

## BIOCHEMICAL AND MOLECULAR MARKERS ASSOCIATED WITH EARLINESS TRAIT IN BREAD WHEAT

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**ABSTRACT:** This study was aimed to find out some biochemical and molecular genetic markers associated with earliness trait in bread wheat (*Triticum aestivum L.*). Six parental genotypes of wheat were used that represent a wide range of variability for earliness trait namely; Sids- 4 and Sakha- line (early), Sakha-93 and Gemmeiza-7 (medium), Gemmeiza-9 and Milan (late). F<sub>1</sub> seeds were obtained for five crosses were chosen to make biochemical analysis using SDS – PAGE Banding Pattern. These crosses were (Sids-4 × Sakha-line), (Sakha-93 × Gemmeiza-7), (Gemmeiza-9 × Milan), (Sids-4 × Milan) and (Sids-4 × Gemmeiza-7). The obtained results showed that, there are a biochemical and molecular markers associate with earliness trait in wheat. Two bands with molecular weight ranged from (90.276 to 77.181 KD) in the cross (Sids-4 × Milan), that appeared only in F<sub>1</sub>. So, these bands could be used as a selective biochemical marker for earliness trait in wheat.

Regarding to the molecular analysis, only one cross (Sid-4 × Milan), was chosen for the wide variation between its parents. RAPD-PCR analysis was carried out using F<sub>2</sub> bulked segregant analysis technique. The obtained results showed that, the primer (OPB-10) exhibited only one negative marker with 637bp that appeared in late parent and late bulked F<sub>2</sub>. In addition, in primer (OPD-05) one positive marker with 1463bp that appeared in early parent and early bulked F<sub>2</sub>. These two bands could be used as marker for earliness in wheat breeding programs.

**Key words:** Wheat, earliness, molecular markers, RAPD, SDS- PAGE.

## INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the major staple foods all over the world. In Egypt, wheat is the main cereal crop. Breeding for high yielding cultivars is an ongoing process for increasing the wheat production. However, we are needed for early cultivars with high yielding. Characterization of the new genotypes should include their performance, DNA and/or protein patterns. Molecular markers are the best tools for determining genetic relations of domestic cultivars in a short period of time. In wheat, RAPD technique is mainly used to mark and locate target genes; identify and mark fragments of (alien) chromosomes; determine the genetic relationships between *Triticum sp.* and related species; and analyze the genetic diversity of wheat varieties (Yu *et al.*, 2004) Several studies have been carried out to determine genetic variability and/or similarity using RAPD molecular markers (Suvarna, 2001). And (Svetlana *et al.*, 2007) determined the levels of genetic polymorphism in the collection of spring common wheat genotypes based on RAPD -PCR analysis. In addition, proteins are usually considered as the direct product of genes and the environment does not influence their expression. Among the

biochemical techniques, SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm. The analysis of storage protein variation in wheat has proved to be useful tool not only for diversity studies but also to optimize variation in germplasm collections. SDS- PAGE can be used as a promising tool for distinguishing cultivars of particular crop species (kaleem *et al.*, 2008).

The objective of this investigation was to develop some molecular and biochemical markers, using SDS-PAGE protein banding pattern and RAPD markers, associated with earliness trait in wheat genotypes employing bulked segregant analysis technique.

In addition, the relationship between some wheat genotypes was also studied.

## MATERIALS AND METHODS

This study was carried out at Experimental Farm of Gemmeiza, Agricultural Research Station, Egypt through three successive seasons of 2004/2005, 2005/2006 and 2006/2007. Chemical analysis were carried out at the Molecular Genetic Lab. Genetics Dept., Fac. of Agriculture, Zagazig University and Agriculture Genetic

Engineering Research Institute (AGERI) Giza, Egypt. Six parental genotypes of spring wheat (*Triticum aestivum* L.) were used in this study namely; Sids-4, Sakha- line, Gemmeiza-7, Gemmeiza-9, Sakha-93 and Milan were chosen which represent a wide range of earliness variability. The name, origin and pedigree of these cultivars are presented in Table 1. In the first season, the parental genotypes were planted in three different sowing dates. Simultaneously, pair crosses (half diallel), were performed to obtain the F<sub>1</sub> seeds. In the second season, the hybrid seeds were sown. Meanwhile, F<sub>1</sub> plants were self-pollinated to produce F<sub>2</sub> seeds. In third season, the obtained seeds of these populations i.e., P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub> and F<sub>2</sub> for the cross (Sids-4 × Milan) is evaluated using a randomized complete block design. Each plot consists of 30 individual guarded plants for P<sub>1</sub>, P<sub>2</sub> and F<sub>1</sub>, and 300 plants for the F<sub>2</sub> and the following analysis were done:

