EPISTASIS AND MOLECULAR MARKERS LINKED TO EARLINESS IN BREAD WHEAT

A. M. M. Al-Naggar[1], S. R. S. Sabry[2] and Kh. I. M. Gad[2]

1- Department of Agronomy, Faculty of Agriculture, Cairo University, Giza, Egypt
2- Wheat Research Department, FCRI, Agriculture Research Center, Giza, Egypt

ABSTRACT

A better understanding of type of gene action and molecular markers linked to earliness would help wheat breeders to efficiently improve early maturing high yielding cultivars. One early (Sids 4) and one late (Sunval) wheat (Triticum aestivum) cultivar (P_1 and P_2, respectively) were crossed and the F_1 was selfed to produce F_2 and backcrossed parents. The six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) were evaluated in the field for earliness and yield traits. Significant and positive additive (a) and dominance (d) gene effects occurred for days to heading (DTH), days to maturity (DTM), grain filling period (GFP) and grain filling rate (GFR), indicating an enhancing effect for these traits due to these types of gene effects. Dominance was larger in magnitude than additive in most studied traits. Significant digenic epistatic gene effects were exhibited for all the three types of epistasis (aa, ad and dd) in DTH, GFP and grain yield/plant (GYP) and for aa and dd in GFR and spikes/ plant (SPP), indicating that interallelic interaction was important in the inheritance of these traits. The aa was the highest in magnitude for DTH and GYP while dd was the highest for GFP, SPP and GFR. The genetic variability among five wheat populations that differ in earliness trait (P_1, P_2, F_1, early F_2 bulk and late F_2 bulk), were investigated using 10 RAPD primers. The number of polymorphic amplicons was 46 out of a total of 75 amplicons, thus revealing a level of 61.33% polymorphism. All the five wheat populations were characterized by 21 positive and/or negative RAPD markers. Bulked segregant analysis was used to rapidly identify markers associated to earliness. Out of 10 primers, only two primers revealed two positive and one negative markers associated with earliness trait. Further studies are required to confirm the linkage between these RAPD markers and earliness.

Key words: *Epistasis, Earliness, Wheat, Molecular markers, Bulked segregant analysis, RAPD, Generation mean analysis*

INTRODUCTION

A better understanding of earliness inheritance and type of gene action would help wheat breeders to efficiently improve early maturing high-yielding cultivars. Additive gene action is evidently accounted for a large amount of the variation for number of days to heading (Avey *et al* 1982 and Menshawy 2005), number of days to maturity (Menshawy 2005) and grain filling duration and rate (Rasyad and Van Sanford 1992, Beiquan and Kronstad 1994 and Mou and Kronstad 1994). But dominance also was important (Avey *et al* 1982 and Menshawy 2005) for earliness traits, while epistasis was reported in few studies (Ketata *et al* 1976 for earliness and Przulj and Mladenov 1999 for grain filling traits).

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 that can be used to construct linkage maps to identify varieties (He *et al* 1992) and to assess genetic diversity in wheat (He *et al* 1992, Dhaliwal *et al* 1993, Cao *et al* 2002, Munshi *et al* 2003, Maric *et al* 2004 and Abd-El-Haleem *et al* 2009). It is characterized by its low technical input and small quantity of plant DNA needed for the analysis (Manabe *et al* 1999).

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 F_2 population are bulked for the development of molecular markers associated with a given characteristic. Based on this bulked segregant analysis, 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) determine the intra- and inter-allelic gene interactions controlling the inheritance of wheat earliness and yield traits and (2) identify RAPD markers associated with earliness in wheat *via* bulked segregant analysis.

MATERIALS AND METHODS

Plant materials

Two bread wheat (*Triticum aestivum* L.) cultivars, showing clear differences in earliness were chosen to be used as parents of this study. These two parents included one Australian very late (Sunval) (P_1) and one Egyptian early (Sids 4) (P_2) cultivar. The F_1 and F_2 cross between P_1 and P_2 and BC_1 and BC_2 were produced as well as the early and late F_2 bulks.

In the first growing season (2004/2005) the two parental genotypes (P_1 and P_2) were planted on three different sowing dates of 15-days intervals in order to match between early and late genotypes in flowering. One cross was performed between the late and the early parents, i.e Sunval × Sids 4 and F_1 seeds were obtained. In the second season (2005/2006), the F_1 hybrid seeds of this cross were sown and the F_1 plants were back crossed to each of parents and seeds of the two backcrosses BC_1 and BC_2 were obtained. At the same time, F_1 plants were self-pollinated to produce F_2 seeds.

