African Journal of Biotechnology Vol. 10(77), pp. 17793-17804, 5 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2726
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Seed storage protein electrophoretic profiles in some Iranian date palm (*Phoenix dactylifera* L.) cultivars

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Accepted 31 October, 2011

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops in the south of Iran. However, little knowledge is currently available about the molecular characterization of date palm cultivars in this country. Determination of genetic variability and cultivar identification in date palm are two major important factors in breeding programs, characterization of germplasm, and conservation purposes. The genetic variation of seed proteins was assayed by SDS-PAGE for 12 cultivars in two regions; Bam and Jiroft in Iran. Seed proteins profiles were evaluated as a chemical character to clarify the taxonomic complexity and genetic variability in the cultivars. SDS-PAGE seed proteins electrophoregrams showed a range of peptides varying between 11.673 to 369 KDa. The relative similarity between cultivars were estimated by Jaccard's similarity index and clustered in UPGMA and NJ for the mode of exposure. Cultivars distance in each region and ward minimum variance for the total of cultivars were moreover calculated. The cultivars from Bam and Jiroft were separated and this might have been done due to their unique genetic build-up. In order to make this assumption precise, data were computed to perform a PCA. Cluster analysis and PCA demonstrated their validity in establishing genetic diversity. When PCA was studied, the previously described results about ward's method clustering of Jaccard's similarity matrix were also visualized.

Key words: Cultivar identification,genetic variation, *Phoenix dactylifera*, SDS-PAGE, seed protein profile.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), a species of the Arecaceae family, is widely distributed in different districts of the world. It constitutes the most important plant in arid and desert areas where it provides favorable conditions for both human and animal habitats and establishes adjacent fruit crops and many other vegetables. Probably domesticated since more than 5000 years ago in Mesopotamia, date palm is cultivated for fruit production and all parts of the tree are used for many industrial purposes such as timber, furniture, rope, and packing material (Elhoumaizi et al., 2002). In fact, date palm as an irreplaceable tree in irrigable desert lands can grow under unfavorable conditions where many of the other fruit species may not grow. It also provides protection for other crops situated under it from heat, wind, and even cold weather; and plays a big role to stop desertification and gives life to desert areas (Baliga et al., 2011; Mohamed Ahmed et al., 2011). Iran is the major producer and exporter of date palm in the world, and four hundred types of date palm cultivars have been reported to exist in Iran (Barreveld, 1993; Dowson, 1964). Bam and Jiroft are two main and important areas of palm cultivation in southeast Kerman province in Iran. Geographically, Bam is situated at latitude 29°10'78" N and longitude 58°36'19" E at an altitude of 1057 m above sea level. The weather

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Abbreviations: SDS-PAGE, Sodium dodecyl sulphate polyacrylamide gel electrophoresis; **PCA,** principal component analysis; **Tris base,** tris (hydroxymethyl) aminomethane; **SDS,** sodium dodecyl (lauryl) sulfate; **TCA,** trichloroacetic acid; **UPGMA,** unweighted pair group method of arithmetic means; **NJ,** neighbor joining; **MW,** molecular weight; **RF,** relative mobility; **OD,** optical density; **No.,** number of band.

in Bam is hot and dry but due to exposure to various desert climate, sometimes the hottest summers and coldest winters have been reported. Average annual rainfall is 68 mm. On the other hand, Jiroft is situated at latitude 28°12'00" N and longitude 57°13'00" E at an altitude of 650 meters above sea level. In the same way, the weather in Jiroft is hot and humid, and the temperature in summer reaches over 50°C. Average annual rainfall is 140 mm.