### SDS – PAGE Analysis

Protein analysis was performed using sodium dodecyl sulphate polyacrlamide gel electrophoresis (SDS–PAGE) according to Laemmli (1970) for five crosses (Sids 4× Sakha line), (Sakha 93 × Gemmeiza 7), (Gemmeiza 9 ×

Milan), (Sids 4 × Milan) and (Sids-4 × Gemmeiza-7).

### RAPD DNA Analysis

DNA analysis was performed using RAPD technique. Six random primers used against the template DNA presented in Table 2. Fresh leaves were collected from parents, F<sub>1</sub>, bulked from early F<sub>2</sub> plants and bulked late F<sub>2</sub> plants at heading stage. DNA was extracted according to (Dellaporta *et al.*, 1983).

### Statistical Analyses

The data was computed using (numerical taxonomy and multivariate analysis system according to (Rohlf exeter software, 2000).

## RESULTS AND DISCUSSION

### Protein Analysis

Data in Fig. 1 and Table 3 illustrate SDS–PAGE protein profiles of the studied wheat genotypes. The electrophoretic profiles of the studied genotypes revealed that, the total number of protein banding patterns was thirty one, distributed widely among wheat entries, and having a range of molecular weights of 34.237 to 133.994 KD. Twenty eight bands (90.323%) out of the thirty one

**Table 1. The name, origin and pedigree of wheat genotypes used in this study**

	<b>Name</b>	<b>Origin</b>	<b>Pedigree</b>	<b>Character</b>
<b>1</b>	<b>Sids 4</b>	<b>Egypt</b>	Maya"s"/ Mons"s">// CMH74A.592/3/ Sakha 8*2 SD/000/-2SD-2SD-0SD	<b>Early</b>
<b>2</b>	<b>Sakha Line</b>	<b>Egypt</b>	Sakha12/5/Kvz//CNO67/Pj62/3/YD"s"Bios"s"/4/K13 4(60)VEE S14665-4S-1S-0SY-0S	<b>Early</b>
<b>3</b>	<b>Sakha 93</b>	<b>Egypt</b>	Sakha92/ TR81032 S8871-1S-2S-1S-0S	<b>Medium</b>
<b>4</b>	<b>Gemmeiza 7</b>	<b>Egypt</b>	CMH74 A.630/Sx//Seri82/3/Agent CGM4611-2GM-3GM-1GM-0GM	<b>Medium</b>
<b>5</b>	<b>Gemmeiza 9</b>	<b>Egypt</b>	Ald"s"/Huac"s"//CMH74 A.630/Sx CGM583-5GM-1GM-0GM	<b>Late</b>
<b>6</b>	<b>Milan</b>	<b>Mexico</b>	VS73.600/MRL/3/BOW//YR/TRF CM75113.B-5M-2Y-3B-0Y-0CF-0M-0CHL-0AP	<b>Late</b>

**Table 2. Sequence and operon codes of the random primers used to detection of variation in wheat**

Primer codes	Sequence (5' to 3')
OPB-10	CTG CTG GGA C
OPD-05	TGA GCG GAC A
OPC-20	ACT TCG CCA C
OPA-11	CAA TCG CCG T
OPB-18	CCA CAG CAG T
OPB-05	TGC GCC CTT C

