

COMPARISON OF RAPD-PCR AND SDS-PAGE TECHNIQUES TO EVALUATE GENETIC VARIATION AMONG NINE BARLEY VARIETIES (*Hordeum* spp)

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ABSTRACT

Genetic variability of barley (*Hordeum* spp) considered very important, reducing their genetic diversity affects negatively their tolerance to stress factors, causing the great loose of yield and implies the risk of their genetic vulnerability. Thus, the knowledge of genetic diversity of barley germplasm is important for breeding programmes and their conservation. Random amplified polymorphic DNA (RAPD) and SDS-PAGE techniques were used to examine the genetic variation among barley varieties. To maximize the genome coverage nine barley varieties from diverse origins were selected and analyzed with 18 selected RAPD markers. A total of 116 amplified bands and 63 polymorphic allelic variants were detected, ranging from 3 to 6 per locus with an average of 3.937. Genetic distance calculated from RAPD data ranged from 0.357 to 0.767. The dendrogram based on genetic distance estimation and PCA was produced two groupings that generally consistent with available genetic information. Zanbaka and Tadmor/roh6 were found to be closely related. Their genetic relationship was also confirmed by the facts that they shared a common ancestor and they were clustered together. A total of 14 unique bands (8 positives and 6 negatives) were identified out of 116 bands which could be considered for marker assisted selection. A slight range of variation in SDS-protein banding patterns was observed. A total number of 12 bands with different molecular weights were observed in SDS-PAGE, among these, only 5 bands were polymorphic. The dendrogram tree and PCA analysis showed that Elkheir and Canela were genetically distanced from the other genotypes. According to the results of RAPD and SDS-PAGE, a wide range of genetic diversity was observed among all varieties, indicating them as prime candidates of selective breeding for specific traits and broadening the genetic base.

Key words: Barley, RAPD-PCR, SDS-PAGE, Genetic Diversity

INTRODUCTION

Barley (*Hordeum* spp) is one of the oldest domesticated cereals and its origin is in the territory of Fertile Crescent in the Middle East around 8000 BC (Badr *et al.*, 2000). Since ancient times, it was used as human food and as an ingredient in fermented beverages (Kling *et al.*, 2004). The identification of varieties of crop plants had become increasingly important to the documentation of genetic resources and for the protection of breeder's interests. It is especially important for the malting and brewing industries, as different varieties of barley (*Hordeum vulgare* L. ssp. *vulgare*) have different qualities and characteristics (Velicevici *et al.*, 2012). Farmers also have their proprietary rights on the utilized varieties for sustainable

production. Examination of grain morphological characteristics was the standard method for identifying barley cultivars, however not all varieties could be distinguished on that basis (Giancarla *et al.*, 2012). Traditional methods of plant breeding have made significant contributions to crop improvement but they proved to be slow in targeting complex traits like grain yield, grain quality, and drought or salinity tolerance. Among the earliest markers, morphological traits were used for germplasm management, but they have a number of limitations including low polymorphism, low heritability, late expression and vulnerability to environmental influences (Smith and Smith, 1992). To meet the growing needs to increase food production, biotechnology offers novel and powerful tools to assist and complement breeding efforts (Abdel-Tawab *et al.*, 2003).

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Molecular markers have been established to be valuable tools for characterization and evaluation of genetic diversity within and between species and populations (Velicevici *et al.*, 2012). The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as random amplified of polymorphic DNA (RAPD) (An *et al.*, 2009; Zeng 2002), simple sequence repeats (Microsatellite) (Matus *et al.*, 2002), the sequence-tagged sites (STS), random amplified microsatellite polymorphism (RAMP), and inter-simple sequence repeat polymorphic DNA (ISSR) (Rashal *et al.*, 2004). Molecular markers have been used in barley for detecting genetic diversity (Ciulca, 2010), genotype identification, and genetic mapping (Todorovska *et al.*, 2003). Genetic analysis with RAPD markers is relatively easy, fast and efficient; therefore it has been extensively used for determining genetic diversity among barley varieties and for identifying the best quality for malting (Diaz-Perales *et al.*, 2002; Todorovska *et al.*, 2003). Determination of genetic variability among genotypes is useful for both practical applications in a breeding program and cultivar protection (Sozen, 2010). A variety of approaches from morphological data to molecular markers including RAPD (Bibi *et al.*, 2009), AFLP (An, 2009), SSR (Hina *et al.*, 2008) and ISSR (Guasmi *et al.*, 2012) markers have been applied for the assessment of genetic variability among genotypes. Among various approaches, analysis of barley grain storage proteins, hordeins by polyacrylamide gel electrophoresis at acidic pH or by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), was recommended in 1988 as an official method for barley variety discrimination (Canci *et al.*, 2003). The aim of this study was to identify and differentiate 9 barley varieties using RAPD and SDS-PAGE techniques.

