

## GENETIC DIVERSITY OF SOME WHEAT (*Triticum aestivum* L.) CULTIVARS

A.A. Guirges<sup>(1)</sup>, E. A. El Absawy<sup>(2)</sup>, H. A. El Rabey<sup>(2)</sup>,  
K. F. Abdellatif<sup>(3)</sup> and M.S. Abdel Aal<sup>(3)</sup>

<sup>(1)</sup>Molecular Biology Dept., <sup>(2)</sup>Bioinformatics Dept., <sup>(3)</sup>Plant Biotechnology Dept.,  
Genetic Engineering and Biotechnology Institute, Minufiya University, PO Box 79  
Sadat City, Egypt

(Received: May 28, 2009)

**ABSTRACT:** *The genetic diversity of twelve Egyptian hexaploid wheat (Gemmiza 7, Gemmiza 9, Sids1, Giza 168, Gemmiza 10, Giza 170, Sakha 93, Sakha 94, Sakha 61, Sakha 69, Gemmiza 3 Gemmiza 5) cultivars was investigated based on eleven morphological characters and molecular (RAPD-PCR) markers. The two-way hierarchical cluster analysis of morphological traits revealed that the wheat cultivars were clustered in two main clusters. The first cluster consists of Gemmiza 7 and Gemmiza 9 cultivars and the second cluster contains Sids1, Giza 168, Gemmiza 10, Giza 170, Sakha 93, Sakha 94, Sakha 61, Sakha 69, Gemmiza 3 and Gemmiza 5 cultivars. The most related cultivars are Gemmiza 3 and Gemmiza 5. 115 RAPD-PCR markers were resulted from fingerprinting the 12 wheat cultivars under study using 11 arbitrary primers, 88 bands out of them were polymorphic and the other 27 were common. The resulted data were analyzed using NTSYS-PC2 program in order to address the intercultivars relationships. According to the RAPD cluster analysis, Sakha 93 was separated alone distant from all studied wheat germplasms. The other samples were clustered in two main groups. In the first group, Sids 1 was separated alone distant from the other members of this group that were divided in two clusters, the first clusters consisted of Giza 168, Giza 170, Sakha 61 and Sakha 69; Giza 168 and Giza 170 were highly related to each other and Sakha 61 and Sakha 69 were also relatively related to each other. The second cluster consists of Gemmiza 3, Gemmiza 5 and Gemmiza 7. Gemmiza 3 and Gemmiza 5 were highly related to each other than Gemmiza 7. The second group consisted of Sakha 94, Gemmiza 10 and Gemmiza 9. The latter germplasm appeared distant from Sakha 94 and Gemmiza 10. The results of this study showed that, morphological traits and molecular markers are relatively consistent with each other. Thus, it can be concluded that both morphological and molecular markers (RAPD) could be used in determination of genetic diversity and intercultivars relationships for wheat genotypes under study.*

**Key words:** *Wheat, genetic diversity, RAPD-PCR, morphology.*

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## INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to the tribe *Triticeae* of the grass family *Poaceae*. In this tribe, wheat belongs to the sub-tribe *Tritineae*, which is of recent origin, and contains about 35 genera including *Triticum*, *Aegilops*, *Thinopyrum*, *Dasypyrum*, *Lophopyrum* and *Secale*. The various species of these genera are easily hybridize with each other resulting in either a direct exchange of genetic material or polyploidy (Caligari and Brandham, 2001). Wheat is one of the most important cereal and forage crops in the world for human and animal as a source of energy. Most of food technology depends on seed storage proteins of wheat for quality of products (i.e, baking qualities of wheat) (Jones, 1987 ). Ninety-five percent of grown wheats today are of the hexaploid type comprising three genomes A, B and D. Each of these genomes has seven chromosome pairs. The remaining 5% is durum (*Triticum turgidum* L., var. *durum*) tetraploid wheat (Heun *et al.*, 1997).

