# Genetic diversity and bulked segregant analysis for earliness in bread wheat

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## ABSTRACT

*The genetic variability among six bread wheat genotypes (two of early matutity, i.e Sids 4 and Line 126, two of late maturity, i.e Sunval and Gemmeiza 9 and two of medium maturity, i.e Giza 168 and the $F_1$ of Sunval × Sids 4) was investigated using 11 RAPD primers. The number of polymorphic amplicons was 41 out of a total of 91 amplicons, thus revealing a level of 45.05 % polymorphism. The genetic relationships among the 6 wheat genotypes were examined using the Dice coefficient and a dendrogram was constructed according to the UPGMA analysis. Four out of the six wheat genotypes were characterized by 23 positive and/ or negative RAPD markers. Bulked segregant analysis (BSA) was also used to rapidly identify markers associated to earliness of five wheat populations (Sunval, Sids 4, their $F_1$ cross, early $F_2$ bulk and late $F_2$ bulk). Out of 10 primers, only 2 primers revealed two positive and one negative marker associated with earliness trait. Further studies are required to confirm the linkage between these RAPD markers and earliness.*

**Key words:** *Wheat, earliness, RAPD, bulked segregant analysis, dendrogram, dice coefficient, polymorphism.*

## INTRODUCTION

Traditionally, the assessment of the genetic variation in crop plants has been conducted on the basis of phenotypic and cytogenetic characters, which frequently lack the resolving power needed to identify individual genotypes (Teshale *et al.*, 2003). In the last decade, molecular markers such as RFLP, RAPD, ISSR, AFLP have been used to assess genetic variation at the DNA level, allowing an estimation of degree of relatedness between individuals without the influence of environmental variation (Gupta *et al* .,1999).Randomly amplified polymorphic DNA (RAPD) is a useful method for generating molecular markers (Welsh and McClelland, 1990) that can be used to construct linkage maps, to identify varieties (He *et al.*1992) and to assess genetic diversity (Koller *et al.*, 1993). It is characterized by its low technical input and small quantity of plant DNA needed for the analysis (Hernandez *et al.*, 1999 and Manabe *et al.*, 1999). Also, RAPD based fingerprinting was used successfully in wheat to assess genetic diversity (He *et al.*,1992, Dhaliwal *et al.*,1993; Cao *et al.*,1999 Kudriavtsev *et al.*,2003; Munshi *et al.*,2003 ; Maric *et al.*, 2004 and Abd-El-Haleem *et al.*, 2009).

Michelmore *et al.* (1991) developed the bulked segregant analysis (BSA) of $F_2$ plants

as a simpler alternative to isogenic line analysis, where the highest and lowest extreme groups of the $F_2$ population are bulked for the development of molecular markers associated with a given characteristic. Based on this BSA, RAPD was used to identify DNA markers associated with drought tolerance (Malik *et al.*,2000 ; Abdel-Twab *et al.*, 2003 and Al-Naggar *et al.*,2004), yield and some stress physiological traits (Nachit *et al.*, 2000), resistance to *Fusarium* head blight (Sun *et al.*, 2003), and salinity tolerance (Mehboob-ur-Rahman *et al.*, 2004). Therefore, the objectives of this investigation were to: (1) assess the genetic diversity at the molecular (DNA) level among wheat genotypes that differ in earliness traits, and (2) identify RAPD markers associated with earliness in wheat *via* bulked segregant analysis (BSA).

## MATERIALS AND METHODS

### Plant materials

Based on the results of previous experiments conducted by the Wheat Research Department, ARC, six bread wheat (*Triticum aestivum* L.) genotypes, showing clear differences in earliness were chosen for this study. These six genotypes included two early genotypes (Sids 4, Line 126), two late ones (Sunval and Gemmeiza 9) and two intermediate (Giza 168 and $F_1$ of Sunval × Sids 4). The first five genotypes are presented in Table (1). Randomly ampilified polymorphic (RAPD) analysis was used in the present study to perform two types of analysis, i.e., to investigate the genetic diversity among six bread wheat genotypes and to identify markers associated to earliness *via* the bulked segregant analysis approach using five populations of the cross between Sids 4 (early) and Sunval (late) *i.e* Sids 4, Sunval, their $F_1$ cross, the early $F_2$ bulk and the late $F_2$ bulk. These experiments were carried out in the Molecular Genetics and Genome Mapping (MGGM) Laboratory at the Agricultural Genetic Engineering Research Institute, ARC, Giza, Egypt.

***Table (1): Name, pedigree, origin and maturity of the parent bread wheat genotypes used in this study.***

| Genotype | Pedigree | Origin | Maturity |
|---|---|---|---|
| Line 126 | BCH"S"//HORK"S"/4/7C/PATO(B)/3/LR/INIA/BB/5/CNO/ GII//BB/INIA/3/NAPO//TOB66/SPROW"S" | Egypt | Early |
| Sids 4 | Maya"S"Mon"S"/CMH74.A592/3/Sakha8* | Egypt | Early |
| Gemmeiza 9 | ALD'S'/HUAC'S'//CMH74A.630/SX | Egypt | Late |
| Sunval | COOK*2/VPM-1//3COOK(1345) | Australia | Very late |
| Giza 168 | MRL.1BUC//SERI CM 93046. | Egypt | Medium |

