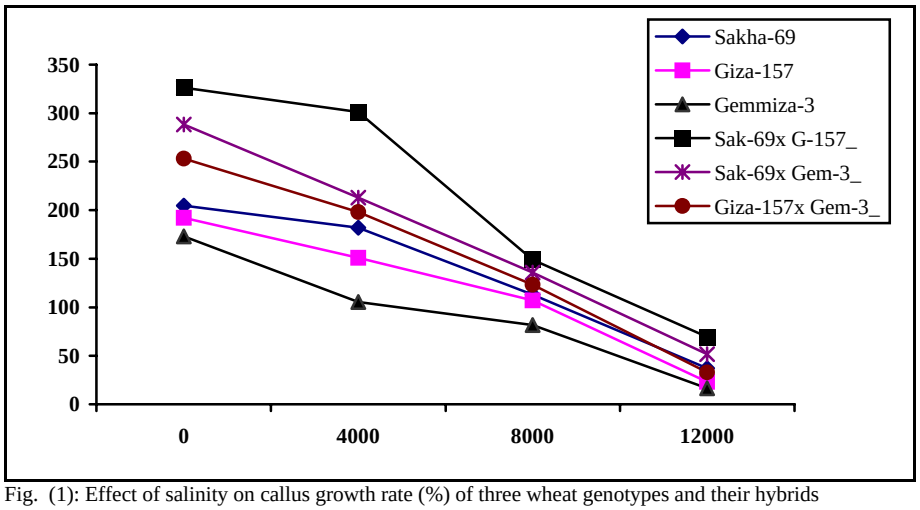

Fig. (1): Effect of salinity on callus growth rate (%) of three wheat genotypes and their hybrids

Among the genotypes, Sakha-69 was the most tolerant for salinity and gave the highest growth rate value (134.3%), while Gemmiza-3 was the most sensitive genotype to salinity scoring the lowest growth rate (94.2%).

All hybrids scored higher averages in callus growth rate than its parents. The hybrid (Sakha-69 x Giza-157) produced the highest growth rate (211.6%) over the other two hybrids, while (Giza-157 x Gemmiza-3) had the lowest rate (151.9%). So, the tested genotypes and their hybrids differed in their ability to tolerate high salinity depending on the genetic make up of callus. Similar results were obtained by Rashed (1989), El-Hennawy (1996), Abdel-Hafez *et al.* (1999) and Abdel –Hafez and Hamad (2000) who reported that callus weight of hybrids was less decreased as salt concentration was increased compared with that of parents.

Effect of salinity on plant regeneration

Salinity treatments gradually decreased the ability of callus to induce plantlets, where the plant regeneration means highly significantly decreased from 57.8% (control) to 26.4% and 16.1% for 8000 and 12000 ppm NaCl, respectively (Table 3 and Figure 2). Also, there was a reduction at 4000 ppm NaCl, but not enough to reach the level of significance. These results reflect that the plant regeneration trait is an essential parameter to evaluate the effect of salinity *in vitro* on enotype rather than grown calli.

The ability of regeneration for tested genotypes and their hybrids decreased with increase of salinity level from 0 and up to 12000 ppm NaCl (Table 3).So, Sakha-69 and hybrid (Sakha-69 x Giza –157) were most tolerant and gave high means 39.7% and 53.9% in plant regeneration, respectively.

Table (3) : Effect of salinity on plant regeneration (%) of three wheat genotypes and their hybrids.

Genotypes	Salinity level (ppm) NaCl				Genotypes means
	0	4000	8000	12000	
Sakha –69	62.5	46.1	31.3**	18.8**	39.7
Giza-157	56.3	41.8	18.3**	12.5**	32.2
Gemmiza –3	43.8	31.3	12.5**	6.3**	23.5
Sak-69 x G-157	66.1	74.2	43.8**	31.3**	53.9
Sak-69 x Gem-3	60.5	50.4	33.5**	19.0**	40.9
G-157 x Gem-3	57.3	42.8	18.8**	8.5**	31.9
Treatment means	57.8	17.8	22.4**	12.1**	

L.S.D.	5%	1%
Genotypes (G)	7.3	9.7
Salinity (S)	11.0	14.5
G x S	22.0	29.1

It could be concluded that the tested genotypes and their hybrids differed in their ability to tolerate high salinity depending on the genetic make up of the callus. Collins *et al.* (1990) and Abdel-Hady *et al.* (2001) showed that regeneration in wheat was severely depressed in cell line grown on high level of NaCl.

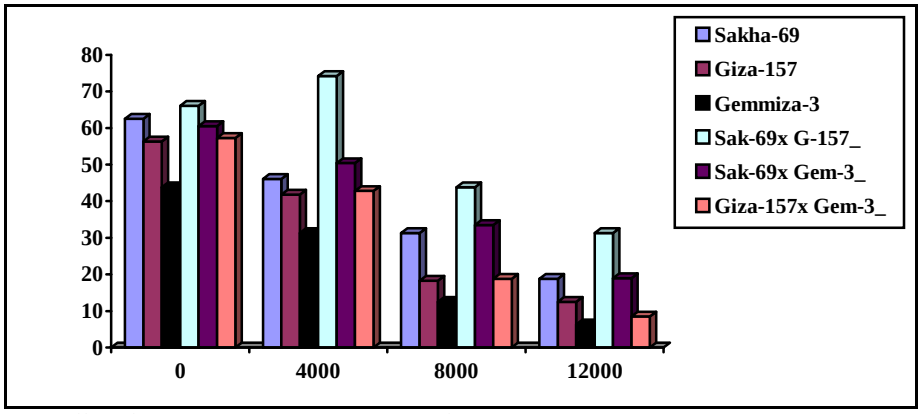

Fig.2: Effect of salinity on plant regeneration (%) of three wheat genotypes and their hybrids.

