

## GENETIC RELATIONSHIPS OF SOME EGYPTIAN AND YEMENI WHEAT VARIETIES DETECTED BY RAPD AND ISSR MARKERS

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### ABSTRACT

Two types of molecular markers, Randomly Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR), were assayed to determine the genetic diversity of four hexaploid Egyptian wheat varieties [(Giza 164, Giza 168, Sakha 93 and Sakha 94) (*Triticum aestivum*)], and four tetraploid Yemen's wheat genotypes [Behows 14, Smara'a, Mysany and Saba'a (*Triticum durum*)]. In RAPD analysis, six used primers displayed RAPD profiles with polymorphic bands. A total of 44 bands out of 68 bands for the Egyptian and Yemeni varieties were polymorphic (64.3% of polymorphism) consist of DNA fragments with molecular weights ranged from 112 to 1170 base pairs. The Yemeni genotypes were distinguished via OP-A 23 and HB-11 primers that generated two bands at molecular weight of 103.22 and 374.16 base pairs, respectively. The Egyptian varieties were distinguished via OP-A 10 and HB-12 primers that generated two bands at molecular weight of 483.98 and 707.79 base pairs, respectively. In ISSR analysis, six used primers generated variable banding patterns with amplified bands ranged from 44 to 1119 base pairs. Thirty five bands out of 71 bands were polymorphic for the Egyptian varieties and Yemeni genotypes with 49.3% of polymorphism. Primer 19-B gave a specific marker for the Yemeni genotypes with molecular weight of 247.40 base pairs. While primer HB-10 gave a specific marker for the Egyptian varieties with molecular weights of 202.69 and 200.00 base pairs. Dendrogram was constructed for the tested cultivars using UPGMA algorithm based on RAPD and ISSR analysis. The dendrogram indicated that the Egyptian varieties were grouped in one cluster and the Yemeni ones were separated in the other cluster. The present study showed that RAPD and ISSR analyses are useful tools for generating candidate specific markers of genetic characterization and distinguishing between the tetraploid and hexaploid wheat genotypes and necessity for protection of Egyptian royalties of the landraces.

Key Words: *Wheat, Genetic diversity, Molecular markers, RAPD marker, ISSR marker.*

### INTRODUCTION

Wheat is considered as the most important gramineae plants. The past limitation associated with pedigree data and morphological, physiological and cytological markers for assessing genetic diversity in cultivated and wild plant species have largely been circumvented by the development of DNA markers such as Random Amplified Polymorphic DNAs (RAPD) (Williams *et al* 1990). This method has proven to be useful for germplasm

identification and elucidation of genetic relationships of numerous plant cultivars and species (Williams *et al* 1990; Halward *et al* 1992 and Levi and Rowland 1997).

Molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations; different markers might reveal different classes of variations (Russell *et al* 1997). The generated DNA polymorphism reflects both the distance between two annealing sites and the pattern of their distribution throughout the genome of a particular cultivar or species (Williams *et al* 1990).

The advent of the Polymerase Chain Reaction (PCR) favored the development of different molecular techniques such as RAPD and Inter-Simple Sequence Repeat polymorphic DNA (ISSR) etc. (Nagaoka and Ogihara 1997). RAPDs proved to be useful as genetic markers in the case of self pollinating species with a relatively low level of intra-specific polymorphism, such as tetraploid, hexaploid wheat (Joshi and Nguyen 1993 and Ramiz *et al* 2007) and cultivated barley (Tinker *et al* 1993). RAPD and ISSR techniques do not require the use of radioactive materials and are simple to use and able to detect a significant degree of polymorphism (Williams *et al* 1990). Many authors referred to the validity of RAPD analysis for taxon characterization. In this regard, Gregor *et al* (1994) used RAPD analysis to fingerprint some *Prunus domestica*. They stated that comparing DNA from various cultivars of the same species have proved the reliability of this method. Also, Dollo *et al* (1990) suggested that RAPD analysis can provide a new alternative for cultivar identification and classification, and can be effectively incorporated into breeding programs. ISSR markers, have become good DNA molecular markers for research on populations of the same species, and could be used to assess genetic diversity (Qian *et al* 2001). ISSRs have been used for cultivar identification in maize (Pejic *et al* 1998), potatoes (Prevost and Wikinson 1999), wheat (Ammiraju *et al* 2001), and barley (Tanyolac 2003).

