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Development of RAPD and ISSR Markers Associated with Salt Tolerance in Bread Wheat using *in Vitro* Culture

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ABSTRACT

Ten double haploid (DH) genotypes and five varieties of bread wheat were grown in high salinity soil and then germinated in Petri dishes under different NaCl concentrations. Five DH genotypes and two varieties (Giza 168 and Sakha 93) that were characterized by their high salinity tolerance were selected to use as sources of mature embryos to produce calli. the growing calli were transferred to callus culture medium and salinized with NaCl to a final concentration of 0, 0.9 or 1.2%. Two of the five selected DH genotypes and the two wheat varieties successfully induced calli under salinity stress. RAPD analysis using three random primers showed that 19 of 34 total amplified fragments were polymorphic with 58.3% polymorphism under 0.9 and 1.2% Nacl, whereas 66.7% polymorphism using primer B-08 was higher than that observed for the other two primers, B-05 and B-11 (61.5 and 46.7%, respectively). The four wheat genotypes revealed 11 specific markers for salinity tolerance with 38.5% polymorphism. ISSR analysis using four different primers for salinity tolerance revealed 31 polymorphic fragments with 58.4% polymorphism from a total of 56 amplified fragments under salinity stress. The four wheat genotypes revealed 22 specific markers for salinity tolerance with 41% polymorphism, whereas Sakha 93 and DH-1 had the highest number of salinity-stress specific markers at both NaCl concentrations. RAPD and ISSR techniques are useful methods to detect specific markers for salinity tolerance and could be used in marker-assisted selection (MAS) of wheat breeding programs to predict the most tolerant genotypes.

Keywords: double haploid lines, *in vitro* selection, NaCl, PCR-markers

INTRODUCTION

Soil salinity is one of the major abiotic stresses reducing agricultural productivity. The levels of salt that negatively affect plant growth affect large areas of the world. It is estimated that more than a third of all of the irrigated land in the world is presently affected by salinity. This is exclusively in regions classified as arid and desert lands, which comprise 25% of the total land of our planet. The loss of arable farmland due to salinization is directly in conflict with the needs of the world population which is projected to increase by 1.5 billion in the next 20 years (Blumwald *et al.* 2004). Therefore, increasing the yield of crop plants in optimal soils and in less productive lands, including salinized lands, is essential for feeding the world (Flowers *et al.* 2000). One way of increasing productivity in stressful environments is to breed crops more tolerant to stress. However, success in breeding for tolerance has been limited because tolerance to stress is controlled by many genes and their simultaneous selection is difficult (Flowers *et al.* 2004), complexity of the several tolerance mechanisms involved, tremendous effort is required to eliminate undesirable genes that are also incorporated during breeding (Richards 1996) and there is a lack of efficient selection procedures particularly under field conditions (Ribaut *et al.* 1997).

DNA molecular markers based on PCR such as random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSRs) have become excellent tools for plant breeders (Lima-Brito *et al.* 2006). In contrast to RAPD amplification, the ISSR markers are more feasible and reproducible, and the distribution of ISSRs in the eukaryotic genome makes them highly informative (Bornet *et al.* 2002). Reddy *et al.* (2002) confirmed that ISSR is a simple, cost-efficient, robust, multilocus marker method which is extremely useful in determining genetic variability.

RAPD and ISSR markers have proved to be the most polymorphic markers in wheat and hence are highly useful markers for various applications in wheat (Roder *et al.* 1998). Apart from using them in diversity analysis, ISSR markers have been shown to be associated with various agronomically important traits such as leaf rust resistance (Feuillet *et al.* 1995), pre-harvest sprouting tolerance (Roy *et al.* 1999), protein content (Prasad *et al.* 1999), kernel traits (Campbell *et al.* 1999) and seed size in wheat (Ammi Raju *et al.* 2001). RAPD markers have also shown to be associated with various traits contributing to kernel hardness in bread wheat (Galande *et al.* 2001). Molecular markers can be used for selection of linked traits of agronomic importance which would increase the efficiency and precision of breeding and thus molecular breeding will become a common agricultural practice in coming years. The aim of the present study was to develop RAPD and ISSR markers associated with salt tolerance in calli of bread wheat genotypes.

MATERIALS AND METHODS

Plant material

Hexaploid bread wheat (*Triticum aestivum* L.) materials used in this study were comprised of 10 double haploid (DH) genotypes (DH-1 through DH-10) that were kindly provided by Dr. Tarek Hewezi from Laboratoire de Biotechnologie et d'Amélioration des Plantes (BAP), Castanet Tolosan, France and five varieties (Giza 157, Giza 168, Sakha 93, Sakha 8, Sids 1) provided by the Agricultural Research Center, Giza, Egypt.

Selection of salinity-tolerant wheat genotypes

Wheat materials were grown in high salinity soil with 8.35 EC ds

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m^{-1} and under ground saline water with 4000 to 6000 ppm soluble salts at the Experimental Station of the National Research Center, El-kalyobia governorate from November 2006 through March 2007. The row length was 2.5 m and rows were 30 cm apart with hills spaced at 10 cm allowing a total of 25 plants/row. The wheat genotypes were then germinated in Petri dishes on Whatman paper in the lab under different NaCl concentrations (0, 0.9, 1.2, 1.5, 2.0 and 2.5%) for two weeks under a 16/8 h light/dark photoperiod, in order to determine the appropriate concentrations of NaCl and to verify the most salinity-tolerant genotypes, which will be used in callus induction and plant regeneration. From former field and lab experiments, five of ten DH genotypes i.e., DH-1 to DH-5 and two of five wheat varieties (Giza 168 and Sakha 93) that were characterized by their high salinity tolerance were selected to use as sources of mature embryos.

