II.10 USING MARKER-ASSISTED SELECTION TO IMPROVE ALLELOPATHIC ACTIVITY IN SPRING WHEAT

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Abstract

The major goal of the present study was to detect the genetic polymorphism among six spring wheat (Triticum aestivum L.) cultivars due to allelopathy activity with molecular markers and subsequently identify RAPD markers linked to wheat allelopathy (weed suppression) gene by using marker-assisted selection. Results showed genetic variation in allelopathic activity in wheat, thereby providing a sufficient gene pool for the development of allelopathic wheat cultivars in order to suppress weeds. RAPD markers were used to detect DNA polymorphism among these cultivars which included commercial and old Egyptian wheat cultivars and the crosses between strongly allelopathic activity and intermediate cultivars were accomplished; Giza 160 x Sakha 61 and Giza 167 x Sids 1 to detect RAPD marker linked to this trait. The dendrogram presented in this study clearly demonstrated the ability of RAPD to detect a large amount of genetic diversity and to identify intergroup differences. The total number of fragments resulted from 14 RAPD primers was 798. Thirty-five alleles were polymorphic among the cultivars with an average of 1.5 allele per RAPD locus, using the 35 polymorphic RAPD alleles. Nei's genetic distance showed two main clusters for the studied cultivars ranging from 0.64 to 0.87 with an average of 0.75. Bulk Segregant Analysis (BSA) in F_2 for the cross between Giza 160 (high activity cultivar for allelopathy) and Sakha 61 (intermediate cultivar for allelopathy) and Giza 167 (high activity cultivar for allelopathy) and Sids 1(intermediate cultivar for allelopathy), was used to identify the RAPD markers linked to weed suppression genes. Out of the 14 primers used, the two RAPD, A11 and C7 with Giza 160 x Sakha 61 cross, while B12 and C7 with Giza 167 x Sids 1 cross, showed polymorphism between strongly and weakly bulked DNA. Both primers could be analyzed as dominant markers. In conclusion, the present study indicated that RAPD markers, combined with bulk segregant analysis, can be used to identify molecular markers linked to Avena fatua suppression gene in wheat, which can be used in breeding as a selection tool in early generations.

Keywords: *Triticum aestivum* L., RAPD technique, allelopathy, bulk segregant analysis.

INTRODUCTION

In wheat breeding programs, the choice of parents is the most important step in the development of adapted cultivars to biotic and abiotic stresses. Weeds are one of the biotic stresses and the major constraints to wheat production. Wheat farmers have

become increasingly reliant on synthetic herbicides for weed control in their farming systems. However, the extensive use of herbicides has resulted in the rapid development of herbicide resistance in weeds. The ineffectiveness of herbicides on resistant weed species and environmental imperatives has prompted the search for non-herbicidal innovations to manage weed populations (Wu *et. al.,* 1999). Wheat can compete well with weeds, but strong weed competition reduces yield. Good weed control is essential to minimize yield losses, and to prevent weed seed contamination at harvest. Allelopathy in wheat refers to the fact that wheat can chemically affect the growth of other plants by secondary metabolites exudation into the surrounding environment (Zhang *et. al.*, 2004). Allelopathy may also affect the growth of wheat plants themselves, a phenomenon known as the auto-toxic effect (Wu *et. al.,* 2001). Wheat cultivars differed significantly in their allelopathic effects on the establishment of the following wheat crop (Guenz *et. al.,* 1967; Kimber 1967). Weed suppression by crop allelopathy during the early establishment period could reduce the need for commercial herbicides to early season application, with late season weed control provided by the heightened advantages of crop competitiveness.

One of the important applications of DNA markers is estimating the genetic diversity among genotypes and calculating the similarity and dissimilarity values. Studying genetic diversity based on DNA level is the most important step to choose extreme genotypes and establishing an effective breeding program. RAPD loci, also referred to Randomized Amplified Polymorphic DNA have proved to be a valuable source of highly polymorphic DNA markers (El-Maghraby *et. al.,* 2010). Bulked Segregant Analysis (BSA) is a method to identify molecular markers linked to a gene of interest without having to construct a map of the genome (Michelmore et. al., 1991), which is considered the first goal in marker-assisted selection. BSA has been used successfully to detect Quantitative Trait Loci (QTL) linked with wheat tolerance to weed competition (Louise Bach Jensen *et. al.,* 2001 and Hanwen Wu, 2005).

The aim of the present research was to detect the genetic polymorphism among some Egyptian wheat genotypes with molecular markers to improve allelopathic activity in traditional plant breeding.

MATERIALS AND METHODS

Plant material

The studied wheat (*Triticum aestivum* L.) genotypes were selected from the germplasm bank of Wheat Research Program, Agricultural Research Center, Egypt, which represents a wide genetic background. The name, pedigree and origin of these genotypes are presented in Table 1. PCR analysis were carried out using genomic

DNA from the six wheat cultivars (Sakha 61, Giza 160, Giza 167, Giza 168, Gemmiza 1, and Sids 1) as shown in Table 1. The F_1 hybrids derived from cross of the four wheat (*Triticum aestivum* L.) parental genotypes is presented in Table 2

DNA extraction

Leaves were collected from two-week old plants of the ten wheat parental genotypes and subjected to liquid nitrogen. DNA extraction was performed using Murray and Thompson (1980) method.

Determination of the concentration of DNA

The concentration of DNA was calculated, assuming that DNA at a concentration of 50 µg ml^{-1} has an OD of 1 at 260 nm as follows:

$$\text{DNA concentration (µg µl}^{-1}) = \frac{OD_{260} x 50 D.F x 50 \text{mg/ml}}{1000}$$

PCR amplification

Fourteen RAPD primer pairs developed and provided by AGERI Lab, Giza were used to create the molecular marker(s). The primer pairs ID are presented in Table 3. Amplification reaction volume was 30 µl, containing, 1x PCR buffer with $MgCl_2$ (50 mM KCl, 10 mM Tris-HCl (pH=9.0), 1.75 mM $MgCl_2$, 150 µM each of dNTP (dATP, dCTP, dGTP, and dTTP, 0.15 µM primer, 50ng template DNA and 1.0 µl of Taq polymerase(5 units $µl^{-1}$). Reaction mixtures were exposed to the following conditions: 95^0C for 3 min, followed by 35 cycles of 40 sec. at 94^0C, 40 sec. at 50^0C (depending on the annealing temperature of primers), 1 min. at 72^0C, and a final 10 min. extension at 72^0C.

