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Genetic Selection for Salt Tolerance in Some Egyptian Wheat Genotypes (*Triticum aestivum* L.) *via* Tissue Culture

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Key words: Triticum aestivum, Callus induction, Plant regeneration

Abstract

Five wheat genotypes and their hybrids under four salinity (sea water) levels were considered for tissue culture and randomly amplified polymorphic DNA (RAPD). The genotypes and the hybrids differed in their ability to callus induction, callus fresh weight and regeneration. Among the genotypes, Sakha 93 (P_1) followed by Line WB19 (P_5) was the most tolerant genotypes for salinity and gave the highest growth rate (46.6%) and (46.3%), respectively while Giza 168 (P_3) was the most sensitive one to salinity with lowest growth rate (26.6%). All hybrids scored higher averages in callus growth rate than their parents. $P_1 \times P_5$ followed by $P_1 \times P_2$ and $P_1 \times P_4$ produced the highest growth rate 75.6, 59.1 and 52.6% over hybrids while $P_3 \times P_4$ had the lowest rate 28.5%, respectively. The hybrid $P_1 \times P_5$ gave the highest percentage of plant regeneration over all genotypes and their hybrids followed $P_2 \times P_5$, $P_1 \times P_2$ and $P_4 \times P_5$. The highest number of RAPD specific markers scored for hybrid $P_1 \times P_5$ was (6 markers), while Line WB19 (P_5), $P_1 \times P_2$ and $P_2 \times P_5$ were (4 markers). These markers can be verified as the RAPD markers associated with salt tolerance.

Introduction

Wheat is considered as a main staple food crop for more than one third of the world population and the main food for Egypt. There is a huge shortage of wheat production in Egypt. More than 50 per cent wheat is imported for annual consumption. Due to extreme increase in population Egypt needs to increase its wheat production through its cultivation in the new reclaimed soils especially under saline conditions (Salam 2002).

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Salinity is one of the major factors responsible for low yield and restricted economic utilization of land and water resources both in arid and semi-arid regions of the world. Ninety five per cent of the cultivated bread wheat is hexaploid. Wheat is classified as a semi tolerant crop to salinity. One way to alleviate the problem is the breeding of salt tolerant genotypes that perform better than the existing sensitive varieties under moderate to high salinity stress (Arzani 2008).

Plant tissue culture technology has potential application for select on regenerated tolerant to salinity materials (Abdel-Hady 1999, Hala et al. 2012, Salma et al. 2013 and Mona Ismail 2014).

These markers can be verified as being genetic markers associated with salt tolerance in the three wheat genotypes and help in marker-assisted selection breeding program. Reda Moghaieb et al. (2011). The present investigation was carried out with the following objects to: (i) *In vitro*, selection of five wheat genotypes and their hybrids under different salinity levels, (ii) determine the genetic markers related to salt tolerance in wheat genotypes and their hybrids using randomly amplified polymorphic DNA (RAPD).

Materials and Methods

The present investigation was carried out in Biotechnology Research Group, Tissue Culture Laboratory, Botany Department, National Research Centre, El-Dokki, Giza, Egypt during the period from 2012 to 2015. Four local cultivars and one introduced line of bread wheat (*Triticum aestivum* L.) differing widely in agronomic traits were chosen as parents.

Mature embryos of Sakha 93, Gemmiza 10, Giza 168, Sids 12, Line WB19 and ten F_1s hybrids cultured on media containing four concentrations sea water. Callus cultures for the four genotypes, one introduced line and their F_1 were induced from mature embryos following procedures outlined as described by Ozias-Aktins and Vasil (1983).

Mature embryos were rinsed in 70% ethanol for one minute, sterilized in Clorox (2.25%) for 5 minutes and washed with sterile distilled water for several times. The grains then soaked in sterile water for 16 hrs. Mature embryos were excised and cultured with the scutellum in contact with medium.

The culture medium contained the inorganic components of (MS), plus 2 mg/l 2, 4-D, 3% sucrose, 150 mg/l L-asparagine, 0.5 mg/l thiamine-HCl, 250 mg/l myo-inositol, 1 g/l casein hydrolysate and 0.8% agar. The pH 5.8 was adjusted and autoclaved for 15 minutes at 121°C and the cultures were incubated at 23°C with photoperiod of light/dark (16/8 hrs). Callus was subcultured at four weeks

intervals until enough callus 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) as described by Abdel-Hady et al. (2003).