In vitro selection of salinity-tolerant cell lines

mature seeds were harvested from main spikes, surface-sterilized in 70% (v/v) ethanol for 10 min and in commercial bleach (5% sodium hypochlorite) for 20 min and then washed several times in sterile distilled water. They were aseptically excised from the caryopsis and placed with the scutellum upwards on solid agar medium in sterile Petri dishes for 14 days at 26 ± 1°C in continuous darkness. According to Birsin *et al.* (2001) and Rashid *et al.* (2002), the agar medium contained Murashige and Skoog (1962) (MS) mineral salts, 30 g/l sucrose, 2 mg/l 2,4-D and 7 g/l agar (Phyto-Tech. Laboratores, USA). The media was adjusted to pH 5.8 and autoclaved for 20 min at 121°C at 1.1 kg/cm^2. After callus induction, calli were subcultured at four-week intervals on fresh MS medium. The growing calli were transferred to callus culture medium and salinized with NaCl at final concentrations of 0, 0.9 and 1.2% (w/w). The calli were maintained on their respective treatments for two subcultures (4 weeks each) and incubated in darkness at 24°C. Two of the five selected double haploid genotypes and the two wheat varieties successfully induced calli (**Fig. 1**) under salinity stresses.

DNA extraction, RAPD and ISSR

DNA extraction using CTAB method was performed from the calli of four wheat genotypes according to Doyle and Doyle (1987). RAPD analysis was performed using three 10-mer random primers (Metabion, Martinsried, Germany) and ISSR primers were procured from Integrated DNA Technologies Inc. (San Diego, CA, USA) based on core repeats anchored at the 5′ or 3′ end as shown in **Table 1**.

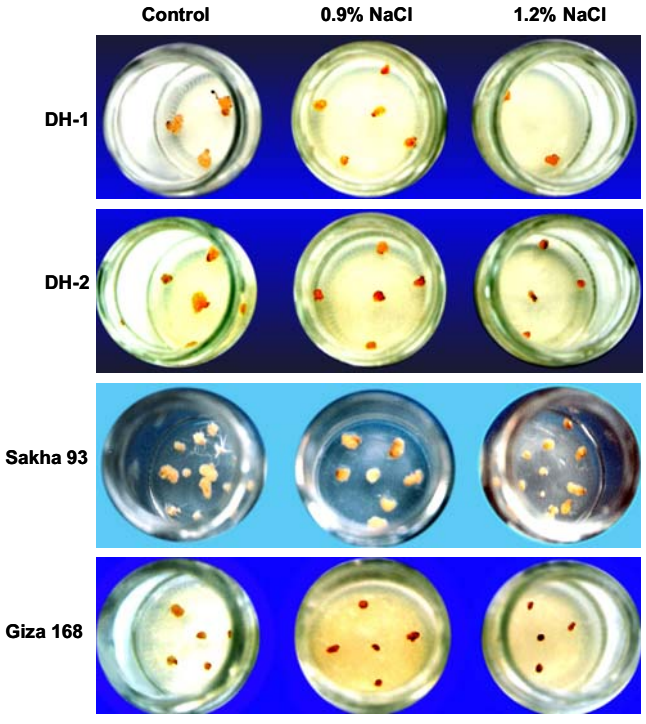

Fig. 1 Callus induction from mature seeds of four wheat genotypes under different NaCl concentrations (0, 0.9 and 1.2%).

Table 1 Names and sequences of RAPD and ISSR primers used for PCR analyses.

Primers	Name	Sequences
RAPD	B-05	5′-TGC GCC CTT C-′3
	B-08	5′-GTC CAC ACG G-′3
	B-11	5′-GTA GAC CCG T-′3
ISSR	17899-A	$(CA)_6$ AG
	814-B	$(CT)_8$ G
	HB-14	$(CTC)_3$ GC
	HB-15	$(GTG)_3$ G

Amplification reactions for RAPD and ISSR analyses were used in a final volume of 25 µl containing 10X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, pH 9.0), 2 mM dNTPs, 10 mM primer, 50 ng of template DNA and 0.5 U of *Taq* polymerase (Promega, USA). Reactions were performed in a thermocycler (Biometra, GMBH). RAPD-PCR was performed according to Williams *et al.* (1990) as one cycle of 94°C for 2.5 min (denaturation), 30 cycles of {94°C for 1 min, 37°C for 1 min and 72°C for 1 min (annealing)} and a final extension of 10 min at 72°C. ISSR amplification was performed according to Zietkiewiez (1994) with an initial denaturation of 2 min at 94°C, followed by 40 cycles of 94°C for 30 sec, annealing at 52°C for 45 sec, extension at 72°C for 2 min and a final extension at 72°C for 7 min. PCR products were analyzed using 1.2% agarose gel electrophoresis and visualized with 10 µg/µl ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 100 to 2000 bp (MBI, Fermentas).

RESULTS AND DISCUSSION

The results of callus induction and *in vitro* selection of salinity-tolerant genotypes were previously reported by Abdelsamad *et al.* (2007).