It is clear that numerous species-specific markers are required for documentation of the Egyptian and Yemeni wheat genotypes. This study demonstrated that molecular markers are needed to identify wheat cultivars and differentiate between the Egyptian and Yemeni wheat varieties. DNA molecular markers have received considerable attention in the recent years. ISSR marker has been utilized in gramineae plants to identify markers associated with seed size in wheat (Ammiraju *et al* 2001) and fingerprinting in rice (Blair *et al* 1999) as well as in other crops, e.g., in fingerprinting cashew (Archak *et al* 2003).

The objective of this study was to detect molecular genetic fingerprints and to determine the genetic relationships for four hexaploid Egyptian and

four tetraploid Yemeni wheat varieties using two types of molecular markers; RAPD and ISSR.

## MATERIALS AND METHODS

### Plant materials

This study was carried out at the Genetic Department, Faculty of Agriculture, Ain Shams University, during 2008. Four hexaploid Egyptian wheat varieties were kindly provided by Field Crop Research Institute, Agricultural Research Center (ARC). Other four tetraploid Yemeni genotypes were kindly provided by Faculty of Agriculture, Sana'a University. The names and ploidy levels of these genotypes are listed in Table 1.

**Table 1. Names and ploidy levels of the hexaploid Egyptian and tetraploid Yemeni wheat genotypes.**

No.	Egyptian varieties	Ploidy level	No.	Yemeni genotypes	Ploidy level
1	Giza 164	Hexaploid	5	Behows 14	Tetraploid
2	Giza 168	Hexaploid	6	Smara'a	Tetraploid
3	Sakha 92	Hexaploid	7	Mysany	Tetraploid
4	Sakha 71	Hexaploid	8	Saba'a	Tetraploid

### Extraction of genomic DNA

The DNA was extracted from grains of the eight Egyptian and Yemeni wheat entries using DNeasy plant Mini Kit (QIAGEN).

### RAPD-PCR Analysis

PCR amplification was performed using six random 10-mer arbitrary primers synthesized by Operon biotechnologies, Inc. These primers and their nucleotides sequences are listed in Table 2. Amplification was conducted in 20 µl reaction volume containing the following reagents: 2.0 µl of dNTPs (2.0 mM), 2.0 µl of MgCl<sub>2</sub> (2.0 mM), and 2.0 µl of 10x buffer, 3.0 µl of primer (1.0 pmol), 3.0 µl of DNA template (20 ng/µl), 1 µl of *Taq* polymerase (1 U/µl) and 10.0 µl of sterile dd H<sub>2</sub>O. The DNA amplifications were performed in an automated thermal cycle (model Techno 012) programmed for one cycle at 94°C for 5 min followed by 40 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. The reaction was finally stored at 72°C for 10 min. Amplified products were size-fractionated [using 100 bp ladder marker (Axygen Biosciences)] by electrophoresis in 1.0% agarose gels in TBE buffer at 120 V for 1 h. The bands were visualized by ethidium bromide under UV fluorescence and were photographed.

### ISSR-PCR Analysis

Selected primers based on dinucleotide, tetranucleotide or pentanucleotide repeats were used for ISSR analysis. PCR amplification was performed using six ISSR primers that produced clear and reproducible bands. These primers and their nucleotide sequences are listed in Table 2. Amplification was conducted in 20 µl reaction volume containing the following reagents: 2.0 µl of dNTPs (2.0 mM), 2.0 µl of MgCl<sub>2</sub> (2.0 mM), and 2.0 µl of 10x buffer, 3.0 µl of primer (1.0 pmol), 3.0 µl of template DNA (20 ng/µl), 1 µl of *Taq* polymerase (1 U/µl) and 12.0 µl of sterile dd H<sub>2</sub>O. The PCRs were programmed for one cycle at 94°C for 5 min to pre-denature, followed by 30 cycles of 1 min at 94°C, 1 min at 57°C, and 2 min at 72°C. The reaction was finally extension at 72°C for 5 min, and eventually stored at 4°C. Amplified products were electrophoresed in 1% agarose gel with 0.5x TBE buffer. After staining the gel with ethidium bromide, band patterns were visualized with an UV transilluminator. Fragments sizes were estimated with the 100 bp ladder DNA marker.

