# Genotypic diversity assessment of some durum wheat (*Triticum durum*) genotypes using RAPD analysis

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**Abstract.** *Bousba R, Gueraiche S, Kanouni MR, Bounar R, Djekoune A, Khammar H, Nadia Y. 2020. Genotypic diversity assessment of some durum wheat (Triticum durum) genotypes using RAPD analysis. Biodiversitas 21: 2696-2701.* Knowledge of genetic variability in durum wheat (*Triticum durum* Desf.) is of major importance in the development of breeding programs and the preservation of local landrace resources. The objective of this study is to highlight the molecular polymorphism among six durum wheat genotypes from different origins and characterized by different sensitivity to drought tolerance (tolerant, semi-tolerant, and non-tolerant). 15 Random Amplified Polymorphic DNA (RAPD) markers were used to assess the molecular diversity of these genotypes. Our results show a total of one hundred and sixty-nine amplicons, where one hundred and twenty-four are polymorphic bands. The number of bands per primer ranged from two (OPJ-06) to fifteen bands (primers OPE-13 and OPB-01). The values of the Allelic frequency varied from 1 (OPJ-06) to 0.20 (OPA-17) and 0.19 (OPE-13, OPO-06, B-19 and OPA-20). Also, the values of the coefficient of genetic similarity range from 0.69 to 0.80, these results indicate a large variation between tested genotypes. According to the dendrogram generated by the RAPD approach, we obtained four distinct groups: the first one (G1) contains GTADUR and KORIFFLA x SHAM, the second group (G2) contains the local genotype BIDI-17. However, the genotype WAHA was in the third group (G3), the fourth and fifth groups contained the genotypes CIRTA and TELL, respectively. A complementary analysis was done to estimate genetic differentiation, using CPA Analysis that indicated four groups among the six genotypes, where, the local genotypes BIDI-17 and CIRTA were classified together. For allele's richness, the local genotypes show in this investigation, the highest values in comparison to the introduced genotypes, which suggested the performance of the RAPD markers (high polymorphism and fast genetic analysis). The molecular markers RAPD-PCR type, despite their non-specific characteristics, has shown a strong aptitude for genetic characterization in durum wheat and a high level of polymorphism, which makes it possible in a preliminary study to make exploitable discrimination. These results may be helpful in the improvement and varietal selection, and useful in accelerating breeding programs of durum wheat.

**Keywords**: Abiotic stress, adaptation, genetic diversity, RAPD marker, *Triticum durum*

## INTRODUCTION

Plant genetic resources from the biological basis for global food security, is the raw material used by plant breeders and biotechnology specialists to produce new adapted varieties to different environments and growing conditions (Khan et al. 2015). The various local landraces and the genetic diversity they contain play a key role in economic, social, and cultural development. The intensification agriculture which based on the use of a limited number of improved genotypes affects several local landraces that have been marginalized and are currently very rare to find or even if it's lost. One of the major bases of genetic erosion, according to the FAO report (2011), is the substitute for local genotypes with modern ones.

Thereby, the exploitation of genetic variability has long been based on the use of unique morphological characters and/or agronomic interest (Huang and Han 2014). These were the only tools available to trace the history of populations. However, the consistency of these markers has proven to be insufficient over time. Certainly, it often happens that phenotypic variations are not only linked to the evolutionary history of populations but are also determined by environmental factors.

Access to other levels of study of genetic diversity such as those of DNA dates back to the last three decades. Molecular markers have many advantages compared to morphological and biochemical markers, in particular, their independence from the organ analyzed and the stage of development of the plant; also they are not influenced by environmental fluctuations. Molecular markers can be extremely varied for plants with the same morphology and physiology. They allow identifying the polymorphism between families, genera, species, varieties, populations and even between individuals (Elangovan 2016), which makes them very effective indicators of genetic variability in molecular phylogeny, in studies of genetic diversity and genetic identification: material selection and varieties (Bousba et al. 2013; Anamika et al. 2018).

