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GENETIC MARKERS FOR GRAIN PROTEIN PERCENTAGE IN EGYPTIAN WHEAT (*Triticum aestivum* L.)

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Abstract: Randomly amplified polymorphic DNA (RAPD) markers were used to study the genetic relationships and similarity among four wheat genotypes (two local cultivars, namely Giza-168 and Sakha-69 and two recombinant inbred lines one with low grain protein percentage "GPP" and the other with high grain protein percentage. Amplifications using five primers produced RAPD fingerprints with varying numbers of bands which ranged in size from 196 bp to 2519bp.The number of amplified DNA fragments per primer ranged from 4 to 10 with a mean of 8.6 bp. The primers have amplified 43 bands, out of which 33 bands (76.7%) were polymorphic. Twenty RAPD markers were found to be genotype specific with eight being specific for the low GPP line, six markers for the high GPP line, four markers for Giza-168 and two markers for Sakha-69. The mean genetic similarity among the four genotypes was 0.714 ranging from 0.656 to 0.829. The genetic relationships among the four genotypes estimated by UPGMA cluster analysis based on the RAPD-derived data indicated that a relatively small number of RAPD primers could be used for distinguishing wheat genotypes according to protein percentage. The results suggested that identification of RAPD molecular markers associated with genes influencing GPP would allow wheat breeders in Egypt to select for GPP independent of enviro-nmental effects.

Key words: RAPD, Wheat, Grain protein percentage.

Introduction

Wheat (*Triticum aestivum L.*) occupies an important place in the crop breeding in Egypt. The demand for this crop is increasing as a consequence of rapid population growth. Although wheat production in Egypt has been greatly improved during the recent years, continual improvement in productivity is highly desirable. Therefore, yield improvement in wheat varieties remains the prime objective of most breeding efforts. Genetic improvement of grain quality, which is determined by the storage proteins and other constituents of endosperm, has always been an important target for enhancing the nutritive value and the technological properties

of the wheat flour (*Dhaliwal, 1977*).

The remarkable success in the genetic improvement of grain yield of common wheat varieties over the past 50 years was not accompanied with comparable progress in grain quality *(*Van Lill and Purchase, *1995*). According to Zlatska (2005) grain protein content in wheat was among the characters that suffered from intense selection, the narrow genetic base of germplasm in use has been considered the main reason. Knowledge of genetic diversity patterns allows plant breeders to better understand the evolutionary relationships among accessions, to sample germplasm in more systematic fashions, and develop strategies that incorporate useful diversity in their breeding programs (Bretting and Widrlechner, 1995) .Information about genetic diversity and relatedness in the available germplasm and among elite breeding material is a fundamental element in plant breeding.

The future of plant breeding programs depend upon the availability of genetic variability to increase productivity. Traditional assessment of genetic diversity has been based on differences in morphological and agronomic traits or pedigree information for the different crops .

Recently, a powerful strategy to increase efficiency of selection in plant breeding is the use of molecular markers. The PCR-based DNA marker techniques seem to provide the means for generating useful information on polymorphism, genetic relatedness, and diversity.

The random amplified polymorphic DNA (RAPD) markers are extensively used in gene mapping (Chalmers *et.al*; 2001) and for identification of markers linked with useful traits (*Bai et.al; 2003).*

Due to its technical simplicity and speed, RAPD methodology has been used for diversity analysis in soybean (Zenglu and Randall; 2001). In the present study RAPD technique was used for (1) assessment of genetic similarity and polymorphism in four wheat genotypes varied significantly in grain protein percentage (GPP) (2) identification of DNA specific markers for high and low grain protein percentage cultivars.

Material and Methods

The plant material:

The plant material used in the study consisted of tow local cultivars, namely Giza-168 and Sakha-69 and two F8 recombinant inbreed lines, one with high and the other with low grain protein percentage (GPP) derived from a divergent

selection program for high and low GPP among 100 F7 long spike-multifloreted recombinant inbred lines (RIL_S) of bread wheat (*Triticum aestivum* L.). The 100 F7 RIL_S were derived by the single-seed descent breeding method from segregates which appeared in the progeny of a black glumed landrace (WK-12) which was among the landraces collected from farmer's fields in remote dry area in Upper Egypt (Omara, 1994). Seeds of the four genotypes were kindly provided by. Prof. Dr.M.R.Omara, Genetics Department, Assiut University. The grain protein percentages were 22.89 and 16.99 for the selected high and low lines, respectively, 14.95 for Giza-168 and 14.97 for Sakha-69 respectively.