Salt stress was carried out on the calli of five genotypes and their F_1 using the same medium supplemented with different concentrations of sea water (control, 4000, 6000 and 8000 ppm). Ten jars of each genotype and their hybrids were used for each concentration of sea water. The salt tolerant calli were transferred to MS containing 0.1 mg/l 2, 4-D and free-hormone for root formation.

Percentage of plantlet formation was calculated as described by Abdel-Hady et al. (2003). The fresh weight of callus on different concentrations of sea water was determined before and after four weeks.

`Ten random primers were used to differentiate four parents Sakha 93 (P_1), Gemmiza 10 (P_2), Giza 168 (P_3), Line WB19 (P_5) and five hybrids $P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_5$, $P_2 \times P_5$ and $P_3 \times P_5$ by RAPD analysis.

`The tissue culture experiments were subjected to completely randomized design. Variance analysis of data was carried out using statistical package for the social sciences (SPSS) program. The differences among means for all treatments were tested for significance at 5% level by using DMRT. Means followed by the same letter are not significantly different at $p \leq 0.05$. Analysis of variance and LSD values were estimated according to Wynne et al. (1970).

Table 1. Names and sequences of the ten random primers used for RAPD analysis.

No.	Name	Sequence (5'..... 3')
1	OP-AQ15	TGC GAT GCG G
2	OP-AX06	AGG CAT CGT T
3	OP-C03	GGG GGT CTT T
4	OP-C10	TGT CTG GGT G
5	OP-G05	CTG AGA CGG A
6	OPM-05	GGG AAC GTG T
7	OPN-10	ACA ACT GGG G
8	OPQ-14	GGA CGC TTC A
9	OPN-13	AGC GTC ACT C
10	OPN-04	GAC CGA CCC A

Results and Discussion

Data showed that percentage of callus induction and callus fresh weight (mg) of the five genotypes Sakha 93 (P_1), Gemmiza 10 (P_2), Giza 168 (P_3), Line WB19 (P_5)

and their hybrids of bread wheat from mature embryos. Sakha 93 (P_1) gave the highest percentage (95), in callus induction and callus fresh weight (426.2 mg), followed by Line WB19 (P_5) 93% and (393 mg) respectively. Giza 168 (P_3) had the lowest one (79%) and (296.5 mg), respectively. Hybrids showed that the highest callus induction was 100% and (651.1 mg) on callus fresh weight was $P_1 \times P_2$ followed by hybrid $P_1 \times P_2$, while hybrid $P_3 \times P_4$ records the lowest one 79% and (443.8 mg), respectively. Similar results were obtained by Barakat and Abdel-Latif (1995), in wheat, Castillo et al. (1998) in barley, Abdel-Hady et al. (2001), Abdel-Hady et al. (2003), Abdel-Hady et al. (2007) in wheat and Salama et al. (2013) in wheat.

Effect of salinity treatments on 0, 4000, 6000 and 8000 ppm/l sea water on callus growth rate of the genotypes and their F_1s shown in (Figs 1, 2). However, all salinity treatments highly decreased significantly on growth rate of the five tested genotypes and their hybrids compared with control (0.0 ppm sea water). Among the genotypes, Sakha 93 (P_1) followed by Line WB19 (P_5) was the most tolerant for salinity and gave the highest growth rate of 46.6 and 46.3%, while Giza 168 (P_3) was the most sensitive to salinity scoring the lowest growth rate (26.6%).

All hybrids scored higher averages in callus growth rate than their parents. $P_1 \times P_5$ followed by $P_1 \times P_2$ and $P_1 \times P_4$ producing the highest growth rate 75.6, 59.1 and 52.6% over the other hybrids while $P_3 \times P_4$ had the lowest rate 28.5%.