Among the molecular markers, the RAPD technique is one of the most useful methods for identifying genetic polymorphism in plants (Hartl 1988; Bhanupriya et al. 2014). Williams et al. (1990) described a genetic marker inspired by PCR allowing random amplification of DNA segments using a single small primer (generally 10 nucleotides), they called this technique Random Amplified Polymorphic DNA (RAPD) or Arbitrary Primer PCR (AP-PCR). The RAPD technique is a simple method which consists of an enzymatic amplification of DNA using small primers chosen at random, it requires little technical investment and makes it possible to quickly obtain various fragments of genomic DNA providing a lot of polymorphism (Aydogan and Yagdi 2012; Dilipan et al. 2017). RAPD technique is one of the most useful methods for identifying genetic polymorphism in plants (Przyborowski and Sulima 2010; Santos et al. 2015; Wang et al. 2016).

RAPD analysis has been extensively used to document genetic variation in wheat (*Triticum durum* Desf) (Barcaccia et al. 2002; Aliyev et al. 2007; Najaphy et al. 2012; Pandey et al. 2012;). RAPD markers have also been used for cultivar identification (Aydogan and Yagdi 2012; Kanupria et al. 2012), fingerprinting of genomes and for tagging of genes (Dong et al. 2010; Khanna et al. 2014).

The present work aims to characterize some durum wheat (*Triticum durum* Desf.) genotypes by analyzing the existence of genetic diversity; using as molecular polymers analysis tools the RAPD molecular markers type (random amplified polymorphic DNA) which is a variant of the PCR (polymerase chain reaction) technique. The advantage of this technique compared to others is that it only uses a few nanograms of DNA and does not use radioactivity; moreover, it does not require prior knowledge of the sequence of the template DNA to be amplified.

## MATERIALS AND METHODS

### Plant material, DNA extraction and quantification

Six genotypes of durum wheat (*Triticum durum* Desf), from different origins (Table 1), were used in our study. Genomic DNA was extracted from young leaves, using Cetyltrimethylammonium bromide (CTAB), as described by (Saghai-Maroof et al. 1984). Total DNA was isolated from 100 to 150 mg of fresh young leaf samples; surface sterilized with 20% Chlorex solution in the presence of liquid nitrogen, where they were ground using pastel and mortar. 1 ml of extraction buffer was added to the powder,

then incubated at 60°C in the bath marry for 1 h with manual and frequent shaking by reversing tubes, then after centrifugation at 12000 rpm for 15 min, an equal volume of phenol/chloroform/isoamyl alcohol mixture 25:24:1, were added to the supernatant, the whole is agitated and centrifuged at 10000g for 10 min at 4°C. were added to aqueous phase 500 µL of 24:1 chloroform/isoamyl alcohol mixture were added, then after-shaking, the set is centrifuged at 10000 g for 10 min at 4°C, at the aqueous phase, 2/3 of isopropanol volume was added, the mixture was kept at -20°C for 30 min, after precipitation, the set was centrifuged at 10 000 g for 10 min at 4°C, after the removal of the supernatant, the set was dried in a vacuum centrifugal concentrator evaporator (Speed Vac Savant Instruments) for 5 min; 500 µL of chloroform/isoamyl alcohol mixture (24:1) was then added, tubes manually shaken, were centrifuged at 10000 g for 10 min at 4°C, at the aqueous phase was added 2/3 of isopropanol volume the mixture was kept at -20°C for 30 min, after precipitation, the set was centrifuged at 10 000 g for 10 min at 4°C, after supernatant removal, the DNA tip was vacuuming dried for 5 min and resuspended in 50 µL of TE (10 mM Tris (pH 8), 20 mM Na2EDTA) overnight at 4°C. The quantity and quality of DNA (1.5 µL) were estimated by Nanodrop spectrophotometer and purity by agarose gel electrophoresis (0.8%).

