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The Suitability of RAPD Markers in Identifying Some Hexaploid Wheat Crosses

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Abstract: Ten random primers were used to study the suitability of RAPD technique for characterization of four wheat crosses from their parents. From this study, the patterns of molecular markers were classified into seven types included in three categories: The first category included, Type I markers shared bands in both parents and offspring, Type III markers shared bands in female parent and offspring, Type IV markers shared bands in male parent and offspring. The second category also included three types, Type II markers shared bands in male and female parents, Type V markers were presented in the female parent only, Type VI markers were present in the male parent only. Third category contained Type VII markers which were present in offspring only. The shared marker (category 1) ranged from 77.22% for cross number 2 to 96.2 for cross number 1. While, the percentage of markers not shared in the studied crosses (category 2) ranged from 2.53 for cross number 1 to 21.25 for cross number 3. While, unique markers related to the third category were observed in three crosses, which considered as a good marker to identifying new crosses to protect the rights of plant breeders. However, RAPD markers are a powerful tool to detect different molecular markers in wheat crosses.

Key words: RAPD markers % Wheat crosses % Random primers % Parents

INTRODUCTION

Wheat is one of the most important agricultural crops and is a basis for human nutrition in Egypt and worldwide. Therefore, there is an urgent need to increase productivity level of wheat to reduce gap resulting from population explosion. Different types of markers were used in breeding applications. Morphological and cytological markers are not useful for breeding analysis [1]. Although isozyme markers are useful to characterize genetic diversity [1, 2] and to identify the crosses between cultivars [1], the paucity of isozyme loci restricts their usefulness in breeding [3]. One of the limitations of protein and isozyme markers is a relatively less polymorphism that may exist between the two parents; this limits the total number of markers, which can be actually scored in a given cross. This has shifted the focus to DNA-based molecular markers [4].

DNA markers have been used to manipulate marker-assisted selection (MAS) and to guide the introgression of target genes from related species by restriction fragment length polymorphism (RFLP) in the past several years [5]. However, RFLP is labor intensive and costly. One of the most used techniques, random amplified polymorphic DNA (RAPD), was used due to its simplicity; require a small quantity of DNA and the ability to generate numerous polymorphisms [6]. RAPD technique has been successfully used for the assessment of genetic diversity in hexaploid wheat [7-12]. According to McDonald [13], using of RAPD technique in the genetic purity testing has important advantages compared to the other systems. The information obtained through germplasm characterization by RAPD is extensively used for identification of germplasm, screening of duplicates, assessing genetic diversity and monitoring the genetic stability of conserved germplasm. Recently, RAPD markers have been widely used for crosses identification and testing genetic purity in several crops such as Chrysanthemum [14], barley [15], chilli [16], cotton [17], black pepper [18] and sorghum [4].

The main objective of this investigation was studying the possibility of using RAPD markers for identification of wheat crosses from their parents.

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MATERIALS AND METHODS

Plant Materials: The present study was carried out at the Agriculture Research and Experiment Station, Faculty of Agriculture, Cairo University, Giza, Egypt and the Genetics and Cytology Department, National Research Center, Dokki, Giza, Egypt, during the period from 2010 to 2012. Four wheat crosses i.e.: H_1 (Sids 4 x Assiut 216), H_2 (Sakha 93 x Assiut 249), H_3 (Gemmiza 9 x Line 1457) and H_4 (Assiut 230 x Line 1457). These crosses were chosen according to their differences in there agronomic characteristics [19].

DNA Extraction: Five seeds per each wheat genotype (parents and F_1 crosses) were germinated in moist filter papers for 2 weeks. Genomic DNA was extracted from young leaves of the studied genotypes according to the Bio-Flux kit protocol. RAPD analysis was performed using 10 random primers (Table 1) produced from Operon Technologies Inc. (Alabameda, CA).

RAPD Reaction: PCR reaction was carried out in a final volume of 25 µl containing 12.5 µl of Master Mix (Bioteke), 2.5 µl of 5 µM of each primer, 50 ng of template DNA [20]. Reactions were performed in a thermocycler (Biometra T1, G mbH), as follows : one cycle of 95°C for 5 min (denaturation), 36 cycles of 94°C for 1 min, 36°C for 1 min (annealing) and 72°C for 1 min (extension) and a final extension of 2 min at 72°C. PCR products were analyzed using 1% agarose gel electrophoresis and visualized with ethidium bromide staining.

