

Genetic Diversity Studies on Wheat Landraces in Palestine Using RAPD Markers in Comparison to Phenotypic Classification

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Abstract

Wheat is a highly economic agricultural crop world wide. Profitability of wheat can be increased through selection by farmers, which showed an increase in grain and straw production. Description of landraces is essential in order to reserve farmer's rights in their landraces which they maintained for several years. Estimates of genetic relationship are important in designing crop improvement programs. Information on genetic diversity is also valued for the management of germplasm and for evolving conservation strategies. Molecular markers are the best tools for determining genetic relations to domestic cultivars. This study was undertaken to examine the extent of genetic variation among the diverse individuals of important crops such as wheat and to evaluate RAPD as a molecular marker for genetic classification of cultivars of wheat and compare this approach with the authentic data collected from the same cultivars cultivated in Palestine. The results of this work clearly indicated the level of genetic diversity and similarities expressed in clusters of the landraces analyzed. The RAPD technique could be used effectively to demonstrate valuable results for farmers in recognition of landraces and their original sources.

Key words: Wheat, Genetic diversity, RAPD

INTRODUCTION

The conservation and sustainable use of dry land crop diversity in Palestine aims to promote conservation of agrobiodiversity using the *In-situ* strategies. On-farm is one of the strategies of *In-situ* conservation which deals in conserving landraces of crops in farmer's fields. To reach such goal, the project encouraged the informal seed sector in which farmers produce their own seeds. The project worked with farmers in two parallel paths, on-farm and on-station. To evaluate the farmer's selection, the project concentrated on planting the wheat landraces on two agricultural stations, one in north of West Bank (Bet-Qad) near town of Jenin, and the other on the south of West Bank (Al-Aroub) near city of Hebron.

Wheat (*Triticum* sp.) is considered as one and even the first domesticated crop in the world. This is because of the importance of the crop in food security at global level. Many studies conducted by scientists and field breeding employed by farmers are intensively aimed to develop more productive cultivars with desirable characters being targeted. These characters include adaptation to extreme environmental conditions, for example adaptation to dry weather, and resistance to pathogens causing heavy losses in production. Desirable characters are more dominant or present in wild relatives of cultivars, which can be detected by determining the specific genes responsible for the character. Then, genes can be isolated and manipulated to cultured cells of targeted crops by biotechnological applications [1].

Determination of genetic relatedness is important in designing crop improvement programs through genetic transformation. Information on genetic diversity is also valued for the management of germplasm and for evolving conservation

strategies. DNA markers have proven to be the best tools for exploring genetic relatedness.

Many types of marker systems have been used to study plant biodiversity. These include restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) [2][3] and random amplification of polymorphic DNA (RAPD) [4] [5].

However, most of these techniques are labor intensive, time consuming and prior information about genome is necessary before performing these techniques. On the other hand, despite the problems associated with RAPD reproducibility [6], this technique gained importance due to its simplicity, efficiency, relative ease to perform and non-requirement of sequence information [4].

In this study and for the first time in Palestine we have used DNA based technique such as RAPD to examine the relatedness of 12 cultivars of wheat collected from different locations and compared the results with phenotypic classification.

MATERIAL AND METHODS

Plant material

Plant seeds of wheat landraces were collected from Jenin and Hebron of West Bank in Palestine and planted in the two agricultural stations namely Al-Aroub and Biet Gad (Table 1 and 2). The national team of Biodiversity Project helped the farmers in recording data over the replanting of the chosen specimens. Morphological characteristics of the chosen specimen were recorded during three consecutive years. The vegetative specimens of wheat were air dried and conserved for morphological and DNA analysis.