MATERIALS AND METHODS

Plant materials

The present work was carried out at the University of Sulaimani, Kurdistan region-Iraq. Nine Iraqi barley varieties (*Hordeum distichum* L. and *Hordeum vulgare* L.) were used for applying RAPD and SDS-PAGE techniques, they were:

1. Canela (Full name: Canela/Zheadar/2/Limon/3/Msel, Origin: Cimmyt, 2-row)
2. ATACO (Full name: ATACO/ Bermejo/Higo/3/Cln-B/80, Origin: Cimmyt, 6-row)
3. ABN (Full name: ABN-B/KA-B/Raisa/3/Aleli/4/Limon/5, Origin: Cimmyt, 2-row)
4. Quinn (Full name: Quinn/Aloe/Cardo/3/Ciru, Origin: Cimmyt, 6-row)
5. Zambaka (Origin: State Board of Seeds Testing and Certification. Ministry of Agriculture. Iraq, 6-row)
6. Elkheir (Origin: State Board of Seeds Testing and Certification, Ministry of Agriculture. Iraq, 6-row)
7. Arta/3/avar (Origin: State Board of Seeds Testing and Certification, Ministry of Agriculture. Iraq, 6-row),
8. Tadmor/roh6 (Origin: State Board of Seeds Testing and Certification, Ministry of Agriculture. Iraq, 6-row)
9. Arivat (Origin: State Board of Seeds Testing and Certification, Ministry of Agriculture. Iraq, 6-row).

The varieties were collected from the Field crops department of the Ministry of Agriculture and Water Resources in Kurdistan region of Iraq. The varieties used in this study are representing the wide range of barley crop in the region.

DNA extraction

Seeds were grown in pots and put in greenhouse, at the University of Sulaimani, Faculty of Agricultural Sciences. Watering was done once a day and after three weeks, the healthy leaves were harvested. Fresh leaves were bulked from five individuals of each variety for extracting the genomic DNA, using Cetyltrimethyl ammonium bromide (CTAB) protocol of Doyle and Doyle (1987) as described by Smykal (2006).

Determination of DNA Concentration by Spectrophotometer

The DNA samples were diluted 100 times using TE buffer. The optical density (OD) of each sample was read at wavelength of (260 and 280 nm) with a spectrophotometer. The DNA concentration was calculated by the equation below:

$$\text{DNA concentration } (\mu\text{g}/\mu\text{L}) = \frac{50 \mu\text{g}/\text{mL} \times \text{ODA}_{260} \times \text{dilution factor}}{1000}$$

The OD of diluted DNA at 260 nm was measured. The dilution factor was 100 (10 μL of extracted DNA in 990 μL of high pure water or ddH₂O). The D_{260/280} ratio for pure DNA was 1.78-1.85 (Sambrook *et al.*, 1989). The final concentration of extracted DNA was 0.5-0.6 $\mu\text{g}/\mu\text{L}$. A solution of 20 ng/ μL was prepared by adding of 20 μL of extracted DNA in 480 μL of ddH₂O. Five μL (100 ng) of the diluted solution was used for PCR reaction.

Table 1. RAPD primers, their sequences, and annealing temperature

Primer	Sequence 5'-3'	Annealing temperature (°C)
OPD02	GGACCCAACC	37
OPD10	GGTCTACACC	37
OPD18	GAGAGCCAAC	37
OPD20	ACCCGGTCAC	37
OPG12	CAGCTCACGA	37
OPG14	GGATGAGACC	37
OPG10	AGGGCCGTCT	37
OPJ10	AAGCCCGAGG	37
OPF03	CCTGATCACC	37
OPH13	GACGCCACAC	37
OPC07	GTCCCGACGA	38
OPC15	GACGGATCAG	38
OPD08	GTGTGCCCCA	38
OPF13	GGCTGCAGAA	38
OPF14	TGCTGCAGGT	38
OPJ13	CCCACTACC	38
OPB11	GTAGACCCGT	38
OBP14	TCCGCTCTGG	38

RAPD-PCR reaction

Eighteen primers used in this study were generated the amplicons, Table 1 (Raoudha *et al.*, 2010). The reaction mixture (25 µL) contained 10x assay buffer, 2.5 mM MgCl₂, 400 µM dNTP's (Fermantas), 5pmoles of primer, 100 ng template DNA and 1 U of Taq DNA Polymerase (Fermantas). Amplification was carried out in a Thermo-cycler (Master cycler) for 35 cycles, each consisting of a denaturation step at 94°C for 1 min, annealing at 35°C for 50 seconds and an extension step at 72°C for 2 min. An initial denaturation step at 94°C for 5 min and a final synthesis step of 5 min at 72°C were also included. Amplified products were electrophoresed on a 1.5% agarose gel stained with Ethidium Bromide (0.5 µg/mL) and subsequently visualized using a gel documentation system (Ugenius).