The tested genotypes and their hybrids differed in their ability to tolerate high salinity depending on the genetic makeup of callus. Similar results were reported by El-Hennawy (1996) who found that the rate of growth of callus decreased as the salinity level increased. Abdel-Hady (1999), Abdel-Hady et al. (2001), Abdel-Hady et al. (2003) and Abdel-Hady et al. (2010) reported that there were highly significant differences among the genotypes and their hybrids in response to salt stress in callus growth rate in wheat.

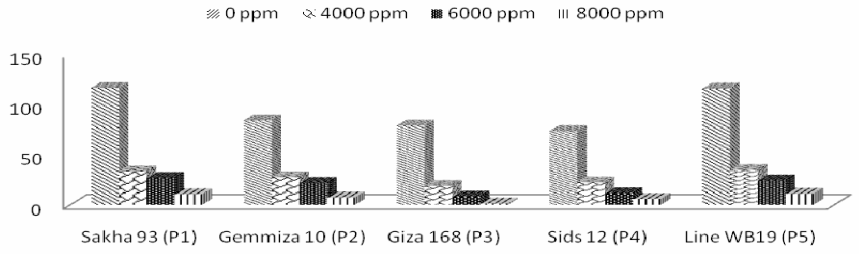

Fig. 1. Effect of different salinity concentration (sea water) on callus growth (%) for five parents wheat genotypes.

Salinity treatments gradually decreased the ability of callus to induce plantlets from 52.4 (Control) to 38.2, 20.9 and 11.6% for 4000, 6000 and 8000 ppm sea water, respectively (Table 2 and Fig. 3).

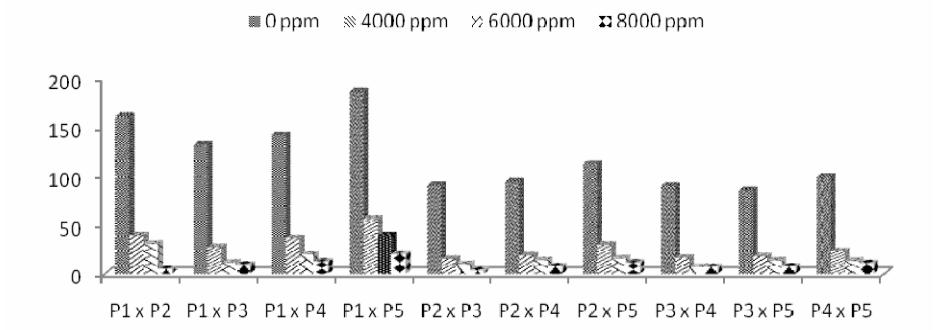

Fig. 2. Effect of different salinity concentration (sea water) on callus growth (%) for 10 hybrids.

The ability of regeneration for genotypes and their hybrids decreased with increase of salinity level from 0 and up to 8000 ppm sea water (Table 2). So, Sakha 93 (38.5%) followed by Line WB19 (37.1%) and $P_1 \times P_5$, $P_2 \times P_5$, $P_1 \times P_2$, $P_1 \times P_4$ and $P_4 \times P_5$ were most tolerant and gave high means 57.0, 41.0, 37.7 and 33.1% in plant regeneration, respectively. It could be concluded that the genotypes and their hybrids differed in their ability to tolerate high salinity depending on the genetic makeup of the callus. On the other hand, the interaction between genotypes, their hybrids and salinity was highly significant.

Plant regeneration on medium containing 0.0 ppm sea water produced the highest percentage in all genotypes and their hybrids.

At 4000 ppm sea water, there was reductions in plant regeneration ability. $P_1 \times P_5$ gave the highest percentage of plant regeneration (65.4%) over all genotypes followed by $P_2 \times P_5$, $P_1 \times P_2$ and $P_4 \times P_5$ scoring 44.6, 41.4 and 36.2%, respectively.

At 6000 ppm sea water, there was significant decrease in plant regeneration of all genotypes and their hybrids compared to control. Sakha 93 (P_1), Line WB19 (P_5) and $P_1 \times P_5$, $P_2 \times P_5$ scored the highest percentage 30.6, 29.4, 50.1 and 37.6, respectively over the other genotypes and their hybrids.

At the highest level of sea water concentration 8000 ppm, there were highly significant decrease in plant regeneration compared to control.