Table 1. Evaluation of production of wheat cultivars at Al Aroub, Hebron. West Bank. Palestine

no	Variety	Treatments	Production Kg/du		% of increase in	
			grain	straw	grain	straw
1	Debiya soda	before selection	160	300	12.5	3.3
		after selection	180	310		
2	Debiya bida	before selection	240	640	8.3	4.7
		after selection	260	670		
3	Nab Al-Jamal	before selection	360	640	13.9	1.6
		after selection	410	650		
4	Anbar	before selection	440	1020	31.8	32.4
		after selection	580	1350		
5	870	before selection	200	440	10	17.0
		after selection	220	530		
6	Arael	before selection	280	500	14.3	6
		after selection	320	530		

DNA isolation

DNA was isolated from dried leaf material of wheat cultivars following the application of cetyl trimethyl ammonium bromide (CTAB) method [7]. Briefly 0.1-0.5 g of dry wheat leaves were surface sterilized with 20 % detergent (chlorox) solution and were placed in liquid nitrogen and then grinded using pestle and mortar. 1ml of extraction buffer was added to the powder, then incubated at 65 °C for 30 min, centrifuged at 1000 rpm, potassium acetate was added so that the final concentration were 1 M, incubated at 0 °C for 40 min, then centrifuged at 12000 rpm for 15 min. An equal volume of phenol/chloroform was added, mixed well by inverting slowly then centrifuged at 3000 rpm for 10 min. The supernatant was transferred to new tube and phenol/chloroform step was repeated twice. DNA sample was decanted (about 100 µl).

For DNA precipitation 1ml of isopropanol were added and incubated for 10 min at -80 °C, then centrifuged at 12000 rpm for 20 min. The pellet was re-suspended twice in 70% cold ethanol and centrifuged at 10000 rpm for washing. Finally the pellet

was dried for 30 min at room temperature then resuspended in 50 µl 10 mM Tris HCl/EDTA (TE) buffer.

Selection of the appropriate primers

Five different primers were tested on all wheat cultivars using the same PCR reaction conditions with the only change in the annealing temperature, the best primer number 4 (P4-TCAGGACGCTAC) produced good DNA banding pattern.

PCR reaction and RAPD analysis

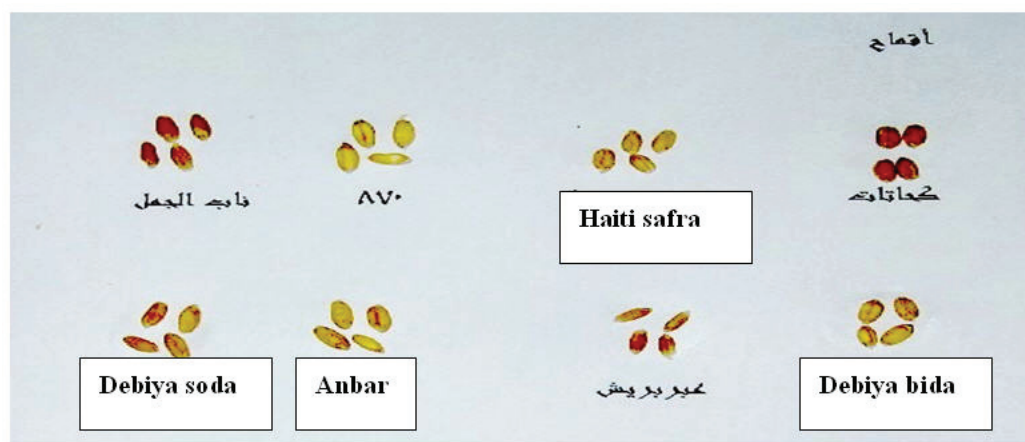
The PCR reaction was performed in a final volume of 25 ml containing 1X *Taq* polymerase buffer, 1.0 units of *Taq* polymerase (Gibco, BRL), 100 mM of each dNTPs (Promega), 50 pg of random primer (Operon technologies), 2.5 mM MgCl₂ and about 50 ng of total genomic DNA. The reaction mixture was overlaid with 25 ml of mineral oil and PCR was performed using the following cycling parameters: initial step of 2 min at 94 °C, followed by 40 cycles of 30 sec at 94 °C, 15 s at 37 °C, 1 min at 72 °C; and a final step of 72 °C for 10 min.