RAPD Data Analysis: Amplified RAPD markers were scored as present (+) or absent (-) for each sample. Ambiguous bands that could not be easily distinguished were not scored [20]. The similarity of samples was calculated as follows: Similarity = $2\ N_{AB}/N_A+N_B$ where N_{AB} is the number of bands shared by individuals A and B and N_A and N_B are the number of bands in individuals A and B, respectively [21, 22]. The monomorphic and polymorphic bands were recorded. The monomorphic bands are those which are present in both parents and may or may not be expressed in the cross. Polymorphic bands are those that are present in either of parents and also expressed in their cross or they are expressed either only in male/female or in the cross.

The RAPD profiles obtained in the present study were classified into seven types of markers according to presence or absence of bands according to Akhare *et al*. [4], Chung *et al*. [14] and Mehetre *et al*. [17]. For testing applicability of RAPD profile for identification of the four wheat crosses from their parents, RAPD profiles of each cross were compared with its respective parents.

RESULTS AND DISCUSSION

All used primers produced multiple band profiles with the studied wheat genotypes, parents and their crosses. The four crosses produced a total number of bands ranging from 65 for H_3 to 74 bands for H_1. The number of amplified fragments per primer ranged from 12 (Primer OPN-06) to 39 bands (Primer OPX-11), with a mean of 27.3 bands per primer. Primer (OPX-11) with the genomic DNA of crosses H_1, H_2 and H_3 as well as primer (OPX-17) with genomic DNA of cross number 2 were generated the highest number of amplicons (10). While, primer (OPN-06) with cross number 4 exhibited the lowest number of amplicons (1). The size of amplified fragments varied with the different primers, ranging from 146 to 1363 bp (Fig. 1). Hussien *et al*. [23] discussed the possibility of RAPD markers to distinguishing wheat diallel crosses from their parents. In this paper we discussed the possibility of the same marker in identifying wheat crosses that differ in their parents. Based on the presence or absence of bands in each cross and its respective parents, three categories could be distinguished, including seven types of RAPD markers as follows:

First Category: included bands common in a cross and both of its parents (Type 1 marker) and bands common in cross and its female parent (Type 3 marker) or its male parent (Type 4 marker). The bands of marker Types 1, 3 and 4 are good markers to confirm that the cross is of its respective parents. In addition, bands of Type 4 marker are especially important markers to identify the true cross.

Table1: List of random primers used in RAPD analysis and their nucleotide sequences.

Primer	Sequence	Primer	Sequence
OPX-11	5'-GGAGCCTCAG-3'	OPW-04	5'-CAGAAGCGGA-3'
OPT-08	5'-AACGGCGACA-3'	OPN-06	5'-GAGACGCACA-3'
OPC-19	5'-GTTGCCAGCC-3'	OPA-03	5'-AGTCAGCCAC-3'
OPX-17	5'-GACACGGACC-3'	OPC-15	5'-GACGGATCAG-3'
OPD-13	5'-GGGGTGACGA-3'	OPN-04	5'-GACCGACCCA-3'