**Table 2. Primers and their nucleotide sequences used for RAPD and ISSR analyses.**

RAPD Primers			ISSR Primers		
No.	Name	Sequence	No.	Name	Sequence
1	OP-A-03	5' AGT CAG CCA C 3'	1	44-A	5' (GT) <sub>3</sub> GG 3'
2	OP-A-07	GAA ACG GGT G	2	89-B	(CA) <sub>3</sub> GT
3	OP-A-10	GTG ATC GCA G	3	99-B	(CA) <sub>3</sub> GG
4	OP-B-07	GGT GAC GCA G	4	HB-10	(GA) <sub>3</sub> CC
5	OP-B-11	GTAGACCCGT	5	HB-11	(GT) <sub>3</sub> CC
6	OP-B-12	CCT TGA CGC A	6	HB-12	(CAC) <sub>3</sub> GC

Data analysis for RAPD and ISSR fragments were scored for presence (+) or absence (-) by using the Phoretix 1D image analysis system (Phoretix International, London) to integrate the data. The similarity matrix and the dendrogram among the eight wheat genotypes were determined using Unweighted Pair Groups Method with Arithmetic average (UPGMA) cluster analysis program.

## RESULTS AND DISCUSSION

### Molecular genetic fingerprints of wheat genotypes

#### RAPD Analysis

Six random primers (OP-A<sup>1</sup>, OP-A<sup>2</sup>, OP-A<sup>3</sup>, OP-B<sup>1</sup>, OP-B<sup>2</sup> and OP-B<sup>3</sup>) were used in the present study to identify the tested eight wheat genotypes (Fig. 1). Thirty four monomorphic and twenty four polymorphic distinct fragments (41.3% of polymorphism) were detected (Table 3). The results of RAPD analysis showed that all used primers were polymorphic. OP-A<sup>2</sup> primer showed the highest polymorphism level among the tested cultivars (60% of polymorphism), while OP-A<sup>1</sup> primer showed the lowest polymorphism level (33.3% of polymorphism) (Fig. 3). The compiled data for the six used primers recorded 41.38% of polymorphism.

Some genotype(s)-specific markers were detected among the eight studied wheat cultivars as shown in Table 4. OP-A<sup>1</sup> primer gave a specific marker for Saba'a Yemeni genotype with molecular weight of 880.08 base pairs. Primer OP-A<sup>1</sup> and HB-11 primer gave two specific markers for the tetraploid Yemeni varieties with molecular weights of 103.22 and 274.16 base pairs, respectively. OP-A<sup>2</sup> and OP-A<sup>3</sup> primers showed two specific markers for Smara'a genotype with molecular weights of 1170.13 and 1072.12 base pairs, respectively. Also OP-A<sup>3</sup> and HB-12 primers showed two specific markers for the hexaploid Egyptian varieties with molecular weights of 483.98 and 707.79 base pairs, respectively. Finally, HB-12 primer showed two specific markers for Behows<sup>1</sup> with molecular weights of 362.20 and 221.23 base pairs. Shehata (2004) studied genotyping variability of some wheat cultivars including Giza<sup>1</sup> and Giza<sup>2</sup> using eight RAPD primers (OP B-1, -2, -3, 4 and OP I-1, -2, -3 and -4). A total of 64 polymorphic bands out of 86 ones were generated by these eight primers. OP I-1 primer generated four unique bands for Giza<sup>1</sup>. Abdollahi (2008) used 22 random operon primers of OPA, OPB, OPC and OPD series for exploring the polymorphism among some Indian wheat cultivars. OP A<sup>1</sup> primer generated only 26 polymorphic bands. Moreover, OP A<sup>2</sup> primer was used with other RAPD primers to generate polymorphic bands and distinguishing between some diploid and tetraploid wheat cultivars (Aliyev *et al* 2007).