Table 2. Evaluation of production of wheat cultivars at Bet-Qad agricultural station, Jenin. West Bank. Palestine

no	Variety	treatment	Production Kg/du		% of increase in	
			grain	straw	grain	straw
1	Debiya soda	before selection	113	252	15	9.1
		after selection	130	275		
2	Debiya bida	before selection	170	300	24.7	20.0
		after selection	212	360		
3	Kahatat	before selection	165	245	24.2	10.2
		after selection	205	270		
4	Haiti safra	before selection	210	277	9.5	8.3
		after selection	230	300		
5	Anbar	before selection	284	365	5.6	4.1
		after selection	300	380		
6	Wheat 870	before selection	240	315	4.2	1.2
		after selection	250	320		
7	Arael	before selection	233	300	7.3	7.7
		after selection	250	323		
8	Menka	before selection	145	223	24.1	37
		after selection	180	260		
9	Nemra 8	before selection	176	275	8	12.3
		after selection	190	310		

Table 3: Phenotypic classification of wheat

Var.	Plant height cm	seed/spike	Wt.1000 seed gm	seed			awn		spike length cm	Productivity kg/du(1000m ²)	
				shape	size	color	color	length cm		Grain	straw
Kahatat	80-90	40-45	35-40	round	med	redesh	yellow	8	4	200-300	380-500
Debiya bida	80-85	35-40	38	oval	med.	brown (honey color)	yellow	10	5	180-280	380-450
Nab Al-jamal	80-85	40-45	60	elongated	med.	yellow	yellow	9	7	250-350	400-600
Debiya soda	80-90	30-40	35	oval	med.	redesh	black	10	6	150-220	300-400
Haiti safra	85-90	32-37	42	oval	small-med.	yellow	yellow	9	5	200-300	350-500
Arael	75-80	40-50	30-35	oval	small-med.	yellow	yellow	4.5	7	350-400	500-600
Nemra 8	90-100	40-45	40	oval	med.-big	brown (honey color)	yellow	11	7	230-350	470-650
Wheat 870	70-75	45-50	43	oval	med.-big	yellow	yellow	11	6	350-450	450-600
Menka	75-80	33-39	40-45	elongated	med.	redesh	black	9	4.5	200-350	400-550
Anbar	90-100	40-50	40-45	oval	med.-big	brown (honey color)	black	11	6	300-450	646
Sham 1	90	38-40	45	elongated	med.	brown (honey color)	black	12	6	350-400	500
Sham 3	80	40	33.5	elongated	med.	brown (honey color)	yellow	12	7	300-450	450-600
Sham 5	90	45	50	elongated	med.	brown (honey color)	yellow	10	5	350-400	500

**Figure 1.** Seed specimens, some of which were used in the study

Agarose gel electrophoresis

The PCR products were separated on a 2% agarose gel. The gels were stained with ethidium bromide (2 mg/liter) and visualized using the Nighthawk (pdi), dark room system incorporating UV transilluminator and CCD camera.

Data analysis

Bands were scored as 1 for their presence or 0 for their absence across the cultivars to generate a matrix. A genetic similarity (GS) was computed based on Jaccard's coefficient of similarity [8].

The data was subsequently used to construct a dendrogram using the un-weighted pair group method of arithmetical averages (UPGMA) algorithm. All the computations were carried out using the RAPDistance software; Version 1.04 for the Analysis of Patterns of RAPD Fragments.

RESULTS

Phenotypic analysis

The results of the chosen specimens of landraces production before and after selection during the seasons 2001/2002, 2002/2003 at Al-Aroub and Bet-Qad agricultural stations respectively as shown in Table 1 & 2.

Wheat Morphology: The morphological characteristics are shown in Table 3, Fig 1, 2A, 2B & 2C).

Genotypic analysis

RAPD detected polymorphism, which was correlated well with the cultivars data. Nearly 65% were polymorphic for wheat as shown in the dendrogram below.