Linneaus (1953) recognized seven species within the genus *Triticum* while the wild relatives were included in the genus *Aegilops*. Taxonomists upheld this dichotomy for over 200 years after which Stebbins (1956) initiated the merger of two genera into a single genus *Triticum*, which was subsequently supported by Bowden (1959) and Kimber and Feldman (1987). Analysis of genetic relationships is an important component for plant breeding programs, as it provides information about genetic diversity (Mohammadi and Prasanna, 2003) and sources of genetic variation. It is vital for plant breeding programs to have sufficient genetic diversity available in order to develop new varieties that are aimed towards the increase of crop productivity and to withstand damage from biotic and abiotic factors. In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by calculating the genetic similarity (GS) or genetic distance (GD) between the parents since the latter can be used as an estimation of expected genetic variance in different sets of segregating progenies derived from different crosses (Korzun, 2003).

Genetic diversity is a statistical concept referring to the variations within the individual gene loci / among alleles of a gene, or gene combinations, between individual plants or between plant populations. Genetic diversity has several 'indicators', which are measured using various tools such as classical or Mendelian genetic analysis, that can be employed to evaluate variation in single known gene (qualitative traits), such as resistance to disease (Smale and McBride, 1996). On the other hand, multivariate analysis can be used to analyze variation in quantitative traits. Also, pairwise coefficients of parentage are calculated from pedigree information and used as indicators of genetic diversity (Cox *et al.*, 1996). The classical methods of diversity studies are based on morphological characters which are influenced by various environmental factors. However, the molecular markers, which are unrestricted in number and not influenced by the

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environment, have the ability of sampling diversity directly at the genome level. The molecular biology tools provide detailed information about the genetic structure of natural population which was not available in the past (El Rabey, 2004 and Hussein, 2009).

The present study aimed to address the genetic diversity and the genetic relationships among the studied wheat cultivars both in the field by determination of some morphological traits and in the lab by analyzing the molecular markers as revealed by RAPD-PCR.

### **MATERIALS AND METHODS**

Breeder, basic and certified grains of twelve Egyptian hexaploid bread wheat cultivars (*Triticum aestivum* L.) were used in this study and were kindly provided by the Agricultural Research Center, Giza, Egypt. The name and pedigree of these cultivars are presented in Table 1.

Table 1: The twelve Egyptian wheat cultivars used for the study and their pedigrees.

No	Cultivar	Pedigree
1	Sids1	HD2172/p'avon "S" 1/1158.57/MAYA74 "S"
2	Giza 168	MRUBUCI/SERI
3	Giza 170	KAUZI/AL T AR84/AOS
4	Sakha 61	Inia IRL4220117C/Y "S"
5	Sakha 69	Inia-RL4220 x 7C/yr'S' CM1540-25.65.0S
6	Sakha 93	Sakha 92/TR 810328
7	Sakha 94	ICHArS" /5/CROW "s"
8	Gemmiza 3	Bb/7C*2//Y50/Kal*3//Sakha8/4/Prv/WW/5/3/Bg"s"//On
9	Gemmiza 5	Vee "S" /SWM6525
10	Gemmiza 7	CMH74 A. 630/5x//Seri 82/3/Agent
11	Gemmiza 9	Aid "S" /Huac "S" /CMH74A.630/SX
12	Gemmiza 10	MAYA74 US" IONI/1160147/3/BB/GLU4

### **Field experiment**

Grains of each cultivar were planted at the Genetic Engineering and Biotechnology Research Institute farm during the seasons 2003/2004 and 2004/2005 in order to discriminate among them. The cultivars were arranged in a randomized complete block design with three replicates, each replicate contained thirty plots and each plot was 4.2 m<sup>2</sup> (3.5/ m long and 1.2/ m wide). The following data were scored: Plant height (cm), Flag leaf area (cm), Number of days to flowering, Number of days to maturity, Length of main spike (cm), Weight of main spike (gm), Number of spikelets / spike, Number

of grains / spike, Weight of 1000 grain (gm), Number of spikes /m<sup>2</sup> and Grain yield / plot (kg). Each plot was harvested and threshed to determine the grain yield.

