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Assessment of Genetic Diversity Among Wheat Varieties in Sulaimanyah using Random Amplified Polymorphic DNA (RAPD) Analysis

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Abstract

Genetic diversity among 11 durum and bread wheat genotypes was studied using random amplified polymorphic DNA (RAPD) analysis. A total of 70-75 DNA fragments were amplified with 10 random decamer primers 40% (bread , and 35.7% (durum wheat) of which were polymorphic. Genetic similarity matrix based on Dice index detected coefficients ranging from 0.5 to 0.952 (bread wheat) and 0.102 to 0.917 (durum wheat). These coefficients were used to construct a dendrogram using unweighted pair group of arithmetic means (UPGMA). The bread wheat genotypes were clustered into two major groups; first group includes Cham 4 and Cham 6; and the second includes Tammuz, Aras, and Rabia. The durum wheat genotypes were classified into two groups: group 1 presents the varieties Acsad 65 and Cemmitto, and group 2 contains Creso Kurde, Creso Italy, Ovanto, and Bakrajo 1. The highest similarity among the wheat varieties was observed between Cham 4 and Cham 6 for bread wheat and Bakrajo 1 and Ovanto for the durum wheat. The most distant genotype in the dendrogram was Acsad 65 and Aras. It has been clearly shown that most of the varieties possessed narrow genetic background. The information would be helpful for future genome mapping programs as well as for the application of intellectual breeder rights. The study will also work as indicator for wheat breeders to evolve varieties with diverse genetic background to achieve sustainability in wheat production in the country

الملخص

تمت دراست الاختلاف الوراثى بين اصناف من الحنطة الناعمة والخشنة باستخدام التضخيم العشوائي للتعدد الأشكال الحمض النووي الريبي منقوص الأكسجين. ووجدت 70-75 قطعة من الحمض النووي الريبي منقوص الأكسجين،كما وجدت الاختلاف الوراثى بنسبة 40% فى الحنطة الناعمة و 37.5% فى حنطة الخشنة. وان التشابه الوراثى تتراوح بين 0.5-0.952 فى الحنطة الناعمة و 0.102-0.917 فى الحنطة الخشنة. وتم استخدام التشابة الوراثى لتكوين شجرة التنوع الوراثى. وباسناد على نتائج الشجرة التنوع الوراثى، قسمت الحنطة الناعمة الى مجموعتين رئيسيتين : المجموعة الاولى يشمل شام 4 وشام 6، والثانية تشمل تموز،أراس وربيعة. و صنفت الحنطة الخشنة الى مجموعتين: المجموعة الاولى يشمل اكساد 65 و سيميتو والثانية تشمل كريزو كردى، كريزو ايطالى، اوفانتو وبكرجو. كما لوحظت اعلى نسبة التشابة بين الصنفين شام 4 وشام 6 فى الحنطة الناعمة والصنفين اوفانتو و بكرجو فى الحنطة الخشنة. واظهرت اعلى نسبة من الاختلاف الوراثى فى الاصناف أراس و اكساد 65مقارنة بالاصناف الاخرى. ولقد ثبت بوضوح أن معظم الاصناف يمتلك الخلفية الضيقة. وان نتائج هذة الدراسة سوف تكون مفيدة فى المجال تكوين الخرائط الكروموسومية والبرامج التربية و التحسين فى العراق.

Key words: Wheat; DNA; RAPD; Genetic Diversity

1. Introduction

Wheat is a staple food; and is one of the important agricultural crops, which is a basis for human nutrition; and is of enormous economic importance to both Iraq and worldwide. Historically, many genetic markers such as morphological markers (Porter and Smith, 1982) and bio-chemical markers such as isozymes and seed storage proteins (Miller et al., 1989) have been used to monitor and maintain germplasm biodiversity. These markers were more prone to environmental effect and limited by small number of loci (Tanksley, 1983; Tanksley et al., 1989). DNA markers have facilitated genetic studies in plant, animal, and prokaryotic genomes (Mullis, 1990; Erlich et al., 1991). Among the several DNA based techniques, random amplified polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams et al., 1990) gained importance due to its simplicity, efficiency, and non

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requirement of sequence information (Gepts, 1993; Karp et al., 1997).