### Selection of the appropriate primers

RAPD analysis was performed using 15-mer random primers (Operon Technologies, USA) (Table 2), tested on all wheat cultivars using the same PCR reaction conditions with the only change in the annealing temperature, as one cycle of 95°C for 5 min (denaturation), 36 cycles of {94°C for 1 min, 36°C for 1 min and 72°C for 1 min (annealing)} 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. The sizes of the fragments were estimated based on the DNA ladder of 100 bp.

### DNA amplification and electrophoresis

The PCR allows, using a thermocycler, to amplify specific DNA sequences (molecular markers or other genome sequences). In this work tested primers, were decamer RAPD primers with a GC nucleotide composition exceeding 50%. The volume for the final PCR reaction was 25 µL and contained Multiplex Master Mix, 5µM of primer sense and anti-sense, and 50 ng of DNA.

**Table 1.** Origin, pedigrees and release information of studied varieties.

| Varieties | Origin | Pedigrees | Release information |
|---|---|---|---|
| Bidi17 | Algeria | Old local landrace | 1930 |
| Cirta | Algeria | Hedba3/GDOVZ 619 | 2000 |
| Waha | ICARDA | PELICANO/RUFF//GAVIOTA/ROLETTE | 1979 |
| Gta dur | Cimmyt | Gaviota /durum | 2007 |
| KORIFLA X SHAM-3 | Syria | DURUM-DWARF-S-15/CRANE//GEIER, T R.DR [1281] | 1926 |
| TELL 76 | Algeria | CRANE (SIB)/F3-TUN//(SIB) ANHINGA/3/(SIB) FLAMINGO | 1976 |

**Table 2.** The nucleotide sequences of the 15 primers used for RAPD

| Primer name | Sequence (5'-3') |
|---|---|
| OPG-09 | CTGACGTCAC |
| OPF-20 | GGTCTAGAGG |
| OPA-17 | GACCGCTTGT |
| OPC-05 | GATGACCGCC |
| OPE-13 | CCCGATTCGG |
| OPO-03 | CTGATACGCC |
| OPO-05 | CCCAGTCACT |
| OPO-06 | CCACGGGAAG |
| B-19 | ACCCCCGAAG |
| OPA-20 | CTTCGCATCC |
| OPH-20 | GGGAGACATC |
| OPJ-06 | TCGTTCCGCA |
| OPA-10 | GTGATCGCAG |
| OPA-13 | CAGCACCCAC |
| OPB-01 | GTTTCGCTCC |

The conditions of PCR in an Applied Biosystems thermocycler were as follows: denaturation at 95°C and activation of Taq for 5 min and 30 cycles from 30 sec at 94°C, 30 sec at 52°C and 1 min at 72°C, followed by a final elongation at 72°C for 7 min.

The amplified PCR product was analyzed on 2% agarose gel electrophoresis, prepared in 1x TBE buffer. Gels are visualized using the nighthawk (pdi), darkroom system incorporating UV transilluminator and CCD camera E-BOX VX2. Data analysis bands were scored as 1 for their presence or 0 for their absence across the cultivars to generate a matrix.

**Molecular data analysis**

For all genotypes, each primer was recorded as presence (1) and absence (0) of the band throughout the amplified profile with primer. For each RAPD primer, the total number of amplified bands, the number of monomorphic bands, the number of polymorphic bands, and the percentage of polymorphism by the E-Capt Software were calculated. After calculating the allelic frequency, the PIC value was calculated as follows:

$$PIC = 1 - \sum Pij^2 \text{ (Botstein et al. 1980)}$$

Pij = the frequency of the $i$th allele in the $j$th genotype for each locus.

The number of alleles present at a given locus represents the allelic richness of a genotype. The Principals Components Analysis (PCA) (p <0.01), and the genetic similarities were performed by GenAlex software. This analysis makes it possible to highlight differences or similarities between cultivars. The hierarchical cluster analysis (HCA), this leads to the construction of a classification tree (or dendrogram). The PCA is a fundamental method in multidimensional descriptive statistics, and it allows for the simultaneous processing of any number of quantitative variables (or qualitative variables with numerical recoding). The purpose of the PCA is to obtain the most relevant summary of the initial data. The program calculates the covariance matrix, diagonalizes it, and extracts its own values and vectors. The matrix's own vectors define the factorial axes. The data in the collapsed table are then projected on these axes. Since the correlation coefficients are always between – 1 and +1, then the program displays the correlation circle.