Extraction of total protein and SDS-PAGE

Variability of total seed storage proteins was investigated using SDS-PAGE. Protein was extracted from dry seeds. After the fine grinding of the seeds an appropriate quantity (0.01 g) of the powder was taken into a 1.5 mL Eppendorf tube. Protein extraction buffer (500 µL) containing 0.05 M Tris-HCl (pH 8.0), 0.4% SDS, 5 M Urea, 2.5% glycerol, 1.5% 2-Mercaptoethanol, 1 mM EDTA and 0.01% (w/v) bromophenol blue was added to the tube. The sample was vortexed for 5 min, followed by the incubation for 3 hours at room temperature. The samples were centrifuged at 12000 rpm for 10 min at room temperature. The proteins extracted in the supernatant were size fractionated using SDS-PAGE.

The soluble seed proteins were subjected to SDS-PAGE in gel slabs of 1.5 mm thickness (3.5 cm, 4.5% stacking and 15.5 cm, 15% resolving gels) as described by Laemmli (1970). Electrophoresis was performed with a discontinuous buffer system in a UVP vertical electrophoresis unit (Cambridge, UK). The gel was run at 30 mA until the bromophenol blue marker had reached the bottom of the gel. The gel was stained in a staining solution (10% methanol, 6.6% acetic acid and 0.05% Coomassie Brilliant Blue R-250) for overnight. The gel was de-stained in de-staining solution (40% methanol, 7% acetic acid) for overnight until the color of background disappeared. Then, the gel was rinsed in water several times until the electrophoresis protein bands were clearly visible (Jin *et al.*, 2006).

Data analysis

The RAPD bands were scored in a binary form as presence or absence (1/0) of bands for each sample. SPSS software (version 21) was used to calculate Jaccard's coefficient (Aida *et al.*, 2007). A hierarchical cluster analysis was performed using the average linkage method and Jaccard's coefficient. The dendrogram and principal component analysis (PCA) based on the RAPD data or total grain protein patterns of barley varieties was constructed using SPSS software (Aida *et al.*, 2007).

RESULTS AND DISCUSSION

RAPD analysis

Genomic DNA was extracted from bulk sampling for each variety (Figure 1). The advantages and inconvenience of the bulk analysis have been discussed by Michelmore *et al.* (1991). Studies conducted on different primers (Table 1) were established that the minimum length of primers to produce detectable level of amplification might be of nine bases, while in case of decamer primers, guanine and cytosine should be at least 40% (Williams *et al.*, 1990). These primers are generally universal, can amplify different loci of several barley genomes which make this technique being being an effective in assessing DNA polymorphism of different genotypes (Velicevici *et al.*, 2012). Sixteen primers generated 116 bands; however the primers varied in number of bands (Table 2). The primers OBP11 and OBP14 did not generate any bands. Figure 2 shows the polymorphic bands among barley varieties amplified by primer "OPF03". Among the tested RAPD primers, 16 primers generated polymorphic bands. The number of polymorphic bands obtained from 16 primers was 63 having the ratio ranged from 71.4% (for primer OPD18 and OPG12) to 33.3% (for primer OPG14). The number of polymorphic bands in this study

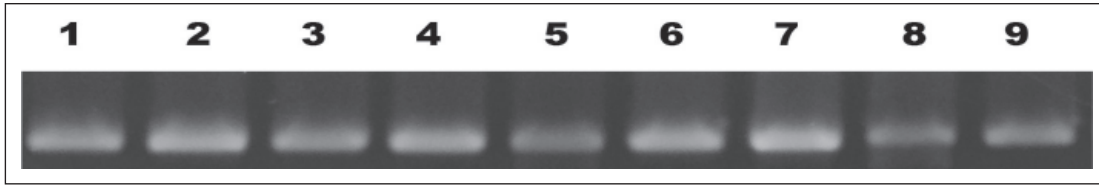


Fig. 1. Genomic DNA extracted from leaves of barley varieties. 1=Arivat, 2=Tadmor/roh6, 3=Arta/3/avar, 4=Elkheir, 5=Zambaka, 6=Quinn, 7=ABN, 8=ATACO, 9=Canela, respectively.