#### ISSR Analysis

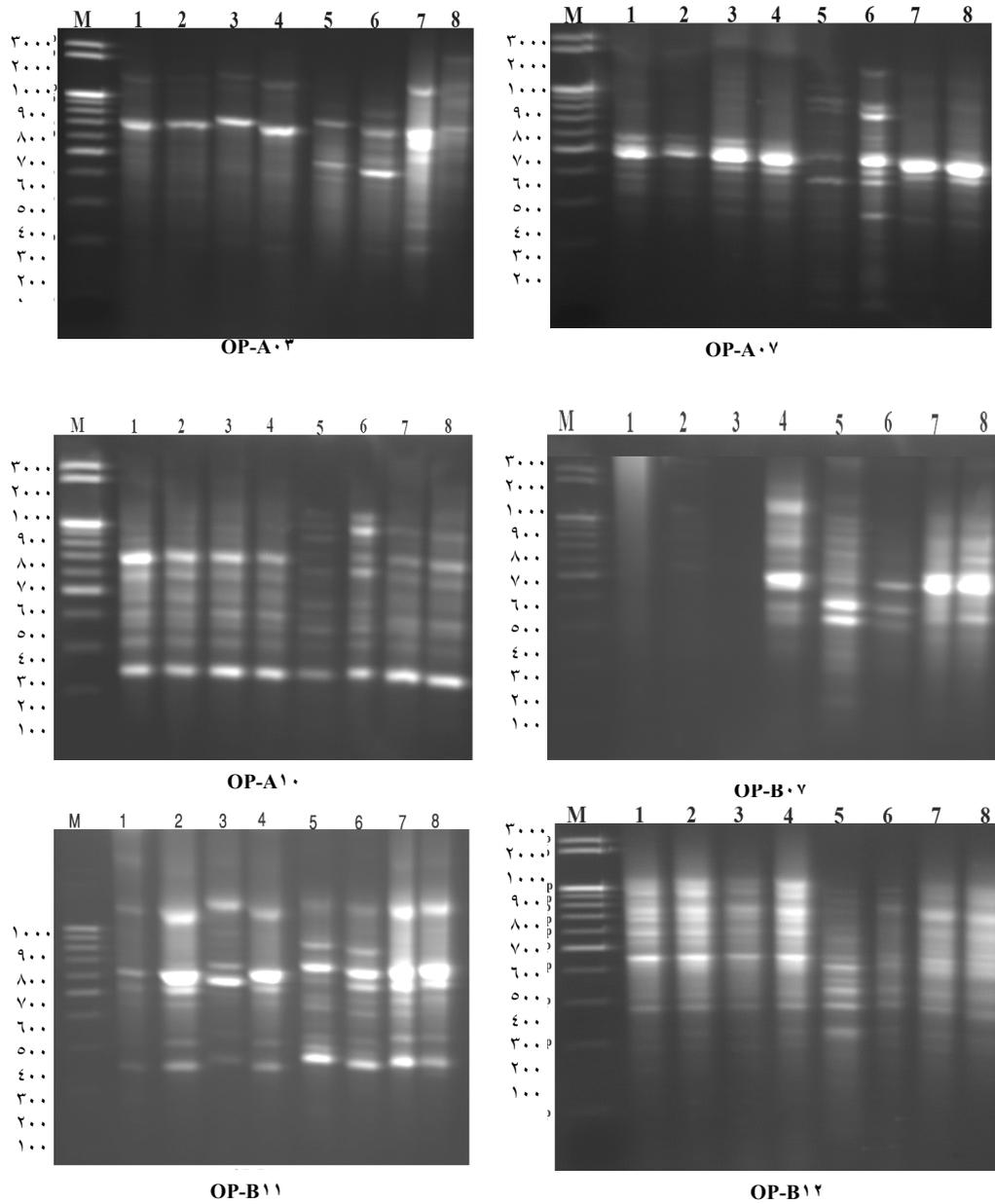
Six ISSR primers (44-A, 49-B, 49-B, HB-10, HB-11 and HB-12) were used in the present study to identify the eight wheat varieties (Fig. 2). Thirty six monomorphic and thirty five polymorphic distinct fragments (49.3% of polymorphism) were detected (Table 3). The results of ISSR

analysis showed that all used primers were polymorphic. HB-12 primer showed the highest polymorphism level among the tested varieties (72.73% of polymorphism), while HB-11 primer showed the lowest polymorphism level (33.33% of polymorphism) (Fig. 3). The compiled data for the six used primers recorded 49.30% of polymorphism.