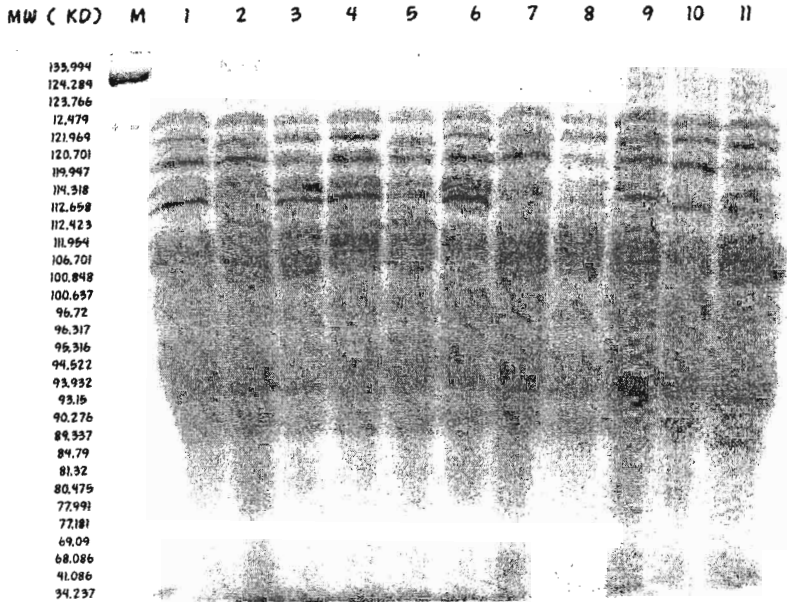
bands were polymorphic Table 4. Otherwise, the three monomorphic bands were no. 1, 30 and 31 exerted in molecular weight 133.994, 41.149 and 34.237 KD. Some unique amplified bands characterize these genotypes such band no. 5 (Mw= 121.969), no. 6 (Mw = 120.701), no. 8 (Mw = 114.318), no. 9 (Mw = 112.658), no. 13 (Mw = 100.848), no. 15 (Mw = 96.72), no. 16 (Mw = 96.317), no. 17 (Mw = 95.316), no. 20(Mw = 93.15), no. 21 (Mw = 90.276) , no. 22 (Mw = 89.337), no. 23 (Mw = 84.79), no. 24 (Mw = 81.32), no. 25 (Mw =80.475), no. 26 (Mw = 77.991) and no. 27 (Mw = 77.181) Table 5. Regarding the number of observed bands among the genotypes while Table 3 showed that the presence of light

variation between the six parents (8 and 9 bands). Meanwhile the number of bands observed belong different crosses showed a wide variation. The highest number of bands was 9 bands for (Sids-4 × Gemmeiza-7) and the lowest number of bands was 6 bands observed in (Sids-4 × Sakha line). The obtained results for crosses could be attributed to the interactions between the parents as well as the crosses. Also, Table 3. showed that some specific bands restricted in early parent (Sids-4, no. 4) as well as (band no. 29 for Sakha line) these specific bands restricted only in parents but not appeared in F<sub>1</sub>, this results can be attributed to recessive or masking behavior for this region. Moreover this table no. 3 showed an interesting

Table 3. SDS-PAGE protein bands of wheat genotypes as appeared in Fig. 1

Band no.	1	2	3	4	5	6	7	8	9	10	11
1	+	+	+	+	+	+	+	+	+	+	+
2	-	-	-	-	-	+	+	+	-	-	-
3	-	-	+	+	-	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-	-	-	-
5	-	-	-	-	+	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	+	-	-
7	-	-	-	-	-	-	-	-	-	+	+
8	-	-	-	-	-	-	+	-	-	-	-
9	-	-	-	-	-	+	-	-	-	-	-
10	+	+	+	+	+	-	-	-	-	-	-
11	-	-	-	-	-	-	-	+	+	-	-
12	-	-	-	-	-	-	-	-	-	+	+
13	-	-	-	-	-	-	-	+	-	-	-
14	+	+	+	+	+	+	-	-	-	-	-
15	-	-	-	-	-	-	-	+	-	-	-
16	-	-	-	-	-	-	-	-	+	-	-
17	-	-	-	-	-	-	-	-	-	-	+
18	-	-	-	-	+	+	-	-	-	-	+
19	-	-	+	+	-	-	-	-	-	-	-
20	+	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	+	*
22	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	+	-	-	-	-	-	-
24	-	+	-	-	-	-	-	-	-	-	-
25	-	-	+	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	+
27	-	-	-	-	-	-	-	-	-	+	*
28	+	-	-	+	+	+	+	+	+	+	+
29	-	+	+	-	-	-	-	-	-	-	-
30	+	+	+	+	+	+	+	+	+	+	+
31	+	+	+	+	+	+	+	+	+	+	+