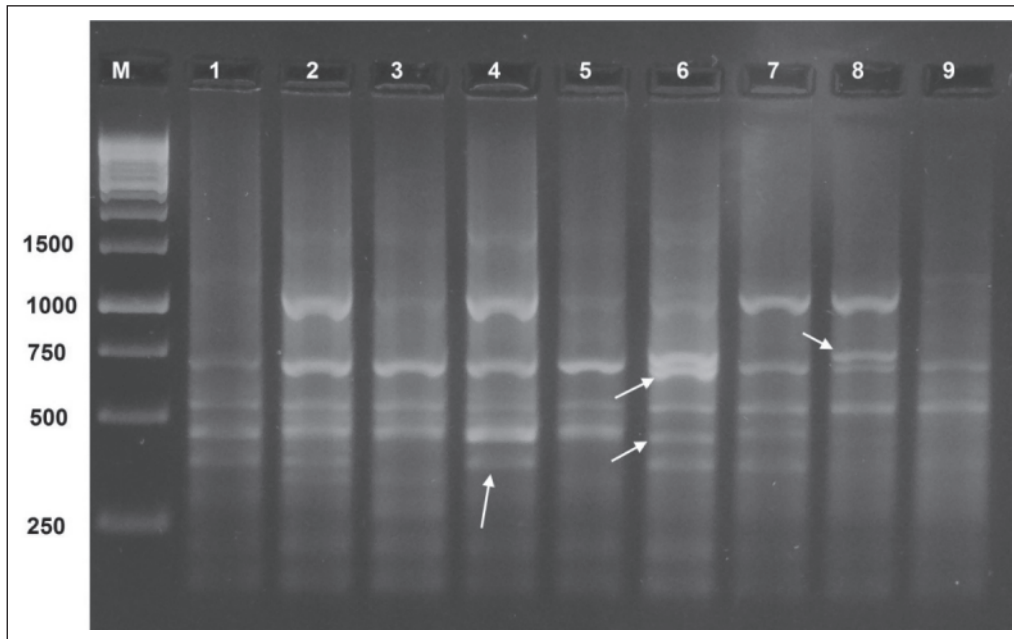


Fig. 2. Result of amplified PCR product analysis using primer OPF03 (M lane=1000 bp markers (DNA ladder), 1–9 lanes Amplified DNA fragment of 1=Arivat, 2=Tadmor/roh6, 3=Arta/3/avar, 4=Elkheir, 5=Zambaka, 6=Quinn, 7=ABN, 8=ATACO, 9=Canela, respectively). The white arrow shows some polymorphic bands.

Table 2. Number of amplified DNA-fragments, polymorphic bands and size of amplified fragments (bp) in barley varieties investigated with RAPD primers

Primer	Total amplified bands	Number of polymorphic bands	Polymorphism %	Range of fragment size (bp)
OPD02	6	3	50.0	500-1500
OPD10	6	4	66.6	500-1000
OPD18	7	5	71.4	400-1800
OPD20	7	3	42.8	400-1200
OPG12	7	5	71.4	350-1350
OPG14	9	3	33.3	250-1900
OPG10	9	4	44.4	600-1500
OPJ10	6	3	50.0	300-1400
OPF03	9	4	44.4	300-750
OPH13	5	3	60.0	500-1300
OPC07	9	5	55.5	350-1500
OPC15	7	4	57.1	400-1250
OPD08	7	3	42.8	300-750
OPF13	6	4	66.6	300-1000
OPF14	7	4	57.1	400-1900
OPJ13	9	6	66.6	300-1000
Total	116	63	54.31	
Average	7.25	3.93		

Table 3. Distance matrix based on Jaccard's coefficient analysis showing the relationship among various barley varieties. 1: Arivat, 2: Tadmor/roh6, 3: Arta/3/avar, 4: Elkheir, 5: Zanbaka, 6: Quinn, 7: ABN, 8: ATACO, 9: Canela

Varieties	1	2	3	4	5	6	7	8	9
1	0	0.452	0.636	0.55	0.595	0.575	0.59	0.676	0.556
2		0	0.429	0.438	0.357	0.549	0.531	0.68	0.532
3			0	0.444	0.439	0.532	0.574	0.755	0.609
4				0	0.368	0.511	0.587	0.689	0.652
5					0	0.581	0.628	0.767	0.667
6						0	0.512	0.682	0.644
7							0	0.6	0.361
8								0	0.528
9									0