RAPD provides virtually limitless set of descriptors to compare individual plants and the population. With this innovative tool, genetic diversity can be estimated (Demeke et al., 1996), and equally it is possible to carry out large scale screening of genetic resources held in gene banks, natural populations, ecosystems, and natural reserves with this quick and rapid technique. Analysis of RAPD is based on the amplification of DNA fragments with the polymerase chain reaction (PCR) starting from primers with arbitrary sequences (Williams et al., 1990). This technique is considerably faster and simpler than some other molecular techniques. RAPD markers have been used to examine both interspecific and intraspecific variations in a number of plant species (Kazan et al., 1993; Bai et al., 1998). The analysis of SSR, based on the PCR, is also easier to perform than other molecular analysis, and is highly amenable to automation. RAPD analysis has been extensively used to document genetic variation in *Triticum* (Cao et al., 1998; Sun et al., 1998; Bedo et al., 2000; Czaplicki et al., 2000; Gerashchenkov et al., 2000; Gupta et al., 2000; Yuejin and Lin, 2000), suggesting a narrow genetic base. RAPD markers have also been used for cultivar identification (Hu and Quirose, 1991; Malik et al., 1996), fingerprinting of genomes (Nybom et al., 1989, Welsh and McClelland, 1990), and for tagging of genes (Klein-Lankhorst et al., 1991; Martin et al., 1991; Rafalski et al., 1991; Kelly et al., 1993). The objective of this study was to analyze RAPD-based genetic variance among 11 varieties of wheat.

Table 1. Nucleotide sequences of the 10 primers used in this study (Naghavi, 2004).

Primer name	Sequence 5 ⟶ 3
UBC1	CCTGGGCTTC
UBC3	CCTGGGCTTA
UBC9	CCTGCGCTTA
UBC13	CCTGGGTGGA
UBC104	GGGCAATGAT
UBC105	CTCGGGTGGG
UBC106	CGTCTGCCCG
UBC108	GTATTGCCCT
UBC109	TGTACGTGAC
UBC110	TAGCCCGCTT

2. Materials and Methods

2.1. Plant Materiel and DNA Extraction

Seeds of 5 bread wheat varieties and 6 durum wheat varieties were obtained from the Department of Agriculture in Sulaimanyah. DNA was isolated from bulks containing equal quantities of leaf tissue from 10 plants. Leaves were collected and frozen in liquid nitrogen, and then crushed to make a fine powder. One hundred milligram of fine powder was used for DNA extraction. Five hundred micro-liter of CTAB buffer (1.4 M NaCl,

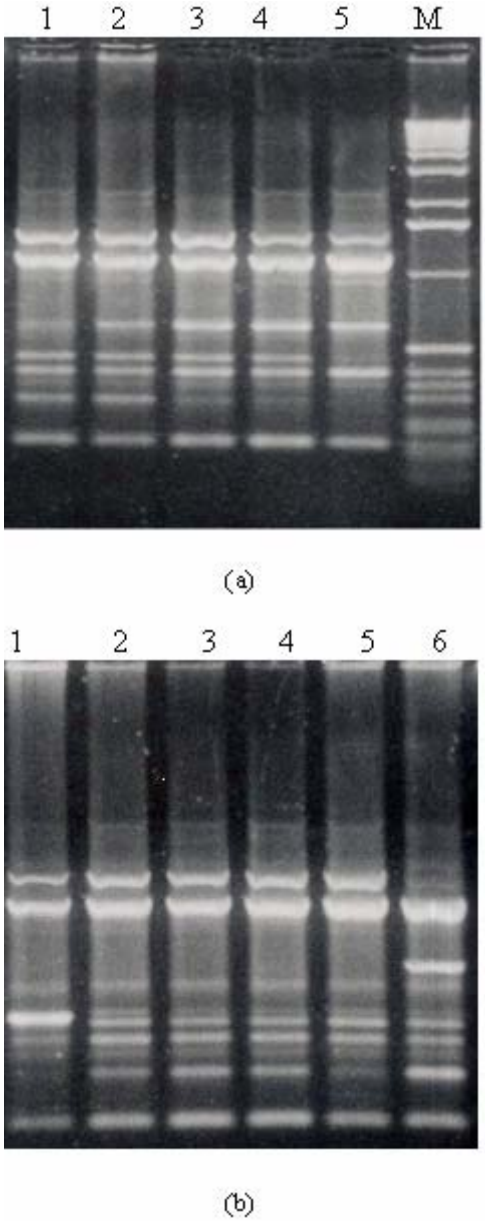


Figure 1: An example of RAPD banding pattern obtained from primer UBC3 on 11 genotypes of wheat; **a.** Bread wheat : 1= Tammuz, 2= Rabia, 3=Aras, 4=Cham 6, 5=Cham 4, M: marker size; **b.** Durum wheat: 1=Acsad 65, 2=Bakrajo 1, 3=Cemmitto, 4=Creso Kurde, 5= Ovanto, 6= Creso Italy.