Date palm is a dioecious perennial, monocotyledon fruit tree and its heterogeneous genetic form makes its progeny strongly heterogeneous and variable. Multiplication of date palm is mainly done vegetatively (Ahmed et al., 2011; Salem et al., 2001; Torres and Tisserat, 1980). Date palm cultivars are divided to three main types according to their fruit moisture content as soft, semi-dry and dry cultivars (Besbes et al., 2004; Elhoumaizi et al., 2002). Therefore, individuals with economically important traits are easily cloned. Clonally propagation of elite cultivars with known high performance is highly desirable in the world. The main limitation for this type of propagation is the minimal production of offshoots, some of which naturally die when separated from the mother plants. Over the years, many varieties have been transplanted to areas other than the original regions, and they may have been adapted and cultivated with different names. As a result, a variety may have a different name in different plantation areas or even two genetically varieties may have the same name (Torres and Tisserat, 1980). This matter also reduces the genetic diversity of the cultivars, making them vulnerable to biotic and abiotic stresses. The morphological markers such as fruit characteristics have been used to describe the varieties, but the environment significantly affects these markers. In general, the identification and evaluation of genetic diversity between cultivars based on morphological markers are very difficult and time-consuming to determine because the onset of fruiting takes between 3 to 5 years. Furthermore, characterization of varieties requires a large set of phenotypic data that are normally difficult to collect and interpret, due to the environmental effects (Al-Khalifah and Askari, 2003; Azeqour et al., 2002; Masmoudi-Allouche et al., 2009). Biochemical markers (isozymes and proteins) have proven to be effective in varietal identification as well (Arzate-Fernandez et al., 2005; Bennaceur et al., 1991; Salem et al., 2001). However, they give limited information and are usually approached indirectly for detecting genomic variation. The molecular markers are useful tools of identification and phylogenetic analysis of different plant species and cultivars. Genetic fingerprinting using molecular purity and verification of labeling and identity of plants is used in production and marketing systems (Thawaro and Te-chato, 2009; Xu and Crouch, 2008).

Knowledge of genetic diversity is a useful tool in genebank management and planning experiments as it facilitates efficient sampling and utilization of germplasm by identifying and/or eliminating duplicates in the gene stock. One practical application of knowledge of genetic diversity is in the design of populations for genome mapping experiments (Alves et al., 2010). Characterization of germplasm using biochemical techniques (fingerprinting) has received attention because of the increased recognition of germplasm resources in crop improvement and the selection of desirable genotypes to be used in breeding programs. Genetic markers are useful for screening germplasm with the minimum cost in time and labour (Bhargava et al., 2005; Capraro et al., 2008; Ghafoor et al., 2002; Ladizinsky and Hymowitz, 1979). Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic, evolutionary relationships among crops, their wild relatives, and determine genetic homology at the molecular level. They can also be used for distinguishing cultivars of particular crop species (Emre, 2009; Juan et al., 2007; Xynias et al., 2006; Yuzbasioglu et al., 2008). However, a few studies shown that cultivars could not be identified by the SDS-PAGE method, as the electrophoretic patterns of proteins were similar among cultivars.

Among the biochemical techniques, SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm. Seed storage proteins have been used as genetic markers in four major areas: (i) analysis of genetic diversity within and between accessions, (ii) founding genome relationships, (iii) plant domestication in relation to genetic resource conservation and breeding, and (iv) as a tool in crop improvement. SDS-PAGE is considered to be a practical and reliable method because seed storage proteins are largely independent of environmental fluctuations (Bertozo and Valls, 2001; Stoyanova and Boller, 2010; Takac et al., 2011).

The aim of the present study was to investigate the differentiation of 12 date palm cultivars grown in two regions, Bam and Jiroft in Iran by the numerical analysis of seed storage protein profiles obtained by using SDS-PAGE technique.

MATERIALS AND METHODS

Plant material

Twelve date palm cultivar seeds, pollinated with specific 'Mazafati' male cultivar were collected from two date cultivation regions in Iran namely Jiroft and Bam (Bam and Jiroft Agricultural Research Center) (Table 1). The seeds were stored in 8°C until extraction. The homogeneity of each accession was determined by using 10 seeds at the level of individual seed by SDS-PAGE independently (Ehsanpour et al., 2010). The comparison of cultivars was not performed until we were sure about the homogeneity of protein patterns for each cultivar.

Chemicals

All chemicals used were of the highest obtainable grade.

Table 1. List of the date palm cultivars analysis.

Parameter	Region	
	Jiroft	**Bam**
Cultivar (lable)	Bazmani sefid (Bw.Ji)	Bazmani sefid (Bw.Ba)
	Mahminai (Mah.Ji)	Mazafati (Ma.Ba)
	Gordial (Go.Ji)	Khorbak syah (Khs.Ba)
	Kharok (Kha.Ji)	Khosh kang (Khk.Ba)
	Almehtari (Al.Ji)	
	Mordar sang (Mo.Ji)	
	Kaluteh (Ka.Ji)	
	Halilehi (Hal.Ji)	

Acrylamide, bisacrylamide, TEMED, Tris base, SDS, were purchased from Merck Co. β-mercaptoethanol was obtained from Sigma Co. All other chemicals used were standard analytical pure reagents. Molecular weight (MW, 14. 2 × 10^3 to 205 × 10^3 Da) of protein markers for SDS-PAGE was purchased from Fermentas Life Sciences Company.