## RESULTS AND DISCUSSION

Genetic diversity and the genetic distance were studied using fifteen RAPD (Di-mer) primers; they generated 169 bands, which ranged in size from 100-1000 bp; with an average of 11 bands per primer; where 124 of them were polymorph which means 74% of total polymorphism. The total numbers of fingerprinting bands are summarized in Table 3.

**RAPD analysis**

All the fifteen RAPD primers proved to be highly effective in discriminating the six durum wheat genotypes. In this analysis, a total of 169 bands ranging from 100 bp to 1000 bp, were screened among all primers; of which 124 were polymorphic (74%) across all tested genotypes, and the highest value was recorded with OPC-05, OPE-13, OPA-20, OPH-20, OPA-10 and OPB-01 Primers (13, 12, 12, 11, 11 and 10, respectively). However, the lowest value was registered in OPJ-06 primer, with the percentage of polymorphism changed from 40% (OPF-20) to 100% (OPH-20/OPA-10/OPA-13), with an average of 69.14%. The number of bands generated per primer ranged from 2 to 15 alleles. So the OPB-01and OPE-13 primers gave the highest number of fragments (15 amplicons were detected), while OPJ-06 primer revealed the least number (2 amplicons) (Table 3). The values of allelic frequency ranged from 1 (OPJ-06) to 0.63 (OPA-10) with an average of 0.79 high PIC values of 0.35 (OPA-10) and low PIC value of zero (OPJ-06), with an average of 0.21 (Table 3). RAPD technology seems to have at the genetic level a high potential because it reveals high polymorphism. Besides, primers are universal across group taxonomies. Technology RAPD is fast spread because it gives a tool for the quick genetic analysis of biological systems that did not benefit before from molecular markers (Khan et al. 2010). Compared with other studies on molecular polymorphism using markers RAPD, the average of polymorphism, was from 0 to 100% (Saleh et al. 2017), while it was 77% (Zamanianfard et al. 2015) among 25 durum wheat genotype.

**Table 3.** Number of DNA bands generated by individual RAPD primers used and their genetic parameters. Monomorphic bands: MB, Polymorphic bands: PB, Allelic frequency: AF

| Primer name | Sequence (5'-3') | Total of bands | MP | PB | % P | AF | PIC |
|---|---|---|---|---|---|---|---|
| OPG-09 | CTGACGTCAC | 11 | 5 | 6 | 54.5 | 0.83 | 0.17 |
| OPF-20 | GGTCTAGAGG | 10 | 6 | 4 | 40 | 0.90 | 0.10 |
| OPA-17 | GACCGCTTGT | 12 | 4 | 8 | 66.7 | 0.80 | 0.20 |
| OPC-05 | GATGACCGCC | 10 | 3 | 7 | 70 | 0.83 | 0.19 |
| OPE-13 | CCCGATTCGG | 15 | 5 | 10 | 66 | 0.79 | 0.21 |
| OPO-03 | CTGATACGCC | 13 | 2 | 11 | 84.6 | 0.79 | 0.23 |
| OPO-05 | CCCAGTCACT | 11 | 5 | 6 | 54 | 0.83 | 0.17 |
| OPO-06 | CCACGGGAAG | 13 | 6 | 7 | 53.9 | 0.78 | 0.21 |
| B-19 | CCCCCGAAG | 12 | 3 | 9 | 75 | 0.82 | 0.21 |
| OPA-20 | CTTCGCATCC | 13 | 2 | 11 | 84.6 | 0.85 | 0.21 |
| OPH-20 | GGGAGACATC | 12 | 0 | 12 | 100 | 0.65 | 0.32 |
| OPJ-06 | TCGTTCCGCA | 2 | 2 | 0 | 0 | 1 | 0 |
| OPA-10 | TGATCGCAG | 12 | 0 | 12 | 100 | 0.63 | 0.35 |
| OPA-13 | CAGCACCCAC | 8 | 0 | 8 | 100 | 0.75 | 0.28 |
| OPB-01 | GTTTCGCTCC | 15 | 2 | 13 | 86.7 | 0.71 | 0.28 |
| TOTAL | - | 169 | 45 | 124 | 69.14 | 0.79 | 0.21 |

