STUDY OF GENETIC DIVERSITY AMONG SIX IRAQI WHEAT GENOTYPES USING RANDOM AMPLIFIED POLYMORPHIC DNA

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Abstract

The degree of genetic divergence was estimated in six Iraqi soft wheat genotypes, four exotic genotypes, one produced by hybridization and one produced by gamma irradiation, through random amplified polymorphic DNA methodology. A total of 42 DNA fragments were generated by the 3 random primers, with an average of about 7.4 bands per primer. The 42 fragments showed polymorphism among the six wheat genotypes. Jaccard similarity matrix ranged from 25 to 66.7%, which indicated a high genetic diversity among the genotypes. We conclude that random amplified polymorphic DNA analysis can be used for the characterization and grouping of wheat genotypes; these results will be helpful in our wheat breeding program.

دراسة التغاير الوراثي بين ستة اصناف من الحنطة العراقية بأستعمال تقنية التضخيم العشوائي لتعدد اشكال الحامض النووي الرايبي المنقوص الاوكسجين

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الخلاصة

تمت دراسة الاختلاف الوراثي بين ستة أصناف من الحنطة العراقية الناعمة ، اربعة منهـا مـستوردة وواحدة منتجة ببرامج التهجين والاخر منتج بالتشعيع، بأستعمال تقنية التضخيم العـشوائي لتعـدد اشـكال الحامض النووي الرايبي المنقوص الاوكسجين. وتم الحصول على ٤٢ حزمة DNA بأستعمال ٣ بادئـات وبمعدل ٧,٤ حزمة لكل بادئ اظهرت الحزم تغايرا بين الاصناف الـستة اذ اظهـرت مـصفوفة التـشابه (Jaccard) نسب تشابه تراوح بين ٢٥–٦٦,٧ % مما يؤكد وجود تغاير كبير بين الاصناف. بـذلك ثبـت بوضوح كفاءة تقنية التضخيم العشوائي لتعدد اشكال الحامض النووي الرايبي المنقـوص الاوكـسجين فـي تشخيص وتصنيف الطرز الوراثية للحنطة مما يعزز برامج التهجين.

Introduction

Being a staple food, wheat occupies an important place in the crop husbandry of Iraq. But wheat production in Iraq has been decrease during recent years than it was previously, continued disimprovement in productivity conceder a highly risk because of increasing demand by the still-growing human population. However, during the last few years, yield improvement in wheat varieties has not been substantial; the narrow genetic base of the germplasm in use has been

considered the main reason. Knowledge of diversity patterns allows plant breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion, and to develop strategies to incorporate useful diversity in their breeding programs(1). 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 our breeding program depends upon the availability of genetic variability to increase productivity. Traditionally, assessment of genetic diversity has been based on differences in morphological and agronomic traits or on pedigree information for the different crops (2; 3). Recently, restriction fragment length polymorphisms and isozyme markers have been used for diversity studies and for genetic mapping of these crop species (4; 5). But their use has remained limited, as they revealed low levels of polymorphism and isozyme expression was found to be highly influenced by environmental conditions(6). However, PCR-based DNA marker techniques seem to provide the means for generating useful information on polymorphism, genetic relatedness and diversity. The PCR-based random amplified polymorphic DNA(RAPD) markers are dominant markers and are extensively used in genetic mapping(7) and for the identification of markers linked with useful traits(8). Due to its technical simplicity and speed, RAPD methodology has been used for diversity analyses in several crops (9). Wheat is characterized by a large genome size (approximately 17,000 Mb) and little or no sequence information is available for the wheat genome. We made an RAPD analysis of six genotypes of wheat to estimate their genetic diversity and relatedness and to compare the different source genotypes. The information gathered will be helpful for our breeding programs.