100 mM Tris, 20 mM EDTA, pH 8, 2% CTAB) was added to each eppendorf tube containing the crushed leaf material; and was thoroughly mixed by pipetting. The mix was incubated for 60 min at 60°C. Equal volume (500 µl) of chloroform: isoamyl alcohol (24:1) was added, and tubes were then shaken until a homogenous mixture was obtained. Samples were then centrifuged at 10000 rpm for 7 minutes in a bench centrifuge. The aqueous phase was transferred to a fresh tube. Ammonium acetate [0.08 volume of cold 7.5 M (32 µl)] and cold isopropanol [0.54 volumes of (233 µl)] were added in the tube and were mixed gently to precipitate the DNA at -80 °C for one hour. Samples were centrifuged at 10000 rpm for 7 minutes to pellet the DNA. After discarding the supernatant, the pellet was washed three times with 70%

ethanol. Pellet was dried at room temperature for one hour and re-suspended in 40 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). To remove RNA, DNA was treated with 40 µg RNAse-A at 37°C for one hour and was stored at 4°C until used. To use in PCR, 1:5 dilution of DNA was made in double distilled deionized and autoclaved water.

Table 2. Effectiveness of RAPD marker in detecting polymorphism of wheat varieties.

	Bread wheat	Durum wheat
Number of assay units (primer)	10	10
Total bands scored	75	70
Polymorphic fragment scored	30	25
Percentage of polymorphism	40	35.71
Minimum polymorphism scored per pair primers	1	1
Maximum polymorphism scored per pair primers	4	5
Average polymorphism scored per pair primer	3	2.5

2.2. PCR (Polymerase Chain Reaction) Amplification

The amplification reaction contained 1XPCR mix, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 50 ng of each primer, 1 U Taq polymerase and 20 ng template DNA. Amplifications were performed in a Biometra gradient thermocycler with the following cycling profile: an initial denaturation at 92°C for 5 min, followed by 40 cycles of 1 min at 92°C, 1 min at 37°C and 2 min at 72°C with a final extension for 6 min at 72°C. PCR products were mixed with 1/5 vol of loading buffer and separated on a 2% (w/v) agarose gel in 1X TBE at 70 V for 3 h. The gels were then stained in a 0.4 μg/mL ethidiumbromide bath and the DNA fragments visualized under UV light.

2.3. DATA Analysis

The data obtained with the technique RAPD w scored in a binary form as the presence or absence (1/0) of bands for each sample. SPSS was used to calculate DICE similarity coefficient. The similarity matrices were converted into distances matrices; and used to generate dendrograms by UPGMA.

3. Results and Discussion

In order to increase the confidence level of the fragments included in the matrices (for RAPD), Using this approach, it is possible to lose more than one useful information, but the aim was to obtain reproducible and clear data.

Electrophoresis of PCR products on 2% agarose gels containing ethidium bromide (Figure 1A and B) revealed different degrees of polymorphism for different primers (Table 3). In the RAPD analysis, ten-mer primers were used to amplify all of the genotypes, ll primers showed reproducible and well-resolved bands. These primers produced fragments ranging from about 400 bp to 3,500 bp in size. A total of 70-75 fragments were observed from these primers. In total, 30 polymorphic bands for bread wheat and 25 polymorphic bands for durum wheat were detected. The highest number of polymorphic and scorable bands was obtained by primer UBC13, the lowest by primers UBC105, and UBC106 (Table 2).

The RAPDs generated were used to determine the genetic distances between the wheat varieties. The relationship of these varieties, as identified by the classification, has been represented as a dendrogram (Figures 2 and 3).

The genetic similarity for pairs of species was calculated using Dice coefficients (Tables 3 and 4). The similarity matrix based on all possible pairs of varieites ranged from 0.105 to 0.917 for bread wheat and 0.5 to 0.952 for durum wheat (Tables 3 and 4). The lowest pair-wise similarity matrix value was between Tammuz and Cham 4 (0.105) for bread wheat and between Acsad 65 with Italy and Cemmitto (0.5). This reveals a relatively high degree of genetic variability within the species. The highest pair-wise similarity was between Cham 6 and Cham 4 (0.917) for bread wheat and between Bakrajo 1 and Ovanto (0.952) for durum wheat. The reason for this higher similarity was that two varieties have the same parents.

The dendrograms were constructed to express the similarity among the varieties based on the RAPD (Figure 2). Cluster analysis was carried out by the UPGMA method on the Dice similarity coefficients. The position of the genotypes in different clusters is presented in Figure 2. The dendrogram constructed with UPGMA revealed that 11 genotypes fell into different distinct groups. The dendrogram divided the bread wheat varieties into two groups: group 1 includes Tammuz, Aras, and Rabia while group 2 includes Cham 4 and Cham 6. The varieties Cham 4 and Cham 6 showed high similarity (Figure 2). The most distant genotype in the dendrogram was Aras. On the other hand, the dendrogram revealed two groups for durum wheat. group 1 contains the Creso Kurde, Creso Italy, Ovanto and Bakrajo 1. The second group includes Acsad 65 and Cemmitto (Figure 3). The varities Ovanto and Bakrajo 1 showed high similarity, and the variety Acsad 65 revealed the highest distance.