### Extraction and purification of genomic DNA

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al.* (1997) was adopted for obtaining good quality total genomic DNA. Young green leaves from each genotype were collected from one-week-old seedlings germinated from seeds of each genotype and quickly frozen in liquid nitrogen and then ground using mortar and pestle. Five ml of CTAB extraction buffer (60°C), 50 mg PVP (polyvinyl pyrolidone) and 15 µl β-mercapto-ethanol (0.3%) were added to a half gram of leaf powder. The tubes were mixed by inversion and incubated at 65°C for one hour. Then, 6 ml of chloroform: Isoamyl alcohols (24:1) were added and the contents were

mixed by inversion to form an emulsion. The tubes were centrifuged at 5000 rpm for 20 min at room temperature. The top aqueous layer was further centrifuged at 5000 rpm after addition of 6 ml of chloroform: isoamyl (24:1). Half-volume of 5 M NaCl and two volumes of cold absolute ethanol were added to the supernatant and mixed well. The tubes were incubated at –20ºC overnight, then centrifuged at 8000 rpm for 15 min. The supernatant was discarded, the pellet was washed with 70% cold ethanol, and dried in a speed vacuum for 10 min. The pellet was dissolved in 300 µl TE buffer (pH 8.0) overnight at 4-6ºC, then transferred to 1.5 ml centrifuge tube. To remove RNA contamination, 4 µl (10 mg/ml) RNase A (Sigma Co., USA) were added to the DNA solution and incubated at 37ºC for 2 hours. The extracted DNA was deproteinized by adding 4 µl (1mg/ml) proteinase K (Sigma Co., USA) and incubating at 37ºC for 2 hours. Three hundred µl of Tris-saturated phenol-chloroform were added, and mixed by inversion. Tubes were centrifuged at 14000 rpm for 15 min in a microfuge (Eppendorf, USA). The upper layer was transferred to new tubes using wide bore pipette tip and 150 µl of TE buffer was added to the phenol phase, mixed and spun for 10 min.Then the upper layer containing the DNA was removed and added to the sample. DNA was precipitated overnight at –20ºC using 0.1 volume 3 M sodium acetate (pH 8.0) and two volumes of chilled absolute ethanol. The samples were centrifuged at 14000 rpm at 4ºC for 15 min. The DNA was washed with 70 % ethanol, briefly air-dried and re-dissolved in TE buffer.

**Estimation of DNA concentration**

DNA concentration was determined by diluting the DNA 1:5 in $dH_2O$. The DNA samples were electrophoresed in 0.7% agarose gel against 10ug of a DNA size marker (Lambda DNA digested with *HindIII* and Phi × 174 DNA digested with *HaeIII*). This marker covers a range of DNA fragment size between 23130bp and 310bp, and a range of concentrations between 95 ng and 11 ng. Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

**Detection of polymorphism among wheat genotypes**

RAPD analysis was employed to detect the polymorphism among the four wheat genotypes (Line 126, Sids 4, Gemmeiza 9, Sunval) in addition to two other genotypes (Giza 168 and the $F_1$ derived from the cross Sunval × Sids 4). These genotypes were chosen to represent early (Sids 4 and Line 126), medium (Giza 168 and $F_1$ of Sunval × Sids 4) and late (Sunval and Gemmieza 9) maturity. A set of eleven random 10-mer arbitrary primers (Table 2) was used in the detection of polymorphism among the six wheat genotypes. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) at AGERI. RAPD assay was preformed as described by Williams *et al.* (1990) with some modifications. The amplifications reactions were carried out in a volume of 25 µl containing 20ng genomic DNA, 25 pmoles primer, 2mM dNTPs, 2mM $MgCl_2$ and 2 U Taq polymerase (Fermentas) with, 1 × PCR buffer.

**Thermocyling profile and detection of the PCR products**

PCR amplification was performed in a Perkin-Elmer /GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an

annealing step at 36°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

***Table (2): Sequence of the eleven decamer arbitrary primers used in RAPD analysis to detect polymorphism among six wheat genotypes.***

| Primer | Sequence (5'-3') |
|---|---|
| OPA-10 | GTGATCGCAG |
| OPA-18 | AGGTGACCGT |
| OPB-03 | CATCCCCCTG |
| OPB-07 | GGTGACGCAG |
| OPB-10 | CTGCTGGGAC |
| OPB-16 | TTTGCCCGGA |
| OPB-17 | AGGGAACGAG |
| OPD-06 | ACCTGAACGG |
| OPE-04 | GTGACATGCC |
| OPO-02 | ACGTAGCGTC' |
| OPP-01 | GTAGCACTCC |

**RAPD data analysis**

The banding patterns generated by RAPD-PCR markers analyses were compared to determine the genetic relatedness of the genotypes. Clear and distinct amplification products were scored as '1' for the presence and '0' for the absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973).

Dice formula: GSij = 2a/(2a+b+c), where GSij is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called unweighted pair group method using arithmetic average (UPGMA) according to Sneath and Sokal (1973).

**Bulked segregant analysis (BSA)**

This method is used to rapidly find markers associated to a specific trait (Michelmore *et al.*, 1991). The essence of this procedure is the creation of bulk sample of DNA for analysis, by pooling DNA from individuals with similar phenotypes regarding the total trait. In this study, earliness is the main trait of interest for conducting bulked segregant analysis.