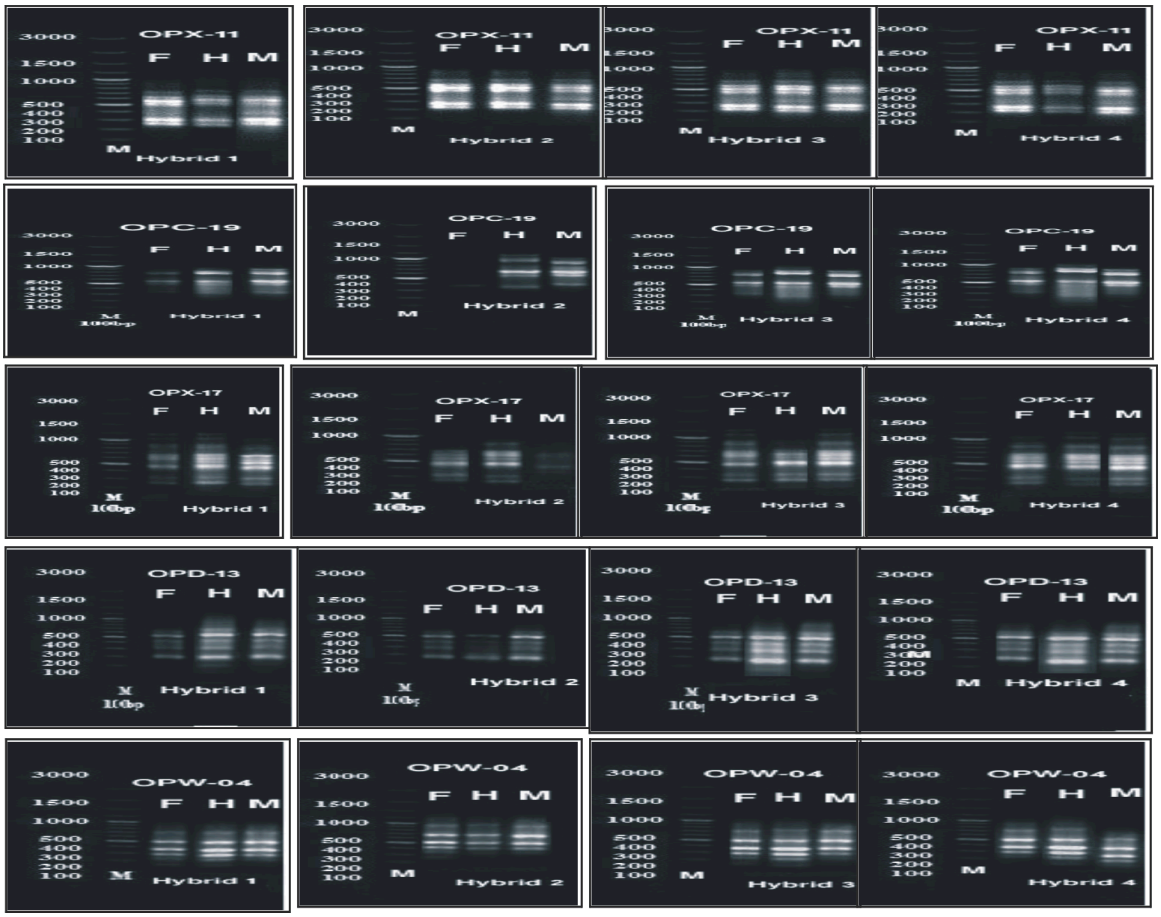

Fig. 1: RAPD profiles of wheat hybrids and their parents resulted from five primers, F= Female parent, M= Male parent, H= Hybrid, M= DNA marker.

Second Category: included bands found in either or both parents but not shared with the cross. These were bands of Type 2 markers, which were monomorphic for parents but absent in cross. Bands of Type 5 marker are expressed only in female and bands of Type 6 expressed only in male parent.

Third Category: included non-parental bands, which expressed only in crosses, such cross-specific bands are useful for the identification of specific crosses.

Results in this study are presented separately for each cross as follows:

Cross Number (1) and its Parents (& Sids 4 X Assiut % 216): The RAPD profile of cross number (1) revealed 65 shared markers with its parents of Type 1, category 1 (Table 2). The primers (OPX-11, OPX-17 and OPA-03) gave 9 shared bands, while the primer OPN-06 gave the lowest number of shared markers (4). These markers are useful for ascertaining that the cross is truly between its respective parents. The number of bands of Type 3 of the category 1, that were produced with primers OPX-11, OPN-06 and OPC-15 were 1, 2 and 2 bands, respectively, while with Type 4 marker, they were 1, 2 and 3 bands produced by primers OPT-08, OPC-19 and OPN-04, respectively. These bands that belong to this category are good markers to confirm that the cross is truly among its respective parents and the bands from Type 4 markers are especially important markers to identify the true cross. This cross had only two bands (1 band with each of primers OPC-19 and OPC-15) that belonging to Type 2 of category two. For the non-parental bands but expressed in the cross only, cross 1 had only one band from the third category with primer OPC-19. Such cross-specific bands are useful for the identification of specific cross (Fig. 1).