Amplification products were visualized along with DNA standard marker, on 2.5% agarose gel with 1x TBE buffer and detected by staining with an ethidium bromide solution for 30 min. Gels were then distained in de-ionized water for 10 min. and photographed using Polaroid films under UV light.

Data handling and cluster analysis

Data were scored for computer analysis on the basis of the presence (1) or absence (0) of the amplified products for each primer. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients, according to Jaccard (1908). The similarity coefficients were then used to construct dendrograms (Nei, 1972), using the unweight pair group method with arithmetic averages (UPGAMA) employing the SHAN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.1 Program (Rolhf, 2000).

Table 1. Name and pedigree of the studied bread wheat genotypes.

No	Cultivar	Pedigree
1	Sakha 61	Inia/RL 4220//7C/Yr "S" CM 15430-2S-55-0S-0S
2	Giza 160	Chenab / Giza 155
3	Giza 167	AuUP301//G11/SX/Pew'S"/4/Mal'S"/May'S"//Pew'S'CM67245-C-1M-2Y-1M-7Y-1M-0Y
4	Giza 168	MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B
5	Gemmiza 1	Maya 74/On//1160.147/3/Bb/1991 Gall/4/Chat'S" CM58924-1GM-OGM
6	Sids 1	HD2172/Pavon "S"//1158.57/Maya 74"S" Sd46-4Sd-2Sd-1Sd-0Sd

Table 2. The F1 hybrids derived from cross of the four wheat (Triticum aestivum L.) parental genotypes.

No.	Cross Parent*i* x Parent*j*
1	Giza 160/Sakha 61
2	Giza 167/Sids 1

Table 3. Primers used for RAPD analysis.

No.	Primer Pair	Sequence
1	A2	TGCCGAGCTG
2	A3	AGTCAGCCAC
3	A6	GGTCCCTAGC
4	A7	GAAACGGGTG
5	A11	CAATCGCCGT
6	B1	GTTTCGCTCC
7	B3	CATCCCCCTG
8	B12	CCTTGACGCA
9	C2	GTGAGGCGTC
10	C3	GGGGGTCTTT
11	C4	CCGCATCTAC
12	C5	GATGACCGCC
13	C7	GTCCCGACGA
14	C8	TGGACCGGTG

RESULTS AND DISCUSSION

Genetic relationship using RAPD markers

Random Amplified Polymorphic DNA (RAPD) experiment was conducted using 14 RAPD primer pairs developed and provided by Agricultural Genetic Engineering

Research Institute (AGERI). The initial name and sequence are presented in Table 3 for the RAPD markers, which were used to find out the genetic relationships among cultivars and to create the molecular-marker data. The Jaccard's similarity coefficient was calculated among the six wheat cultivars.

Jaccard's similarity coefficient

Similarity between all pairs of cultivars are illustrated in Table 4. This table showed that the similarity matrix of genetic distance ranged from 0.64 between Sakha 61 with each of Giza 168, Gemmiza 7 and Sids1 to 0.87 between Giza 167 and Giza 168. The other pairs of cultivars showed varied values of similarity ranges from 0.65 between Giza 169 with Sids 1 to 0.78 between Giza 160 and Giza 167. The average similarity among cultivars was 0.76. In the present investigation, a fundamental question concerning the genetic relationship of a representative sample of Egyptian wheat cultivars was addressed. This genetic relationship information can be used to gain insight into cultivar divergence, which is an important step towards an exploitation of genetic resources for breeding. To answer this question, study the genetic divergence based on Random Amplified Polymorphic DNA (RAPD) technique has been performed.

Cluster analysis

Fourteen RAPD loci were used in the presenjt study, their sequences are illustrated in Table 3. Among these 14 RAPD loci, nine RAPD markers (64%) generated polymorphism among cultivars with a total of 204 fragments. Of the total scorable RAPD bands 133 alleles were detected for polymorphism (Table 5). This represented an average of 1.5 alleles per locus detected by a single RAPD locus. Nei's genetic distance (GD) was measured using the 35 polymorphic RAPD alleles, (Fig.s 2-4).

Nei's genetic distance (Fig. 1) showed that the genetic distance for the different cultivars combinations ranged from 0.69 to 0.87 and the studied cultivars formed two main clusters; the first main cluster separated at genetic similarity of about 0.72 and created two sub-clusters. The first sub-cluster included Sakha 61 at a genetic similarity of about 0.72. The second sub-cluster included Giza 160 at a genetic similarity of about 0.77 as a group and Giza 167 with Giza 168 in another group at genetic similarity of about 0.87. In relation to the second main cluster, Fig. (1) showed that this cluster formed two cultivars at a genetic similarity of about 0.77 including Gemmiza 7 in one group and Sids 1 in another group. Tables 5 and 6 showed the molecular weight and usefulness of RAPD markers to detect the polymorphism among the Egyptian cultivars based on the presence (+) and absence (-) for PCR-products. Several approaches have been suggested to saturate genomic regions of interest with molecular markers. Bulked segregant analysis

provides a rapid, technically simple alternative for identifying markers linked to specific genes. The only prerequisite is the existence of a population resulting from a cross that segregates for the gene of interest. The success of the approach will depend on the genetic divergence between the parents in the target region. In wheat, using of molecular markers was necessary to identify the markers, which are linked to weed suppression genes and are simple to be used.