Material and Methods

The plant material: The plant material used in the study consisted of six genotypes of wheat:

Table 1: The wheat varieties that used in study

Variety	Method of production	Origin
Al-nedaa	γ-rad 15Kr	Saber beak X maxibac
Al- tahady	Hybridization	Saber beak X maxibac
Al-noor	Exotic	Ecarda
Al-hashimia	Exotic	Ecarda
Um rabee'a	Exotic	Jori69 X Haw
Wahat al-Iraq	Exotic	Pic "S"Roffs X Rhexta

All genotypes were planted in pots in a growth chamber.

DNA extraction:

The wheat genotypes were grown in plastic containers (250 mL), 0.2-0.3 g leaf tissues were obtained from the 6-day-old seedlings. 100mg of newly grown leaf used to extract DNA by CTAB lyses as listed in (10).

RAPD analyses:

DNA concentrations in the working solution of approximately 15ng/µL in d.H2O were confirmed by spectrophotometer. For RAPD analysis (11), 1X PCR buffer, 200 µM of each dNTPs, 10 pMol random primers and 1.25 U of *Taq* DNA polymerase, concentration of genomic DNA and $MgCl_2$ were optimized. The 10-base oligonucleotide primers obtained from alfa DNA Canada as listed in table 2. DNA amplification reactions were performed in a thermal cycler (GeneAmp 9700, ABI). The PCR profile was: one cycle of 94°C for 5 min, 40 cycles of 94°C for 1 min, 38°C for 1 min, and 72°C for 1 min, and a final extension for 10 min at 72°C.

Table 2: The primers that used in study

OPA 05	5'-AGGGGTCTTG-3'
OPB-05	5'-CCTTCACGCA-3'
OPC-05	5'-GATGACCGCC-3'

Analyses of RAPD data:

The RAPD fragments were analyzed by electrophoresis on 1.5% agarose gels with ethidium bromide (10 ng/100 mL of agarose solution in Tris borate EDTA buffer). The bands were counted by starting from the top of the lanes to the bottom. All visible and unambiguously scorable fragments amplified by the primers were scored under the heading of

total scorable fragments. Amplification profiles of the six 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 (Table1) on the basis of number of shared amplification products (12). 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 (Figure 1) constructed using unweighted pair group of arithmetic means UPGMA (13).

Results and Discussion

DNA of six varieties of wheat was amplified with 3 different random primers, as listed in table 2, fragments were generated by the 3 primers, with an average of about 7.4 bands per primer (range = 3-11). The number and size of the DNA fragments were strictly dependent upon the sequence of the primer. Reactions were repeated from two to three times to check the consistency of the amplified products; only easily resolved and bright DNA bands were counted. All the genotypes differed, based on their amplification profile with these 42 DNA bands. 32 fragments were polymorphic (76%) in these six wheat varieties. Ten individual plants of each genotype were tested separately; all showed similar banding patterns, indicating that the genotypes were highly homozygous. All the six wheat varieties could be identified with a single primer. These results suggest that RAPD markers provided substantial information for the identification of wheat genotypes. Among the six wheat genotypes, Al-tahady produced the largest number of DNA-amplified fragments (12 bands), while the smallest number (4 bands) was produced by Al-noor.

The reproducibility of the RAPD technique can be influenced by variable factors, such as primer sequence, template quality and quantity, the type of thermocycler, and polymerase concentration (7). However, the use of a standardized RAPD protocol can ensure a reproducible RAPD pattern.

The concentrations of $MgCl_2$, *Taq* DNA polymerase and concentrations of template DNA were optimized for PCR conditions. DNA concentrations of 5, 10, 15, 20, and 25 ng/25 µL in each reaction were assayed. The concentration of 10 ng/25 µL was found to produce the most consistent and reproducible banding patterns. Tahir(14) used RAPD technique to evaluate Iraqi wheat mutants and found that 3mM $MgCl_2$ was the optimum concentration for amplification. same results have been obtained. Higher than 3mM $MgCl_2$ produced nonspecific amplification. Similarly, one unit concentration of *Taq* DNA polymerase was found optimum for amplification of genomic DNA. Other reaction conditions were also kept constant, and the results were found to be consistent and reproducible. All of the amplified bands were identical in each repetition.