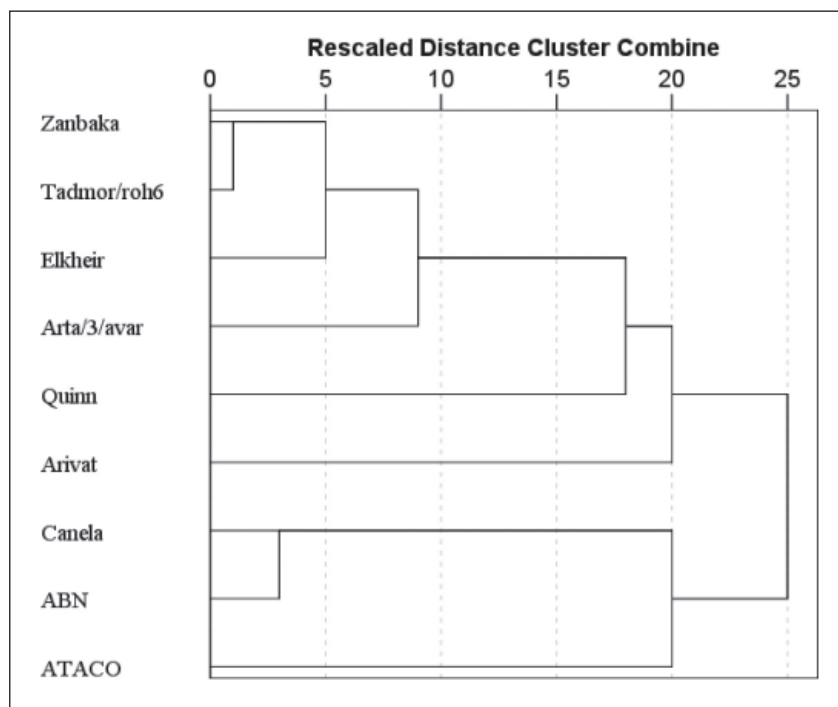


Fig. 3. Grouping of barley varieties using hierarchical cluster analysis (average linkage and Jaccard's coefficient distance) based on RAPD data.

ranged from 3 (OPJ02, OPD20, OPJ10, OPG14, OPH13 and OPD08) to 6 (OPJ13). The size of bands ranged from 250 to 1900 bp, while the polymorphism among various molecular weights is given in ranges (Table 2). The average number of polymorphic bands per primer was 3.93. This polymorphic ratio indicates the presence of different alleles for barley varieties used, suggesting the pose of reasonable variation between these varieties. The monomorphic bands for primers used were not resolve any diversity issue and they were discarded from the analysis.

The average genetic distances ranged from 0.357 (Zanbak and Tadmor/roh6) to 0.767 (Zanbaka and ATACO) (Table 3). The genetic variation found in this study (0.746) was similar to an earlier report

by Chen *et al.* (2000) using RAPD markers to analyze the genetic variation of different barley populations from China. The dendrogram based on RAPD-PCR data to estimating genetic distances, was distinct all varieties into two clusters (Figure 3). The first cluster was divided into five sub-clusters; including Zankaka and Tadmor/roh6 together, Elkheir, Arta/3/avar, Quinn and Arivat, respectively. The second cluster contained Canela, ABN and ATACO. In consequence, the usage of some genitors from different groups and subgroups presented will offer the opportunity to follow the segregation of target loci in their hybrids.

To visualize the genetic relatedness among barely varieties in details, PCA was made for 63 polymorphic bands generated by 16 primers. The

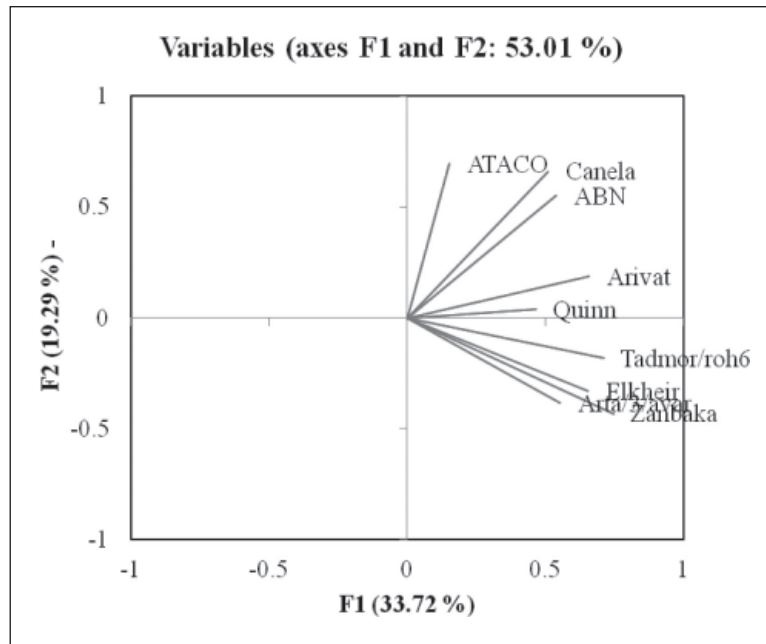


Fig. 4. Principal component analysis (PCA) of barley varieties constructed by RAPD markers. Variance percent for each principal component (PC) are listed beside each axis in parentheses.