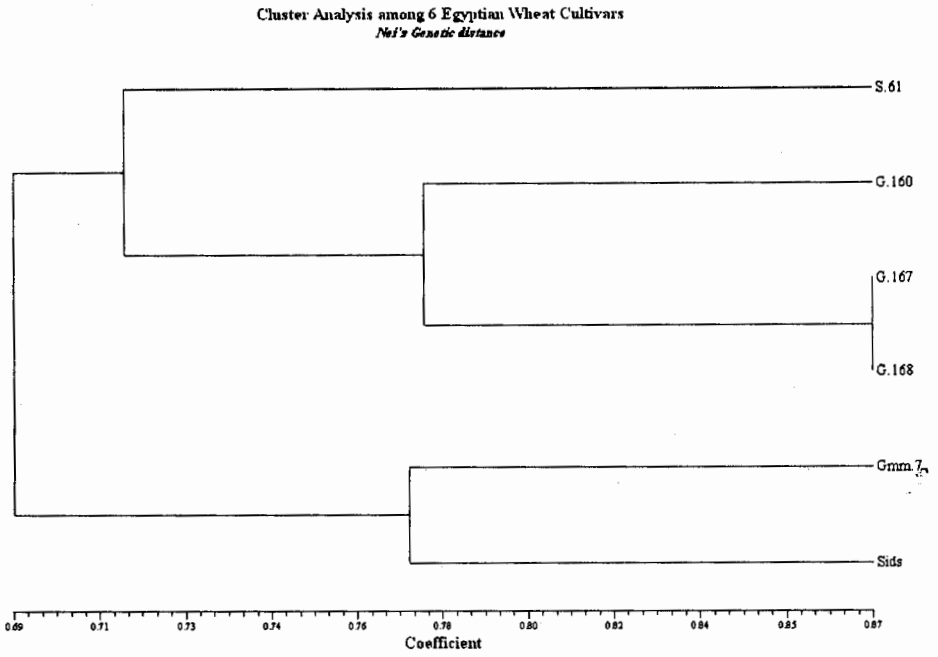
Bulked segregant analysis (BSA)

Bulked Segregant Analysis involves screening for differences between two pooled DNA samples derived from a segregating population that originated from a single cross. Each pool, or bulk, contains individuals selected to have identical genotypes for a particular trait or genomic region.

DNA was bulked from F_2 individual-homozygous plants which resulted from a single cross between Giza 160 and Sakha 61 and the second cross between Giza 167 with Sids 1. Each of the two bulks consisted of 10 F_2 homozygous individuals and were employed to identify RAPD markers linked to competitive ability gene against *Avena fatua* using 14 RAPD primer pairs (Table 6). Out of the 14 RAPD primers, three primers only (Table 7) showed polymorphism among the two crosses and their parents as shown in Fig.s (5-8). Three primers, A11, B12 and C7, generated amplification products that were present in one bulk but not in the other. The 700bp fragment, amplified by A11 was present in Giza 160 and Bulked Intermediate and Low Activity but absent in the Sakha 61 and Bulked Competitive Activity as shown in Fig. (5). The 1500bp fragment, amplified by C7 was absent in Giza 160 and Bulked Competitive Activity but present in Sakha 61 and Bulked Intermediate and Low Activity as shown in Fig. (6). Regarding the second cross between Giza 167 and Sids 1, the 100bp fragment, amplified by B12 was present in Giza 167 and Bulked Intermediate and Low Activity but absent in both of Sids 1 and Bulked Competitive Activity (Fig. 7). Similarly, the 1500bp and 500bp fragments, amplified by C7 were absent in Giza 167 and Bulked Competitive Activity but present in both of Sids 1 and Bulked Intermediate, and Low Activity (Fig. 8).

The trend of these results have been emphasized by El-Maghraby et al. (2005) by using eight parental diallel cross and SSR markers. Similar results were obtained by employing SSR in bread wheat as Motiul Quader, et. al. (2000), Louise Bach Jensen et al. (2001) and Jian et al. (2003). On the other hand, M. Olofsdotter (2001) has observed the polymorphism in rice (*Oriza Sativa)* and use the obtaining results to identify QTL controlling allelopathy as we obtained. Wu et. al. (2003 and 2005) analyzed the linkage of genetic markers (SSR, RFLP, AFLP) by using DH and QTL, found that improving the allelopathic activity might be possible through MAS in wheat breeding as we found in this work.

In conclusion, this study indicates that RAPD markers, combined with bulk segregant analysis, can be used to identify molecular markers linked to *Avena fatua* suppression gene in wheat. Once these markers are identified they can be used in wheat breeding program.



breeding as a selection tool in early generation

Figure 1. Dendrogram obtained from UPGMA cluster based on RAPD wheat cultivars.

Fig. 1. Cluster analysis for six of Egyptian wheat cultivars using Nei Genetic Distance

Table 4. Similarity matrix of genetic distance for the six Egyptian wheat cultivars based on Jaccard's coefficient, using 14 RAPD primers.

Cultivar	Sakha 61	Giza 160	Giza 167	Giza 168	Gemmiza 7	Sids 1
Sakha 61	1.00					
Giza 160	0.77	1.00				
Giza 167	0.72	0.78	1.00			
Giza 168	0.64	0.76	0.87	1.00		
Gemmiza 7	0.64	0.65	0.75	0.73	1.00	
Sids 1	0.64	0.65	0.69	0.73	0.77	1.00

Table 5. Usefulness of RAPD markers in different genetic background, tested on six wheat cultivars (+ presence of PCR products, - absence)

Marker	MW(bp)	Sakha 61	Giza 160	Giza 167	Giza 168	Gemmiza 7	Sids 1
A2	200	-	-	-	+	-	-
A3	1000	+	+	+	+	-	+
A6	1500	+	+	-	-	-	-
	600	+	+	-	-	+	+
A7	500	-	-	+	+	+	+
A11	1500	+	+	+	-	-	-
	1000	-	-	-	+	+	+
	900	-	+	+	+	+	+
	400	-	-	-	-	+	-
B1	300	-	+	-	-	-	-
B12	1000	-	-	+	+	+	+
	400	+	-	-	-	+	+
C2	2000	-	-	-	-	-	+
	1500	+	+	+	+	+	-
	1200	-	+	+	+	+	+
	1000	+	-	-	-	+	+
	700	-	+	+	+	+	-
	500	-	+	+	+	+	+
	100	-	+	+	+	+	-
C3	2000	+	+	+	+	+	-
	1500	-	+	+	+	-	-
	700	+	+	+	+	+	-
	600	-	-	+	+	-	-
	500	+	+	+	+	-	-
C4	1000	+	-	+	+	+	+
	500	-	-	-	+	-	-
C5	700	-	-	+	-	+	-
C7	1500	+	-	+	-	-	-
	1000	+	+	-	-	-	-
	600	-	-	+	+	+	+
	500	+	-	+	-	+	-
C8	2000	+	+	+	+	-	+
	1500	-	-	-	-	-	+
	1000	+	+	+	+	-	-
	700	+	+	+	+	-	+

- Total Scorable bands=133, Total fragments= 798 (Data not shown)
- Total polymorphic bands=35, Total polymorphic fragments= 210 (as shown in the table)

Table 6. Molecular weight for different fragments in six wheat cultivars and two Bulked DNA.