1- Sids4  
 2- Sakha line  
 3 - Sakha93  
 + = present,  
 4- Gemmeiza7  
 5- Gemmeiza9  
 6- Milan  
 - = absent  
 7- Sids4 x Sakha line  
 8- Sakha93 x Gemmeiza7  
 9- Gemmeiza9 x Milan  
 \* = protein marker  
 10- Sids4 x Milan  
 11- Sids4 x Gemmeiza7



**Fig. 1. SDS – PAGE profiles of wheat genotypes associated with earliness trait in wheat**

- |                                |                                 |
|--------------------------------|---------------------------------|
| <b>1. Sids-4</b>               | <b>2. Sakha-line</b>            |
| <b>3. Sakha-93</b>             | <b>4. Gemmeiza-7</b>            |
| <b>5. Gemmeiza-9</b>           | <b>6. Milan</b>                 |
| <b>7. Sids-4 x Sakha line</b>  | <b>8. Sakha-93 x Gemmeiza-7</b> |
| <b>9. Gemmeiza-9 x Milan</b>   | <b>10. Sids-4 x Milan</b>       |
| <b>11. Sids-4 x Gemmeiza-7</b> |                                 |

**Table 4. Protein banding polymorphism for wheat genotypes and their crosses**

<b>Monomorphic bands</b>	<b>3</b>
<b>Polymorphic (without unique)</b>	12
<b>unique bands</b>	16
<b>Polymorphic (with unique)</b>	28
<b>Total number of bands</b>	31
<b>Polymorphism (%)</b>	90.323%
<b>Mean of band frequency</b>	0.258

bands observed in F<sub>1</sub> for the cross (Sids-4 × Milan) represent the early parent (Sids -4) and the late parent (Milan) these bands were no. 21 and 27 at the zones of molecular weight varied from (90.276KD to 77.181 KD) that appeared in F<sub>1</sub> and not observed in either these parents, that can be attributed to the interaction between the two different genotype. These results were agreement with those obtained by Altenbac *et al.* (2001), Dovřáček and Čurn (2005) Shauib *et al.* (2007) and Kaleem *et al.* (2008).

Regarding to the dendogram that demonstrates the relationships among the wheat genotypes based on data recorded from polymorphism of protein patterns. Fig. 2 illustrated that, the tested

entries of wheat were divided basically into two main groups. First group included 5 parents Sids 4, Sakha line, Sakha - 93, Gemmeiza -7 and Gemmeiza - 9, meanwhile, the second group included the 6<sup>th</sup> parents and all crosses.

The protein banding patterns of wheat resulted from direct genetic control and in general not affected by environmental conditions. In addition, because wheat is essentially a self-pollinated crop, protein composition of the different varieties of wheat remains stable for plant generation (El-Morshidy *et al.*, 2003). Therefore, the protein composition of wheat can be characterized and thus to identify its variety.



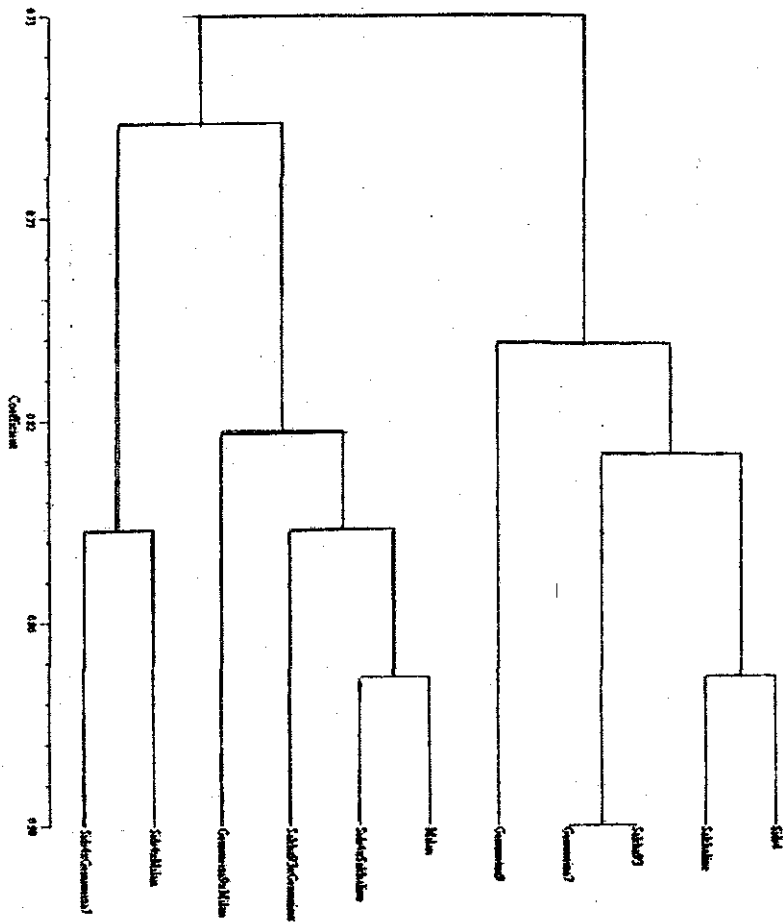
Table 5. Relative frequencies and polymorphism distribution for SDS-PAGE bands in different wheat genotypes