DNA extraction and RAPD-PCR:

The wheat genotypes were planted in plastic containers (250 ml). The leaf tissues were obtained from 6-days old seedlings. DNA was extracted following the protocol of Dellaporta *et al* (1983). As RADP technique the concentration of genomic DNA, 10x PCR buffer, with $(NH4)_2 SO_4$, $MgCl_2$, dNTPS (dATP, dTTP, dGTP), and dCTP), 10 mere random primer and taq polymerase were optimized as described by (Williams *et.al;* 1990).

Ten-base oligonucleotide primers were obtained from Operon Ine Alamada CA (USA). The code number of the primers used in the present investigation is OPE-7, OPY-7, OPO-12, OPI-2 and OPC-18. The taq DNA polymerase was purchased from GIBICO BRL,

DNA amplification reactions were performed in a thermal cycler (Perkin Elmer co). The PCR profile was one cycle at 96 ^oC for 5 m, 40 cycles at 94 ^oC for 1 m, 36 ^oC for 1 min, 72 ^oC for 3min and a final extension for 10 min at 72 ^oC.

Analysis of RAPD data:

The RAPD fragments were electrophoresed on 1% agarose gel stained with Ethidium bromide (20ng/50ml of agarose gel in Tris borate EDTA buffer) .The 100bp standard DNA (Life technologies) was used for estimating RAPD product sizes. The PCR were runs generally performed once; if there was a reaction failure for a particular primer in one or more lanes, the run was repeated with all four DNA samples. The bands were visualized by UV-illumination and counted starting from top to bottom lane.

Amplification profiles of the four genotypes were compared with each other, and bands of DNA fragments were scored as present or absent.

The data of the primers were used to estimate genetic similarity on the basis of the number of shared amplification products (Nei and Li, 1979). The equation used was: No. of shared amplification products = 2 X (No. of common bands between any two lanes) / (total No. of bands in the same two lanes*).*

Genetic relationship among the genotypes was estimated with the dendrogram constructed using DICE computer package to estimate the pairwise differences matrix and plot the phonogram among genotypes. Unique bands detected in a particular genotype but not in others were used as positive DNA markers. The absence of a common band for a given genotype was referred to as a negative specific marker.

Results

RAPD markers:

All examined primers reacted with the four investigated wheat genotypes producing a unique banding pattern for each genotypes.

Amplifications using five primers produced RAPD fingerprints with varying numbers of bands ranging in size from 196 bp to 2519bp.

Depending on the primers, the number of bands varied between 24 to 36. From a total of 139 scorable bands, only 10 bands were conserved among all genotypes. The primers have amplified 43 bands (Fig 1 and Table 1), out of which 33 bands were polymorphic with a percentage of 76.7%. The five primer DNA fragments which were detected in particular genotypes produced multiple band profile with a number of amplified DNA fragment ranging from 4 to 10 with a mean of 8.6 (Table 1).

DNA specific markers:

The DNA specific markers of the four wheat genotypes are listed in Table (2). Twenty RAPD markers were found to be genotype specific markers (11 positive + 9 negative markers), eight out of which (6 positive + 2 negative) were specific for the low GPP line. The specific DNA markers detected in the four genotypes were six markers (2 positive+4 negative) for the high GPP line, four markers (2 positive+2negtive) for Giza-168 and two markers (1 positive + 1 negative) for Sakha-69. The base pair number of the detected DNA specific markers ranged from 275 to 1796. The largest number of genotype-specific DNA markers was generated by OPY-7 (6markers=2 positive +4 negative) followed by primers OPI-2, (5 markers), OPO-12 and OPE-7 (4 markers) and OPC-18 (one marker).