### **RAPD analysis**

DNA was extracted immediately using a modified CTAB method (Saghai-Marooif *et al.* 1984). The RAPD analysis was carried out using 15 random primers (10-mer) for wheat fragment amplification. The primers were selected according to literature and they are as illustrated in Table 2.

**Table 2: Nucleotides sequence of the 11 RAPD primers used in fingerprinting the twelve wheat cultivars under study.**

<b>Primer name</b>	<b>Primer sequence</b>
OPB-20	GGACCCTTAC
OPE-02	GGTGCGGGAA
OPN-00	GACCGACCCA
OPN-05	ACTGAACGCC
OPN-08	ACCTCAGCTC
OPO-04	AAGTCCGCTC
OPO-05	CCCAGTCACT
OPO-06	CCACGGGAAG
OPO-12	CAGTGCTGTG
OPO-15	TGGCGTCCTT
OPAN-16	CAAGGTGGGT

### **PCR amplification condition**

A total volume of 20 µl PCR reaction was used which consisted of 1.0 µl (50 ng) template DNA, 0.2 µl dNTPs (10 mM), 1.6 µl MgCl<sub>2</sub> (25 mM), 2.0 µl 10X buffer (10 mM tris, pH 8.0, 50 mM KCl and 50 mM ammonium sulphate), 4.0 µl primer (15 pmole) and 0.1 µl *Taq* polymerase (5U/ µl). The volume was brought up to 20 µl by sterilized double distilled H<sub>2</sub>O. The PCR cycling condition involved initial denaturation at 94°C for 3 min. followed by 35 cycles of amplification under the following parameters, template denaturation at 94°C for 1 min, primer annealing at 36°C for 1.5 min. and primer extension at 72°C for 2 min. A final extension step at 72°C for 7 min. was given, followed by storage at 4°C. The products were separated on 2% agarose gel electrophoresis.

### **Statistical analysis**

For morphological traits, the collected data from field experiments were statistically analyzed using JMP 7 software (SAS, 2007) and then analyzed by the Two Way Cluster Analysis program for addressing the genetic relationships among the studied genotypes as constructed using Ward's method (Milligan, 1980). For the RAPD data, gels were scored as 0/1 for

absence/presence of the bands, respectively and the resulting markers were analyzed using the NTSYS-pc2.0 software (Rohlf, 1998). Similarity coefficient matrices were calculated using simple matching similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrogram was constructed using the UPGMA method (Unweighted Pair-Group Method with arithmetical algorithms Averages (Sneath and Sokal, 1973).