Band no.	RF	Mw	Frequency	Polymorphism
1	0.133	133.994	1	*
2	0.169	124.284	0.273	***
3	0.171	123.766	0.182	***
4	0.176	12.479	0.182	***
5	0.178	121.969	0.091	**
6	0.183	120.701	0.091	**
7	0.186	119.947	0.182	***
8	0.209	114.318	0.091	**
9	0.216	112.658	0.091	**
10	0.217	112.423	0.455	***
11	0.219	111.954	0.182	***
12	0.242	106.701	0.182	***
13	0.269	100.848	0.091	**
14	0.270	100.637	0.545	***
15	0.289	96.72	0.091	**
16	0.291	96.317	0.091	**
17	0.296	95.316	0.091	**
18	0.300	94.522	0.182	***
19	0.303	93.932	0.182	***
20	0.307	93.15	0.091	**
21	0.322	90.276	0.091	**
22	0.327	89.337	0.091	**
23	0.352	84.79	0.091	**
24	0.372	81.32	0.091	**
25	0.377	80.475	0.091	**
26	0.392	77.991	0.091	**
27	0.397	77.181	0.091	**
28	0.45	69.09	0.818	***
29	0.457	68.086	0.182	***
30	0.698	41.086	1	**
31	0.786	34.237	1	**

\* Monomorphic fragment

\*\* Unique fragment

\*\*\* Polymorphic fragment



**Fig. 2.** Dendrogram demonstrating the relationships among the wheat genotypes based on data recorded from polymorphism of protein pattern

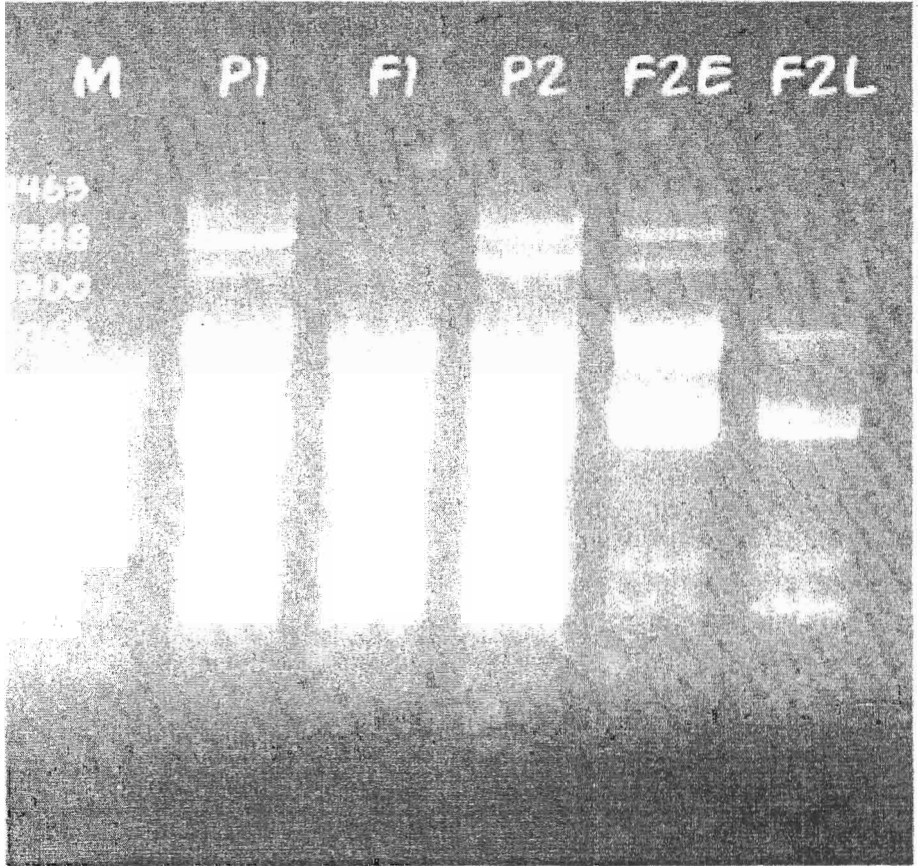
The previous view of protein patterns in the studied wheat genotypes could be used as a selective marker for desirable trait as suggested by (Lin *et al.*, 1999).