**Table 4.** Matrix of genetic distances using RAPD markers

| Varieties | V1 | V2 | V3 | V4 | V5 | V6 |
|---|---|---|---|---|---|---|
| BIDI17 | 1 | | | | | |
| CIRTA | 0.442 | 1 | | | | |
| WAHA | 0.213 | 0.259 | 1 | | | |
| GTA DUR | 0.315 | 0.412 | 0.169 | 1 | | |
| KORIFFLAXSHAM-3 | 0.352 | 0.334 | 0.176 | 0.447 | 1 | |
| TELL | 0.314 | 0.335 | 0.131 | 0.25 | 0.397 | 1 |

**Genetic variability and cluster analysis**

The purpose of this analysis is to classify all the genotypes into a reduced number of groups or clusters, where the formation of these groups can follow common characteristics of genotypes (Bautista-Salas 2009). This method determines the level of similarity or divergence between individuals using the mean link procedure and Euclidean distance to construct such homogeneous classes. In our investigation, Similarity indices were developed based on the obtained amplified fragments of the six wheat genotypes using the fifteen RAPD primers. The genetic similarity values ranged from 0.131 to 0.447 (Table 4). This reflects the existence of wide genetic diversity which characterizes the six studied genotypes. The highest value was found between GTADUR and KORIFFLA x SHAM, while the lowest genetic similarity was observed between WAHA and TELL varieties. The result registered for genotypes that have the highest similarities introduce suggests that these last consist of closely linked groups, although, the lowest similarities translated some divergence between these varieties. Our results are in agreement with the results of Shirnasabian et al. (2014), who used molecular markers to characterize 18 durum wheat genotypes. Ecological and geographical differentiations are two important factors that influence breeding in cereal crops. The genetic diversity detected in the present study may be due to all these prevalent background factors as the studied genotypes are from different origins. Similar results were registered by Aydogan and Yagdi (2012), who used

17 RAPD polymorphic molecular markers to characterize the genetic relationship among 16 bread wheat genotypes. Guasmi et al. (2012), reported that RAPD and ISSR techniques are very useful to assay the genetic diversity among 80 barley genotypes and the percentage of polymorphism was 66.67%. Also, some studies reported a high level of genetic variability using RAPD markers (Saleh et al. 2017, Koteswara et al. 2020).

**The Principal Component Analysis (PCA)**

Principal Components Analysis (PCA) is one of the most important methods of ordination analysis. It constructs a new set of orthogonal components axes so that the projection of points (varieties) onto them has a maximum variance.

According to the generalized data of 15 primers, percentage of variation explained by the first 2 principal components was 49.27% of the total genetic variance (27.46% for PC1 and 21.80% for PC2) which indicates the reliability of the PCA as data mining analysis in genetic diversity studies. It can be noted that two-dimensional PCA was able to separate all the studied varieties in the first two principal coordinates (Figure 1).