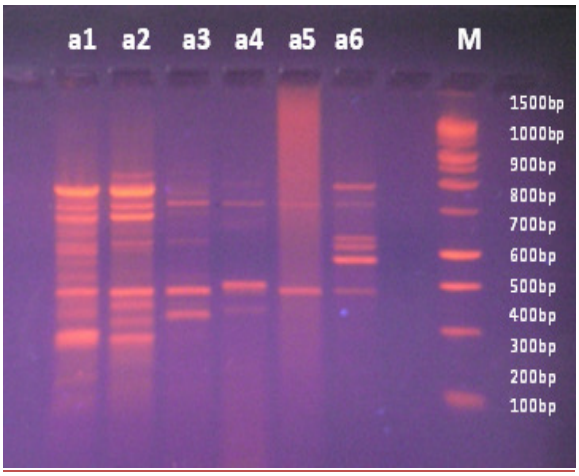

Figure 1: An example of RAPD banding pattern obtained from primer OPB-05 on 15% agarose gel, 5V/cm at 1hr. for 6 genotypes of wheat, a1: Al-nedaa, a2: Al-tahady, a3: Al-noor, a4: Al-hashimia, a5: Um rabee'a, a6: Wahat al-Iraq and lane M represented the molecular marker (100bp DNA Ladder Promega).

Table 3: Similarity Matrices computed with Jaccard coefficient for 6 wheat varieties* obtained from RAPD-PCR showing the relationship between verities.

Similarity coefficient	Matrix	computed	with	Jaccard	
	a1	a2	a3	a4	a5	a6
a1	1	0.643	0.167	0.250	0.364	0.364
a2		1	0.214	0.286	0.286	0.286
a3			1	0.500	0.286	0.500
a4				1	0.667	0.250
a5					1	0.250
a6						1

***a1: Al-nedaa, a2: Al- tahady, a3: Al-noor, a4: Al-hashimia, a5: Um rabee'a, a6: Wahat al-Iraq**

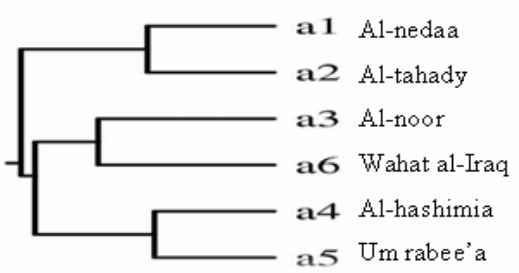

Figure 2: Dendrogram of six wheat varieties showing genetic similarity based on RAPD data by using UPGMA cluster analysis, showing the relationship between verities.

The line Al-Nedaa was found to be 64.3% similar to Al- Tahady. Both these genotypes will be has the same breeding program, the difference between them is due to the gamma
irradiation which may cause changes in there genomics. Similarly, Al-Noor was 50% similar to line Wahat al-Iraq, as it is seen in table (1) both lines is exotic and may share the same origin. Al-Hashimia and Um rabee'a found to hase 28.6% similarity. The last four genotypes clustered in second group. Al-Nedaa and Al- Tahady are placed in first group. This clustering of the genotypes might be due to the selection from a single population; the same was observed with other wheat cultivars (15). The low similarity of Al-Nedaa and Al- Tahady with the rest of the genotypes seems to be due to the fact that they are only local varietis. Bibi *et al*. (16) compared hemp varieties using RAPDs. They found a mean of 97.1% polymorphism over all varieties and loci. Mukhtar *et al*. (15) observed 445 DNA fragments amplified with 50 random primers in 20 varieties; they found 64.38% polymorphism. In our study, 42 DNA fragments were amplified with 3 random primers, with an average of 7.4 bands /primer. There was 45% polymorphism in the six wheat varieties. Overall, a high genetic base was found, with 16.7 to 64.3% similarity among the six genotypes. 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 Iraq. These findings would also contribute to choose parents for our breeding program.

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