Table 2: Number of detected bands in the three categories, included the seven types of RAPD markers of cross number (1) and its parents.

	1st category			2nd category			3rd category
Primer name	Type 1	Type 3	Type 4	Type 2	Type 5	Type 6	Type 7
OPX-11	9	1	-	-	-	-	-
OPT-08	6	-	1	-	-	-	-
OPC-19	5	-	2	1	-	-	1
OPX-17	9	-	-	-	-	-	-
OPD-13	7	-	-	-	-	-	-
OPW-04	6	-	-	-	-	-	-
OPN-06	4	2	-	-	-	-	-
OPA-03	9	-	-	-	-	-	-
OPC-15	6	2	-	1	-	-	-
OPN-04	4	-	3	-	-	-	-
Total	65	5	6	2	0	0	1

Table 3: Number of detected bands in the three categories, included the seven types of RAPD markers of cross number (2) and its parents.

	1st category			2nd category			3rd category
Primer name	Type 1	Type 3	Type 4	Type 2	Type 5	Type 6	Type 7
OPX-11	9	-	-	-	-	1	-
OPT-08	6	-	-	1	1	-	-
OPC-19	1	-	6	-	-	-	1
OPX-17	3	4	-	-	-	-	3
OPD-13	3	-	-	2	1	-	-
OPW-04	4	-	-	2	-	-	-
OPN-06	3	-	-	-	3	-	-
OPA-03	9	-	-	-	-	-	-
OPC-15	6	-	-	-	-	3	-
OPN-04	4	-	3	-	-	-	-
Total	48	4	9	5	5	4	4

Table 4: Number of detected bands in the three categories, included the seven types of RAPD markers of cross number (3) and its parents.

	1st category			2nd category			3rd category
Primer name	Type 1	Type 3	Type 4	Type 2	Type 5	Type 6	Type 7
OPX-11	9	-	-	-	1	-	-
OPT-08	7	-	-	-	-	-	-
OPC-19	6	-	1	1	1	-	-
OPX-17	8	-	-	2	-	-	-
OPD-13	7	-	-	-	-	-	-
OPW-04	5	1	-	-	-	-	-
OPN-06	2	-	-	4	-	-	-
OPA-03	2	-	-	7	-	-	-
OPC-15	6	-	2	-	1	-	-
OPN-04	6	-	1	-	-	-	-
Total	58	1	4	14	3	0	0

Cross Number (2) and its Parents (& Sakha 93 X % Assiut 249): This cross revealed 48 common parental bands (Type 1), four bands of Type 3 and nine bands of Type 4, these types of markers belong to category 1 (Table 3). Number of Type 2 bands, belonging to the second category, ranged from one (OPT-08) to two for each of (OPD-13 and OPW-04) with a total number of 5 bands. Type 5 showed five bands common with its female parent, but Type 6 had four bands. Four bands related to the third category (Type 7 marker) were observed in this cross, which are useful for the identification of specific crosses (Fig. 1).

Cross Number (3) and its Parents (& Gemmiza 9x % Line 1457): When comparing cross number (3) with its parents we found that the total number of bands was 58 bands related to Type 1, one band related to Type 3 and four bands related to Type 4 (Table 4). All these

Table 5: Number of detected bands in the three categories, included the seven types of RAPD markers of cross number (4) and its parents.

	1st category			2nd category			3rd category
Primer name	Type 1	Type 3	Type 4	Type 2	Type 5	Type 6	Type 7
OPX-11	9	-	-	-	-	-	-
OPT-08	6	-	-	1	-	-	-
OPC-19	6	-	-	1	-	-	1
OPX-17	9	-	-	1	-	-	-
OPD-13	6	-	1	-	-	-	-
OPW-04	5	1	-	-	-	-	-
OPN-06	1	-	-	5	-	-	-
OPA-03	8	-	-	1	-	-	-
OPC-15	6	-	2	1	-	-	-
OPN-04	7	-	-	-	-	-	-
Total	63	1	3	10	0	0	1

Table 6: Total number of bands and their percentages in the three categories for the studied wheat crosses.