### DNA Analysis

RAPD-PCR analysis was used to determine the genetic variation and to obtain molecular markers for wheat genotypes. Depending upon the previous results, which were detected from the field experiments and protein profiles, cross (Sids-4 × Milan) was chosen represent the extremes for earliness trait to continue the molecular analysis. The five genotypes represent by P<sub>1</sub>, P<sub>2</sub>, and F<sub>1</sub>, bulk F<sub>2</sub> early and bulk F<sub>2</sub> late were carried out. Six primers (OPB-10, OPD-05, OPC-20, OPA-11, OPB-18 and OPB-05) were used to differentiate between the five genotypes Fig. 3. A total of 46 DNA bands varied from 257 bp (OPA-11) to 1627 bp (OPB-05) were amplified through the six random primers. Six bands out of the forty six bands (13.04%) were polymorphic Table 6. The number of RAPD-PCR bands generated by each primer varied from seven bands (OPB-10, OPC-20 and OPB-18) to nine bands (OPD-05) with an average of 7.66 Table 7.

Also the number of polymorphic bands through each primer ranged from 1 band (OPB-10) to 2 bands (OPB-05) with an average 1 band/primer. The highest number of amplified and polymorphic bands were generated by (OPD-05) (9, 2) followed by primer (OPB-18) (7, 2), respectively. On the other hand, the lowest primer was (OPB-10) (7, 1). The polymorphic band no. 1 with size 1463bp showed banding pattern by application of primer (OPD-05) from the early parent (Sids 4) and early bulked F<sub>2</sub> but not detected in bulked late F<sub>2</sub>. In addition, the primer (OPB-10) exhibited only one negative band no. 6 with size 637bp that appeared in late parent (Milan) and late bulked F<sub>2</sub>. These results were agreement with (Joshi and Nquyen, 1993), Lin *et al.* (1999), Gerashchenkov *et al.* (2000), El-Morshidy *et al.* (2003), Mari *et al.* (2004), Bhutta *et al.* (2006), Nalini *et al.* (2006), Halima *et al.* (2007), Hanocq *et al.* (2007) and Kunpu *et al.* (2008).

So, these bands could be used as a selective molecular marker associated with earliness gene (s) for wheat breeding to select the early genotypes.



**Fig. 3. RAPD-PCR DNA banding patterns of wheat genotypes associated with earliness trait in wheat using primer OPD-05**

P<sub>1</sub>: Sids-4

P<sub>2</sub>: Milan

F<sub>1</sub>: Sids-4 x Milan

F<sub>2</sub>E: Bulk of F<sub>2</sub> early

F<sub>2</sub>L: Bulk of F<sub>2</sub> late

**Table 6. RAPD-PCR bands of wheat genotypes using primers OPB-10**

NO.	bp	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L
1	1583	+	+	+	+	+
2	1442	+	+	+	+	+
3	1333	+	+	+	+	+
4	1192	+	+	+	+	+
5	837	+	+	+	+	+
6	637	-	+	+	-	+
7	317	+	+	+	+	+

**Table 6. Cont.**

**OPD-05**

NO.	bp	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L
1	1463	+	-	-	+	-
2	1388	+	+	+	+	-
3	1300	+	+	+	+	+
4	1063	+	+	+	+	+
5	983	+	+	+	+	+
6	815	+	+	+	+	+
7	751	+	+	+	+	+
8	444	+	+	+	+	+
9	350	+	+	+	+	+

**Table 6. Cont.**

**OPC-20**

NO.	bp	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L
1	1100	+	+	+	+	+
2	848	+	+	+	+	+
3	706	+	+	+	+	+
4	631	+	+	+	+	+
5	580	+	+	+	+	+
6	357	+	+	+	+	+
7	260	+	+	+	+	+

Table 6. Cont.