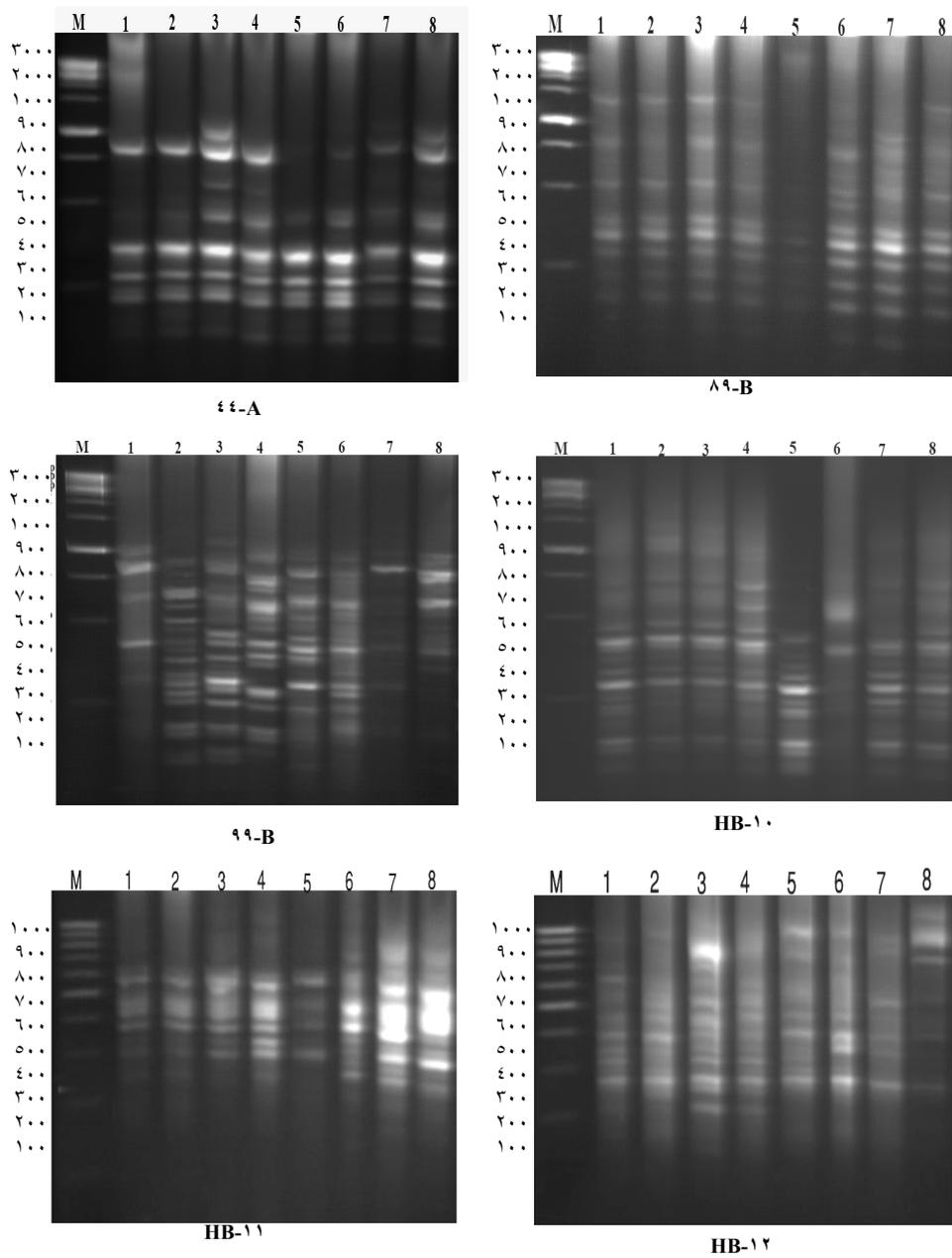
**Table 3. Levels of polymorphism detected with each RAPD and ISSR primer tested in the eight studied wheat varieties.**

RAPD	Primer name	OP-A.3	OP-A.7	OP-A.10	OP-B.7	OP-B.11	OP-B.12	Total
	Monomorphic bands	6	4	7	0	0	7	34
	Polymorphic bands	3	6	4	4	3	4	24
	<b>Total</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>4</b>	<b>3</b>	<b>11</b>	<b>58</b>
Polymorphism %	33.33	60.00	36.36	44.44	37.50	36.36	49.38	
ISSR	Primer name	44-A	89-B	99-B	HB-10	HB-11	HB-12	Total
	Monomorphic bands	0	7	7	8	6	3	36
	Polymorphic bands	0	4	8	7	3	8	30
	<b>Total</b>	<b>0</b>	<b>11</b>	<b>15</b>	<b>15</b>	<b>9</b>	<b>11</b>	<b>71</b>
Polymorphism %	0.00	36.36	53.33	46.67	33.33	72.73	49.30	