On the other hand, the interaction between genotypes and salinity was highly significant as shown in Table (3). Plant regeneration on medium containing 0.0 ppm NaCl produced the highest percentage in all genotypes and their hybrids. At the level of 4000 ppm NaCl, there were reductions in plant regeneration ability, but not enough to reach the significant level except in hybrid (Sakha-69x Giza –157), where it was increased by 8.1% as compared to control. The hybrid (Sakha-69 x Giza-157) gave the highest percentage of plant regeneration (74.2%) over all genotypes and their hybrids. At the level of 8000 ppm NaCl, there were significant decreases in plant regeneration of all genotypes and their hybrids as compared to the control. Both (Sk-69 x G-157) and (SK-69 X Gem-3) hybrids scored the highest percentage 43.8% and 33.5%, respectively over the other genotypes and their hybrids, although these percentages of plant regeneration were reduced as compared to control. At the highest level of 12000 ppm NaCl, there were highly significant decrease in plant regeneration as compared to control. Hybrid (Sak-69x G-157) was the most tolerant hybrid and able to regenerate (31.3%) on the highest level of salinity 12000 ppm NaCl while, (G-157 x Gem –3) gave the lowest regeneration (8.5%).

It was clear from the results obtained that prediction for salt tolerance on genotypes could be early measured in tissue culture. Similar results were obtained by Abdel-Hafez and Hamad (2000) and Abdel-Hady *et al.*(2001).

Proline content

Proline content in the stressed calli was different from the non-stressed. There were highly significant increases from 0.54 (control) to 1.31, 2.56 and 3.85µ Mole /g F.W. for 4000, 8000 and 12000 NaCl, respectively. Both Sakha-69 and hybrid (Sak-69 x G-157) were the most tolerate and gave the highest content of proline 2.38 and 3.27 µmol/g F.W. over the other genotypes and their hybrids. Results in Table (4) revealed

that the interaction between genotypes and salinity levels in relation to proline content was highly significant and proline content increase by increasing salinity level. The three genotypes (Sak-69, G-157 and Gem-3) gave increment in proline content, but not enough to reach significant level at 4000 ppm NaCl. The hybrid (Sak-69 x G-157) scored the highest proline content (6.17 μ Mole/g F.W.) at 12000 ppm NaCl , while Gem-3 had the lowest content (1.75 μ Mole/g F.W.) at the same level.

The aforementioned results supported the conclusion that proline was more accumulated in the salt-tolerant genotype, and may be useful as a possible salt injury sensor in wheat. This variation of proline could be useful in selection for salt-tolerance and used as a marker of salt tolerant
plants. Similar results were obtained by Shen and Shen (1992). They observed that under high NaCl concentrations, the percentage of free proline in total amino acids markedly increased in barley seedlings. Abdel- Hady *et al.* (2001) reported that the free proline is a good parameter to evaluate the effect of salinity.

Table (4): Free proline content (μ Mole/g) fresh weight of three wheat genotypes and their hybrids as influenced by NaCl levels

Genotypes	Salinity level (ppm) NaCl				Genotypes means
	0	4000	8000	12000	
Sakha –69	0.65	1.31	2.82**	4.75**	2.38
Giza-157	0.53	1.09	2.14**	3.23**	1.75
Gemmiza –3	0.48	0.96	1.23**	1.75**	1.11
Sak-69 x G-157	0.60	2.21**	4.09**	6.17**	3.27
Sak-69 x Gem-3	0.55	1.63**	3.00	4.17**	2.34
G-157 x Gem-3	0.42	0.67	2.06**	3.05**	1.55
Treatment means	0.54	1.31**	2.56	3.85**	

L.S.D.	5%	1%
Genotypes (G)	0.308	0.41
Salinity (S)	0.462	0.61
G x S	0.924	1.23

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التنبؤ بالقدرة على تحمل الملوحة فى القمح باستخدام مزارع الأنسجة

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قسم النبات – المركز القومي للبحوث – الجيزة – مصر

أجرى هذا البحث بهدف التعرف على إمكانية استخدام تكنيك مزارع الأنسجة فى التنبؤ بقدرة التحمل للملوحة وذلك بزراعة الأجنة الناضجة من ثلاثة أصناف من القمح والهجن بينها على بيئة موراشيج وسكوج مضافاً إليها تركيزات مختلفة من كلوريد الصوديوم (4000، 8000، 12000 جزء فى المليون) وتتلخص أهم النتائج المتحصل عليها فيما يلى:

1- أظهرت النتائج وجود معنوية مرتفعة فى نقص معدل نمو الكالس فى الأصناف تحت الدراسة والهجن الناتجة منها بزيادة مستويات الملوحة.
2- أظهرت النتائج وجود نقص فى معدل نمو الكالس وإنتاج النباتات فى الهجن الناتجة بزيادة مستويات الملوحة بنسبة أقل من الآباء.
3- سجل الصنف سخا 69 والهجين (سخا 69 × جيزة 157) أعلى نسبة فى إنتاج النباتات المتحملة للملوحة.
4- أوضح التحليل الإحصائى وجود فروق معنوية عالية فى تجمع البرولين فى كالس الأصناف والهجن الناتجة منها بينما أعطى الهجين (سخا 69 × جيزة 157) أعلى تجمع للبرولين فى جميع معاملات الملوحة تحت الدراسة.

يتضح من ذلك إمكانية استخدام تقنية زراعة الأنسجة كأختبار مبكر لمساعدة المربى على التنبؤ بقدرة الأصناف والهجن على تحمل الملوحة.