## OPA-11

NO.	bp	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L
1	1350	+	+	+	+	+
2	1160	+	+	+	+	+
3	909	+	+	+	+	+
4	631	+	+	+	+	+
5	569	+	+	+	+	+
6	496	+	+	+	+	+
7	315	+	+	+	+	+
8	257	+	+	+	+	+

Table 6. Cont.

## OPB-18

NO.	bp	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L
1	1482	+	+	+	+	-
2	1273	+	+	-	+	+
3	1191	+	+	+	+	+
4	1036	+	+	+	+	+
5	863	+	+	+	+	+
6	652	+	+	+	+	+
7	589	+	+	+	+	+

Table 6. Cont.

## OPB-05

NO.	bp	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L
1	1627	-	+	+	+	+
2	1409	+	+	+	+	+
3	1273	+	+	+	+	+
4	1100	+	+	+	+	+
5	981	+	+	+	+	+
6	793	+	+	+	+	+
7	612	+	+	+	+	+
8	505	+	+	+	+	+

+ = band present,

- = band absent.

**Table 7. Number of amplified and polymorphic DNA fragments for 5 tested wheat genotypes**

Primers	Genotypes					No. of amplified bands	No. of polymorphic bands
	P <sub>1</sub> E	F <sub>1</sub>	P <sub>2</sub> L	F <sub>2</sub> E	F <sub>2</sub> L		
OPB-10	6	7	7	6	7	7	1
OPD-05	9	8	8	9	8	9	2
OPC-20	7	7	7	7	7	7	0
OPA-11	8	8	8	8	8	8	0
OPB-18	7	7	6	7	6	7	2
OPB-05	7	8	8	8	8	8	1
Total	44	45	44	45	44	46	6
Mean	7.33	7.5	7.33	7.5	7.33	7.66	1

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## الواسمات البيوكيميائية والجزئية المرتبطة بصفة التبكير في قمح الخبز

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تهدف هذه الدراسة الى التعرف على بعض الواسمات البيوكيميائية والجزئية المرتبطة بصفة التبكير في قمح الخبز. وذلك باستخدام ٦ آباء من القمح والتي بها مدى واسع من الاختلافات بالنسبة لصفة التبكير وهي: سدس ٤، والسلالة سخا (مبكر)، سخا ٩٣، جميزة ٧ (متوسط)، جميزة ٩، ميلان (متأخر). وتم الحصول على حبوب الـ  $F_1$  بالنسبة للخمسة هجن التي تم اختيارها لإجراء التحليل البيوكيميائي باستخدام تحليل الـ SDS-PAGE. banding pattern هذه الهجن كانت (سدس ٤ × السلالة سخا)، (سخا ٩٣ × جميزة ٧)، (جميزة ٩ × ميلان)، (سدس ٤ × ميلان)، (سدس ٤ × جميزة ٧). أظهرت النتائج المتحصل عليها وجود واسمات بيوكيميائية وجزئية مرتبطة بصفة التبكير في القمح. وجود حزميتين ذات وزن جزئي يتراوح بين (٧٧,١٨١ الى ٩٠,٢٧٦ كيلو دالتون) في الهجين (سدس ٤ × ميلان) والتي ظهرت فقط في الجيل الأول لذلك يمكن اعتبار هذه الحزم كواسم بيوكيميائي لصفة التبكير. بالنسبة للتحليل الجزئي تم اختيار الهجين (سدس ٤ × ميلان) فقط لإجراء التحليل لوجود الاختلافات الكبيرة بين الأبوين. تم إجراء تحليل الـ RAPD-PCR مستخدماً تقنية Bulk segregant analysis. وأظهرت النتائج المتحصل عليها أن البائد (OPB-10) اظهر حزمة سالبة ذات حجم ٦٢٧ في الأب المتأخر ونباتات الـ  $F_2$  المتأخرة بالإضافة إلى أن البائد (OPD-05) اظهر حزمة موجبة ذات حجم ١٤٦٣ في الأب المبكر ونباتات الـ  $F_2$  المبكرة. وبذلك يمكن اعتبار هاتين الحزميتين كواسمات لصفة التبكير في برامج تربية القمح.