Sakha 93 × Line WB19 was the most tolerant hybrid, followed by Gemmiza 10 × Line WB19 and able to regenerate 33.6, 20.8%, respectively while hybrid Gemmiza 10 × Giza 168 gave the lowest regeneration (6.2%). Similar results were obtained by Collin et al. (1990), Abdel-Hady et al. (2001), Abdel-Hady et al. (2003) and Salama et al. (2013).

Table 2. Effect of different salinity concentrations (sea water) on plant regeneration (%) of five wheat genotypes and their hybrids.

Parents	Salinity levels (ppm)				Parents mean
	0	4000	6000	8000	
Sakha 93 (P_1)	60.5	46.1	30.6*	16.6**	38.5
Gemmiza 10 (P_2)	54.2	41.8	18.7**	9.9**	31.2
Giza 168 (P_3)	42.1	29.2	12.5**	6.3**	22.5
Sids 12 (P_4)	46.9	30.4	13.1**	8.2**	24.7
Line WB19 (P_5)	58.3	43.6	29.4**	17.1**	37.1
Mean	52.4	38.2*	20.9**	11.6**	
Crosses					Hybrids mean
P_1 x P_2	61.8	41.4	28.1**	19.5**	37.7
P_1 x P_3	46.1	29.2	19.9*	9.3**	26.1
P_1 x P_4	55.6	37.6	26.4**	12.7**	33.1
P_1 x P_5	78.7	65.4	50.1*	33.6**	57.0
P_2 x P_3	37.3	26.1	15.7	6.2**	21.3
P_2 x P_4	44.8	31.5	19.3*	8.3**	26.0
P_2 x P_5	60.7	44.6	37.6**	20.8**	41.0
P_3 x P_4	39.8	27.3	16.2	10.9	23.6
P_3 x P_5	46.1	32.7	20.3*	15.3**	28.6
P_4 x P_5	49.2	36.2	25.2*	13.5**	31.0
Mean	52.0	37.2**	25.9**	15.0**	
L.S.D	0.05	0.01			
Genotypes	7.3	9.7			
Salinity	11.0	14.5			
G × S	22.0	29.1			

*and **significant at 0.05 and 0.01 probability, respectively.

Ten random primers were used to differentiate between four parents Sakha 93 (P_1), Gemmiza 10 (P_2), Giza 168 (P_3), Line WB19 (P_5) from their five $P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_5$, $P_2 \times P_5$ and $P_3 \times P_5$ by RAPD analysis. The primers produced multiple bands with a number of amplified DNA fragments ranging from 8 (primer OP-G05) to 19 (primer OP-A × 06), as shown in Table 3 and Fig. 4.

The total number of reproducible fragments amplified by the ten primers reached 130 bands, from which 101 were polymorphic indicating high level of polymorphism (77.71%). The highest number of RAPD bands were detected for primers OP-AX06, OPM-05 and OP-AQ15 (19, 15, 16 bands respectively polymorphic bands ranged from 50.00% primer OPG05 to 88.89% (primer OPN-

04). Similar results were obtained by Cao et al. (1999), Freitas et al. (2000), Maric et al. (2004) and Guadognulo et al. (2001).

Fig. 3. Effect of different salinity concentrations (sea water) on plant regeneration (%) for five hybrids.

The four parents and five hybrids can be uniquely identified (fingerprinted) with genotype-specific RAPD markers as shown in Fig. 4 and Tables 3, 4. The highest number of unique markers was observed in Sakha 93 (P_1) × Line WB19 (P_5) which scored six unique markers at molecular size (MS) of 861.77 bp, 956.31 bp and 1242 bp of primer OP-AQ15, AX06 and OPN-10 respectively, beside 639, 672 and 1214.73 bp of primer OPN-13, OPN-13 and OP-C10, respectively.

Sakha 93 (P_1) × Gemmiza 10 (P_2) showed four unique markers at band no.3 of primer OPM-05 at MS of 242 bp, band number 10 of primer OP-C03 at MS of 478.37 bp, band number 10 of primer OPN-13 at MS of 639 bp and also at band number 7 of primer OP-AX06 at MS

Gemmiza 10 (P_2)× Line WB19 (P_5) gave four unique markers at band no. 6 of primer OP-AX06 at MS 173.6 bp, band number 7 of primer OP-AQ15 at MS 651.10 bp, band no. 18 of primer OPQ-14 at MS 1560 bp and band no.8 of primer OPM-05 at MS 262 bp, respectively.