Primer	Band	M	Sakha 61	Giza 160	Giza 167	Giza 168	Gemmiza 7	Sids 1	Cross 1	Cross 2
A2	3	500	705.0	686.2	686.2	667.4	686.2	686.2	667.4	648.7
	4	400	517.4	498.7	498.7	479.9	498.7	498.7	479.9	461.2
	7	100	179.9	161.2	161.2	161.2	161.2	161.2	161.2	142.4
A3	3	1000	1000	1074.9	1074.9	1074.9	-	1156.2	-	1241.5
	4	700	559.2	595.6	595.6	595.6	617.1	595.6	641.3	641.3
	6	400	365.8	376.4	376.4	376.4	387.7	387.9	400.0	413.1
A6	2	1500	1653.0	1573.9	-	-	-	-	1369.7	1431.9
	3	1000	1106.6	1106.6	1175.0	1175.0	1078.7	1175.0	985.4	985.4
	4	700	940.0	940.0	960.0	960.0	920.0	920.0	920.0	920.0
		-	700.0	700	-	-	720.0	660.0	660.0	680.0
A7	4	700	572.5	592.1	592.1	592.1	614.2	614.2	639.3	667.8
	5	500	-	-	512.4	487.9	487.9	500.0	525.4	-
	6	400	431.4	420.7	442.2	431.4	442.2	442.2	453.3	-
	7	300	300.0	315.9	315.9	315.9	315.9	315.9	324.3	332.9
A11	2	1500	1122.7	1122.7	1122.7	-	-	-	-	1156.4
	3	1000	-	-	-	1060.3	1030.2	1030.2	-	969.0
	4		-	873.9	905.7	842.5	811.6	781.7	-	752.9
	5	700	725.5	676.3	725.5	700.0	634.7	634.7	634.9	634.7
	6	500	-	-	-	-	546.3	-	-	508.0
	7	400	477.8	477.8	477.8	471.0	464.5	464.5	458.3	452.2
B1	4	700	668.5	668.8	700.0	668.8	700	668.8	700	668.8
	5	500	515.5	500.0	515.5	530.6	530.6	515.5	530.6	530.6
	6	400	400.0	415.4	415.4	415.4	431.9	431.9	431.9	431.9
B3	3	1000	1000	1000	1000	942.8	1000	1000	1000	1000
	4	700	649.2	649.2	649.2	649.2	649.2	673.1	673.1	673.1
	6	400	431.6	431.6	431.6	431.6	448.8	448.8	448.8	448.8
	7	300	336.9	336.9	336.9	344.7	344.7	344.7	344.7	353.4
B12	3	1000	-	-	1056.3	1119.3	1119.3	1188.1	1261.5	1338.6
	4		800.2	800.2	800.2	820	820	844.4	873.9	909.3
	5	700	700	700	700	711.8	711.8	721.2	728.8	741.0
	6	500	500	518.9	518.9	518.9	539.5	539.5	561.2	583.5
C2	2	1500	1648.8	1720.8	1575.3	1648.8	1720.8	-	1791.7	1861.6
			-	1193.9	1193.9	1193.9	1268.3	1345.1	1422.8	1422.8
	3	1000	864.9	-	-	-	905.2	826.4	826.4	826.4
	4	700	-	700	746.4	746.1	787.5	-	-	-
	7	300	247.1	263.2	263.2	280.6	280.6	300	300	321.5
	9	100	-	78.7	88.2	88.2	88.2	-	100	88.2
C3	1	2000	1942.3	1942.3	1884.1	1942.3	2000	-	2062.5	2187.5
	2	1500	-	1426.1	1350.3	1500	-	-	1500	1570.5
	4	700	719.6	730.9	744	759.8	759.8	-	600	600.8
			-	-	571.3	581	-	-	-	525.0
	5	500	552.3	552.3	492.2	508	-	-	-	-
C4	3	1000	1014.6	-	1070.3	1049.4	1049.4	1049.4	1049.4	1049.4
	4	700	847.0	847.0	824.3	824.3	798.7	798.7	798.7	798.7
C5	1	2000	1837.8	1755.7	1755.7	1837.8	1919.1	2000	2000	-
	3	1000	844.3	808.1	808.1	844.3	887.7	887.7	939.3	1000
	4	700	-	-	715.2	-	715.2	-	753.2	715.2
C7	1	2000	1924.8	1924.8	1768.9	1848.3	1685.4	1685.4	1685.4	1596.2
	3	1000	874.0	874.0	-	-	-	-	-	1000
	4	700	718.3	700	772.2	797.6	752.0	752.0	752.0	734.8
			-	-	626.4	678.1	626.4	626.4	626.4	598.7
	5	500	544.7	-	469.6	-	457.7	-	500	469.6
C8	1	2000	2000	2083.3	2000	2000	-	2250	2333.3	2250
	2	1500	-	-	-	-	-	1289.7	1184.9	-
	4	700	662.7	682.9	682.9	682.9	-	713.6	713.6	-