Field evaluation of the 6 populations

In the third season (2006/2007), the obtained seeds of these populations i.e., P_1, P_2, F_1, F_2, BC_1 and BC_2 were sown for evaluation at the field of Giza Res. Sta. of the ARC, using a randomized complete block design (RCBD) with three replicates. Sowing date was 28 November 2006. Each experimental plot consisted of eight rows for F_2 population, two rows for each of the P_1, P_2 and F_1 and three rows for each of the BC_1 and BC_2. The rows were 3.5 m long and 20 cm wide and spaces between plants were 10 cm within each row. Agricultural wheat practices were applied following the recommendations of the Wheat Research Department, ARC. Data of the studied characters were recorded on 30 individual guarded plants for P_1, P_2 and F_1 and 90 plants for BC_1 and BC_2 and 200 plants for the F_2.

Data were recorded on the following traits: (1) days to heading (DTH) as number of days from sowing date to the date at which 50% of main spike awns/ plot have completely emerged from the flag leaves, (2) days to maturity (DTM) as number of days from sowing date to the date at which 50% of main peduncles/ plot have turned to yellow color (physiological maturity), (3) grain filling period (GFP) as number of days from 50% anthesis to 50% physiological maturity, (4) grain filling rate (GFR) in g/day determined by dividing grain yield/plant by the duration of grain filling period, (5) spikes / plant (S/P) as a number of fertile spikes per plant and (6) grain yield / plant (GY) in g as weight of the grains of each individual plant. Analysis of variance of RCBD was performed and LSD estimates were calculated to test the significance of differences among means according to Snedecor and Cochran (1989). Genetic analysis of generation means and estimates of mean effect ($\overline{m}$), additive (a), dominance (d), additive × additive (aa), additive × dominance (ad) and dominance × dominance (dd) effects were computed using the six-parameter model proposed by Gamble (1962). Heritability in both broad and narrow sense was calculated according to Mather (1949). Genetic advance from selection based on 10% selection intensity was computed according to Falconer (1989).

Randomly amplified polymorphic (RAPD) analyses

These experiments were carried out at the Molecular Genetics and Genome Mapping (MGGM) Laboratory at the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt. RAPD was used in the present study to perform two types of analyses, i.e., to investigate the genetic diversity among five bread wheat populations (Sunval, Sids 4, Sunval × Sids 4 (F_1), Early F_2 bulk and Late F_2 bulk) and to identify markers associated to earliness *via* the bulked segregant analysis approach. These genotypes were chosen to represent early (Sids 4 and early bulk), medium (F_1) and late (Sunval and late bulk) maturity.

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 population were collected from one-week-old seedlings 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-mercaptoethanol (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 alcohol (24:1) was added and 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 –20°C overnight, then centrifuged at 8000 rpm for 15 min. The supernatant was discarded, the pellet washed with 70% cold ethanol, and dried in speed vacuum for 10 min. The pellet was dissolved in 300 µl TE buffer (pH 8.0) overnight at 4-6°C, 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 37°C for 2 hours. The extracted DNA was deproteinized by adding 4 µl (1mg/ml) proteinase K (Sigma Co., USA) and incubating at 37°C 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 -20°C 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 4°C 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$_2$O. 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 fragments 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 populations

A set of ten random 10-mer arbitrary primers (Table 1) was used in the detection of polymorphism among the six wheat populations that differ in earliness. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied

Table 1. Sequence of the ten-decamer arbitrary primers used to detect RAPD markers for earliness of wheat.

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

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 94°C. Each cycle consisted of a denaturation step at 94°C 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.

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.

Two bulked samples were prepared from the F_2 population derived from the cross between parents. The two bulk samples represented 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 the same ten decamer random primers given in Table (1). Preparation of RAPD-PCR reactions, thermocycling profile and detection of PCR products were preformed as previously described in detection of polymorphism among wheat populations.

RESULTS AND DISCUSSION

Genetic analysis of the six generations

Analysis of variance

Significant mean squares due to populations (the six populations P_1, P_2, F_1, F_2, BC_1 and BC_2) existed for all studied traits (data not presented). Variances calculated separately for each population are presented in Table (2). The highest magnitude of variance was reported by the F_2 generation for all studied traits followed by that of backcross generations (BC_1's and BC_2's) which is logic due to the fact that maximum heterogeneity exists in F_2 generation. On the other hand, the lowest variance magnitude was manifested by parents (P_1 and P_2) and F_1 populations, which is also logic from the breeding point of view due to the homogeneity of such populations and variance observed in these populations is due to environmental factors. Results of variance magnitude of each of the six populations are logic and suggests the validity of six-parameter model to determine the magnitude of different gene effects for studied characters.