RAPD markers for salinity tolerance in wheat genotypes

A total of 34 scorable amplified DNA fragments ranging in size from 80 to 2000 base pairs were observed using the three primers: B-05, B-08 and B-11 whereas 19 fragments were polymorphic and the other amplified fragments were commonly detected among the four wheat genotypes at 0.9 and 1.2% NaCl. The three primers showed 58.3% mean polymorphism whereas the polymorphism of primer B-08 was higher (66.6%) than the other two primers B-05 and B-11 (61.5 and 46.7%, respectively) (**Table 2**).

Primer B-05 revealed 13 fragments with sizes ranged from 80 to 1200 bp (**Fig. 2, Table 2**), whereas eight fragments were polymorphic and the residual five fragments with molecular sizes 900, 800, 700, 500 and 180 bp regularly appeared among the four wheat genotypes at different NaCl concentrations.

Wheat genotypes treated with 0.9 and 1.2% NaCl exposed two induced amplified bands with 920 and 550 bp at each or at both of the two NaCl concentrations, which did not exist in the controls. For instance, the two induced bands were displayed at 0.9 and 1.2% NaCl in DH-2 and DH-1, respectively but were displayed at each of the two NaCl concentrations among the two other genotypes, whereas the 920 bp band was detected in Sakha 93 at 0.9% NaCl and the 550 bp band was identified in Giza 168 at 1.2% NaCl. primer B-08 revealed lower fragment numbers than the other two primers, where six fragments were detected with sizes ranging from 700 to 120 bp (**Fig. 2, Table 2**). Among them, four were polymorphic and the other two fragments with molecular sizes 290 and 120 bp were not affected by the two NaCl concentrations. Three of the four genotypes displayed one fragment with different molecular sizes at both NaCl concentrations. The 700 bp fragment was

Table 2 RAPD analysis of variable (polymorphic) bands for four wheat genotypes under 0, 0.9 and 1.2% NaCl using three random primers (B-05, B-08 and B-11).

Primer name	*P%	Bs (bp)	Giza 168			Sakha 93			DH-1			DH-2		
			0	0.9	1.2	0	0.9	1.2	0	0.9	1.2	0	0.9	1.2
B-05	61.5	1200							+					
		950	+	+		+	+	+	+					
		920	+	+			+						+	+
		550			+					+	+	+	+	+
		400	+	+	+	+	+	+	+			+	+	+
		300	+	+		+	+	+	+	+	+	+	+	+
		250	+	+	+	+	+	+	+	+		+	+	+
		80				+	+	+	+			+	+	+
	Variable bands = 8		5	5	3	5	6	5	6	3	2	5	6	6
	Total = 13		10	10	8	10	11	10	11	8	7	10	11	11
B-08	66.7	700				+	+	+		+	+			+
		550				+	+		+					+
		420		+	+	+	+	+	+	+	+	+	+	+
		200	+	+	+		+	+	+	+				
	Variable bands = 4		1	2	2	3	4	3	3	3	2	1	1	3
	Total = 6		3	4	4	5	6	5	5	5	4	3	3	5
B-11	46.7	2000	+	+	+	+	+	+		+	+		+	+
		1500			+					+		+	+	+
		1400	+	+	+	+	+			+	+	+	+	+
		600		+	+		+	+		+	+		+	+
		470	+	+		+	+		+	+	+	+	+	
		150					+	+	+	+	+			
		80	+	+	+	+	+	+	+	+	+	+	+	
	Variable bands = 7		4	5	5	4	6	4	3	7	6	4	6	4
	Total = 15		12	13	13	12	14	12	11	15	14	12	14	12
Total variable bands = 19 Overall total bands = 34			Mean polymorphic percentage of the three primers = 58.3*											

+ = Present of amplified bands, Bs = Molecular size by base pair, P% = Polymorphic percentage, Total = Total number of amplified bands.
* (61.5 + 66.7 + 46.7 = 174.9), then Average = 174.9/3 = 58.3%

displayed in DH-1, the 420 bp fragment in Giza 168 and the 200 bp fragment in Sakha 93 while two fragments, 700 and another unique 550 bp band appeared in DH-2 at 1.2% NaCl. Primer B-11 revealed 15 fragments with sizes ranging from 80 to 2000 bp (**Fig. 2, Table 2**) whereas seven fragments were polymorphic and the other eight fragments were not affected by the NaCl treatments and frequently appeared in the four wheat genotypes. RAPD analysis shows some amplified fragments that were newly synthesized due to different salinity stresses and that did not exist in the control. For example, one amplified 1500 bp band was induced at 0.9% NaCl in DH-1, and the same band induced at 1.2% NaCl in Giza 168 while four bands (2000, 1400, 600, and 150 bp) were induced at both 0.9 and 1.2% NaCl. In contrast, the 600 bp fragment appeared in all four wheat genotypes; the 2000 bp fragment appeared in DH-1 and DH-2. However, the 1400 and 150 bp fragments were observed in DH-1 and in Sakha 93, respectively (**Table 2**).

Among the 34 amplified bands, 19 were 58.3% polymorphic and among them 11 bands were specific markers for salinity tolerance either at 0.9 or at 1.2% NaCl with a total average percentage polymorphism of 38.5%. It is interesting to note that the four wheat genotypes varied considerably in their salinity tolerant markers using the three primers, whereas DH-1 revealed the highest number with six markers at 0.9% and five at 1.2% Nacl, followed by Sakha 93 with four markers at 0.9% Nacl and DH-2 with five markers at 1.2% Nacl. However, Giza 168 showed two marker bands at 0.9% and four at 1.2% NaCl. In addition, DH-1 and DH-2 revealed the highest total number of marker bands at both NaCl concentrations with 11 and eight markers, respectively followed by Sakha 93 with a total of seven markers and Giza 168 with six (**Table 4**).