Furthermore, PCA was more advantageous (Evgenidis et al. 2011), because it detected the most important traits for the grouping. As can be seen in Figure 1, the PCA plot shows a clear separation of six genotypes into four groups. The varieties WAHA and TELL diverged alone in the first and second group, WAHA include the primer (OPE-13 and OPO-03) with the highest alleles OPO-06, OPH-20, OPB-01, OPG-09, OPE-13, B-19, OPO-03, and OPA-13. While the genotype TELL includes (OPG-09, B-19) and (OPA-10) was specific to this genotype. The third group, includes BIDI17 and CIRTA. This group includes (CIRTA and BIDI-17) (OPE-13, OPH-20, OPO-06, and (OPO-05 and OPA-20) which were highly polymorphic, the fourth one is grouped as GTADUR and KORIFFLA x SHAM, with OPE-13, B-19, OPO-06 OPH, OPO-05 and OPA-10.
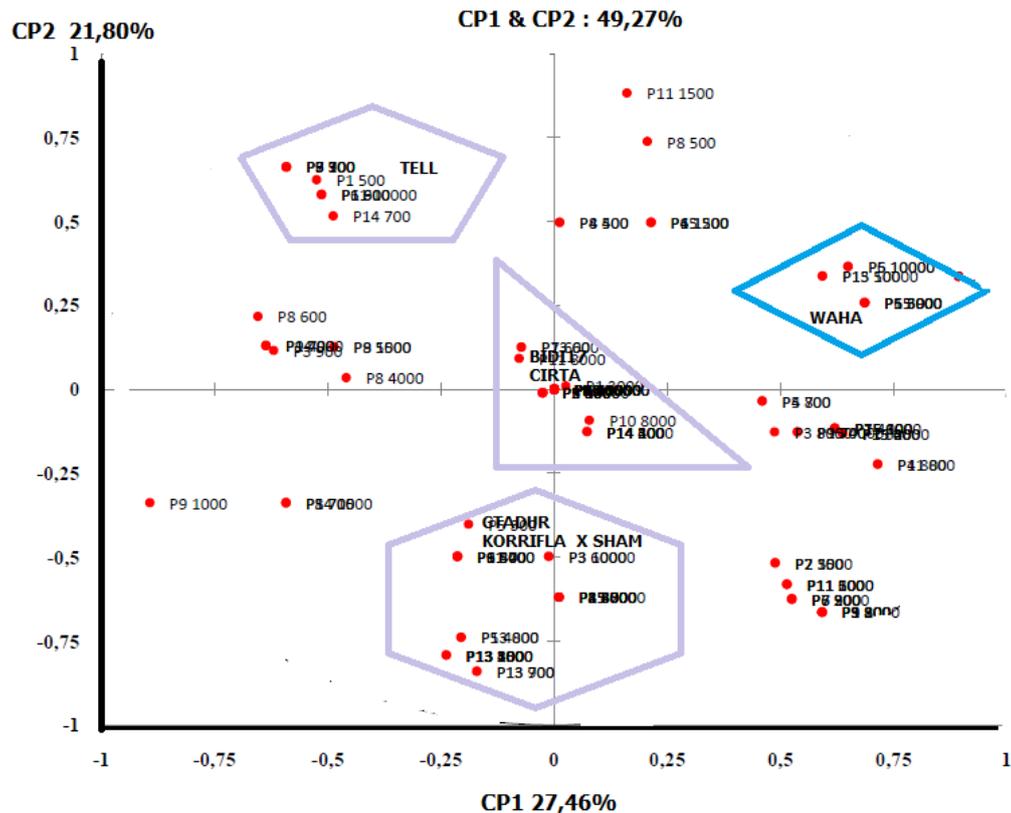
**Figure 1.** Principal components analyses of RAPD (15 primers) data for the six durum wheat genotypes tested

These findings revealed that first two principal components were related to various molecular markers in durum wheat germplasm evaluation, mostly associated with their geographical origin and also these characterizations can identify the diverse genotypes which could be employed in the breeding program for improvement of durum wheat. This technique showed the highest variability between durum wheat genotypes studied.

In conclusion, the present study revealed good variability among durum wheat genotypes tested; these can be considered as the base and step toward further research in developing productive durum wheat generation. The highly polymorphic RAPD primers identified can be used for further genetic diversity in future and serves to facilitate the development of better genotypes and conservation strategies of durum wheat germplasm.

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