## RESULTS AND DISCUSSION

### Morphological characteristics

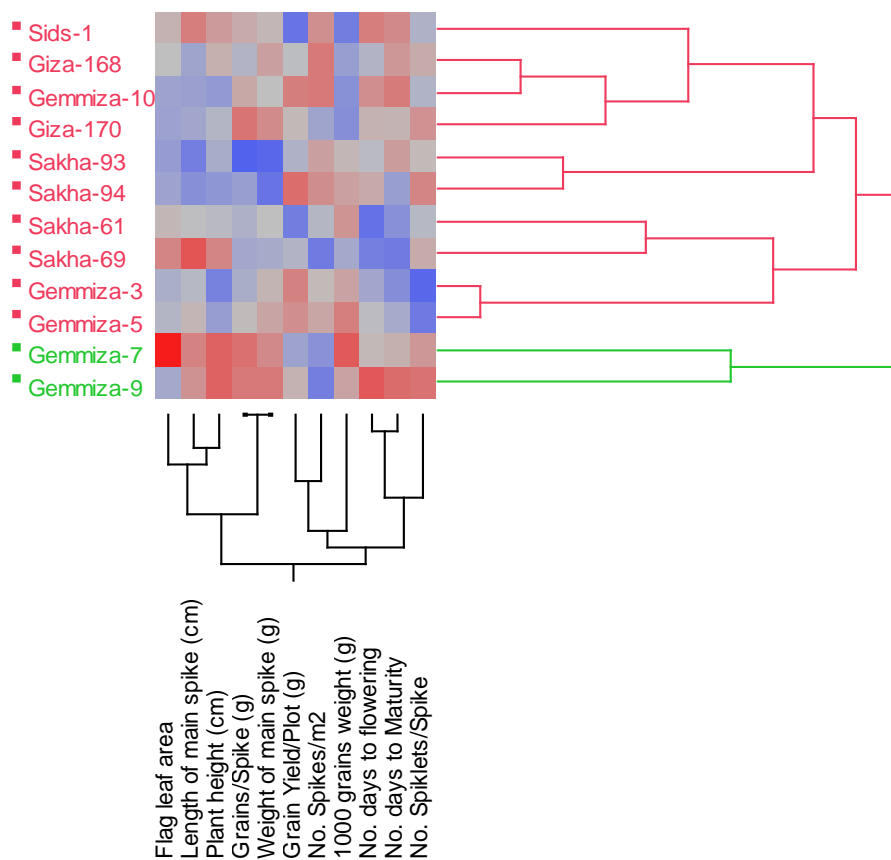
The mean values of the studied morphological traits and field measurements are illustrated in Table 3. Briefly, Gemmiza 7 cultivar had the highest value of plant height (109.62 cm), whereas Sakha 93 cultivar was the shortest (102.88 cm). The highest value of flag leaf area was scored in Gemmiza 7 (59.3 cm<sup>2</sup>) and the lowest value was scored in Sakha 93 (39.9 cm<sup>2</sup>). The longest period to flowering was recorded in Gemmiza 9 (96.46 days), whereas the shortest period was recorded in Sakha 61 (82.7 days). Gemmiza 9 cultivar recorded the longest period to maturity (156 days), while Sakha 69 has the shortest period to maturity (145.9 days). Sakha 69 cultivar showed the highest value for the main spike length (12.15 cm), whereas, Sakha 93 showed the lowest value (10.38 cm). Gemmiza 9 showed the heaviest spikes (5.4 gm) and Sakha 94 revealed the lowest spike weight (4.39 gm). The number of spiklets /spike ranged from 20.85 in Gemmiza 3 to 24.81 in Gemmiza 9. Gemmiza 7 and Giza 170 surpassed the other cultivars in grain number (82.54) while, the lowest number of grains/ spike was observed in Sakha 93 (63.14). Gemmiza 7 recorded the highest 1000-grain weight (53.19 gm), while Sids 1 gave the lowest 1000-grain weight (41.41 g). It was observed that Gemmiza 10 recorded the heighest value (502.73 spikes/m<sup>2</sup>) and Sakha 69 recorded the lowest value (405.21 spikes/m<sup>2</sup>). Gemmiza 10 recorded the highest grain yield (4.1 kg/plot) and Sids 1 gave the lowest grain yield (3.35 kg/plot).

**The Two Way Clustering Analysis of the studied wheat cultivars and the morphological traits**

A two way clustering analysis (Hierarchical Clustering) was used to address the relationships of the 12 wheat genotypes and the eleven studied morphological traits under study (Figure 1). The results of the two-way clustering analysis showed that the wheat cultivars were clustered in two main clusters. The first cluster consists of Gemmiza 7 and Gemmiza 9 cultivars and the second one contains Sids 1, Giza 168, Gemmiza 10, Giza 170, Sakha 93, Sakha 94, Sakha 61, Sakha 69, Gemmiza 3 and Gemmiza 5 cultivars. This cluster was divided into two sub-clusters the first sub-cluster included Sids1, Giza168, Gemmiza 10, Giza 170, Sakha 93 and Sakha 94. Sakha 93 and Sakha 94 appeared highly related to each other and also

**Table 3**

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**Figure 1: Two-way Hierarchical Clustering Analysis of the twelve wheat genotypes and the eleven morphological traits under study.**

Giza168 and Gemmiza 10 are related to each other. The other sub-cluster included Sakha 61, Sakha 69, Gemmiza 3 and Gemmiza 5. Gemmiza 3 and Gemmiza 5 appeared highly related to each other (Figure 1).