RAPD analysis was used to detect molecular markers for salt tolerance in different plant species. For example, in tomato, Foolad and Chen (1998) identified RAPD markers associated with quantitative trait loci (QTLs) conferring salt tolerance during germination. Lee *et al.* (2003) identified two RAPD markers in salt-tolerant rice lines and the transcript involved in the marker showed a higher expression in the salt-tolerant lines than the sensitive lines. Rao *et al.* (2007) identified distinct RAPD patterns for salinity tolerance with the presence of specific amplified products in some sorghum accessions. The results obtained from RAPD analysis in the present study are in agreement with those of Iqbal *et al.* (2007), who emphasized that RAPD analysis provides a rich source of markers for use in wheat improvement and can be used for characterizing and grouping wheat genotypes, which will be helpful in wheat breeding programs.

ISSR markers for salinity tolerance in wheat genotypes

A total of 56 amplified fragments ranging in size from 60 to 2310 bp were observed using the four ISSR primers (17899-A, 814-B, HB-14 and HB-15) whereas 31 fragments were polymorphic and the other amplified fragments were commonly detected among the four wheat genotypes under different salinity stresses (**Table 3**). The four ISSR primers exhibited a mean polymorphic percentage of 58.4%, whereas the polymorphic percentage of primer 814-B was higher (81.8%) than the other three primers (HB-14, 17899-A and HB-15): 66.7, 50 and 35%, respectively.

The primer 17899-A pattern revealed 10 fragments with molecular sizes ranging from 60 to 950 bp (**Fig. 3**) whereas five fragments were polymorphic and the other five were common among the four wheat genotypes (**Table 3**). The four wheat genotypes treated with 0.9 and 1.2% NaCl revealed four distinct induced amplified bands either at high NaCl concentration or at both NaCl concentrations, which did not exist in the controls. For example, Giza 168 exhibited a 275 bp band at 1.2% NaCl while the other three genotypes showed four fragments at 0.9 and 1.2% NaCl. Sakha 93 showed two bands (950 and 890 bp), DH-1 revealed two bands (890 and 275 bp) and DH-2 showed one amplified band (500 bp).