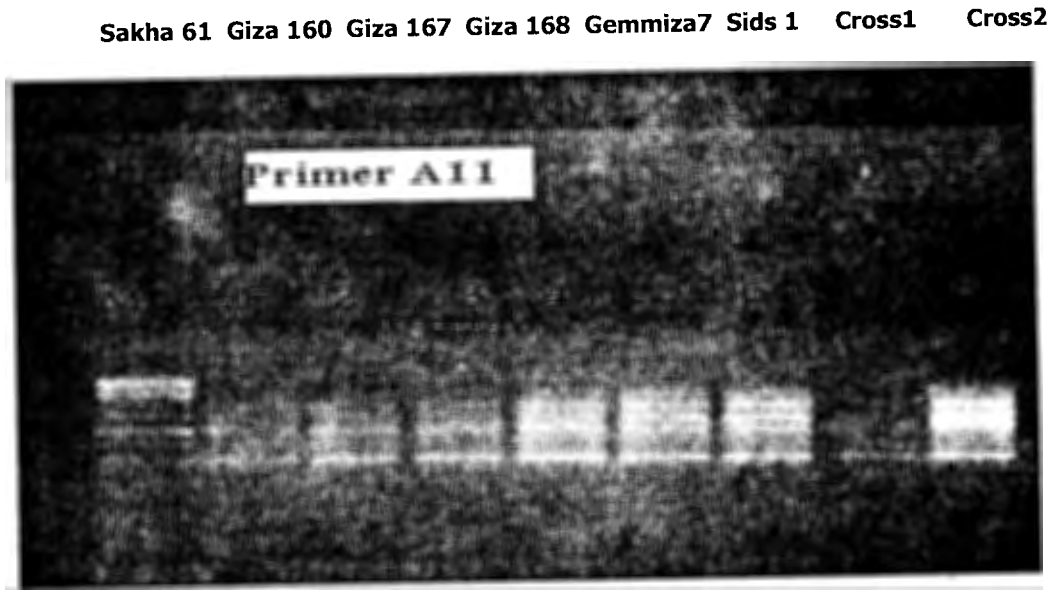


Fig. 2. RAPD patterns obtained with A11 primer.

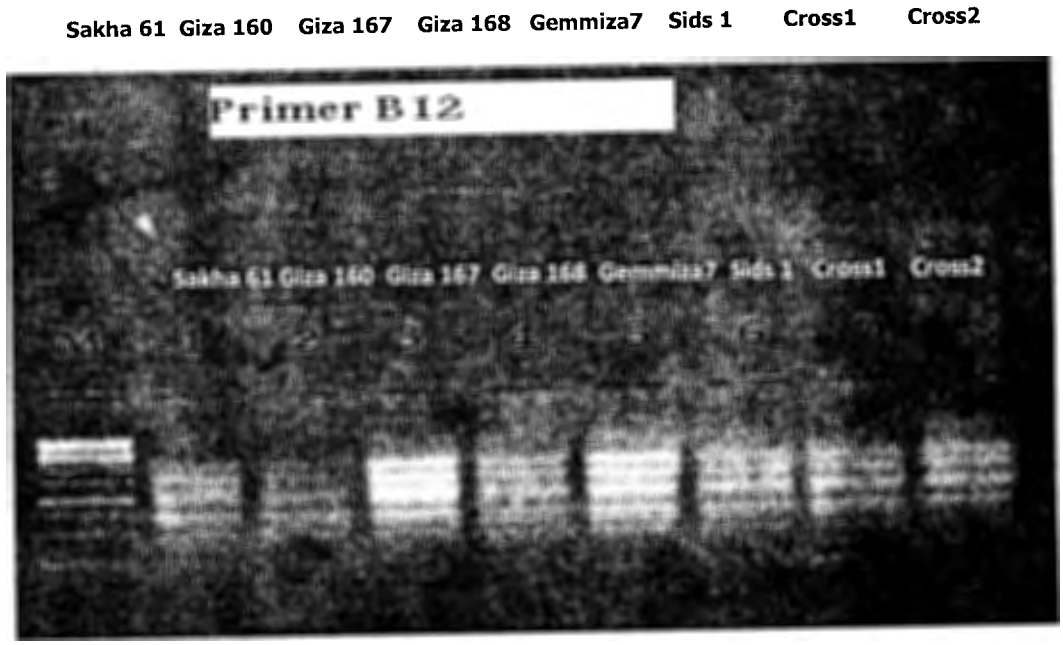


Fig. 3. RAPD patterns obtained with B12 primer.

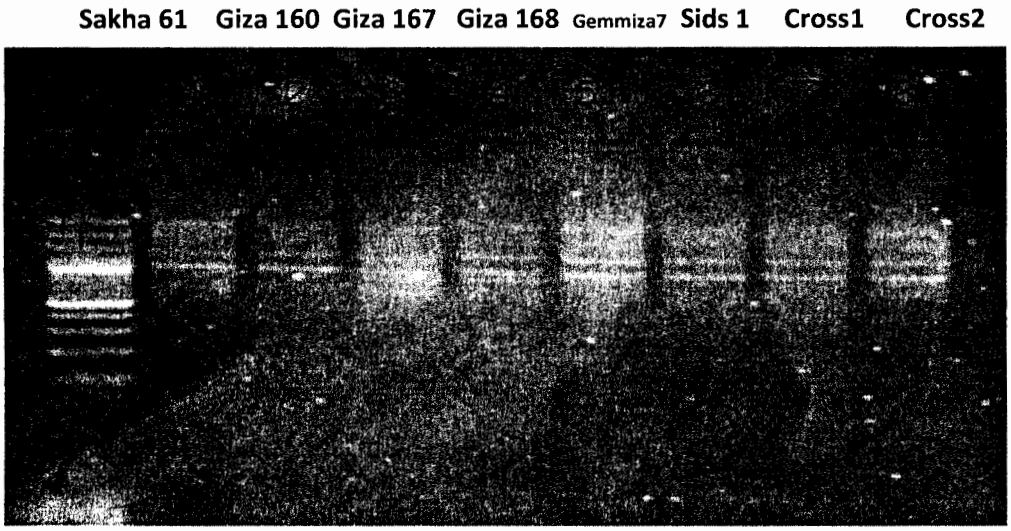


Fig. 4. RAPD patterns obtained with C7 primer.

Table 7. Detecting RAPD markers and molecular weight of bands linked to competitive ability genes against Avena fatua weed in two wheat crosses.