The other way of clustering showed that the morphological traits were divided also into two clusters. Cluster 1 includes five traits; flag leaf area (cm<sup>2</sup>), length of main spike (cm), plant height (cm), number of grains/spike and weight of main spike (gm), while the other cluster includes six traits; grain yield/plot (gm), number of spikes/m<sup>2</sup>, 1000 grains weight (gm), number of days to flowering, number of days to maturity and number of spiklets/spike). The most related traits are grains/spike (gm) and weight of main spike (gm), number of days to flowering number of days to maturity (Figure 1).

The results of the first way of cluster seem to be in disagreement with the pedigree history of the cultivars. This may be related to the response of the morphological traits with the environmental conditions. Cox *et al.* (1996) mentioned that the classical methods of diversity studies based on morphological characters are influenced by various environmental factors. All cultivars of Sakha were aggregated at the middle of the dendrogram while the cultivars of Gemmiza were aggregated near to the edge except Gemmiza 10 cultivar. Thus, it can be concluded that the cultivars relationships based on morphological data could partially reflect the origin of the wheat genotypes as the most related cultivars are clustered together as it was expected.

From the other way of clustering, the morphological traits were clustered into two main groups. The first includes five traits, while the other includes six traits. It can be noticed that some traits are closely related such as grain yield per spike and weight of main spike. This means that some traits can be measured and can be taken as index for other traits. In this case, either of the above mentioned traits can be measured and used as indicators to the other trait. The same result was noticed by Abdellatif (2007), who studied the genetic diversity of 45 wheat cultivars using both morphological and molecular markers.

### **RAPD-PCR analysis for the studied wheat cultivars**

Figure 2 shows examples of RAPD-PCR fingerprint using OPN-0 primer and Table 4 illustrates the statistics of the resulted RAPD fragments using the 11 arbitrary primers. One hundred and fifteen RAPD markers were obtained as a result of fingerprinting the 12 wheat cultivars under study. Twenty-seven bands were common and the other 88 were polymorphic in appearance among the twelve wheat cultivars under study. The number of band/primer ranged from 6 (in OPN-00 primer) to 15 (in OPAN-16 primer). OPN-5 primer revealed the highest polymorphism (13 bands) while OPN-00 primer showed the lowest polymorphism (4 bands).

The cladogram resulting from the analysis of the 115 RAPD-PCR markers using NTSYS-PC2 program (Figure, 3) revealed that Sakha 93 was separated alone distant from all studied wheat germplasms. The other samples were clustered in two main groups. In the first group, Sids 1 was separated alone distant from the other members of this group that were divided in two clusters, the first clusters consisted of Giza 168, Giza 170, Sakha 61 and Sakha 69; Giza 168 and Giza 170 are highly related to each other and Sakha 61 and sakha 69 are relatively related to each other. The second cluster consists of Gemmiza 3, Gemmiza 5 and Gemmiza 7. Gemmiza 3 and Gemmiza 5 are highly related to each other than Gemmiza 7. The second group consisted of Sakha 94, Gemmiza 10 and Gemmiza 9. The latter germplasm appeared distant from Sakha 94 and Gemmiza 10.