An average of 6 bands were generated and a double band was prominent in most of the patterns approximately at the range of 600 bp.

Two clusters of two landraces each (4, 13) and (6, 9) had 100 % similarity (Fig 3 & 4). Specimens 2 and 7, which were bread wheat and wild wheat cultivars respectively, showed great



Figure 2A. Spikes specimens of landraces, some of which were used in the study

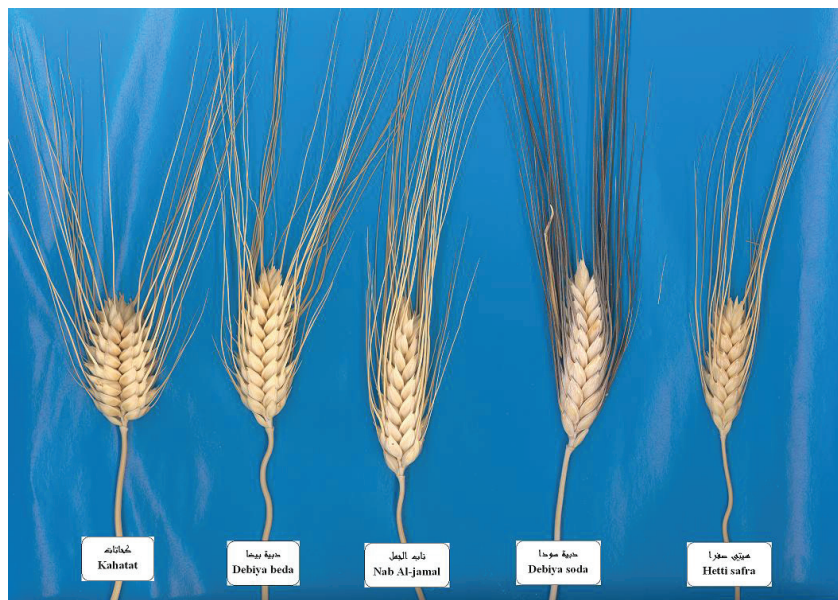


Figure 2B. Spikes specimens of landraces, some of which were used in the study



Figure 2C. Spikes specimens of landraces, some of which were used in the study

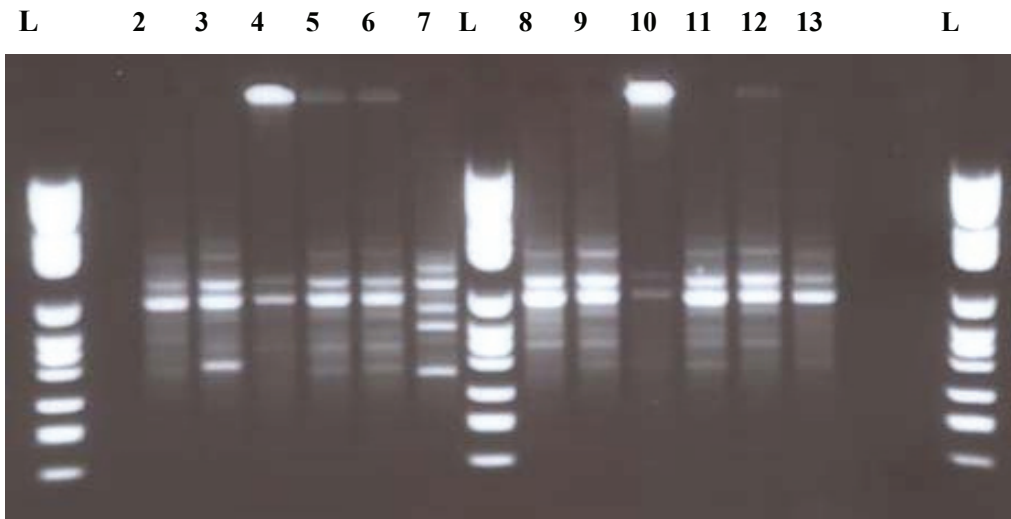


Figure 3. RAPD banding patterns (profiles) of wheat crops using P4 primer. Lanes L, shows the molecular weight marker. Lanes 2 - 13, shows different samples of wheat.

variation from rest of the cultivars (Fig. 4). Specimens 5 and 11 landraces which were obtained from ICARDA, Syria were grouped together with about 97% similarity and were different from others. Rest of the local cultivars were grouped separately as individual types (Fig. 4).