SDS-PAGE analysis

After cleaning and washing the seeds, individual seeds of 12 cultivars were grinded into a fine powder to extract the seed storage proteins. After defatted by washing with 10× n-Butyl alcohol (v/w) and dried by vacuum, 100 mg powder of each bulk seed was added into 1.5 ml plastic Eppendorf with 1 ml 0.22 mol/L Tris-HCI buffer (pH 6.8) extraction solution, mixed thoroughly and stored at 4°C overnight (Chanyou et al., 2006). The mixture was centrifuged at 15000 rpm for 10 min (angle rotor, 01420, 24-place, Boeco centrifuges U-320, Germany), and 200 µl of the supernatant was put in a new tube and was mixed with 800 µl (1:4, v/v) SDS-PAGE disruption mixture (125 mmol/L Tris HCl pH 6.8, 2.5% β-mercaptoethanol, 1% SDS, 5% glycerol, 0.01% bromophenol blue dye) (Laemmli, 1970). The sample Ependorfs was then heated for 5 min at 95°C. The storage protein sample solution with concentration of about 1.85 to 2 mg/ml was prepared for SDS-PAGE after centrifugation for 5 min (Howland, 1996). The stacking and resolving gel concentrations were maintained at 4% and 5 to 11% gradient, respectively. The electrophoresis was carried out under submerged mode using a tank buffer (0.025 M Tris pH 8.3, 0.192 M Glycine, 0.1% SDS) at a constant voltage (50 V at beginning and then 25 V) of 50 mA in a protein gel apparatus. The amount of sample was 50 µl. Gels were fixed with 10% (w/v) TCA solution, stained with 0.25% (w/v) Coomassie brilliant blue R250 and then de-stained with a solution containing 15 ml ethanol 15%, 175 ml acetic acid 5% and 60 ml distilled water till the background became transparent (Howland, 1996). Stock solutions were prepared before making a working solution. The gels were photographed using transilluminator system with a transparent white screen with a Cannon camera and kept wet during photography by addition of 5% acetic acid to avoid drying and trapping air bubbles.

Data analysis

The presence (1) and absence (0) of each band was considered in each cultivar analyzed. The binary data matrix was used to calculate Jaccard's similarity index. The genetic relationships among all cultivars analyzed were surveyed in three dendrograms obtained, respectively using UPGMA and NJ of Total Lab (TL) and ward minimum variance dendrogram according to distance of 1-Jaccard's similarity coefficient of SYSTAT software (Wilkinson, 2010). The standard curve, to calculate molecular weight for each band, was drawn by TotalLabTL software according to MW protein markers and their pixel positions on the gel with quadratic type curve. PCA was performed by computing the data correlation matrix with SPSS programs (ver.19) software.

RESULTS

The analysis of electrophoresis profile of seed storage proteins

The 12 cultivars (4 cultivars from Bam and 8 from Jiroft) showed 14 resolving bands, relative MW mainly ranging from 11.673 to 369 KDa, 11 of which had polymorphisms. Only one heavily stained band was shared by all cultivars (see region γ), its relative mobility (RF) value was 0.8 and MW was about 65 KDa (Figures 1and 2). The numbers of bands varied from 5 to 12 among cultivars, the 'Bazmani sefid' (Bam) and 'Kharok' (Jiroft) ones were observed to have the maximum bands whereas the 'Bazmani sefid'(Jiroft) ones were considered to have the minimum bands (Table 2). The protein bands ran as separate groups with distinct MW regions. For convenient description, the whole bands were classified into four groups designated as α (MW < 20 × 10^3), β (20 × 10^3 < MW < 50 × 10^3), γ (50 × 10^3 < MW > 120 × 10^3) and ω (MW > 120 × 10^3 Da) according to the MW protein markers (Table 2).