Table 2. Variances (S^2) of P_1, P_2, F_1, F_2, BC_1 and BC_2 populations of the wheat cross Sunval × Sids 4 for earliness and yield traits.

Trait	P_1	P_2	F_1	F_2	BC_1	BC_2
Days to heading	1.7	1.8	1.8	18.0	15.1	15.4
Days to maturity	1.6	2.6	1.1	22.0	18.0	14.0
Grain filling period	1.6	2.2	2.0	22.5	21.0	20.0
Grain filling rate	0.5	0.01	0.6	5.0	3.0	2.50
Spikes/ plant	2.3	2.6	2.0	35.0	18.0	15.0
Grain yield/ plant	1.9	1.2	2.1	34.0	17.0	15.0

Generation means

Means of P_1, P_2, F_1, F_2, BC_1 and BC_2, populations of the wheat cross Sunval × Sids 4 for studied earliness and yield traits are presented in Table (3). Significant differences existed between the two parents (P_1 and P_2) for all studied traits. Such differences are prerequisite for the validity of the 6 generation mean analysis. These differences were more pronounced between parents for days to heading, days to maturity and spikes/ plant. The parent Sunval was very late in heading and maturity and had high number of spikes/plant, while the parent Sids 4 showed an opposite direction for the same traits. F_1 generation means were inbetween P_1 and P_2 means for most studied traits, indicating partial to complete dominance as well as additive gene effects. The exceptions were grain filling period and grain yield/plant, where F_1 means were larger than the higher parent, indicating positive overdominance.

Table 3. Means of studied earliness and yield traits for P₁, P₂, F₁, F₂, BC₁ and BC₂ populations evaluated in 2006/2007 season.

Population	Days to heading (No)	Days to maturity (No)	Grain filling period (days)	Grain filling rate (g/day)	Spikes/ Plant (No)	Grain yield/plant (g)
P₁ (Sunval)	123	172	38.5	1.06	34.5	36.0
P₂ (Sids4)	78	144	61.5	0.37	2.9	25.5
F₁ (P₁×P₂)	99	168	62.0	0.56	28.0	50.0
F₂	89	163	67.0	0.41	25.0	45.0
BC₁	115	168	51.0	0.84	32.0	42.0
BC₂	95	158	56.0	0.50	16.0	38.0
LSD 0.05	9	8	6	0.6	9	4

Means of F_2 generation were lower than those of F_1 generation for most studied traits, indicating inbreeding depression. The exception was grain filling period, where F_2 was higher than F_1 mean, indicating inbreeding gain for this trait.

Means of the BC_1 were close to those of their respective female parents (P_1) and means of the BC_2 were close to their respective male parents (P_2) for all studied earliness and yield traits, which is logic from the breeding point of view.

Gene effects

Estimates of gene effects calculated from the six-parameter model of the generation mean analysis of the wheat cross Sunval × Sids 4 for studied earliness and yield traits are presented in Table (4). Significant mean effects (m) exhibited for all studied traits. Significant and positive additive and dominance gene effects occurred for all studied traits, except for grain filling period which showed significant negative additive effects, indicating an enhancing effect for these traits due to both types of intra- allelic gene effects. These results indicated that the potentiality of improving most studied traits by using both pedigree selection and heterosis breeding programs. Only a diminishing effect was acting due to additive type of gene action for grain filling period. Dominance was larger than additive variance for all studied traits, except for spikes/ plant, where the opposite was true effect, a= additive effect, d= dominance effect, aa= additive × additive, ad= additive × dominance and dd= dominance × dominance effects.

Conclusions reported by pervious investigators regarding the relative importance of either additive or dominance in the inheritance of earliness and yield traits in bread wheat are contrasting. Some researchers indicated that dominance was more important than additive effect in the inheritance of days to heading (Avey et al 1982 and Menshawy 2005). However, Beiquan and Kronstad (1994) and Menshawy (2005) reported that additive was more important than dominance effect for grain filling period (Rasyad and Van Sanford 1992, Beiquan and Kronstad 1994, Mou and Kronstad 1994 and

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Table 4. Mean estimates of the six gene effects for studied traits in the wheat studied cross.