Some genotype(s)-specific markers were detected among the eight studied wheat varieties as shown in Table 4. The 89-B primer gave a specific marker for the tetraploid Yemeni genotypes with molecular weight of 287.40 base pairs and two unique bands for Mysany and Smara'a with molecular weights of 408.90 and 207.46 base pairs, respectively. The HB-10 primer gave also two specific markers for the hexaploid Egyptian cultivars with molecular weights of 500.00 and 502.69 base pairs as well as Behows 14 Yemeni variety with molecular weight of 74.74 base pairs. The 99-B primer showed a specific marker for Sakha 71 Egyptian cultivar with molecular weight of 119.11 base pairs. HB-12 primer showed a specific marker for Sakha 92 Egyptian variety with molecular weight of 426.20 base pairs and at molecular weight of 1119.70 base pairs for Saba'a Yemeni genotype. Heikal *et al* (2007) used different RAPD and ISSR primers to fingerprint some Egyptian gramineae species. The ISSR primers (HB-10, HB-11 and HB-12) scored highly polymorphic bands for the tested species.



**Fig. (1):** PCR products by six RAPD primers (OP-A·Ψ, OP-A·Υ, OP-A·Ψ, OP-B·Υ, OP-B·Ψ and OP-B·Υ) for the eight studied wheat genotypes. M DNA marker.

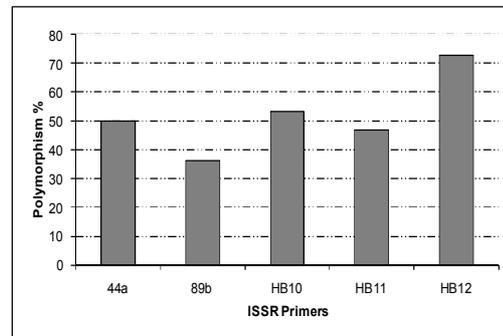
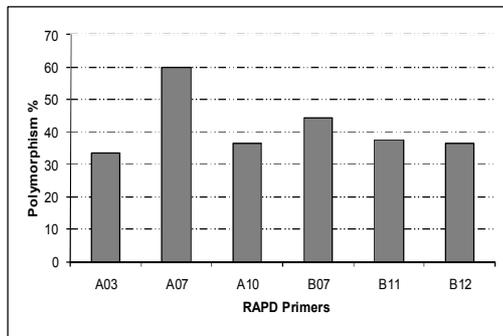


**Fig. (7):** PCR products by six ISSR primers ( $\epsilon\epsilon$ -A,  $\lambda\lambda$ -B,  $\rho\rho$ -B, HB-10, HB-11 and HB-12) for the eight studied wheat genotypes. M DNA marker.

**Table ٤. Variety(s)-specific markers in the eight studied wheat genotypes based on RAPD and ISSR analyses.**

	Primer name	Band No.	MW (bp)	١	٢	٣	٤	٥	٦	٧	٨	Variety(s)-specific marker	
RAPD	OP-A٠٣	٢	٨٨٠.٠٨	-	-	-	-	-	-	-	+	Saba'a (٨)	
		٩	١٥٣.٢٢	-	-	-	-	+	+	+	+	Yemeni* (٥,٦,٧,٨)	
	OP-A٠٧	١	١١٧٥.١٣	-	-	-	-	-	+	-	-	Smara'a (٦)	
		١	١٠٧٢.١٢	-	-	-	-	-	+	-	-	Smara'a (٦)	
	OP-A١٠	٦	٤٨٣.٩٨	+	+	+	+	-	-	-	-	-	Egyptian** (١,٢,٣,٤)
		٦	٣٧٤.١٦	-	-	-	-	+	+	+	+	+	Yemeni (٥,٦,٧,٨)
ISSR	٨٩-B	٤	٧٠٧.٧٩	+	+	+	+	-	-	-	-	Egyptian (١,٢,٣,٤)	
		٨	٣٦٢.٢٥	-	-	-	-	+	-	-	-	Behows'١ (٥)	
		١١	٢٢١.٢٣	-	-	-	-	+	-	-	-	Behows'١ (٥)	
ISSR	٩٩-B	٢	٤٠٨.٩٠	-	-	-	-	-	-	+	-	Mysany (٧)	
		٤	٢٨٧.٤٠	-	-	-	-	+	+	+	+	Yemeni (٥,٦,٧,٨)	
		٦	٢٠٧.٤٦	-	-	-	-	-	+	-	-	Smara'a (٦)	
	HB-١٠	١١	١١٩.١١	-	-	-	+	-	-	-	-	Sakha'١١ (٤)	
		١	٥٥٠.٠٥	+	+	+	+	-	-	-	-	-	Egyptian (١,٢,٣,٤)
		٢	٥٠٢.٦٩	+	+	+	+	-	-	-	-	-	Egyptian (١,٢,٣,٤)
HB-١٢	١٥	٧٤.٧٤	-	-	-	-	+	-	-	-	-	Behows'١ (٥)	
	١	١١١٩.٧٠	-	-	-	-	-	-	-	-	+	Saba'a (٨)	
	٦	٤٢٦.٢٠	-	-	+	-	-	-	-	-	-	Sakha'١٢ (٣)	