Giza 168 (P_3) x Line WB19 (P_5) scored two unique markers both at band no.8 of primer OP-AX06 at MS of 517.91 bp, band no. 5 of primer OPM-05 at MS 161 bp. Sakha 93 (P_1) gave three unique markers; band no. 9 of primer OP-AQ15 at MS 697.18 bp, band no. 2 of primer OP-C10 at MS 650 bp and band no. 12 of primer OP-C03 at MS 517.97 bp.

Gemmiza 10 (P_2) and Giza 168 (P_3) gave one unique marker at band no.5 and no.6 of primer OPQ-14 and primer OP-C10 at MS 1790 bp and 517.97 bp, respectively.

Table 3. Genotype specific RAPD markers.

Genotypes	Markers	Monomorphic bands
Sakha 93 (P_1)	OP-AQ15 (697.18), OP-C10 (650), OP-C03 (517.97).	3
Gemmiza 10 (P_2)	OPQ-14 (1790).	1
Giza 168 (P_3)	OP-C10 (517.97).	1
Line WB19 (P_5)	OP-AX06 (895.94), OPQ-14 (2726), OPM-05 (373), OPN-4 (821.50).	4
Hybrids		
$P_1 \times P_2$	OPM-05 (242), OP-C03 (478.37), OPN-13 (639), OP-AX06 (89.97).	4
$P_1 \times P_3$	OP-AQ15 (651.10).	1
$P_1 \times P_5$	OP-AQ15 (861.77), AX06 (956.31), OPN-10 (1242), OPN-13 (639), OPN-13 (672), OP-C10 (1214.73).	6
$P_2 \times P_5$	OP-AX06 (173.6), OP-AQ15 (651.10), OPQ-14 (1560), OPM-05 (262).	4
$P_3 \times P_5$	OP-AX06 (517.91), OPM-05 (161).	2
Total		25

On the other hand, Line WB19 (P_5) showed that four unique markers; band no. 5 of primer OP-AX06 at MS 895.94 bp, band no. 6 of primer OPQ-14 at MS 2726 bp, band no. 11 of primer OPM-05 at MS 373 bp and band no. 9 of primer OPN-04 at MS 821.50 bp, respectively.