Table 4. Barley varieties characterized by unique positive and/or negative RAPD markers with their sizes (bp)

Varieties	Unique positive bands	Unique Negative bands
Arivat		OPD08 (785 bp)
Tadmor/roh6	OPF03 (375 bp), OPC15 (510), OPJ13 (674 bp)	
Zanbaka		OPG12 (1775 bp)
Quinn	OPD20 (300 bp), OPG12 (450 bp)	
ABN	OPD02 (1010 bp), OPG14 (655 bp)	OPG14 (620 bp)
ATACO	OPJ-13 (485 bp)	OPD02 (400 bp), OPD20 (255bp), OPC07 (1200 bp)

results of this analysis are presented in Figure 4. It is revealed from the results that barley varieties were dispersed on the PCA plot, which evidenced of a good genetic base. It also shows a clear-cut separation of nine varieties into two groups, each group was distinct from others. Group 1 contained Zanbaka, Tadmor/roh6, Elkheir, Arta/3/avar, Quinn and Arivat. Group 2 represented Canela, ABN and ATACO. In general, the result obtained from PCA is in agreement with what was conducted by the dendrogram, which is a further confirmation of genetic relationships determined by cluster analysis.

Table 4 revealed that out of 116 bands overall barley varieties, eight of them were unique positive bands. The two highest unique and positive bands were obtained from OPJ13. One of them (485 bp) was detected in ATACO, and a the second band of 674 bp was in Tadmor/roh6. The primers of OPD02 (1010 bp in ABN), OPD20 (300 bp in Quinn), OPG12 (450 bp in Quinn), OPG14 (655 bp in ABN),

OPF03 (375 bp in Tadmor/roh6), and OPC15 (510 bp in Tadmor/roh6) considered to have one unique band each. On the others hands, there were six unique negative bands: OPD08 (785 bp in Arivat), OPG12 (1775 bp in Zanbaka), OPG14 (620 bp in ABN), OPD02 (400 bp in ATACO), OPD20 (255 bp in ATACO) and OPC07 (1200 bp in ATACO). These results indicated that the unique bands can be used to analyze the genetic diversity among varieties. A total of 14 unique bands were identified here could be considered in marker assisted selection. The sequencing of these positive bands gives us the enough information about the gene related to these markers.

SDS-PAGE technique

For an effective breeding program, information regarding the extent and nature of genetic diversity within a crop species is essential. Protein electrophoresis is a useful method for describing the

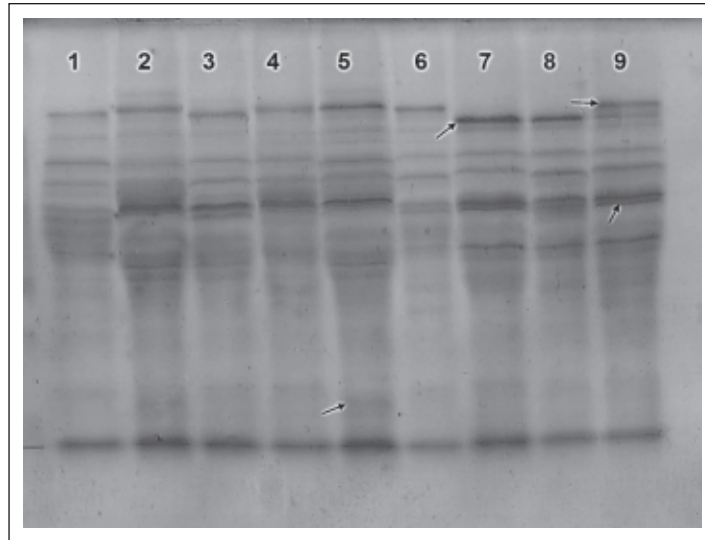


Fig. 5. SDS-PAGE profile of total grain protein of barley varieties separated on 15% SDS-PAGE. Lines 1-10 refer to barley varieties: 1=Arivat, 2=Tadmor/roh6, 3=Arta/3/avar, 4=Elkheir, 5=Zanbaka, 6=Quinn, 7=ABN, 8=ATACO, 9=Canela, respectively. The white arrow shows some polymorphic bands.

Table 5. Distance matrix based on Jaccard's coefficient analysis showing the relationship among various barley varieties using SDS-PAGE data. 1: Arivat, 2: Tadmor/roh6, 3: Arta/3/avar, 4: Elkheir, 5: Zanbaka, 6: Quinn, 7: ABN, 8: ATACO, 9: Canela