**Generation of bulk samples**

Two bulked samples were prepared from the $F_2$ population derived from the cross between Sunval (late parent) and Sids 4 (early parent). The two bulk samples represent an early bulk consisting of DNA from 10 early maturing $F_2$ individuals and the late bulk consisting of DNA from 10 late maturing $F_2$ individual plants.

**Screening of parents and bulks**

BSA involved screening for differences between the two parental genotypes and between the two genotypes and the two pooled DNA samples using ten decamer random primers. The names and sequence of primers are given in Table (3). Preparation of RAPD-PCR reactions, thermocycling profile and detection of PCR products were preformed as previously described in detection of polymorphism among wheat genotypes.

***Table (3): Sequence of the ten-decamer arbitrary primers used to detect RAPD markers for earliness using five wheat populations (Sids 4, Sunval, $F_1$, early $F_2$ bulk and late $F_2$ bulk).***

| Primer | Sequence (5′-3′) |
|---|---|
| OPA-04 | AATCGGGCTG |
| OPA-11 | CAATCGCCGT |
| OPB-14 | TCTGTGCTGG |
| OPB-17 | GACCGCTTGT |
| OPB-19 | CAAACGTCGG |
| OPB-03 | CATCCCCCTG |
| OPB-06 | TGCTCTGCCC |
| OPB-19 | ACCCCCGAAG |
| OPG-05 | CTGAGACGGA |
| OPG-19 | ACGACCGACA |

## RESULTS AND DISCUSSION

**a. RAPD analysis for six wheat genotypes**

**Genetic diversity among wheat genotypes**

Different methods are available for analysis of genetic diversity among germplasm accessions. These methods have relied on morphological, agronomic and biochemical data and recently on DNA-based marker data that allow more reliable differentiation of genotypes. In the present investigation, twenty ten-mer arbitrary primers were initially screened for PCR amplification of the genomic DNA for the six wheat genotypes. Only eleven primers generated reproducible and easily scorable RAPD profiles. The number of amplified fragments from the genomic DNA of each of the six wheat genotypes generated by the different primers is presented in Table (4).

Each of the eleven primers produced multiple band profiles with the six wheat genotypes. The highest number of amplicons (14 amplicons) was generated by the primer OPO02 in the genomic DNA of the cultivar Giza 168. While, the primer OPB16 exhibited the lowest number of amplicons, i.e., two amplicons, which were monomorphic across the six wheat genotypes. As shown in Table (5) the total number of DNA fragments amplified by the eleven primers was 91 with an average of 8.3 amplicons per primer. The number of polymorphic amplicons ranged from 0 to 11. Primer OPD06 amplified the highest number of polymorphic amplicons, while, the primer OPB07 revealed a total of seven amplicons which were all monomorphic across the six wheat genotypes. Therefore, the different primers expressed different levels of

polymorphism, ranging from 0.00% with primer OPB07 to 100% with primer OPB16.

***Table (4): Number of amplified DNA fragments produced by each RAPD primer for the six wheat genotypes.***

| Primer | Gem. 9 | Sids 4 | $F_1$ Sun×Sids4 | Sunval | Giza168 | Line 26 |
|---|---|---|---|---|---|---|
| OPA10 | 5 | 5 | 6 | 6 | 5 | 5 |
| OPA18 | 7 | 6 | 6 | 6 | 7 | 5 |
| OPB03 | 6 | 7 | 9 | 7 | 6 | 6 |
| OPB07 | 7 | 7 | 7 | 7 | 7 | 7 |
| OPB10 | 6 | 7 | 9 | 9 | 10 | 10 |
| OPB16 | 2 | 2 | 2 | 2 | 2 | 2 |
| OPB17 | 4 | 5 | 5 | 5 | 5 | 6 |
| OPD06 | 10 | 10 | 10 | 9 | 12 | 5 |
| OPE04 | 8 | 9 | 9 | 9 | 8 | 5 |
| OPO02 | 12 | 11 | 12 | 10 | 14 | 8 |
| OPP01 | 7 | 6 | 7 | 7 | 5 | 5 |

***Table (5): Total number of amplicons, number of monomorphic and polymorphic amplicons and percentage of polymorphism, as revealed by RAPD primers.***

| Primer | Total # of amplicons | #of mono amplicons | # of Poly amplicons | Polymorphism (%) |
|---|---|---|---|---|
| OPA10 | 6 | 5 | 1 | 16.67 |
| OPA18 | 8 | 3 | 5 | 62.50 |
| OPB03 | 9 | 5 | 4 | 44.44 |
| OPB07 | 7 | 7 | 0 | 00.00 |
| OPB10 | 10 | 6 | 4 | 40.00 |
| OPB16 | 2 | 0 | 2 | 100.0 |
| OPB17 | 6 | 4 | 2 | 33.33 |
| OPD06 | 13 | 2 | 11 | 84.62 |
| OPE04 | 9 | 8 | 1 | 11.11 |
| OPO02 | 14 | 6 | 8 | 57.14 |
| OPP01 | 7 | 4 | 3 | 42.86 |
| Total | 91 | 50 | 41 | |
| Average | 8.3 | 4.5 | 3.7 | 45.05 |