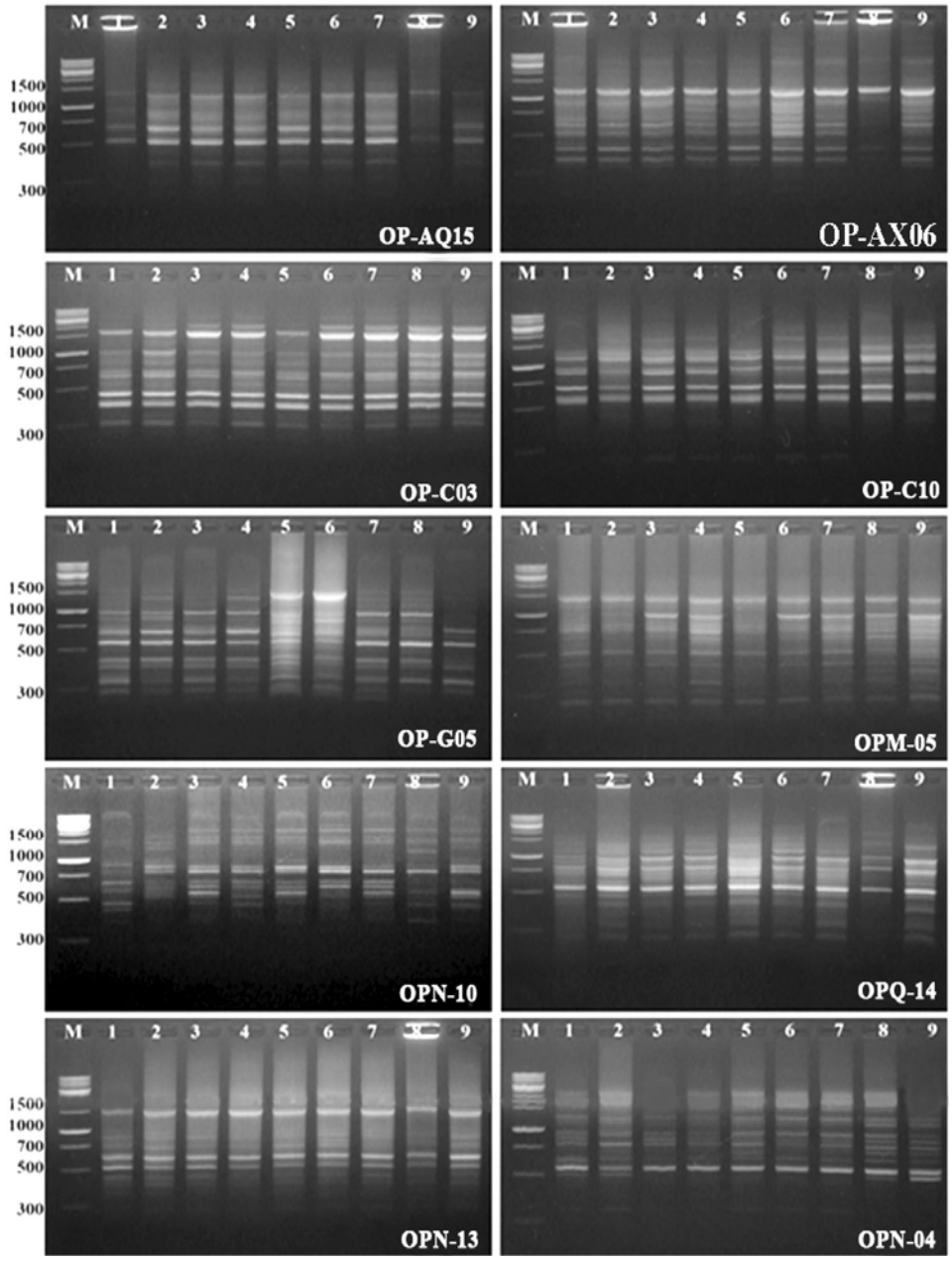

Fig. 4. DNA polymorphism of four genotypes and five hybrids using randomly amplified polymorphic DNA with ten primers 1 - Sakha 93 (P_1), 2 - Gemmiza 10 (P_2), 3 - Giza 168 (P_3), 4 - Line WB19 (P_5), 5 - ($P_1 \times P_2$), 6 - ($P_1 \times P_3$), 7 - ($P_1 \times P_5$), 8 - ($P_2 \times P_5$) and 9 - ($P_3 \times P_5$).

These results indicated that RAPD-PCR markers gave adequate distinctions among all the four parents and five hybrid tested.

Table 4. Primers used in RAPD analysis and their number of bands.

No.	Primer name	Number of scarable bands	Polymorphic bands	Polymorphism (%)
1	OP-AQ15	16	14	87.5
2	OP-AX06	19	13	78.95
3	OP-C03	11	6	54.55
4	OP-C10	9	6	66.67
5	OP-G05	8	4	50.00
6	OPM-05	18	14	77.78
7	OPN-10	15	13	86.67
8	OPQ-14	13	8	61.54
9	OPN-13	12	10	81.25
10	OPN-04	9	8	88.89
Total		130	98	75.38

These unique genotypes specific bands may be associated with some important economic traits such as grain yield and earliness, which could be useful for wheat breeding program. Similar results by Abdel-Tawab et al. (1998), Abdel-Tawab et al. (2001), Mitra et al. (2009), Reda Moghaieb et al. (2011), Aida Rizkalla et al. (2012) the identification markers could introduce a great benefit for breeding wheat without waiting for yield evaluation and could be used as markers assisted selection in breeding and Khavarinejad (2014) showed that analysis of genetic similarity or diversity is fundamental to wheat breeding. The most polymorphism information content (PIC) value and percentage was detected by UBC 350 and UBC 109 with values of 0.53 and 0.50.

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