	1st category		2nd category		3rd category	
Cross number	Total # of bands	% of bands	Total # of bands	% of bands	Total # of bands	% of bands
1	76	96.2	2	2.53	1	1.27
2	61	77.22	14	17.72	4	5.06
3	63	78.75	17	21.25	0	0
4	67	85.89	10	12.82	1	1.29

types of markers are belonging to category 1, which considered as good markers to confirm that the cross is of its respective parents. In contrast, 14, 3 and 0 bands were scored in the Types 2, 5 and 6, respectively. All these three types are related to category 2. As shown in Table 4, the non-parental bands were not expressed in the cross (third category, type 7 marker) that may be useful in the identification of specific cross did not appear in this cross.

Cross Number (4) and its Parents (Assiut 230 X Line 1457): This cross revealed a total number of bands of 63, 1 and 3 bands belonging to Type 1, Type 3 and Type 4, respectively of category 1 (Table 5). While, within the second category, 10 bands were scored for Type 2, no bands were produced for the two Types 5 and 6 within the same category. Also, one band with primer OPC-19 was observed for the identification of this specific cross, which belongs to the third category (Type 7 marker).

RAPD was used to measure the relatedness between parents and offspring [24, 25]. Similarity matrix of the four wheat cross combinations showed that, the male parents of all studied crosses were more similar to their offsprings than the female parents (data not shown). Hussien *et al*. [23] reported that, four crosses were more similar to their male parents, while the other two were more similar to their female parents. These results could not be explained well and might be due to the complexity of wheat genome. From the percentage of each category, it was found that the four studied crosses revealed different percentages of markers shared with their parents (category 1) ranging from 77.22% for cross number (2) to 96.2% for cross number (1) (Table 6). Similar studies carried out in three *Chrysanthemum* crosses by Chung *et al*. [14], revealed that 34.4 to 48.9% of markers were shared with their parents. Also, [17], reported that 55.3% of markers shared with parents in an interspecific cotton crosses. Akhare *et al*. [4] reported that the percentages of markers shared with parents in four sorghum crosses ranged from 61.62 to 76.18%. In crosses obtained from Diallel crossing, the percentage of shared markers ranged from 83.75% to 92.66% as observed by Hussien *et al*. [23]. The percentages of shared markers observed in this study were comparatively higher than those reported in *Chrysanthemum*, cotton and sorghum crosses, but it was closely related to Diallel wheat crosses.

The percentage of markers not shared in the studied crosses (category 2) including in marker types 2, 5 and 6 ranged from 2.53% for cross number 1 to 21.25% for cross number 3. These percentages were lower than the percent of the same category observed in *Chrysanthemum*, which revealed 38.0 to 52.6% as reported by Chung *et al*. [14] and 39.1% for cotton as reported by Mehetre *et al*. [17]. Akhare *et al*. [4] reported that the sorghum crosses

revealed a percentage of markers not shared in crosses reached to 22.98, 24.02, 24.31 and 33.15% in the four studied crosses. Hussien *et al*. [23] reported that, the percentage of markers not shared in wheat crosses reached to 5.33 to 15%. The polymorphism of RAPD markers for these markers category were observed as different sized DNA fragments obtained from amplifications. Therefore, the differences in markers from parents to their crosses may be the results of DNA recombination, mutation or random segregation of chromosomes through meiosis process during hybridization [26, 27].

The percentage of non-parental bands (expressed only in cross) ranged from zero for cross number 3 to 5.06% for the cross number 2. These bands may be generated due to recombination or mutation through meiosis process during hybridization [26, 27], or may be created by hetero duplex formation [28]. The present observations of unique band (s) presence in wheat crosses are lower than of earlier reports on *Chrysanthemum*, which ranged from 11.6 to 13.1% [14]. It is comparable to 5.6% non-parental bands obtained in cotton [17], but is in agreement with that obtained by Chung *et al*. [4], who reported that an average of 10.53, 4.79, 5.22 and 0.83% RAPD markers of third category were detected in the four sorghum crosses. Hussien *et al*. [23] reported that the percentage of bands expressed only in crosses ranged from zero to 6.67 in wheat diallel crossing. Among used primers, it was found that, primers OPC-19 and OPX-17 produced the highest number (3) of non-parental bands (expressed in crosses only). While, the set of the primers did not distinguish the crosses from their parents by producing these specific bands. This may be due to the sequences of the primers used.

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