Cross	Primer	band	M	P1	P2	Bulked High Competitive	Bulked Intermediate Competitive	Bulked Low Competitive
Giza160 x Sakha 61	A11	2	1500	1752.858	1627.5	1564.063	1564.063	1627.5
		3	1000	1057.187	1057.187	1057.187	1000	1000
		4	700	747.043	-	-	700	653.415
		5	500	530.24	530.24	530.24	500	530.24
	C7	1	2000	2000	2083.3	2166.6	2166.6	2250
		2	1500	-	1596.1	-	1685.2	1768.8
		3	1000	928.9	1000	1086.5	1086.5	1086.5
		4	700	731.6	748.3	768.5	768.5	768.5
Giza167 X Sids 1	B12	1	2000	1801.2	-	1852.8	1852.8	1852.8
		2	1500	1276.2	1352.4	1276.2	1276.2	1352.4
		4	700	593.0	593.0	593.0	593.0	629.1
		7	300	321.9	343.6	321.9	343.6	343.6
		9	100	69.4	-	-	75.5	75.5
	C7	1	2000	1924.7	1924.7	2083.3	2000	2000
		2	1500	-	1500	-	1500	1500
		3	1000	870.9	870.9	870.9	928.2	928.2
		4	700	714.0	728.1	728.1	728.1	728.1
		5	500	-	564.4	-	564.4	564.4

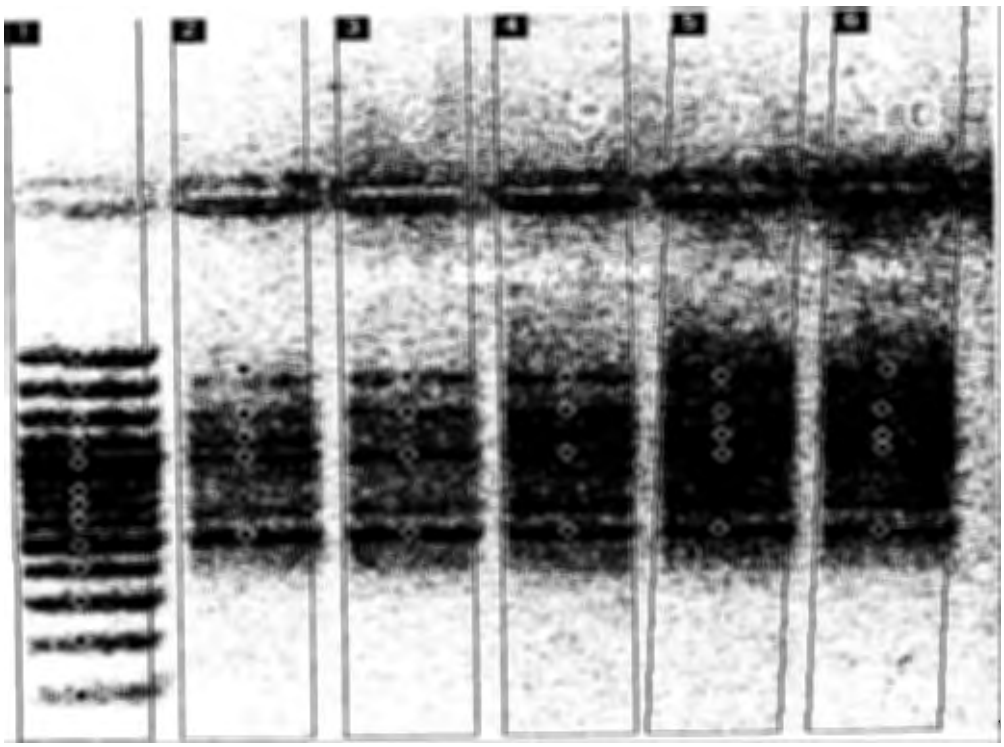


Fig. 5. RAPD markers detecting polymorphism between DNA Bulks in single cross between Giza 160 and Sakha 61 by using A11 RAPD primer.
BHA=bulked highly allelopathy, BIA=bulked intermediate allelopathy and BLA=bulked weakly allelopathy).

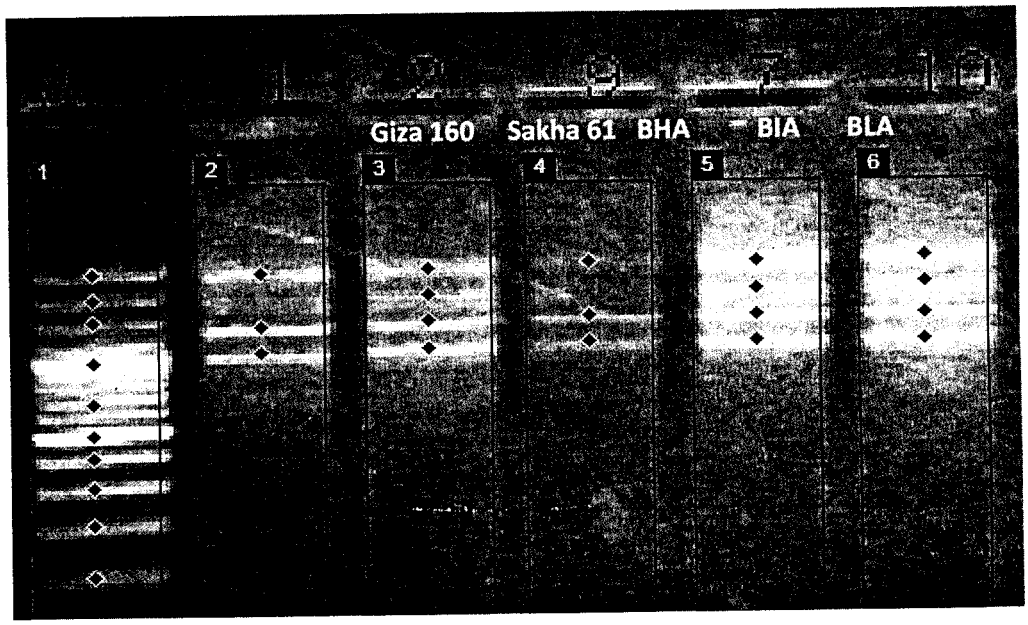


Fig. 6. RAPD markers detecting polymorphism between DNA Bulks in single cross between Giza 160 and Sakha 61 by using C7 RAPD primer.
BHA=bulked highly allelopathy, BIA=bulked intermediate allelopathy and BLA=bulked weakly allelopathy