The total number of polymorphic bands revealed by the eleven primers was 41 and the average number of polymorphic fragments/ primer was 3.7. Thus, the average level of polymorphism was 45.05%. The size of amplified fragments varied with the different primers, ranging from 120 to 1350 bp (Figs. 1 and 2). In this respect, Joshi and Nguyen (1993) investigated the genetic diversity among 15 wheat varieties (*T. aestivum*) using RAPD analysis. Out of 109 amplified DNA fragments, 41 were polymorphic, representing a level of polymorphism of 65%. Perenzin *et al.* (1997) utilized 87 RAPD primers to assay the genetic diversity among wheat genotypes. They reported that 304 polymorphic bands were generated with an average of 3.49 polymorphic amplicon/ primer. Sun *et al.* (1998) used 32 arbitrary primers for RAPD analysis of 46 wheat genotypes, among which 26 primers (81.3%) revealed polymorphism. A total of 279 amplicons was generated and 182 (65.2%) were polymorphic. The number of polymorphic amplicons ranged from 2 to 20 with an average of 7 polymorphic amplicons per primer. Zheng *et al.* (2001) used 55

arbitrary primers in the RAPD analysis of 40 wheat cultivars. Out of 183 amplified fragments, 93 amplicons 50.8% were polymorphic; this represented an average of 1.7 polymorphic amplicons per primer. Moreover, Cao *et al.* (2002) screened 235 random primers against four wheat cultivars to detect RAPD polymorphism. Only, 31 (13.20%) primers produced polymorphism these 31 primers generated a total of 214 reproducible amplified fragments when used with 29 common wheat cultivars. The number of amplified fragments produced by each primer varied from 3 to 12 with an average of 6.9 and an average of 3.10 polymorphic bands per primer. Al-Naggar *et al.* (2004) used 17 arbitrary primers for RAPD analysis of six bread wheat genotypes.

Twelve primers (70.60%) generated polymorphic profiles. The total number of amplicons was 98, of which 34 (34.69%) showed polymorphism. Also, Wjhani (2004) studied the genetic variability among 14 wheat accessions using 39 RAPD primers. The total number of amplicons was 117, including 108 polymorphic amplicons. This represented a level of polymorphism of 92.3 % and an average number of 9 polymorphic bands per primer. The results of the present study are in good agreement with those reported in the literature, and confirm that polymorphism is a general phenomenon in wheat although it is a self- fertilizing species.

**Genetic identification by unique RAPD markers**

Unique markers are defined as bands that specifically identify an accession from the others by their presence or absence. The bands that are present in one accession but not found in the others are termed positive unique markers (PUM), in contrast with the negative unique markers (NUM), which are absent in a specific genotype. These bands could be used for genotype identification (Hussein *et al*, 2003).

As shown in Table (6), the RAPD assay permitted the identification of four out of the six wheat genotypes by unique positive and / or negative markers. These four genotypes , i.e. Gemmmeiza 9, Sunval, Giza 168 and Line 126 were characterized by 18 unique negative markers, while two of them (Giza 168 and Line 126), also revealed five unique positive markers. Line 126 was characterized by the highest number of unique markers (2 positive and 13 negative markers). This was followed by Giza 168 which revealed 3 positive and 1 negative unique markers. Sunval and Gemmeiza 9 revealed only 2 negative unique markers each, while, the two remaining genotypes, Sids 4 and $F_1$ (Sunval × Sids 4) did not exhibit any unique markers. This was expected since RAPD is a dominant marker assay, therefore the $F_1$ genotype should express the bands revealed by any one of the two parents, Sids4 and Sunval. Among the tested primers, 4 exhibited positive unique markers, while 7 revealed negative unique markers. Primer OPD06 revealed the highest number of unique markers (2 positive and 8 negative markers). The RAPD primers generating the different markers and the markers approximate size are listed in Table (6). The size of these unique markers ranged from 120 bp to 1350 bp. certain primers were more informative than others, e.g. OPD06 and OPO02, since each of them identified three out of the six studied wheat genotypes.