The results of ISSR analysis using primer 814-B showed

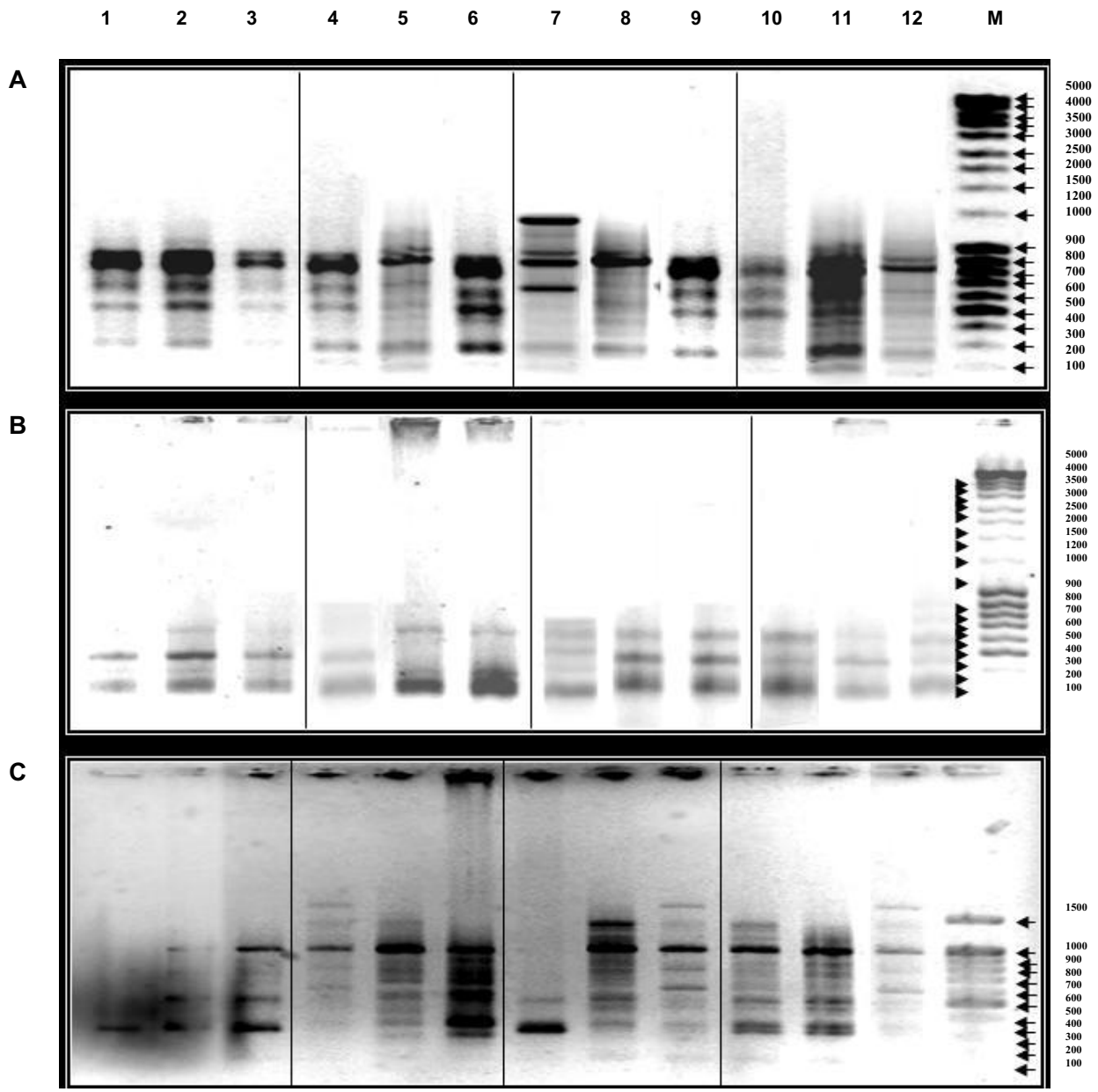

Fig. 2 RAPD amplified products of the calli for four wheat genotypes. Giza 168, Sakha 93, DH-1 and DH-2 at 0, 0.9 and 1.2% NaCl using three random primers: B-05 (**A**), B-08 (**B**) and B-11 (**C**).

11 amplified fragments with molecular sizes ranging from 110 to 1400 bp (**Fig. 3, Table 3**), whereas nine fragments were polymorphic and the residual two fragments ordinary appeared among the four wheat genotypes under all NaCl concentrations. Five induced amplified bands that did not exist in the control were displayed in all four genotypes. for instance, in giza 168 two of the three induced bands (600 and 320 bp) appeared only at 1.2% NaCl while one band (850 bp) appeared at 0.9 and 1.2% NaCl and disappeared in the control. In Sakha 93, three bands (800, 600 and 320 bp) appeared at 0.9 and 1.2% NaCl and disappeared in the control. DH-1 and DH-2 produced two bands at both NaCl concentrations whereas DH-1 displayed 700 and 320 bp bands at 0.9% NaCl while DH-2 showed one of the two induced bands at 1.2% NaCl and the other band at 0.9 and 1.2% NaCl (**Table 3**).

Primer HB-14 revealed 15 fragments with molecular sizes ranging from 80 to 2310 bp (**Fig. 2**). Ten fragments were polymorphic with 66.7% polymorphism while the other residual five fragments were commonly detected among the four wheat genotypes under different NaCl concentrations (**Table 3**). Among the 10 polymorphic fragments, eight were induced under different NaCl concentrations while four bands were affected by salinity stress and consequently disappeared either at the low or high NaCl concentrations. The four genotypes were characterized based on the induced bands which emerged at different NaCl concentrations whereas DH-1 was characterized by four induced amplified bands, three of them which were unique (2310, 1300 and 1130 bp). DH-2 was characterized by three induced amplified bands, one of them which was unique (170 bp) while Sakha 93 was characterized by two induced bands, one of them unique (950 bp) while Giza 168 was characterized by two induced bands (700 and 200 bp).

ISSR analysis using primer HB-15 showed the highest numbers of amplified fragments compared with the other three ISSR primers. Primer HB-15 revealed 20 detectable amplified fragments with molecular sizes ranging from 120 to 2000 bp (**Fig. 3, Table 3**). Seven fragments were polymorphic and the residual 13 fragments ordinarily appeared among the four wheat genotypes under all three NaCl concentrations. Five induced bands were displayed among the four genotypes under salinity stress that had not existed in the control. for instance, at both 0.9 and 1.2% NaCl, Giza 168 and DH-2 showed two induced bands with different molecular sizes in each genotype; Giza 168 showed two unique bands (2000 and 700 bp) while DH-2 showed two unique bands (1060 and 210 bp). DH-1 showed one band

Table 3 ISSR analysis of the variable (polymorphic) bands for four wheat genotypes at 0, 0.9 and 1.2% NaCl using four ISSR primers (17899-A, 814-B, HB-14 and HB-15).

ISSR primers	P%	Bs (bp)	Giza 168			Sakha 93			DH-1			DH-2		
			0	**0.9**	**1.2**	**0**	**0.9**	**1.2**	**0**	**0.9**	**1.2**	**0**	**0.9**	**1.2**
17899-A	50	950					+	+	+			+	+	
		890	+	+	+		+	+		+	+			
		680	+	+	+	+	+	+	+					
		500											+	+
		275			+	+	+	+		+	+	+	+	+
	Variable bands = 5		2	2	3	2	4	4	2	2	2	2	3	2
	Total = 10		7	7	8	7	9	9	7	7	7	7	8	7
814-B	81.8	1400				+	+					+		
		1000				+	+					+	+	+
		850		+	+	+	+	+	+	+	+	+	+	+
		800					+	+						+
		700								+				
		600			+		+	+				+	+	
		320			+		+	+		+			+	+
		190	+	+	+	+	+	+	+	+		+	+	+
		110	+	+	+	+	+	+	+			+	+	+
	Variable bands = 9		2	3	5	5	8	6	3	4	1	6	6	6
	Total = 11		4	5	7	7	10	8	5	6	3	8	8	8
HB-14	66.7	2310								+				
		1650	+			+	+	+	+	+	+	+	+	+
		1300	+	+	+	+	+			+	+	+	+	+
		1130	+	+	+						+	+	+	+
		950					+	+	+	+	+	+	+	+
		850	+	+		+			+	+	+	+	+	+
		700		+	+	+	+	+	+	+	+			+
		400					+	+			+	+	+	+
		200			+				+					+
		170												+
	Variable bands = 10		4	4	4	4	5	4	5	6	7	6	6	9
	Total = 15		9	9	9	9	10	9	10	11	12	11	11	14
HB-15	35	2000		+	+	+								
		1300	+	+	+	+			+	+		+	+	
		1270	+	+	+	+	+		+	+		+	+	
		1060	+	+	+	+	+		+	+	+		+	+
		700		+	+	+	+	+		+	+	+	+	+
		520	+				+		+			+		
		210	+	+	+		+		+				+	+
	Variable bands = 7		5	6	6	5	5	1	5	4	2	4	5	3
	Total = 20		18	19	19	18	18	14	18	17	15	17	18	16
Total variable bands = 31 Overall total bands = 56							Mean polymorphic percentage of the primers = 58.4%*							