Trait	Gene action parameter					
	m	a	d	aa	Ad	Dd
Days to heading	89**	15**	50**	54**	-10**	-61**
Days to maturity	163**	10**	12**	0.0	-4.0 *	-4.0
Grain filling period	67**	-5 *	38.50**	-54**	6.50 *	57**
Grain filling rate	0.41**	0.34**	0.88 *	1.04**	-0.004	1.17**
Spikes /plant	25**	15.0**	7.5 *	18.0**	-1.4	19.20**
Grain yield / plant	28**	15.0**	35.5**	30.0**	7.50*	-43.0 *

*, ** significance at 0.05 and 0.01 levels of probability levels, respectively. m = mean

Menshawy 2005). Differences in conclusions between this study and others with r⋯rd to the relative importance of either additive or dominance effects ma ⌣ ue to differences in genetic background of materials used in different stⳙ ·.

Significⳙant digenic epistatic gene effects (Table 4) were exhibited for al! the three types of epistasis (aa, ad and dd) in days to heading, grain filling period and yield/ plant and for aa and dd in grain filling rate and spikes/plant. This indicates that epistatic gene effects were generally important in the inheritance of studied traits. Few previous reports indicated the existence of epistasis in wheat for earliness (Ketata *et al* 1976) and grain filling traits (Przulj and Mladenov 1999). The aa was the highes: in magnitude among the three digenic epistatic effects for days to heading and grain yield/plant, while the dd was the highest for GFP, GFR and spikes/plant.

Heritability and genetic advance

Very high broad- sense heritability percentages (>86%) were obtained for all studied characters (Table 5), indicating that genetic variance accounted for most of the phenotypic variance. However, narrow- sense heritability percentage was the lowest (17.77 %) for grain filling period, medium (52.63 %) for grain yield/plant and the highest (90.0 %) for grain filling rate. The difference in magnitude between broad- and narrow- sense heritability is attributed to the non- additive genetic effects, i.e. dominance, additive × dominance and dominance × dominance effects. Such differences were very high for grain filling, suggesting that non- additive components accounted for the largest part of genetic variance for most studied traits. Heterosis breeding is a recommended procedure to utilize such non- additive genetic components. On the contrary, grain filling rate showed heritability in narrow- sense of 90 %, indicating that selection for the improvement of this trait in the segregating generations of the studied cross could be considered the method of choice. Percentage of expected genetic advance

Table 5. Heritability % and expected genetic advance under selection for studied traits in the wheat studied cross.

Traits	Heritability %		Genetic advance	
	Broad sense	Narrow sense	Absolute	%
Days to heading	90.08	30.70	2.68	3.01
Days to maturity	91.81	27.27	2.63	1.61
Grain filling period	90.55	17.77	1.73	2.59
Grain filling rate	95.80	90.00	0.04	9.76
Spikes /plant	86.79	24.63	2.26	9.07
Grain yield / plant	94.82	52.63	4.72	16.9

from selection (Table 5) was the highest (16.47 %) for grain yield/plant and the lowest (1.61 %) for days to maturity.

RAPD analysis for five wheat populations

Twenty ten-mer arbitrary primers were initially screened for PCR amplification of the genomic DNA for the five wheat genotypes. Only ten primers generated reproducible and easily scorable RAPD profiles.

The number of amplified fragments from the genomic DNA of each of the five wheat populations generated by the different primers is presented in Table (6). Each of the ten primers produced multiple band profiles with the five wheat populations. The highest number of amplicons (9) was generated by the primer OPA17 in the genomic DNA of the F_2 early bulk. While, the primer OPB06 did not exhibit any amplicon in the DNA of the cultivar Sunval. As shown in Table (7) the total number of DNA fragments amplified by the ten primers was 75 with an average of 7.5 amplicons per primer. The number of polymorphic amplicons ranged from 1 to 9. Primer OPG05 amplified the highest number of polymorphic amplicons, while, the primer OPB06 revealed a total of six amplicons which were all polymorphic across four wheat genotypes. Therefore, the different primers expressed different levels of polymorphism, ranging from 25% with primer OPG17 to 100% with primer OPB06. The total number of polymorphic bands revealed by the ten primers was 46 and the average number of polymorphic fragments per primer was 4.6. Thus, the average level of polymorphism was 61.33%. 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 (2003) used 32 arbitrary primers for RAPD analysis of 46 wheat genotypes, among which 26 primers

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Table 6. Number of amplified DNA fragments produced by each RAPD primer for the 5 population analysis of wheat cross Sunval × Sids 4.