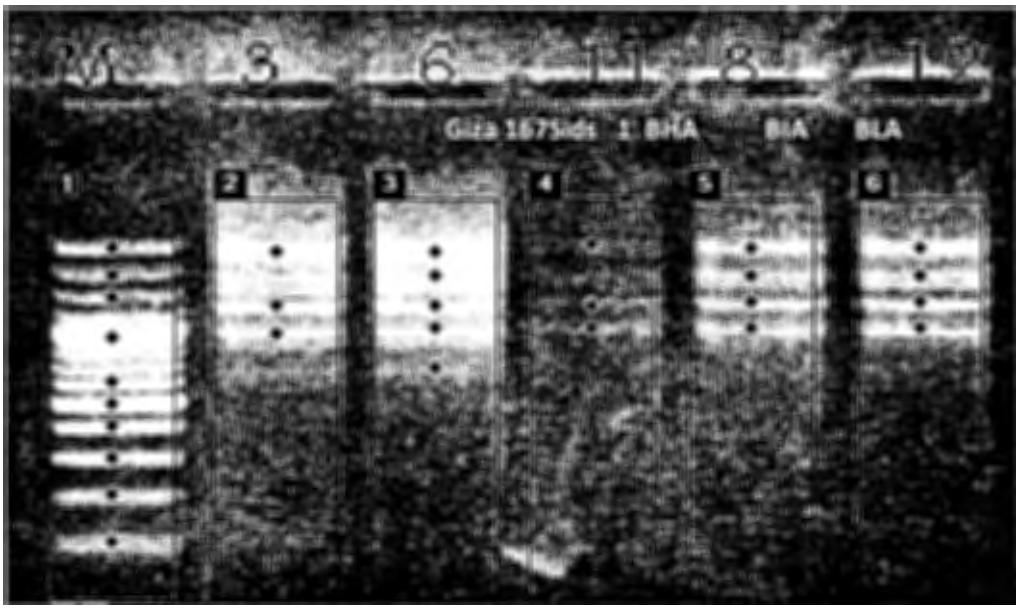


Fig. 7. RAPD markers detecting polymorphism between DNA Bulks in single cross between Giza 167 and Sids1 by using C7 RAPD primer.
BHA=bulked highly allelopathy, BIA=bulked intermediate allelopathy and BLA=bulked weakly allelopathy

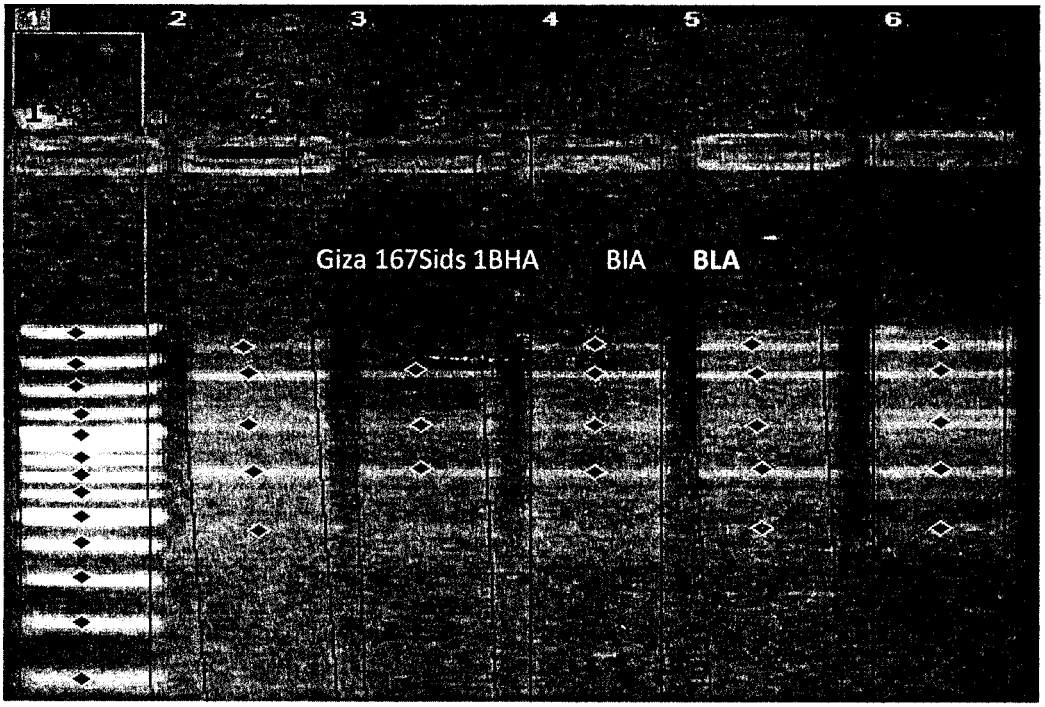


Fig. 8. RAPD markers detecting polymorphism between DNA Bulks in single cross between Giza 167 and Sids1 by using B12 RAPD primer.
BHA=bulked highly allelopathy, BIA=bulked intermediate allelopathy and BLA=bulked weakly allelopathy.

REFERENCES

1. Bach Jensen I, B. Courtois, M. Olofsdoner, L. Shen, R.P. Mauleon. Liz. 1999. Locating genes controlling rice allelopathic effects against *Echinochloa crus-galli*, in "Book of abstracts, second world congress on allelopathy-Critical analysis and future prospects". International Allelopathy Society, Thunder Bay, Ontario.
2. Dilday, R.H., W.G. Yan, K.A.K. Moldenhauer, K.A. Gravois. 1998. Allelopathic activity in rice for controlling major aquatic weeds. In "Allelopathy in rice". (Ed. M. Olofsdotter) pp. 7-26. (IRRI:Los Baros, the Philippines).
3. El-Maghraby, M.A., A.M. EL-Shehawi, M.H. Harby. 2010. Genetic diversity in bread wheat as revealed by RAPD markers and its relationship to leaf rust and hybrid performance. Alex. J. of Agric. Res., Egypt (55).
4. Guenzi, W.D., T.M. McCalla, F.A. Norstadt. 1967. Presence and persistence of phytotoxic substances in wheat, oat, corn and sorghum residues. Agronomy Journal 59, 163-165.
5. Hanwen, Wu. 2005. Molecular approaches in improving wheat allelopathy. Fourth World Congress on Allelopathy, Australia.
6. Kimber, R.W.L. 1967. Phytotoxicity from plant residues. I. The influence of rotted wheat straw on seeding growth. Australlian Journalnof Agricultural Research 18, 361-374.
7. Louise, B.J., C. Brigitte, L. Shen, Z. Li, M. Olofsdotter, and R.P. Mauleon. 2001. Locating genes controlling allelopathic effects against Barnyard grass in Upland Rice. Agronomy journal, 93 (1) :21-26.
8. Olofsdotter, M., L.B. Jensen , B. Courtois. 2001. Improving crop competitive ability using allelopathy — an example from rice . Plant Breeding Volume 121, Issue 1, pages 1–9, Feb., 2002.
9. Michelmore, R.W., I. Paran and R.V. Kasseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genome regions by using segregating populations. Proc. Acad. Sci. U.S.A. 88:9852.
10. Motiul, Q., G. Daggard, R. Barrow, S. Walker and M.W. Sutherland. 2000. Allelopathy, dimboa production and genetic variability in accessions of triticum speltoides . Journal of Chemical Ecology Volume 27, Number 4, 747-760, DOI:10.1023/A:1010354019573