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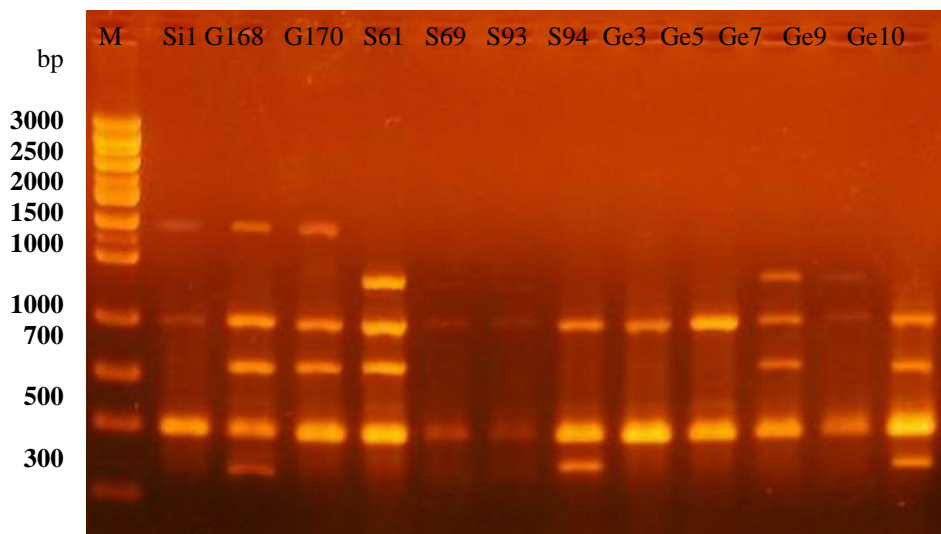
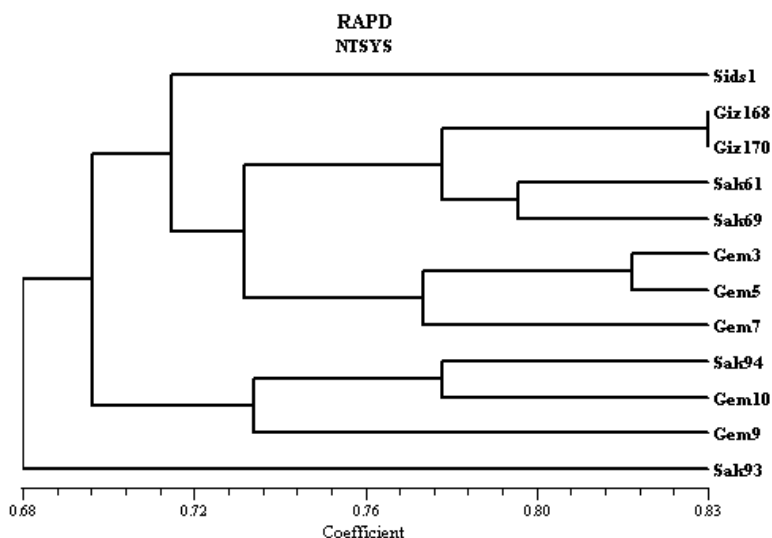


Figure 2: Examples of randomly amplified DNA for the studied 12 wheat cultivars using primer OPN-0

Table 4: Statistics of RAPD analysis for the 12 wheat cultivars under investigation

Primer No.	Serial No.	Monomorphic bands	Polymorphic bands	Fragments range in bp	Total
1	OPB-20	2	6	420-1500	8
2	OPE-02	3	8	342-2000	11
3	OPN-00	2	4	380-2500	6
4	OPN-05	1	13	147-1321	14
5	OPN-08	2	10	277-4000	12
6	OPO-05	1	9	110-1956	10
7	OPO-12	3	6	391-2786	9
8	OPO-04	2	8	265-2065	10
9	OPO-06	2	9	330-1500	10
10	OPO-15	3	6	371-1545	9
11	OPAN-16	6	9	277-1500	15
		27	88	110-4000	115



**Figure 3:** Cladogram showing the genetic relationships of the 12 wheat cultivars under study based on the 115 RAPD-PCR markers. The cladogram was constructed using NTSYS-PC2 program.