\*Yemeni = tetraploid, \*\*Egyptian = hexaploid



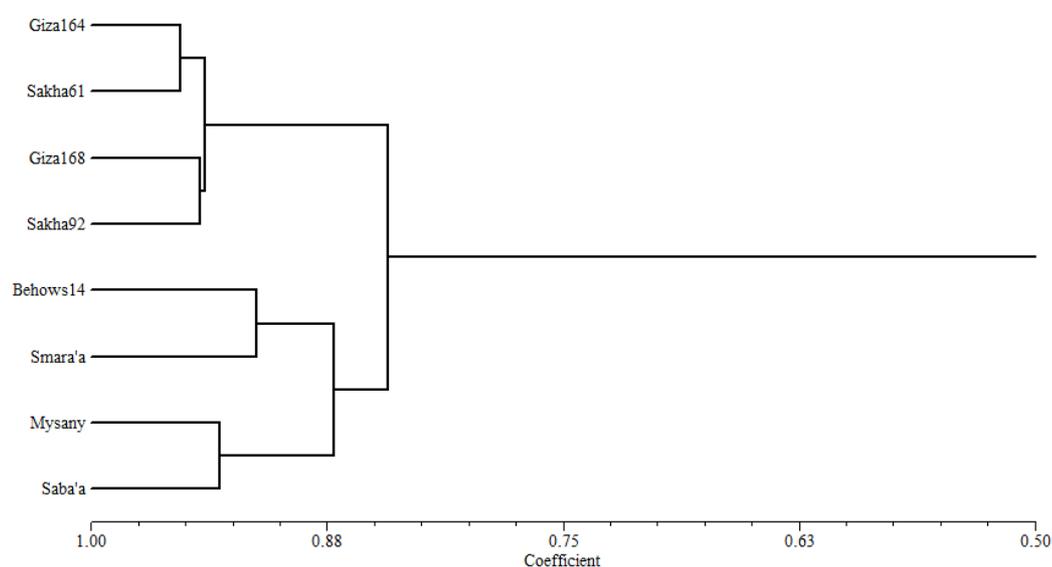
**Fig. (٣): Polymorphism percentage for each primer used with the eight studied wheat varieties.**

### Genetic similarity analysis

The dendrogram tree among the studied wheat varieties based on RAPD, ISSR and overall markers were constructed using UPGMA cluster analysis program depending on the similarity matrix present in Table ٥. The analysis was based on the number of markers that were similar between any given pair of cultivars. The Egyptian hexaploid varieties, Giza'١٦ (٤) and

**Table ٥. Similarity matrix based on the overall marker polymorphism for the studied eight wheat varieties.**

Variety	Giza١٦٤	Giza١٦٨	Sakha٩٢	Sakha٦١	Behows١٤	Smara'a	Mysany	Saba'a
Giza١٦٤	١							
Giza١٦٨	٠.٩٤٦	١						
Sakha٩٢	٠.٩٣٢	٠.٩٤٢	١					
Sakha٦١	٠.٩٠٣	٠.٩٣٢	٠.٩٤٩	١				
Behows١٤	٠.٨٢٣	٠.٨١٣	٠.٨٢٤	٠.٨٣٤	١			
Smara'a	٠.٨٢٨	٠.٨٣٨	٠.٨٢٩	٠.٨٤٩	٠.٩١٣	١		
Mysany	٠.٨٠٤	٠.٨٦٥	٠.٨٣٣	٠.٨٦٥	٠.٨٦٠	٠.٨٨٤	١	
Saba'a	٠.٨٦٣	٠.٨٥٣	٠.٨٤٣	٠.٨٧٣	٠.٨٥٩	٠.٨٨٢	٠.٩٣٢	١