Table 3. Similarity matrix showing the relationship among the bread wheat genotypes based on RAPD data.

Varieties	Tammuz	Rabia	Aras	Cham 6	Cham 4
Tammuz	1,000				
Rabia	0,625	1,000			
Aras	0,421	0,667	1,000		
Cham 6	0,211	0,476	0,500	1,000	
Cham 4	0,105	0,381	0,417	0,917	1,000

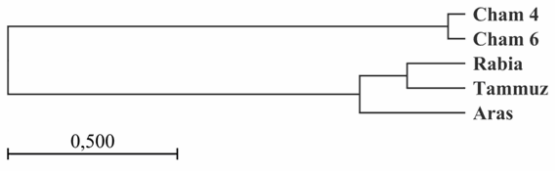


Figure 2. Dendrogram of bread wheat varieties showing genetic similarity based on RAPD data by using UPGMA cluster analysis.

In this study, the polymerase chain reaction (PCR)-based systems (RAPD) have been used and compared for studding the genetic diversity between 11 cultivars of

wheat., RAPD analysis was found to be a valuable diagnostic DNA marker system for evaluating genetic diversity. The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains (Messmer et al., 1992). The number of primers used in the RAPD method should be neither too small, because this could lead to a noninformative or biased analysis, nor too high, which could result in increased cost.

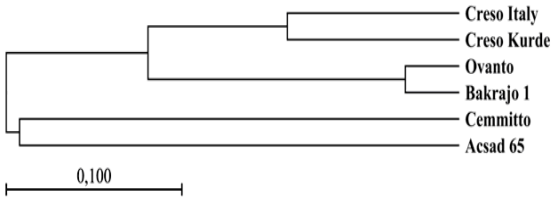
The level of polymorphism for bread wheat (40%) was superior to the level of polymorphism for durum wheat (35%). In this study RAPD markers were able to discriminate Cham 4 and Cham 6, and also this technique able to find some polymorphisms between the Creso Kurde and Creso Italy. This technique showed highest variability between the varieties of bread wheat with comparing with the varieties of durum wheat. This less similarity among the varieties may be attributable to the difference of center, which developed these varieties or a high degree of genetic differences may be the usage of different parents for constructing the varieties.

Table 4. Similarity matrix showing the relationship among durum wheat genotypes based on RAPD data.

Varieties	Acsad 65	Bakrajo 1	Cemmitto	Creso Kurde	Ovanto	Creso Italy
Acsad 65	1,000					
Bakrajo 1	0,667	1,000				
Cemmitto	0,500	0,600	1,000			
Creso Kurde	0,632	0,783	0,571	1,000		
Ovanto	0,706	0,952	0,632	0,818	1,000	
Creso Italy	0,500	0,750	0,545	0,880	0,783	1,000

The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials. Among them, the RAPD markers have been successfully used in wheat germplasm evaluation because of their many advantages. The suitability of the RAPD technique for genetic diversity studies and germplasm evaluations has been shown in many studies. The RAPD technique is quick (Colombo et al., 1998), cost effective (Fugang et al., 2003), and ab to perform analysis without need for prior sequencing of the genome (Huff *et* al., 1993). Although major bands from RAPD reactions are highly reproducible, minor bands can difficult to repeat due to random priming nature of this PCR reaction and potential confounding effects associated with co-migration with other markers (Tessier et al., 1999). Replication slippage is thought to occur more frequently than single nucleotide mutations and insertion\deletion events, which generated the polymorphisms detected by RAPD analysis (Powell et al., 1996). Various numbers of primers have been used in the study of different species of the genus *Triticum* that revealed various degrees of polymorphism. Joshi and Nguyen (1993) used 40 primers in studying wild, and cultivated wheat and revealed 88% polymorphism among all accessions. With 26 UBC primers, Sun et al. (Sun et al., 1998) detected 62.5% polymorphism among 46 genotypes of *T. aestivum* and *T. spelta*. Pujar et al. (1999) tested 81 Operon primers (kit A, F, J, V) and selected 21 primers that produced 3 to 13 polymorphic bands. A 78.2% polymorphism was detected among 64 genotypes of the species *Triticum.*

Figure 3. Dendrogram of durum wheat varieties showing genetic similarity, based on RAPD data by using UPGMA cluster analysis



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