Comparison of the RAPD-PCR data analysis (Figure 3) with that resulted from the morphological data analysis it was appeared that, Giza 168, Giza 170 and Sids 1 were clustered together in one group together with Sakha 69 and Sakha 61 the same like the case of morphological data analysis. Also, Gemmiza 3 and Gemmiza 5 was appeared closely related to each other like in the other analysis. It was also noted that all cultivars related to Gemmiza and Sakha are aggregated beside each other in the dendrogram. These results are relatively similar to that resulted from the morphological data analysis. This result is consistent with Cao *et al.* (2000) who found that there were an agreement between the RAPDs and the morphological classification in wheat. In contrast, Sakha 93 was distant from Sakha 94, the same for Sids 1 which was relatively distant from Gemmiza 10 and Sakha 93 which was separated alone distant from all studied wheat germplasms. This result is in agreement with the previous studies that revealed that relationship among morphological traits and molecular markers sometimes give diverse results (Maric *et al.*, 2004) who reported that there was no significant correlation ( $r=0.12$ ) between RAPD markers and morphological traits of Croatian bread wheat cultivars. Thus it can be concluded that, both morphological and molecular markers (RAPD) succeeded in determination of genetic diversity of wheat genotypes.

In the last decades, many studies were performed using the evidences used in the present study for addressing the genetic diversity and

intercultivars relationships (Joshi and Nguyen, 1993 and Freitas *et al.*, 2000) who used the RAPD markers to estimate the variability within wheat genotypes. Gupta *et al.* (1999) reported that RAPD technology proved to be useful for many crops, but in bread wheat, it has been put to limited use, partly owing to the low level of polymorphism detected and sometimes also partly owing to lack of reproducibility of results.

Finally, it can be concluded that morphological and RAPD data analysis succeeded in assessing the genetic diversity and addressing the intercultivars relationships among the studied wheat germplasms.

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## التنوع الوراثي في بعض أصناف القمح

عادل أبسرخون جرجس<sup>١</sup> - السيد عبد الخالق العساوي<sup>٢</sup> -

حداد عبد السميع الربيعي<sup>٢</sup> - وكمال فؤاد عبد اللطيف<sup>٣</sup> - محمد سيد عبد العال<sup>٣</sup>

(<sup>١</sup>) قسم البيولوجيا الجزيئية (<sup>٢</sup>) قسم المعلوماتية الحيوية (<sup>٣</sup>) قسم البيوتكنولوجيا النباتية - معهد الهندسة الوراثية والتكنولوجيا الحيوية جامعة المنوفية

### الملخص العربي

أقيمت هذه الدراسة لدراسة التنوع الوراثي لعدد ١٢ صنف من أصناف القمح السداسي المصري وهي جميزة ٧ و جميزة ٩ ، سدس ١ ، جيزة ١٦٨ ، جميزة ١٠ ، جيزة ١٧٠ ، سخا ٩٣ ، سخا ٩٤ ، سخا ٦١ ، سخا ٦٩ ، جميزة ٣ و جميزة ٥ وذلك باستخدام احد عشرة صفة مورفولوجية و كذا المعلمات الجزيئية باستخدام تقنية RAPD-PCR باستخدام ١١ بادىء عشوائى.

بتحليل النتائج المتحصل عليها من النتائج المورفولوجية بطريقة التحليل الثنائية أوضحت النتائج أن:

١- الأصناف المدروسة طبقا للصفات المورفولوجية انقسمت إلى مجموعتين ، المجموعة الأولى اشتملت على جميزة ٧ و جميزة ٩ والمجموعة الثانية اشتملت على الأصناف سدس ١ ، جيزة ١٦٨ ، جميزة ١٠ ، جيزة ١٧٠ ، سخا ٩٣ ، سخا ٩٤ ، سخا ٦١ ، سخا ٦٩ ، جميزة ٣ و جميزة ٥ ، وأتضح أيضا أن الصنفين جميزة ٣ وجميزة ٥ مرتبطين بصلة قوية طبقا للمواصفات المورفولوجية و المحصولية المدروسة .