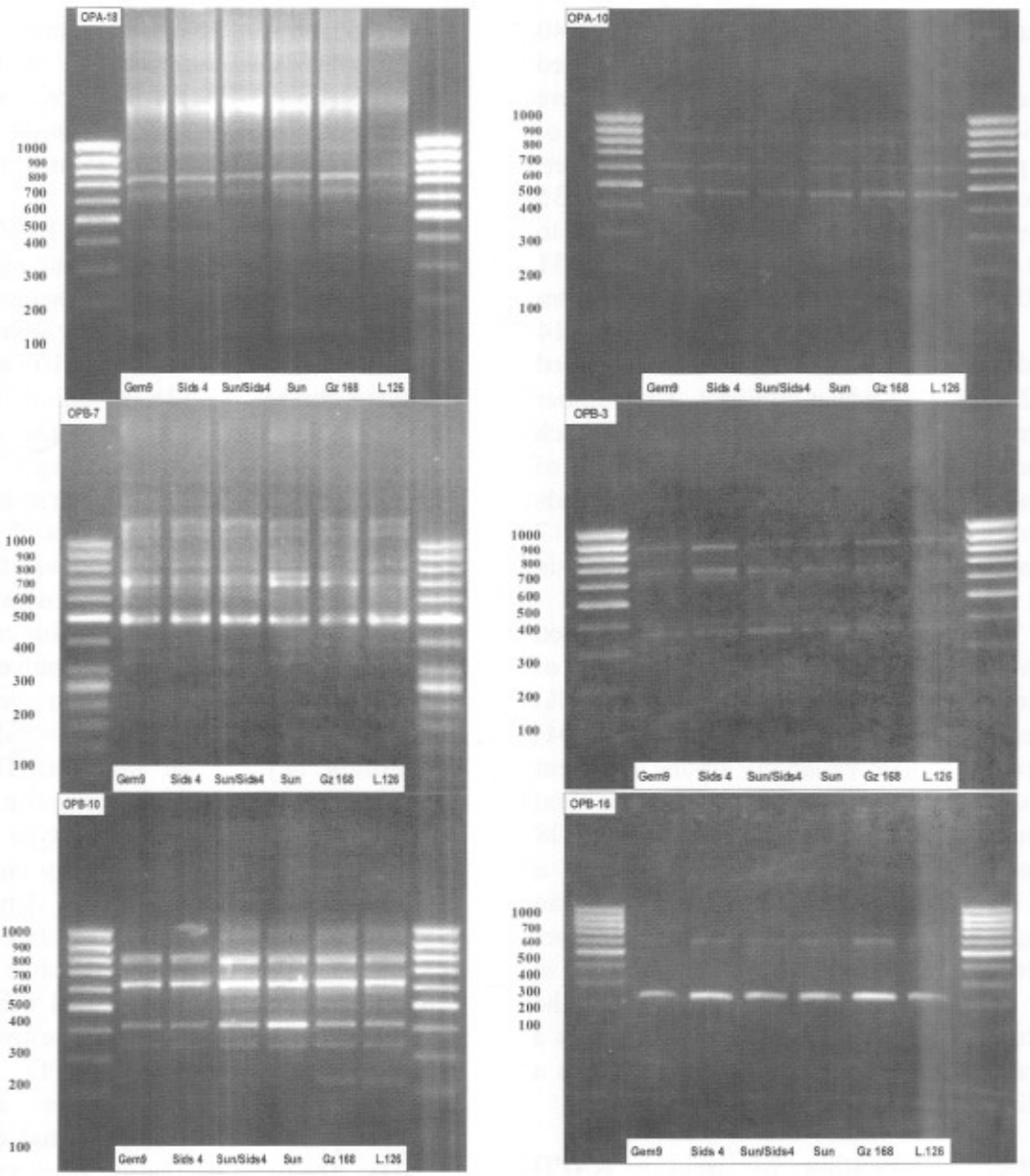


***Fig. (1):RAPD profile of the six wheat genotypes (Gemmeiza9, Sids4, $F_1$ Sun × Sids 4, Sunval, Giza168, L126) amplified with RAPD primers,OPA18,OPA10, OPB07, OPB03, OPB10 and OPB16: MW : 100 bp ladder.***