DISCUSSION

Wheat is an important crop which is commonly grown in the household farms throughout Palestine. Wheat is essential crop and important element in agro biodiversity and food security. The phenotypic classification which local farmers usually depend on to differentiate between wheat landraces is not always correct due to minute phenotypic variations. For accurate characterization of wheat landraces there has to be an accurate and more reliable methods, such as genetic markers in comparison to phenotypic characteristics.

This study is considered as major development in scientific research in Palestine since it is on the road to contribute scientific information on genetic origin and distance between wheat landraces and its wild relatives. The overall results of this study indicated that there was good correlation between the phenotypic and genotypic information. The phenotypic classification revealed that Debya soda (**no. 9**) and Anbar (**no. 6**) were very similar; this was confirmed by RAPD analysis (fig. 4 & table 3). The wild type wheat (**no. 7**) is proven by RAPD to have far genetic differences compared to the two major clusters of wheat landraces (fig. 4). This could be referred to ecological and genetic isolation.

Regarding the relation between Debya beda (**no. 8**) and Arael (**no. 2**) the phenotypic classification showed great similarity, this also was proved by genetic analysis which placed both in the same cluster (fig. 4), although they were collected from two different regions.

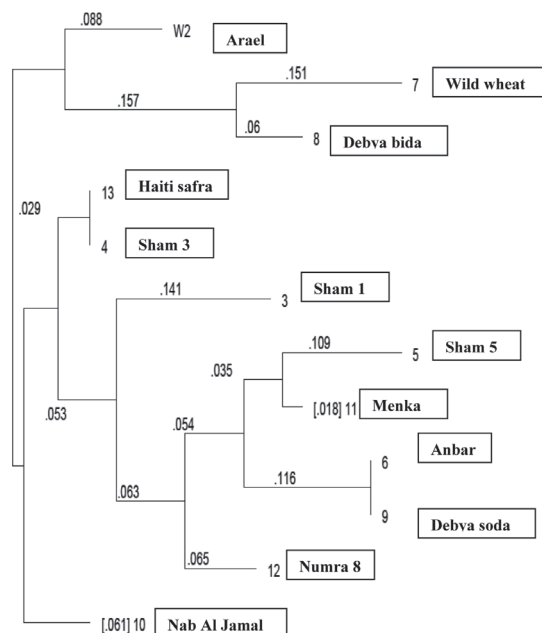


Figure 4. Clustering phylogenetic tree indicating percentage of differences between RAPD types of Wheat samples using P4 primer.

Genetic analysis of Haiti safra (no. 13) and Sham 3 (no. 4) showed 100% similarity but the phenotypic classifications were less correlated (fig. 4 & table 3). The less correlation in these results may be due to lack of information in phenotype analysis such as leaf morphology and length and width of seeds. The ICARDA, Syria landraces (5 & 11) were grouped together and were different from the Palestinian landraces showing the efficiency of RAPD. Nab el Jamal (no 10) was placed based on RAPD in separate cluster although it has close phenotypic characteristics with other wheat landraces except for its weight (table 3, fig.4). The grain weight is important character since it reflects the nutrient content; this desired character can be genetically traced among wheat land races for genetic modification. However, more credible genetic analysis has to be carried out such as AFLP or microsatellite techniques [9] on Palestinian wheat landraces.

It can be concluded that, this study could be considered as base and step toward further research in developing productive wheat landraces and conservation through introduction of plant material to seed and gene banks in Palestine.

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