The diversity of electrophoresis profile of seed storage proteins among cultivars

The genetic diversity of cultivars was significant among 12 cultivars from Bam and Jiroft, the result of which is seen in Table 2. Bam cultivars band numbers were specifically found to be fewer than those of Jiroft cultivars at ω group; in addition, the difference in number of bands at ω group between Bam and Jiroft cultivars was observed to be different on the basis of their molecular weight. On the other hand, intense bands were mainly detected in 50 to 120 × 10^3 Da molecular weight (group γ). 'Bazmani sefid' and 'Halilehi' cultivars from jiroft exclusively lacked bands at ω group.

Table 2. SDS-PAGE seed protein patterns of 4 cultivars from Bam and 8 from Jiroft.

Number	Group (KDa)		Bam				Jiroft							
			Bw.Ba	Ma.Ba	Khs.Ba	Khk.Ba	Bw.Ji	Mah.Ji	Go.Ji	Kha.Ji	Al.Ji	Mo.Ji	Ka.Ji	Hal. Ji
1	α	1	1	1	1	1	1	1	1	1	1	1	1	1
2	(< 20)	2	1	0	1	1	1	1	1	1	1	1	1	1
3		1	1	1	1	1	0	1	1	1	1	1	1	1
4		2	1	1	1	0	0	1	1	1	1	1	1	1
5	β (20 - 50)	3	1	1	1	1	0	1	1	1	1	1	1	1
6		4	1	1	1	1	1	1	1	1	1	1	1	1
7		5	0	1	1	0	0	0	0	0	0	0	0	0
8		1	1	1	1	1	1	1	1	1	1	1	1	1
9	γ	2	1	0	1	0	0	1	1	1	1	0	1	0
10	(50 - 20)	3	0	1	0	0	1	0	0	1	0	1	0	1
11		4	1	1	1	1	0	1	0	1	1	1	1	0
12		1	1	0	0	0	0	0	0	0	0	0	0	0
13	ω (> 120)	2	1	0	0	0	0	1	1	1	1	1	1	0
14		3	1	1	1	1	0	1	1	1	1	1	1	0
Total number of protein bands			12	10	11	9	5	11	10	12	11	11	11	8

The protein bands revealed by the analysis are lumped into 4 groups of molecular weights (α < 20, β= 21 to 50, γ = 51 to 120, ω = 120 KDa) including the number of bands for every cultivar (1 = presence, 0 = absence).

'Bazmani sefid', 'Gordial', 'Halilehi'(Jiroft) and 'Khosh kang'(bam) cultivars included two bands at γ group whereas other cultivars included three and four bands (Figures 1 and 2).

The diversity of electrophoresis profile of seed storage proteins between Bam and Jiroft cultivars

The distribution of bands was identified to be distinctive between Bam and Jiroft cultivars. They could be identified by the No., MW, RF and OD, example a band of 166.283 to 169.908 × 10^3 Da at ω group in 'Bazmani sefid' and 'Khosh kang' could be observed but not in the other cultivars. Additionally, a distinguishable band (MW about 166.283 × 10^3 Da) of 'Bazmani sefid' (Bam) at ω group was a unique one that could be regarded as its 'fingerprint' (Figures 1 and 2).

The diversity of electrophoresis profile of seed storage proteins between Bam and Jiroft cultivars

The distribution of bands was identified to be distinctive between Bam and Jiroft cultivars. They could be identified by the No., MW, RF and OD, e. g. a band of 166.283 to 169.908 × 10^3 Da at ω group in 'Bazmani sefid' and 'Khosh kang' could be observed but not in the other cultivars. Additionally, a distinguishable band (MW about 166.283 × 103 Da) of 'Bazmani sefid' (Bam) at ◆ group was a unique one that could be regarded as its 'fingerprint' (Figures 1 and 2).