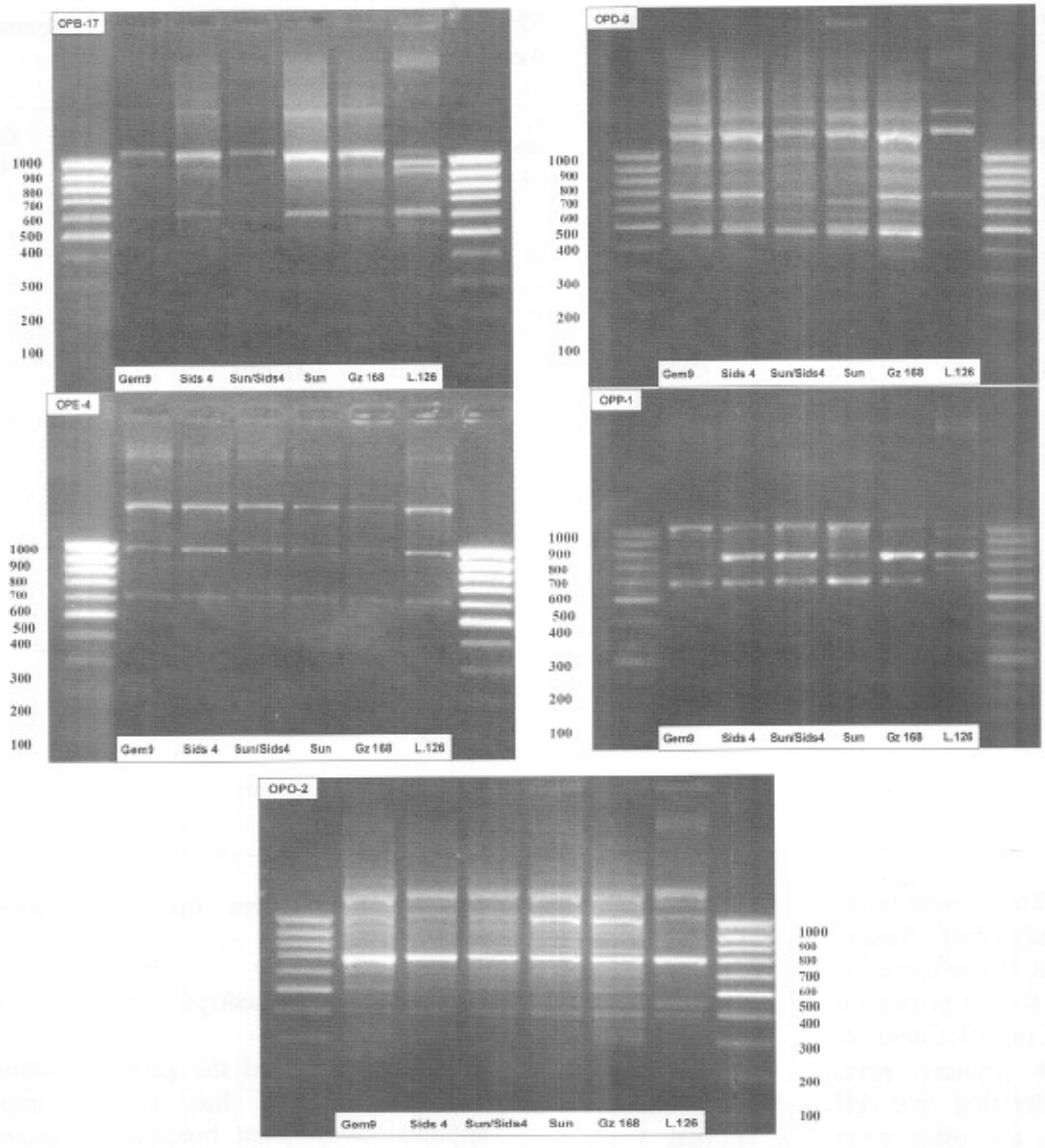


***Fig. (2): RAPD profile of the six wheat genotypes (Gemmeiza9, Sids4, $F_1$ Sun × Sids4, Sunval, Giza168, L126) amplified with RAPD primers, OPB17, OPD06, OPE04, OPPO01, OPO02: MW : 100 bp ladder.***

***Table (6): Positive and negative unique RAPD markers generated for the 6 wheat genotypes, marker size and total number of markers identifying each genotype.***

| Genotype | Positive unique markers | | | Negative unique markers | | | Grand total |
|---|---|---|---|---|---|---|---|
| | Size/ bp | Primer | Total No. | Size/ bp | Prime | Total No. | |
| Gemm.9 | 0 | --- | 0 | 500 | OPB10 | 1 | 2 |
| | | | | 500 | OPB17 | 1 | |
| Sids 4 | 0 | --- | 0 | 0 | --- | 0 | 0 |
| $F_1$(Sunval × Sids4) | 0 | --- | 0 | 0 | --- | 0 | 0 |
| Sunval | 0 | --- | 0 | 700 | OPD06 | 2 | 2 |
| | | | | 1100 | OPO02 | | |
| Giza 168 | 350, 780 | OPD06 | 2 | 650 | OPO01 | 1 | 4 |
| | 370 | OPO02 | 1 | | | | |
| Line126 | 400 | OPA18 | 1 | 120, 420 | OPA18 | 2 | 15 |
| | 950 | OPB17 | 1 | | | | |
| | | | | 500, 550, 650, 800, 970, 1300, 1350 | OPD06 | 7 | |
| | | | | 750, 800, 900 | OPO02 | 3 | |
| | | | | 600 | OPP01 | 1 | |
| Total | | | 5 | | | 18 | 23 |

The present results are in agreement with the findings of other authors in different plant species. Hussein *et al.* (2003) found that 34 out of 40 RAPD primers were able to identify 13 out of the 14 *Citrus* studied accessions. Three RAPD primers revealed unique markers characterizing five different accessions each. While, the other primers identified 1 to 4 accessions. Wjhani (2004) stated that 10 out of 12 RAPD primers identified 6 out of the 14 wheat accessions. Each of two primers revealed unique markers characterizing 2 accessions. While, the other primers identified 1 accession. Hussein *et al.*(2007) characterized 4 cotton genotypes by 24 positive and/ or negative RAPD markers. These unique markers were characterized by 8 out of the 15 RAPD primers used in this investigation and certain primers were more informative than others.

**Genetic relationships among wheat genotypes**

Knowledge of the genetic relationships among genotypes has several important applications in plant breeding programs and plant improvement. It permits the organization of germplasm, including elite lines and provides for more efficient parental selection. To determine the genetic relationships among the six wheat genotypes, the scoring data (1 for presence and 0 for absence) resulting from the eleven tested RAPD primers were used to compute the similarity matrices according to Dice coefficient (Sneath and Sokal, 1973). These similarity matrices were then used in the

cluster analysis to generate a dendrogram using the UPGMA method. As shown in Table (7) the genetic similarity among the six wheat genotypes ranged from 80.3% to 97.4%. The highest genetic similarity revealed by RAPD analysis (97.4%) was between Sunval (Sun) and the $F_1$ genotype derived from the cross Sun × Sids 4. This was followed by 95.9% and 95.4% between Gemmeiza 9 and Sids 4 and between Sids 4 and $F_1$ (Sunval × Sids 4), respectively. While, the lowest similarity (80.30%) was between Line 126 and Gemmieza 9. This reveals a great concordance between the data deduced from the RAPD analysis and the pedigree of these genotypes. The high level of similarity between Sunval and $F_1$ in one hand and Sids 4 and $F_1$ in the other hand reflects the heterozygous genetic constitution of the $F_1$ resulting from the cross between Sunval and Sids4. Similarly, the high level of similarity between Gemmeiza 9 and Sids 4 could be attributed to the presence of the common genotype CMH74 in the genetic background of both cultivars see (Table 1). While, the genetic background of Line 126 and Gemmeiza 9 is totally different and therefore, was reflected in the lowest genetic similarity detected by RAPD analysis.