The similarity matrix of the 4 wheat genotypes obtained from RAPD markers indicated that: the mean of genetic similarity among the four genotypes was 0.714 with a range of 0.656 to 0.829 (Table 3). The highest similarity value was recorded for the two check cultivars Giza -168 and Sakha –69 (0.829), while the lowest value was recorded for the high GPP line and Giza -168 cultivars. The high GPP line was found to be 74.3% similar to Sakha-69, while the low GPP line was 66.7% similar to Giza -168. Figure (2) gives the pherogram from the UPGMA clustering of values given in Table (3).

Table(1): List of primers, their nucleotide sequences, and number of bands of each genotype investigated with five different primers.

primer	Sequence (5→3)	No. of amplified fragment				total	Amplified band	Polymorphic bands
		Giza-168	Sakha-69	High GPP	low GPP			
OPI-2	GGAGGAGAGG	7	8	4	9	28	12	8
OPE-7	AGATGCAGCC	6	8	6	6	26	10	7
OPC-18	TGAGTGGGTG	5	8	7	5	25	9	5
OPY-7	AGAGGCCTCA	9	10	7	10	36	13	8
OPO12	CAGTGCTGTG	5	4	8	7	24	9	5
Total		32	38	32	37	139	43	33

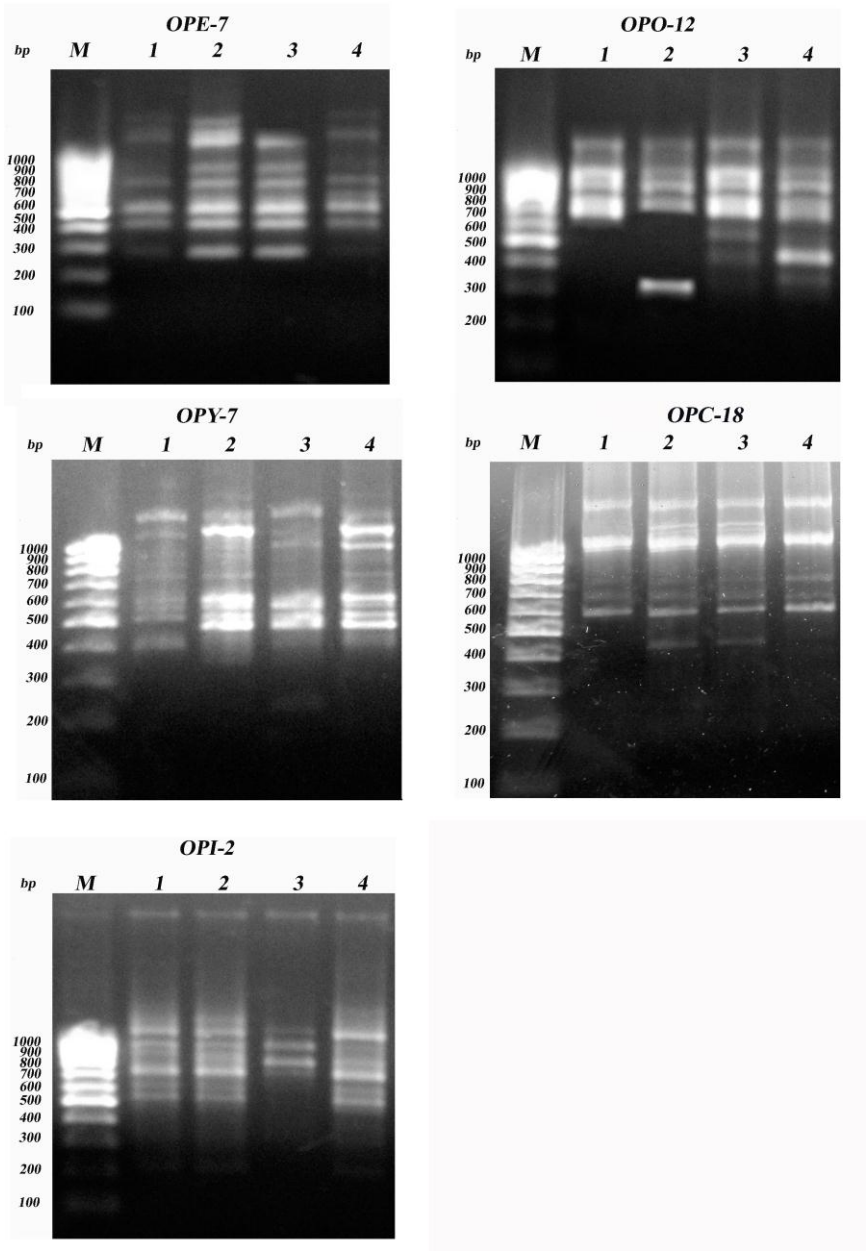

Fig.(1): Agarose gel electrophoresis of RAPD profiles in 4 wheat genotypes (1-4) generated by five RAPD primers. Where (1) Giza-168, (2) Sakha-69, (3) High GPP, (4) Low GPP.

Table(2): Unique DNA fragments (markers) of different wheat genotypes and their molecular size (bp) detected by the different employed primers.

Primer	OPI-2		OPE-7		OPC-18		OPY-7		OPO-12		Total	
	+	-	+	-	+	-	+	-	+	-	+	-
Giza-168							847 416	1027 485			2	2