Total No. = Total number of amplified bands, Bs = Molecular size by base pair, + = Band presence, * = Presence of each amplified fragment.
* (50 + 81.8 + 66.7 + 35 = 233.5), then Average = 233.5/4 = 58.4%

(700 bp) at 0.9 and 1.2% NaCl. However, Sakha 93 displayed two unique induced bands with 520 and 210 bp only at 0.9% NaCl.

Among the 31 polymorphic bands, 22 were newly induced and considered as markers for salinity tolerance either at 0.9 or at 1.2% NaCl with 41% percentage polymorphism (**Table 4**). The four wheat genotypes varied considerably in their salt tolerance markers, whereas at 0.9% NaCl Sakha 93 revealed the highest number (nine) of markers, followed by DH-1 with seven, while both Giza 168 and DH-2 revealed four salinity markers. Moreover, at 1.2% NaCl, Giza 168 and DH-2, which displayed fewer markers at 0.9% revealed the highest number (eight marker bands), followed by DH-1 with 7 markers and then Sakha 93 with six (**Table 4**). On the other hand, ISSR primer HB-14 showed the highest marker percentage (53.3%), followed by primers 814-B and 17899-A with 45.5 and 40%, respectively while primer HB-15 displayed the lowest salinity marker percentage with 35% compared with the other three ISSR primers, as shown in **Table 4**.

ISSRs have been used for detection of polymorphism (Nagaoka and Ogihara 1997) and in genetic mapping of wheat (Kojima *et al*. 1998). They have been used to study genetic diversity and phylogenetic relationships (Qian *et al.* 2001), for DNA fingerprinting (Carvalho *et al.* 2005) and to identify markers associated with yellow berry tolerance in wheat (AmmiRaju *et al*. 2001). Segregation for salinity tolerance and ISSR markers based molecular polymorphism were investigated by (Kaushik *et al.* 2003) in the F_3 plants raised from a cross between salt-tolerant *indica* rice variety CSR10 and salt-susceptible Basmati rice variety Basmati HBC19. Four ISSR amplified bands were presented in only some of the CSR10 × HBC19 F_3 plants and such polymorphic bands stand greater chances of having a linkage with the genes/QTLs for salinity tolerance. Moreover, ISSR markers associated with salt tolerance in wheat (Lang *et al.* 2001) and drought tolerance in rice (Fahmy *et al.* 2007). In addition, the results in the present study confirmed that ISSR was the most useful method to detect molecular markers for salinity tolerance in wheat. This was confirmed by Hou *et al*. (2005) who studied the genetic diversity in barley from west China and reported that the ISSR markers were superior to RAPD markers in the capacity of revealing more informative bands in a single amplification. Similar ISSR results were detected in barley by Fernández *et al.* (2002) and Tanyolac (2003).