***Table (7): Genetic similarity (GS) matrices among the six wheat genotypes as computed according to Dice coefficient from RAPDs.***

| | | | | | |
|---|---|---|---|---|---|
| G 168 | 100 | | | | |
| $F_1$ | 92.5 | | | | |
| Sids 4 | 91.5 | 95.4 | | | |
| Gem 9 | 91.5 | 94.1 | 95.9 | | |
| Sunval | 89.7 | 97.4 | 92.6 | 91.3 | |
| L 126 | 83.3 | 81.9 | 81.8 | 80.3 | 81.4 |

**Cluster analysis of the wheat genotypes as revealed by RAPDs**

The Dice RAPD-based coefficients of genetic similarity among the 6 wheat genotypes were employed to develop a dendrogram using the UPGMA method (Fig.3).The dendrogram separated Line126 from all the other genotypes, thus demonstrating the distinctiveness of the genetic background of this genotype from all the other genotypes. The other wheat genotypes formed a cluster in which Giza 168 was separated from the remaining four genotypes. These four genotypes constituted a subcluster divided into two groups, one group composed of Gemmeiza 9 and Sids 4, while the second group comprised Sunval and the F1 (Sunval × Sids4). Thus, the dendrogram deduced from the RAPD data corresponded well with the pedigree of the studied wheat genotypes.

The results of the present study revealed, therefore that RAPD analysis is an effective tool for detecting polymorphism, distinguishing between wheat genotypes and assessing their phylogenetic relationships. These results agree with Joshi and Nguyen (1993) who found that analysis of the genetic relationships among wheat varieties could distinguish most of the spring and winter wheat cultivars into different clusters in the dendrogram. Sivolap *et al.* (1999) reported that RAPD analysis proved to be one of the most powerful methods of discriminating cultivars. The dendrograms based on RAPD markers most closely conform to the pedigree data. Cao *et al.* (2000) used RAPD marker to assess phylogenetic relationships between 15 wheat accessions. Clusters analysis classified these accessions into five groups in agreement with morphological classification. Sun *et al.* (2003) found that the dendrogram prepared on the

basis of RAPD data corresponded well with the pedigree of two groups of wheat genotypes. Shehata *et al.* (2004) reported that SDS-PAG and RAPD-PCR were successfully used to construct dendrograms to the wheat cultivars into two main groups.

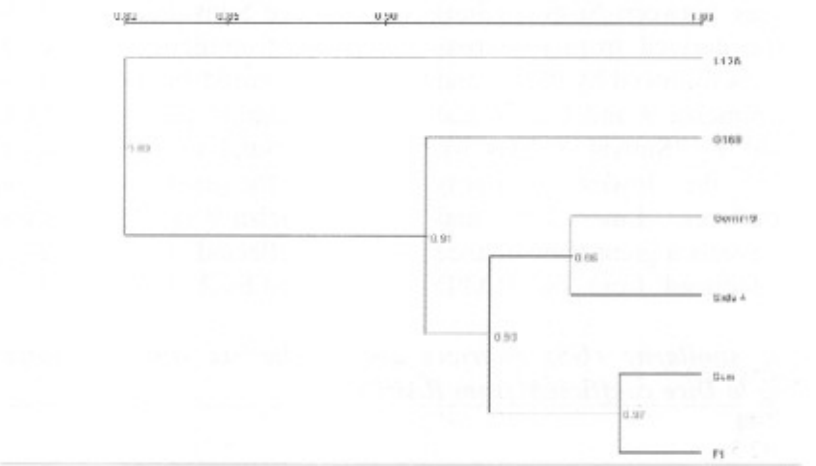

***Fig. (3): Dendrogram for the six wheat genotypes constructed from RAPD data using (UPGMA) according to Dice coefficients.***

**b. Bulked segregant analysis for wheat earliness**

In bulked segregant analysis two parents contrasting for the traits of interest are crossed to generate a segregating population. Then, the DNA samples from different individual progeny in this segregating population are pooled into bulked samples by genotypic or phenotypic class. The success of bulking by phenotypes is dependent on the correspondence of genotype and phenotype. A specific target allele will occur in one bulked sample, but not the other. This pattern of frequency difference will also be seen for any marker or gene that is tightly linked to the target allele. A marker, which shows a clear difference between the bulks, is likely to be linked to the target genes or near by markers. The two bulks show no difference for the rest of the genome. When using RAPD markers in BSA, the low frequency allele will not be amplified. A bulked sample is prepared from the early maturing progeny and another bulked sample is prepared from the late maturing progeny. A DNA marker that is independent of the earliness traits will be represented as a band in both the early and late bulked sample gel lanes. While, a DNA marker linked to the earliness character will be represented as a band in one of the bulked sample lanes, but not the other (Michelmore *et al.*, 1991).

The two bulked genomic DNA samples were employed for PCR amplification to identify RAPD markers associated to the earliness traits. Twenty random 10- mer primers were primarily screened for PCR amplifi-cation of the genomic DNA of the two parental genotypes (Sunval and Sids 4), their

$F_1$ and two bulked $F_2$ samples (early bulk and late bulk). Only 10 out of the 20 primers revealed reproducible, discernible amplification products. Among the 10 primers, two primers (OPB03 and OPG05) revealed amplification products that were present in one bulk but not the other.