**Fig. (٤): Dendrogram tree for the Egyptian and Yemeni wheat varieties based on RAPD and ISSR analyses.**

Sakha ٦١ were highly related to each other with similarity value of ٠.٩٠٣, while variety pairs (Giza١٦٤ and Sakha٩٢) and (Giza١٦٨ and Sakha٦١) showed the lowest similarity value of ٠.٩٣٢. Concerning the tetraploid Yemeni genotypes, the highest similarity value (٠.٩٣٢) was observed between Mysany and Saba'a cultivars, while the similarity value between Behow١٤ and Saba'a was the lowest one (٠.٨٥٩). Concerning the Egyptian and Yemeni varieties, the lowest similarity value (٠.٨١٣) was observed between the Egyptian cultivar Giza١٦٨ and the Yemeni

Behows<sup>١٤</sup>, while the highest similarity value (٠.٨٧٣) was observed between Egyptian variety Sakha<sup>١١</sup> and the Yemeni Saba'a.

Dendrogram tree separated the eight varieties into two major clusters as shown in Fig. ٤. The first cluster was divided into two groups for the Egyptian hexaploid entries which showed variety pairs (Giza<sup>١٦٤</sup> and Sakha<sup>١١</sup>) and (Giza<sup>١٦٨</sup> and Sakha<sup>١٢</sup>). They were highly related to each others. The second cluster was divided into two groups for the Yemeni tetraploid genotypes which showed that pairs (Behows<sup>١٤</sup> and Smara'a) and (Mysany and Saba'a) were remotely related to each others. However, the relationships among the studied wheat genotypes based on RAPD and ISSR analyses were in agreement with the origin ploidy level of these cultivars.

In conclusion, genetic background of the Egyptian varieties revealed that these studied cultivars were related to each other based on RAPD and ISSR analyses. Besides, the results illustrate that tetraploid cultivars could be distinguished by using molecular markers analyses. Moreover, these molecular markers could distinguish unique bands as specific markers for some Egyptian and Yemeni genotypes.

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## تصنيف العلاقات الوراثية لبعض أصناف القمح المصري واليمنى باستخدام نوعين من الكشافات الجزيئية (RAPD & ISSR)

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تم استخدام نوعين من الكشافات الجزيئية (RAPD&ISSR) في هذه الدراسة بهدف تحديد العلاقات الوراثية بين أربعة أصناف من القمح المصري سداسي المجموعة الكروموسومية وهي (جيزة ١٦٤ - جيزة ١٦٨ - سخا ٩٢ - سخا ٦١) وكذلك أربعة أصناف من القمح اليمنى وهي (بحوث ١٤ - سمراء - ميسانى - سبا) اقماح رباعية المجموعة الكروموسومية. و بتحليل نتائج الـ RAPD لقطع الـ DNA باستخدام ستة بادئات كان العدد الكلى لقطع الـ DNA الناتجة من الـ PCR هي ٥٨ قطعة. و كان هناك تعدد مظهري مختلف للقطع الناتجة (٢٤ قطعة للأصناف المصرية و اليمنية كانت ذات تعدد مظهري مختلف بنسبة ٤١,٣٨%) يتراوح وزنها الجزيئى من ١١٣ إلى ١١٧٥ قاعدة.

و بتحليل نتائج الـ ISSR لقطع الـ DNA باستخدام ستة بادئات كان العدد الكلى لقطع الـ DNA الناتجة من الـ PCR هي ٧١ قطعة (٣٥ قطعة DNA في الأصناف المصرية و اليمنية ذات تعدد مظهري مختلف ٤٩,٣%). و كان هناك تعدد مظهري مختلف للقطع الناتجة يتراوح وزنها الجزيئى بين ٧٤ إلى ١١١٩ قاعدة.

الارتباط بين نتائج الـ RAPD لأصناف القمح المصرية و اليمنية اظهر تعدد مظهري مختلف بنسبة ٤٦,٤%.

وضحت شجرة علاقة القرابة باستخدام دليل النشابة بين الأصناف أن بعض الأصناف كانت متقاربة و البعض الآخر غير متقارب.

ومن هذه الدراسة اتضح أن الكشافات الجزيئية (RAPD&ISSR) مفيدة ككشافات خاصة و محددة للتوصيف الوراثى الجزيئى و تحديد العلاقات الوراثية بين أصناف القمح المختلفة ويمكن بواسطتها التمييز بين أصناف القمح ذات المجموعات الكروموسومية الرباعية و السداسية و المحافظة عليها.

المجلة المصرية لتربية النبات ١٣ : ١١ - ٢٣ (٢٠٠٩)