Primer	Sids 4	Sunval	F₁	F₂ early bulk	F₂ late bulk	Total	Mean
OPA04	5	5	5	3	5	23	4.6
OPA11	4	3	4	4	3	18	3.6
OPA14	4	2	4	2	5	17	3.4
OPA17	6	9	8	9	6	38	7.6
OPA19	6	8	6	6	5	31	6.2
OPB03	6	7	4	7	7	31	6.2
OPB06	6	0	6	6	6	24	4.8
OPB19	7	7	6	6	5	31	6.2
OPG05	8	3	5	5	4	25	5.0
OPG17	4	4	4	4	3	19	3.8
Total	56	48	52	52	49	257	51.4
Mean	5.6	4.8	5.2	5.2	4.9	25.7	5.14

Table 7. Total number of amplicons, number of monomorphic and polymorphic amplicons and percentage of polymorphism, as revealed by RAPD analysis for wheat earliness.

Primer	Total No. of amplicons	No. of mono-morphic amplicons	No. of Poly-morphic amplicons	Poly-morphism (%)
OPA04	5	3	2	40
OPA11	5	2	3	60
OPA14	9	3	6	66
OPA17	11	5	6	54
OPA19	8	5	3	37
OPB03	9	2	7	77
OPB06	6	0	6	100
OPB19	8	5	3	37
OPG05	10	1	9	90
OPG17	4	3	1	25
Total	75	29	46	
Average	7.5	2.9	4.6	61.33

(81.3%) revealed polymorphism. A total of 279 amplicons were 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. They 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 band 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 (8), the RAPD assay permitted the identification of all the five wheat populations by unique positive and / or negative markers. Four populations, $i.e.$ F_1, F_2 early bulk and F_2 late bulk and Sunval were characterized by 16 unique negative markers, while three populations (Sids 4, F_2 late bulk and Sunval) revealed five unique positive markers. Sunval was characterized by the highest number of unique markers (2 positive and 10 negative markers). This was followed by F_2 late group which revealed 2 positive and 2 negative unique markers. F_1 and F_2 early bulk revealed only 2 negative unique markers each, while, Sids 4 exhibited only one positive unique marker. 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 8 revealed negative unique markers. Three primers exhibited both positive and negative unique markers. Primer OPB06 revealed the highest number of unique markers (6 positive). The RAPD primers generating the different markers and the marker approximate size are listed in Table (8). The size of these unique markers ranged from 300 bp

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Table 8. Positive and negative unique RAPD markers generated for the 5 wheat populations and their molecular weight (Bulked segregant analysis)

Genotype	Positive unique markers			Negative unique markers			Grand total
	Size/bp	Primer	Total No.	Size/bp	Primer	Total No.	
Sids 4	700	OPB19	1	-----	-----	-----	1
F₁ (Sunval x Sids 4)	-----	-----	-----	400, 300	OPB03	2	2
F₂ early group	-----	-----	-----	600, 500	OPA04	2	2
F₂ late group	650, 450	OPA19	2	800, 600	OPA11	2	4
Sunval	900 750	OPG05 OPA14	2	800 900 900, 850, 800, 700, 600, 400 900 350	OPG05 OPA19 OPB06 OPG17 OPB19	10	12
Total			5			16	21

to 900 bp. Certain primers were more informative than others, e.g. OPB19 and OPA19, since each of them identified two out of the five studied wheat genotypes.

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.

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 nearby 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 the early 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. A total of 20 random 10- mer primers were primarily screened for PCR amplification 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. (1), 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 (Sids4) 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, OPG05 primer amplified one DNA band of molecular size of 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 the late maturity and could be used as a negative RAPD marker for earliness. 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 fragment 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.
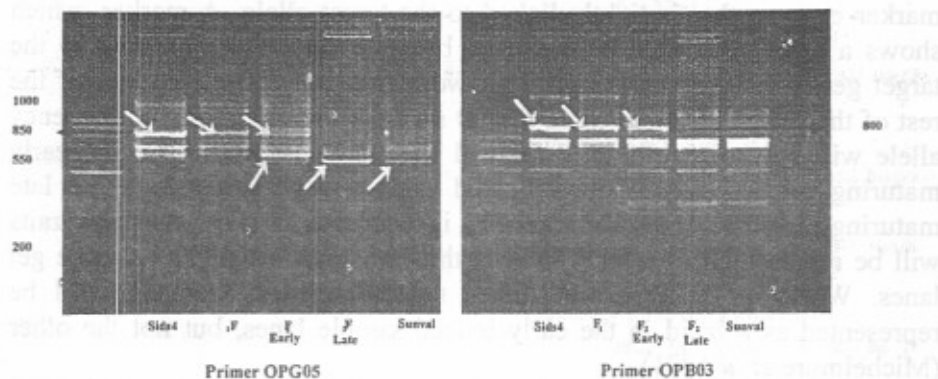
Fig. 1. RAPD profile of the five wheat populations amplified with RAPD primers (OPG05, OPB03) positive unique markers for early genotypes and positive unique marker in late group: MW: 100 bp ladder