As shown in Fig. (4), the early maturing bulked sample revealed two bands: one of molecular size of 800 bp amplified by the primer OPB03 and another band of molecular size of 850 bp amplified by the primer OPG05. These two DNA bands were also amplified by the same primers in the DNA of the early maturing parental genotype (Sids 4) and the $F_1$. While, they were not present in the DNA of the late bulk and the late parental genotype (Sunval). Therefore, these two bands could be considered as positive RAPD markers associated with the earliness trait. Moreover, the primer OPB05 amplified one DNA band of molecular size 550 bp from the DNA of the late bulked sample, the late parent and the $F_1$. While this band was not amplified in the DNA of the early bulked sample and the early parent (Sids 4). Therefore, this band was associated with late maturity and could be used as a negative RAPD marker for earliness.

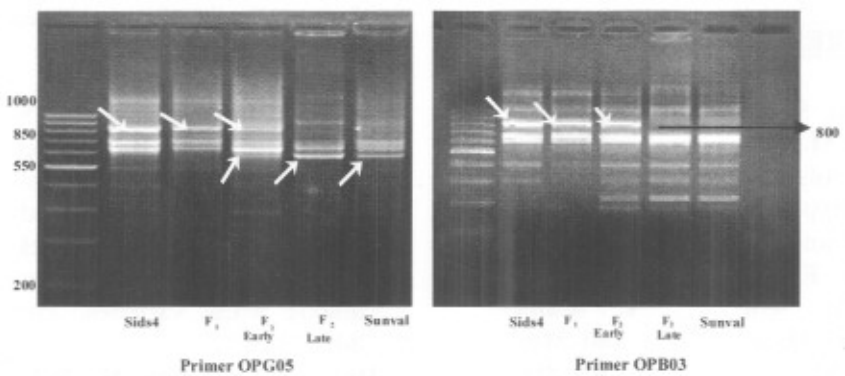


***Fig. (4): RAPD profile of the five wheat genotypes amplified with RAPD primers (OPG05, OPB03) positive unique markers for early genotypes and positive unique marker in late group: MW: 100 bp ladder.***

In this context, Rahman *et al.* (1998) used 74 RAPD primers in an attempt to identify markers linked to salinity tolerance. Four primers produced polymorphic DNA fragments in the two bulked samples. However, RAPD analysis of DNA from individual plants showed that only one polymorphic DNA fragments of 680 bp amplified by primer OPZ10 was associated with salt tolerance. El-Khishin (1999) used bulked segregant analysis for identification of markers associated with resistance to whitefly. Four primers gave positive results between the two bulks for resistance and susceptibility, three of which were mapped to their respective linkage groups. In the present study, two bulked samples were prepared: an early maturing bulk consisting of DNA from the ten earliest $F_2$ individuals and a late maturing bulk consisting of DNA from ten late maturing individual plants. In this respect, Michelmore *et al.* (1991) in lettuce generated two bulks for segregant analysis. They stated that the number of individuals used in each bulk varied between 14 and 20 plants. Naqvi *et al.* (1995) in rice, used DNA from 5 plants to construct

each of the two bulked samples: resistant and susceptible. Hu *et al.* (1997) in wheat used 6 plants to generate the bulked samples. However, since bulked segregant analysis is a rapid assay to determine markers associated to a trait of interest, therefore, the obtained results need further studies to confirm the linkage between the identified markers and the earliness trait. This should be performed by screening the RAPD markers and the earliness trait across all the individuals of the $F_2$ population to confirm the linkage between the polymorphic RAPD markers and the traits of interest on the basis of the recombination frequency.

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## الملخص العربي

### التباعد الوراثي وتحليل الفئات الإنعزالية المجمعة للتبكير في نضج القمح

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تم دراسة التباين الوراثي وعلاقة القرابة بين ستة تراكيب وراثية من القمح متباينة بالنسبة لموعد النضج (اثنين مبكرين فى النضج هما سدس 4، سلالة 126 واثنين متأخرين فى النضج هما سنفال، جميزة 9 واثنين متوسطين فى النضج هما جيزة 168، والجيل الاول للهجين سنفال × سدس 4) وذلك باستخدام 11 بادئ عشوائي من نوع الـ DPAR . حيث أنتجت 91 شظية من الدنا منها 41 شظية أظهرت تباين على مستوى الدنا و كانت نسبة التباين 45,05%. وتم دراسة علاقات القرابة بين الستة تراكيب وراثية باستخدام معامل Dice ورسم الدندروجرام الذي يوضح درجات القرابة وذلك باستخدام تحليل UPGMA . كما امكن تمييز أربعة من الستة تراكيب وراثية تحت الدراسة بواسطة 23 واسم موجب و /أو سالب من نوع الـ DPAR . وتم أيضاً استخدام تحليل الفئات الانعزالية المجمعة Bulked segregant analysis (BSA) كوسيلة سريعة للتعرف على واسمات جزيئية مرتبطه بصفة التبكير لخمسة تراكيب وراثية من القمح هى سدس 4 و سنفال وهجن الجيل الاول بينهما والتجميعة الإنعزالية المبكرة لل 2F والتجميعة الإنعزالية المتأخرة لل 2F . من بين عشرة بادئات من نوع الـ DPAR أظهر بادئان عدد 2 واسم موجب و واسم واحد سالب كانت لها علاقة بصفة التبكير. يلزم إجراء دراسات إضافية لتأكيد ارتباط هذه الواسمات بصفة التبكير.