11. Murray, M. and W.F. Thompson. 1980. Rapid isolation of high molecular-weight plant DNA. Nucleic Acids Res 8: Mu X, Yang C, Wang S. (2005). [Allelopathy of Pseudomonas fluorescens:a preliminary study]. Ying Yong Sheng Tai Xue Bao. 2005 Apr, 16(4):778-9.
12. Nathalie, C., J. Salse, F. Sabot, S. Rahman, A. Bellec, B. Laubin, I. Dubois, C. Dossat, P. Sourdille, P. Joudrier, M.F. Gautier, L. Cattolico, M. Beckert, S. Aubourg, J. Weissenbach, M. Caboche, M. Bernard, P. Leroy and B. Chalhoub. 2005. Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploid wheat species (*Triticum* and *Aegilops*). The Plant Cell 17:1033-1045 (2005).
13. Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.
14. Rolhf, F.J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.02 Exeter Software, Setauket, N.Y.
15. Wu, H., J. Pratley, D. Lemerle, T. Haig. 1999. Crop cultivars with allelopathic capability. Weed Res. 39:171–180
16. Wu, H., T. Haig, J. Pratley, D. Lemerle, and M. An. 2001. Allelochemicals in Wheat (*Triticum aestivum* L.): Cultivar differences in the exudation of phenolic acids. J. Food Chem. 2001, 49:3742-3745.
17. Wu, H., J. Pratley, W. Ma and T. Haig. 2003. Quantitative trait loci and molecular markers associated with wheat allelopathy. TAG, Theoretical and Applied Genetics, Volume 107, Number 8, 1477-1481, DOI:10.1007/s00122-003-1394-x .
18. Zhang, X.K., Y. Jiang, W.J. Liang, C.H. Kong. 2004. Research advances in wheat (*Triticum aestivum* L.) allelopathy. Chin J Appl Ecol 15:1967-1972.

إمكانية استخدام طريقة الانتخاب بواسطة المعلمات الوراثية فى تحسين صفة المنافسة ضد الحشائش فى القمح

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تهدف هذه الدراسة إلى تحديد التصنيفات الوراثية بين عدد ستة أباء من قمح الخبز المصرية باستخدام الدلائل الجزيئية بالنسبة لصفة القدرة على منافسة الحشائش (الزمير) وبالتالى يتبع ذلك التعرف على الدلائل الجزيئية العشوائية المرتبطة بجين منافسة القمح للحشائش عن طريق الدلائل الجزيئية المساعدة المنتخبة. أوضحت الدراسة الحالية أن هناك اختلافات وراثية بين الآباء بالنسبة لصفة منافسة القمح للحشائش مما ينتج عنه توفير وعاء جينى يمكن المربي من تطوير صفة قدرة قمح الخبز على منافسة الحشائش لكي يتم تثبيط نمو الحشائش. استخدم فى هذه الدراسة تقنية الدلائل الجزيئية العشوائية فى تحديد الاختلافات الوراثية بين أصناف قمح الخبز تحت الدراسة وتم إجراء التهجينات بين أصناف ذات قدرة عالية على منافسة الحشائش وأصناف ذات قدرة متوسطة (جيزة ١٦٠ * سخا ٦١) & (جيزة ١٦٧ * سدس ١) لتحديد الدلائل الجزيئية العشوائية المرتبطة بهذه الصفة. أوضح التحليل الشجري المتحصل عليه من هذه الدراسة على قدرة تقنية الدلائل الجزيئية العشوائية المستخدمة على إظهار اختلافات وراثية وكان مجموع القطع الجزيئية العشوائية المتحصل عليها باستخدام ١٤ بادئ عشوائي هو ٧٩٨. من بين هذه القطع المتحصل عليها أعطى ٣٥ أليل اختلافات وراثية بين الأصناف تحت الدراسة بمتوسط ١.٥ أليل لكل موقع عشوائي. وبتقدير المسافات الوراثية بين الأصناف باستخدام معادلة Nei وجد أن هناك مجموعتان رئيسيتان من الأصناف بمسافة تتراوح بين ٦٤% إلى ٨٧% بمتوسط ٧٥%. وقد تم استخدام تقنية BSA في الجيل الثاني للهجن (جيزة ١٦٠ كأب عالي المنافسة مع سخا ٦١ كمتوسط المنافسة) وهجين (جيزة ١٦٧ كأب عالي المنافسة مع سدس ١ كمتوسط المنافسة) للتعرف على الدلائل الجزيئية العشوائية المرتبطة بجين تثبيط نمو الحشائش في القمح. من بين ١٤ بادئ جزيئي عشوائي تم اختبارهم مع الهجينين السابقين أظهر الدليلان A11 & C7 اختلافات كبيرة بين DNA للجيل الثاني بالنسبة للتراكيب ذات القدرة العالية للمنافسة والتراكيب الضعيفة للمنافسة في الهجين جيزة ١٦٠ * سخا ٦١. وبالمثل في الهجين الثانى (جيزة ١٦٧ * سدس١) أظهر الدليلان الجزيئيان B12 & C7 اختلافات كبيرة بين DNA للتراكيب ذات القدرة العالية للمنافسة والتراكيب الضعيفة للمنافسة مما يساعد في تحليل هذين الدليلين الجزيئيين كدلائل جزئية سائدة. وكملخص لنتائج هذه الدراسة تبين أن استخدام تقنية الدلائل الجزيئية العشوائية مع تقنية BSA يمكن من التعرف على جينات منافسة حشيشة الزمير فى قمح الخبز وبمجرد التعرف وتحديد هذه الدلائل الجزيئية يمكن استخدامها من خلال المربي فى الانتخاب لهذه لصفة فى الأجيال المبكرة.