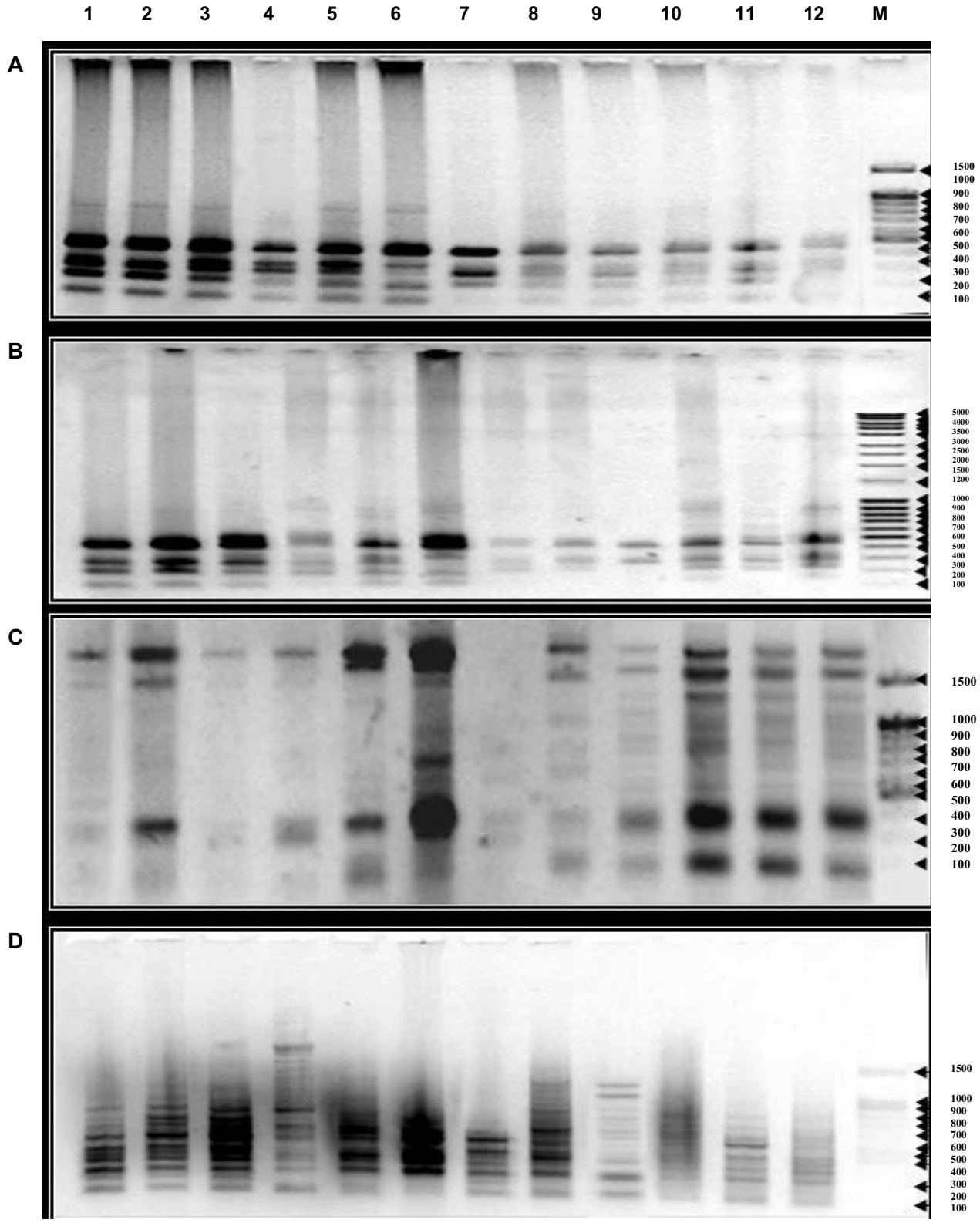

Fig. 3 ISSR amplified products of the calli for four wheat genotypes. Giza 168, Sakha 93, DH-1 and DH-2 at 0, 0.9 and 1.2% NaCl using four ISSR primers: 17899-A (**A**), 814-B (**B**), HB-14 (**C**) and HB-15 (**D**).

REFERENCES

Abdelsamad A, El-Sayed OE, Hayam FI (2007) Biochemical genetic markers assistant selection for *in vitro* salt tolerance in double haploid wheat genotypes. *Journal of Genetic Engineering & Biotechnology (NRC)* in press

Ammi Raju JSS, Dholakia BB, Santra DK, Singh H, Lagu MD, Tamhankar SA, Dhaliwal HS, Rao VS, Gupta VS, Ranjekar PK (2001) Identification of inter-simple sequence repeat (ISSR) markers associated with seed size in wheat. *Theoretical and Applied Genetics* **102**, 726-732

Birsin MA, Önde S, Özgen M (2001) Callus induction and plant regeneration from mature embryos of oat (*Avena sativa* L.). *Turkish Journal of Biology* **25**, 427-434

Blumwald E, Grover A, Good AG (2004) *Breeding for Abiotic Stress Resistance: Challenges and Opportunities*, 4th International Crop Science Congress, Brisbane, Australia, 26 Sep–1 Oct 2004

Bornet BC, Muller FP, Branchard M (2002) Highly informative nature of inter simple sequence repeat (ISSR) sequences amplified using tri- and tetranucleotide primers from DNA of cauliflower (*Brassica oleracea* var. *botrytus* L.). *Genome* **45**, 890-896

Campbell KG, Bergman CJ, Gaulberto DG, Anderson JA, Giroux MJ, Hareland G, Gulch RG, Sorrells ME, Finney PL (1999) Quantitative trait associated with kernel traits in a soft × hard wheat cross. *Crop Science* **38**,

Table 4 RAPD and ISSR markers for salinity tolerance in wheat genotypes at 0.9 and 1.2% NaCl.

Marker type	Primer name	P%	№ of markers with size (bp)	Giza 168		Sakha 93		DH-1		DH-2	
				0.9	1.2	0.9	1.2	0.9	1.2	0.9	1.2
RAPD	B-05	15.4	920			+				+	+
			550		+			+	+		
			Total = 2	0	1	1	0	1	1	1	1
	B-08	66.6	700					+	+		+
			550								+
			420	+	+						
			200			+	+				
			Total = 4	1	1	1	1	1	1	0	2
	B-11	33.3	2000					+	+	+	+
			1500		+			+			
			1400					+	+		
			600	+	+	+	+	+	+	+	+
			150			+	+				
			Total = 5	1	2	2	2	4	3	2	2
Total marker bands = 11				2	4	4	3	6	5	3	5
Mean polymorphism (%) = 38.5*											
ISSR	17899-A	40	950			+	+				
			890			+	+	+	+		
			500							+	+
			275		+			+	+		
			Total=4	0	1	2	2	2	2	1	1
	814-B	45.5	850	+	+						
			800			+	+				+
			700					+			
			600		+	+	+				
			320		+	+	+	+		+	+
			Total = 5	1	3	3	3	2	0	1	2
	HB-14	53.3	2310					+			
			1300					+	+		
			1130						+		
			950			+	+				
			700	+	+						+
			400			+	+		+		
			200		+						+
			170								+
			Total=8	1	2	2	2	2	3	0	3
	HB-15	25	2000	+	+						
			1060							+	+
			700	+	+			+	+		
			520			+					
			210			+				+	+
			Total = 5	2	2	2	0	1	1	2	2
Total marker bands = 22				4	8	9	7	7	6	4	8
Mean polymorphism (%) = 41**											