Varieties	1	2	3	4	5	6	7	8	9
1	0	1	0	1	1	0	0.5	0.5	0.67
2		0	1	1	0	1	1	1	1
3			0	1	1	0	0.5	0.5	0.67
4				0	1	1	1	1	1
5					0	1	1	1	1
6						0	0.5	0.5	0.67
7							0	1	0.34
8								0	0.34
9									0

genetic structure of crop germplasm (Ciaffi *et al.*, 1993). Northwest of Iraq is one of the major centers of origin and diversity for barley progenitors. These ancestors are of great importance in the production of new diversity resources (Adi, 2008). The numerical analysis of SDS-PAGE of seed protein profiles showed slight discriminative protein banding. The total seed protein profiles of 9 barley varieties, obtained by one-dimensional denaturing gel electrophoresis, are shown in Figure 5. The protein patterns of the varieties were inspected visually and compared with each other. SDS-PAGE technique of grain proteins exhibited high similarities between all barley varieties in their low-molecular mass. A total of 12 bands were scored among the 9 barley varieties. Of these 12 bands, five were polymorphic accounting for 42% poly-

morphism. Maximum polymorphism was observed with high molecular weight proteins of all varieties. The average genetic distances ranged from 0.00 to 1.00 (Table 5). The average linkage method and Jaccard's coefficient analysis of distanced cluster analysis generated a dendrogram based on SDS-PAGE profiles of seed proteins (Figure 6), consisting of four major clusters. The cluster I included ABN, ATACO and Canela. The cluster II contained Arta/3/avar, Quinn and Arivat. The cluster III comprised Elkheir. The last cluster included Tadmor/roh6 and Zanbaka. The result of PCA showed a clear separation of nine varieties into four groups, each group distinct from other group (Figure 7). Group 1 included ABN, ATACO and Canela. The second group consisted of Arta/3/avar, Quinn and Arivat. The third group contained Elkheir. The last group

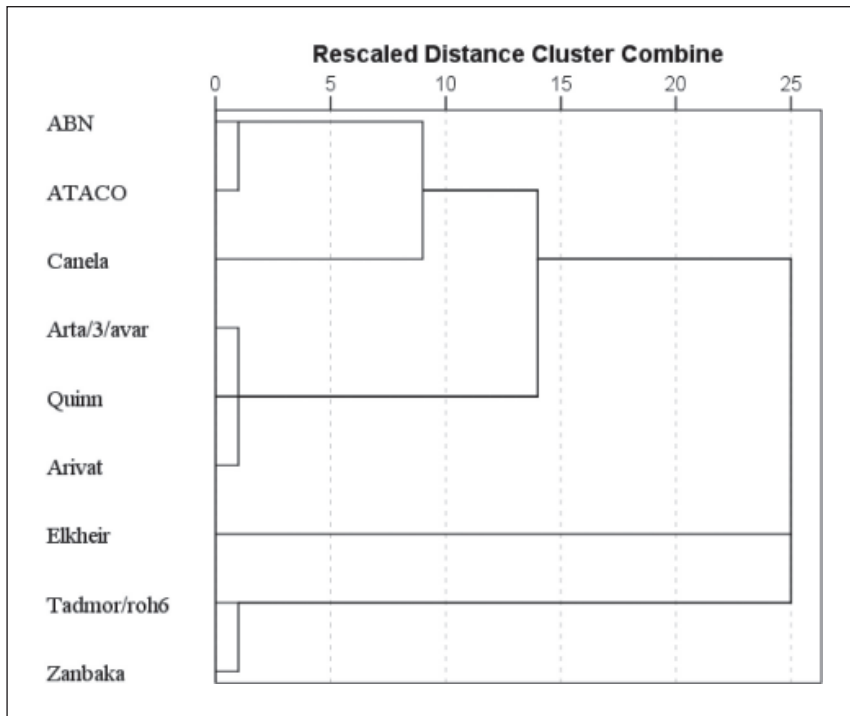


Fig. 6. Grouping of barley varieties using hierarchical cluster analysis (average linkage and Jaccard coefficient distance) based on SDS-PAGE data.