Sakha-69	1281									688	1	1
High GPP		608 529						912 776	544 308		2	4
Low GPP	359 296		2796 659	1774 2775	840				331		6	2
Total	3	2	2	2	1		2	4	3	1	20	

(+) positive markers, (-) negative markers

Table(3): Genetic similarity values calculated from the DNA fragments amplified in the different wheat genotypes using five random primers.

Similarity matrix				
	Giza-168	Sakha-69	High GPP	Low GPP
Giza-168	1			
Sakha-69	0.829	1		
High GPP	0.656	0.743	1	
Low GPP	0.667	0.693	0.696	1

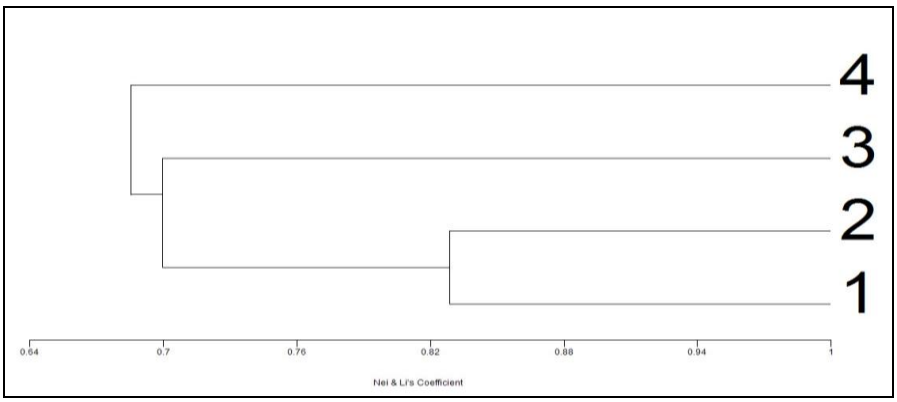

Fig.(2): Dendrogram demonstrating the relationship among the four wheat genotypes based on data recorded from polymorphism of RAPD markers. Where (1) Giza-168, (2) Sakha-69, (3) High GPP, (4) Low GPP.

DISCUSSION

The five primers used in the present study yielded distinct and reproducible RAPD banding profiles that differed among the four genotypes. In previous studies, wheat genotypes have been analyzed using different DNA marker systems to measure the genetic diversity or similarity levels within specific group of genotypes (Eagles *et al*., 2001; Teshala *et al*, 2003 and Hashad *et al*, 2005). These findings, as well as those of the present study clearly demonstrated the reliability, usefulness and efficiency of RAPD markers in analyzing genomic diversity or similarity

The results of the five primers indicated that a high level of polymorphism (76.7%) among the four wheat genotypes which provide wheat breeders with environment-independent DNA markers, to be used in marker-assisted selection.