P% = Polymorphic percentages of marker bands, Total = Total number of markers, + = Presence of marker bands.
* (15.4 + 66.7 + 33.3 = 115.4), then Average = 115.4/3 = 38.5%
** (40 + 45.5 + 53.3 + 25 = 163.8), then Average = 163.8/4 = 41%

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Carvalho A, Matos M, Lima-Brito J, Guedes-Pinto H, Benito C (2005) DNA fingerprint of F1 interspecific hybrids from the *Triticeae* tribe using ISSRs. *Euphytica* **143**, 93-99

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Photochemical Bulletin* **19**, 11-15

Fahmy EM, Doma AA, Hameed AMA, El-Sayed OE, El-Enany MAM (2007) RAPD and ISSR markers related to drought tolerance in rice. *Egyptian Journal of Genetics and Cytology* **32**, 43-63

Fernández ME, Figueiras AM, Benito C (2002) The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theoretical and Applied Genetics* **104**, 845-851

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust locus in wheat (*Triticum aestivum* L.). *Molecular and General Genet*ics **248**, 553-562

Flowers TJ (2004) Improving crop salt tolerance. *Journal of Experimental Bot*any **55**, 307-319

Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR (2000) QTL: their place in engineering tolerance of rice to salinity. *Journal of Experimental Bot*any **51**, 99-106

Foolad MR, Chen FQ (1998) RAPD markers associated with salt tolerance in an interspecific cross of tomato (*Lycopersicon esculentum* × L. *pennellii*). *Plant Cell Reports* **17**, 306-312

Galande AA, Tiwari R, Ammi Raju JSS, Santra DK, Lagu MD, Rao VS, Gupta VS, Misra BK, Nagarajan S, Ranjekar PK (2001) Genetic analysis of kernel hardness in bread wheat using PCR-based markers. *Theoretical and Applied Genetics* **103**, 601-606

Hou YC, Yan ZH, Wei YM, Zheng YL (2005) Genetic diversity in barley from west China based on RAPD and ISSR analysis. *Barley Genetics Newsletter* **35**, 9-22

Iqbal A, Khan AS, Khan IA, Awan FS, Ahmad A, Khan AA (2007) Study of genetic divergence among wheat genotypes through random amplified polymorphic DNA. *Genetic and Molecular Research* **6**, 476-481

Kaushik A, Saini N, Jain S, Rana P, Singh RK, Jain RK (2003) Genetic analysis of a CSR10 (indica) × Taraori Basmati F3 population segregating for salt tolerance using ISSR markers. *Euphytica* **134**, 231-238

Kojima T, Nagaonka T, Noka K, Ogijhara Y (1998) Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theoretical and Applied Genetics* **96**, 37-45

Lang NT, Li Z, Buu BC (2001) Microsatellite markers linked to salt tolerance in rice. *Omonrice* **9**, 9-21

Lee IS, Kim DS, Lee SJ, Song HS, Lim YP, Lee YI (2003) Selection and characterizations of radiation-induced salinity tolerant lines in rice. *Breeding Science* **53**, 313-318

Lima-Brito J, Carvalho A, Marttin A, Heslop-Harison JS, Guedes-Pinto H (2006) Morphological, yield, cytological and molecular characterization of a bread wheat × tritordeum F1 hybrid. *Journal of Genetics* **85**, 123-131

Murashige T, Skoog FA (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and Applied Genetics* **94**, 597-602

Prasad M, Varshney RK, Kumar A, Balyan HS, Sharma PC, Edwards KJ, Singh H, Dhaliwal HS, Roy JK, Gupta PK (1999) A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. *Theoretical and Applied Genetics* **99**, 341-345

Qian W, Ge S, Houng DY (2001) Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics* **102**, 440-449

Rao MVS, Kumari PK, Manga V, Mani NS (2007) Molecular markers for screening salinity response in *Sorghum*. *Indian Journal of Biotechnology* **6**, 274-276

Rashid H, Abdul Ghani R, Chaudhry Z, Naqvi SMS, Quraishi A (2002) Effect of media, growth regulators and genotypes on callus induction and regeneration in wheat (*Triticum aestivum*). *Biotechnology* **1**, 49-54

Reddy MP, Sarla N, Siddiq EA (2002) Title of paper/poster. *4th International Rice Genetics Symposium*, IRRI, Philippines, p 212 (Abstract)

Ribaut JM, Jiang C, de Leon GD, Edmeades GO, Hoisington DA (1997) Identification of quantitative trait loci under drought conditions in tropical maize: Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics* **94**, 887-896

Richards RA (1996) Defining selection criteria to improve yield under drought. *Plant Growth Regulation* **20**, 157-166

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Laroy P, Genai MW (1998) A micorsatellite map of wheat. *Genetics* **149**, 2007-2023

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of a microsatellite on chromosome 6B and a STS on 7D of dread showing association with preharvest sprouting tolerance. *Theoretical and Applied Genetics* **99**, 336-340

Tanyolac B (2003) Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum vulgare* subsp. *spontaneum*) populations from west Turkey. *Genetic Resources and Crop Evolution* **50**, 611-614

Williams JCK, Kubelik AR, Livak, KJ, Rafalski, JA and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531-6535

Zietkiewiez E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**, 176-183