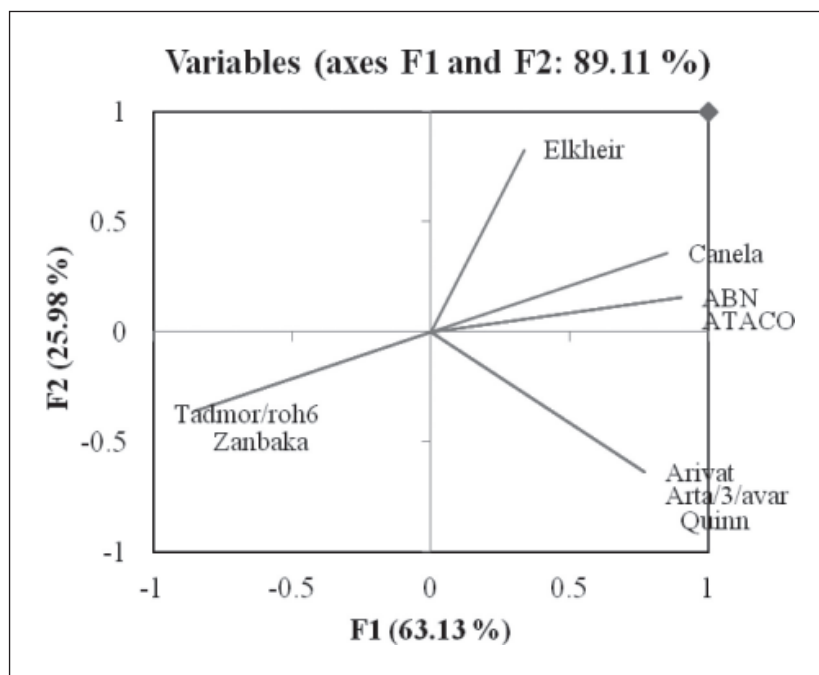


Fig. 7. Principal component analysis (PCA) of barley varieties constructed by SDS-PAGE data. Variance percent for each principal component (PC) are listed beside each axis in parentheses.

included Tadmor/roh6 and Zambaka. The results obtained from PCA are in agreement with the dendrogram, which is a further confirmation of genetic relationships delineated by cluster analysis.

In this study, the inter-specific genomic polymorphisms in nine barley varieties were evaluated through RAPD-PCR and SDS-PAGE techniques. RAPD-PCR technique is currently used

for the discrimination of closely related varieties in order to determine the genetic distance and genetic diversity (Camargo *et al.*, 2010). The number of amplification RAPD bands (116) was more than that of SDS-PAGE (12). The total number of all amplification bands for each primer was between 5 to 9. The variation in number of bands amplified by different primers is influenced by variable factors such as primer structure and less number of annealing sites in the genome (Muhammad *et al.*, 2005). The size (molecular weight) of the amplified fragments ranged from 1900 to 250 bp. This discrepancy may be relate to the genotypes and selection of RAPD primers with scorable bands. The other reason could be the use of more diverse genotypes. Moreover, the total number of polymorphic bands detected by 16 RAPD primers (63) was much higher than SDS-PAGE (5), however the results in this study suggested that the RAPD markers were superior to SDS-PAGE markers for assessing genetic diversity. Tanyolac (2003) observed similar results. Fernandez *et al.* (2002) have studied 16 barley cultivars from different countries and found the high similarity index by ISSRs more than RAPDs. This might be due to highly polymorphic and the abundant nature of microsatellites resulted from slippage during DNA replication.

The genetic matrix between all nine barley varieties was ranged from 0.357 to 0.767. The low genetic similarity and the high genetic distance between the barley varieties indicate that these varieties are separated from each other. The results of genetic distance of RAPD-PCR studies were comparable to the storage protein analysis (Eshghi and Akhundova, 2010), while the genetic dissimilarity ranged from 0.81 to 0.221. Assessment of genetic diversity in local Tunisian barley based on RAPD analysis (Karim *et al.*, 2010) observed the genetic distance, ranging from 0.114 to 0.933. The RAPD and SDS-PAGE dendrograms separated all varieties into three and four clusters, respectively. In this study, it was obvious that the dendrogram based on RAPD markers was not in accordance with that based on SDS-PAGE. The dendrograms of RAPD and SDS-PAGE showed a close relationship between the varieties (Zanbaka, Tadmor/roh6). According to the results of genetic distance and relationships illustrated, the ability to resolve genetic variation among different cultivars may be related to the number of polymorphism detected with marker technique that employed in this study.

Different kinds of electrophoretic methods based on seeds storage protein patterns have been used for the identification and characterization of crop and herbage cultivars (Panella *et al.*, 1993; Karihaloo *et al.*, 2002; Yüzbaşıoğlu *et al.*, 2008).

Some previous studies using different electrophoretic techniques were demonstrated that number of cultivars are indistinguishable based on grain protein profile (Shewry *et al.*, 1978; Marchylo and LaBerge, 1981). Weiss *et al.*, (1991) could not differentiate all 55 European winter and spring barley cultivars by using SDS-PAGE method due to the fact that they were genetically related. However, our findings indicated that SDS-PAGE of seed proteins supplied additional banding patterns for the discrimination of barley varieties, the differentiations were not sufficient to provide a clear distinguishing between the varieties. According to the SDS-PAGE results generated from the study, it can be suggested that Iraqi barley varieties possess a narrow gene pool.

CONCLUSION

This investigation indicated variations among the varieties used in the study. Application of RAPD markers gave an early and accurate result about variations among the varieties, before starting a breeding program. This study indicated that the numerical analysis to profile grain protein alone was not sufficiently enough to differentiate barley varieties. It was also obvious from the results that the dendrogram based on RAPD markers was not in accordance with the dendrogram based on protein markers.

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