The genetic relationships among the four genotypes investigated in the present study as estimated by UPGMA cluster analysis of the genetic distance (1-genetic similarity) matrix based on the RAPD-derived data suggested that a relatively small number of RAPD primers could be used for distinguishing wheat genotypes which varied in protein percentage.

In the present investigation, 20 RAPD specific markers were identified, eight of which were detected in the low GPP line and six were identified in the high GPP lines. Several workers identified DNA markers associated with GPP. The study of Dholakia et.al (2001) revealed nine DNA markers associated with the grain protein concentration (GPC) trait in a number of wheat recombinant inbred lines. They demonstrated that GPC is highly influenced by the environment and pointed out the applicability of inter simple sequence repeat (ISSR) and RAPD markers in finding regions on chromosomes associated with quantitative characters in wheat such as GPC. In addition, QTL (Quantitative trait loci) analysis with a linkage map of 211 markers identified seven loci significantly linked to variations in GPC in barley. These loci accounted for approximately 74% of the total genetic variance for GPC in the population (Emebiril *et al.*, 2005).

Genetic association of DNA markers with important traits can be used for indirect selection of the traits (Briana *et.al*.2001).Our results suggest that identification of RAPD molecular markers associated to genes influencing GPP would allow wheat breeders in Egypt to select for GPP independent of environment effects. In conclusion, RAPD technique was found to be quite

effective in determining the genetic variation among wheat genotypes and could be utilized as DNA fingerprinting for variety identification and for the establishment of plant breeder rights in Egypt. These findings would also contribute to choose parents for the breeding program.

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واسمات وراثية خاصة بالنسبة المئوية للبروتين فى حبوب القمح المصرى

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تم استخدام واسمات الـ RAPD-PCR لدراسة العلاقات الوراثية والتماثل الوراثى بين أربعة تراكيب وراثية من القمح أثنين من الأصناف المحلية هما جيزة-168 وسخا-69 ، بالإضافة إلى أثنين من السلالات المركبة المرباة داخليا إحداهما كانت النسبة المئوية لبروتين الحبوب بها منخفضة والثانية بها نسبة مئوية مرتفعة.

تم استخدام خمسة بادئات Primers تفاعلت مع الأربعة تراكيب وراثية منتجة تحليل RAPD ذو عدد مختلف من الحزم التى تراوحت فى الحجم بين bp196 إلى bp 2519 . وعدد الحزم التى أظهرها كل بادئ تراوح بين 4 إلى 10 حزم بمتوسط bp 8.6 . كان عدد الحزم الكلى 43 شظية DNA كان منها 33 حزمة (76.7 %) ذات صورة متعددة. كان هناك عشرون واسمة RAPD متخصصة منها ثمانية خاصة بالسلالة ذات النسبة المئوية المنخفضة لبروتين الحبوب وستة واسمات خاصة بالسلالة ذات النسبة المئوية المرتفعة لبروتين الحبوب وأربعة واسمات خاصة بالصنف جيزة-168 وواسمين للصنف سخا-69 .

تم تقدير علاقة القرابة بين التراكيب الوراثية عن طريق التحليل العنقودى لنتيجة الـ RAPD . وكان مدى التماثل الوراثى بين الأربعة تراكيب الوراثية يتراوح بين 0.656 إلى 0.829 بمتوسط 0.714 وأظهر التحليل العنقودى أنه يمكن استخدام عدد قليل نسبيا من البادئات للتمييز بين التراكيب الوراثية المختلفة فى القمح بالنسبة لصفة النسبة المئوية لبروتين الحبوب.

وأوضحت النتائج أن: (1) – امكانية استخدام مربى القمح فى مصر للواسمات الجزيئية المتخصصة المرتبطة بالجينات التى تتحكم فى النسبة المئوية للبروتين فى الحبوب فى الانتخاب لهذه الصفة بإستقلالية عن التأثيرات البيئية. (2) - تقنية الـ RAPD كانت فعالة فى تحديد الاختلافات الوراثية بين التراكيب الوراثية المختلفة فى القمح ويمكن استخدمها كبصمة DNA تميز الأصناف ولحفظ حقوق مربى القمح فى مصر.