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 bulked 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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تفاعل الجينات والواسمات الجزينية المصاحبة للتبكير
فى نضج قمح الخبز

أحمد مدحت محمد النجار[1]، سامى رضا صابر صبرى[2] وخالد ابراهيم محمد جاد[2]

١- قسم المحاصيل ، كلية الزراعة، جامعة القاهرة، الجيزة، مصر

٢- قسم بحوث القمح ، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، الجيزة، مصر

إن التفهم الجيد لنوع فعل الجين والواسمات الجزينية المصاحبة للتبكير فى النضج من الممكن أن يساعد مربى القمح لإستنباط أصناف مبكرة وعالية المحصول بكفاءة أعلى. تم تهجين اب مبكر (سدس ٤) مع أب متأخر (سنفال) وإخصاب الجيل الأول F_1 ذاتياً للحصول على الجيل الثانى F_2 وتهجينة رجعياً مع الأب الأول (P_1) للحصول على جيل التهجين الرجعى للأب الأول (BC_1) ومع الأب الثانى (P_2) للحصول على جيل التهجين الرجعى للأب الثانى (BC_2) تم تقييم العشائر الستة ($BC_2, BC_1, F_2, F_1, P_2, P_1$) فى الحقل لصفات التبكير والمحصول. ظهرت تأثيرات جينية معنوية وموجبة للفعل المضيف (a) وللسيادة (d) بالنسبة لعدد الأيام حتى الطرد وعدد الأيام حتى النضج وفترة امتلاء الحبوب ومعدل امتلاء الحبة مما يشير إلى التأثير المحفزلهذه الصفات الذى يرجع لهذين الطرازين من فعل الجين. كان مقدار تباين السيادة اكبر من مقدار التباين المضيف فى معظم الصفات المدروسة. ظهرت تأثيرات جينات التفاعل الثنائى المعنوية لكل الطرز الثلاثة من التفاعل epistasis (مضيف × مضيف،

مضيف × سيادة وسيادة × سيادة) فى صفات عدد الأيام حتى الطرد وفترة امتلاء الحبوب ومحصول حبوب النبات، وللطرازين مضيف × مضيف وسيادة × سيادة فى معدل امتلاء الحبة وعدد سنابل النبات، مما يشير إلى ان تفاعل اليلات الجينات المختلفة كان مهماً فى وراثة هذه الصفات. كان تفاعل المضيف × المضيف الأعلى فى المقدار لصفتى عدد الأيام حتى الطرد ومحصول حبوب النبات بينما كان تفاعل السيادة × السيادة هو الأعلى لصفات فترة امتلاء الحبوب وعدد سنابل النبات ومعدل امتلاء الحبة. تم دراسة التباين الوراثى بين الخمس عشائر وراثية للقمح المختلفة فى التبكير (F_1, P_2, P_1، خلطة الـ F_2 المبكرة وخلطة الـ F_2 المتأخرة) باستخدام ١٠ بادئات عشوائية من نوع الـ RAPD. حيث انتجت ٧٥ شظية دنا منها ٤٦ شظية أظهرت تباين على مستوى الدنا وكانت نسبة التباين ٦١.٣٣%. أمكن تمييز الخمس تراكيب وراثية تحت الدراسة بواسطة ٢١ واسم موجب و/او سالب من نوع الـ RAPD. تم استخدام تحليل الفئات الإنعزالية المجمعة (BSA) كوسيلة سريعة للتعرف على واسمات جزيئية متعلقة بصفة التبكير ومن بين عشرة بادئات من نوع الـ RAPD أظهر عدد ٢ بادئ عدد ٢وأسم موجب و واسم واحد سالب كانت لها علاقة بصفة التبكير. ولكن يلزم اجراء دراسات إضافية لتأكيد ارتباط هذه الواسمات بصفة التبكير.