٢- أوضحت نتائج RAPD-PCR ظهور ١١٥ علامة جزيئية ناتجة من البصمة الوراثية ل ١٢ صنف من القمح تحت الدراسة باستخدام ١١ بادىء عشوائى ، وكان منها ٨٨ علامة جزيئية متباينة الظهور بين الأصناف محل الدراسة و ال ٢٧ علامة جزيئية الأخرى كانت موجودة فى كل الأصناف. كما تم تحليل النتائج المتحصل عليها باستخدام برنامج NTSYS-PC2 لدراسة العلاقات بين الاصناف .

٣- أظهرت النتائج أن الصنف سخا ٩٣ قد انفصل في مجموعة وحيدة بعيدا عن كل الأصناف بينما تجمعت باقى الأصناف فى مجموعتين المجموعة الأولى ضمت الصنف سدس ١ والذى انفصل فى مجموعة وحيدة عن باقى الأصناف بينما توزعت باقى الأصناف فى تحت مجموعتين المجموعة ضمت تحت المجموعة الأولى الأصناف جيزة ١٦٨ ، جيزة ١٧٠ ، سخا ٦١ و سخا ٦٩ و كانت القرابة أقوى بين الصنفين جيزة ١٦٨ و جيزة ١٧٠ ، وضمت تحت المجموعة الثانية جيزة ٣ ، جيزة ٥ و جيزة ٧ وكانت القرابة قوية بين الصنفين جيزة ٣ و جيزة ٥ أكثر منها مع الصنف جيزة ٧. واشتملت المجموعة الثانية على الأصناف سخا ٩٤ جيزة ١٠ و جيزة ٩.

- مما سبق نستنتج أن الدراسة أظهرت نتائج متشابهة تقريبا من خلال دراسة كلا من الصفات المورفولوجية والجزئية لأصناف القمح محل الدراسة وعليه يمكن استخدام كل من الصفات المورفولوجية و الجزئية فى تحديد الاختلافات الوراثية لهذه الأصناف.

**Table 3: The mean values of the measured morphological traits for the 12 wheat germplasms.**

Morphological traits	Sids 1	Giz168	Giz 170	Sak 61	Sak 69	Sak 93	Sak 94	Gem 3	Gem 5	Gem 7	Gem 9	Gem 10
plant height in cm	106.5	105.3	103.5	103.8	107.6	102.8	101.2	99.6	101.7	109.6	109.6	101.4
Flag leaf area in cm <sup>2</sup>	45.7	44.4	40.5	45.4	49.8	39.9	40.7	42.2	43.2	59.3	41.4	40.8
No. of days to flowering	94.1	88.67	90.72	82.7	83.79	89.4	91.24	87.46	89.43	90.31	96.46	92.98
No. of days to maturity	154.39	153.61	152	147.6	145.9	153.3	148.73	147.4	149.67	152.2	156	155.3
Length of main spike in cm	11.8	10.86	10.89	11.18	12.15	10.38	10.6	11.1	11.3	11.74	11.6	10.8
Weight of main spike in g	5.1	5.18	5.3	4.99	4.83	4.29	4.39	5.1	5.16	5.31	5.4	4.99
No. of spikelets /spike	22.78	23.64	24.18	22.96	23.63	23.34	24.43	20.85	21.13	24.02	24.81	22.81
No. of grains/spike	77.24	73.83	82.11	73.27	72.14	63.14	71	72.96	75.75	82.54	81.72	77.5
Weight of 1000 grain in g	41.41	44.24	42.89	49.74	45.1	47.6	48.98	48.8	50.95	53.19	48.67	43.1
No. of spikes/m <sup>2</sup>	489.7	502.9	438.6	454.0	405.2	480.7	491.3	465.5	476.2	423.5	407.6	502.7
Grain yield/plot in kg	3.35	3.8	3.8	3.4	3.74	3.72	4.2	4.08	4	3.63	3.9	4.1