Cluster analysis

According to the statistical analysis of the data in terms of the presence (1) and absence (0) of each band, the Jaccard's similarity index between each cultivar was evaluated. They varied from 0.308 to 1.00%, with a mean of 0.654% (Table 3). UPGMA and NJ dendrogram based on 4 cultivars from Bam and 8 cultivars data from Jiroft (Figures 3 and 4) and ward minimum variance dendrogram according to distance of 1-Jaccard's similarity coefficient for binary data (Table 2) based on 12 cultivars were established (Figure 4). In Figures 3 and 4, the order and distance of cultivars in each region (Bam and Jiroft) was observed based on two methods of UPGMA and NJ. The ward minimum variance dendrogram displayed a single tree at 1.069 distance from all of the possibilities (Figure 5). Two major clusters at 0.7 distance were obtained. The first cluster at 0.375 distance with about 62.5% similarity includes 'Bazmani sefid' and 'Halilehi' Jiroft. The second cluster at 0.636 distance with 62.5% similarity includes 10 members in two subgroups at 0.341 and 0.305 distances. This cluster at 0.341 distance with about 65.9% similarity was made up of three cultivars: 'Mazafati', 'Khorbak syah' and 'Khosh kang'. At 0.305 distance with 69.5% similarity,

Table 3. List of Jaccard's Similarity Index of 12 cultivars according to the data matrix of Table 2.

Parameter	Bw.Ba	Ma.Ba	Khs.Ba	Khk.Ba	Bw.Ji	Mah.Ji	Go.Ji	Kha.Ji	Al.Ji	Mo.Ji	Ka.Ji	Hal.Ji
Bw.Ba	1.000											
Ma.Ba	0.571	1.000										
Khs.Ba	0.769	0.750	1.000									
Khk.Ba	0.667	0.636	0.727	1.000								
Bw.Ji	0.308	0.364	0.333	0.444	1.000							
Mah.Ji	0.917	0.615	0.833	0.727	0.333	1.000						
Go.Ji	0.833	0.538	0.750	0.636	0.364	0.909	1.000					
Kha.Ji	0.846	0.692	0.769	0.667	0.417	0.917	0.833	1.000				
Al.Ji	0.917	0.615	0.833	0.727	0.333	1.000	0.909	0.917	1.000			
Mo.Ji	0.769	0.750	0.692	0.727	0.455	0.833	0.750	0.917	0.833	1.000		
Ka.Ji	0.917	0.615	0.833	0.727	0.333	1.000	0.909	0.917	1.000	0.833	1.000	
Hal. .Ji	0.538	0.636	0.583	0.600	0.625	0.583	0.636	0.667	0.583	0.727	0.583	1.000

seven members at two sub-clusters were identified.

The first subcluster at 0.083 distance with 91.7% similarity included two cultivars 'Kharok' and 'Mordar sang' Jiroft and the remaining cultivars studied constitute the second sub-cluster. Within the second subcluster, two subgroups were distinguished with 100% similarity. The first subgroup included one cultivar 'Gordial' Jiroft, but the other cultivars of second subgroup formed two well-defined clusters with 100% similarity; three from Jiroft: 'Kaluteh', 'Mahminai' and 'Almehtari', and one from Bam: 'Bazmani sefid' (Figure 5). With respect to the last subgroup, 'Bazmani sefid' Bam had differences from other Bam cultivars (average 66.9% similarity) and 'Bazmani sefid' Jiroft (30.8% similarity); whereas, 'Bazmani sefid' Bam showed the most similarity (91.7%) with 'Kaluteh', 'Mahminai' and 'Almehtari' Jiroft (Table 3). The similarity can be explained in term of the populations that appear to have been partly mixed.

Principal component analysis

To evaluate and analyze the data more precisely, a PCA was implemented. The results summarized in Table 4 shows that the three first components represented 82.514% of the total variability. The first component explained 53.216%, the second component 18.729% and the third component 10.57% of total variation. Figure 6 demonstrates the distribution of the cultivars according to the first three components (1, 2 and 3). The results reveal that cultivars were randomly aggregated in the plot, and they suggested that a typical continuous genetic diversity characterizes the Bam and Jiroft date palm cultivars.

Principal component analysis applying correlation matrices among the characters lead to the production of 6 distinct groups (Figure 6). The distribution of cultivars among these groups were characterized as follows: 'Bazmani sefid (Bw.Ji)' and 'Halilehi (Hal.Ji)' were listed in group 1; 'Gordial (Go.Ji)', 'Kaluteh (Ka.Ji)', 'Almehtari (Al.Ji)' and 'Mahminai(Mah.Ji)' were placed in group 2; 'Bazmani sefid (Bw.Ba)' was a member of group 3; 'Kharok(Kha.Ji)' and 'Mordar sang(Mo.Ji)' were categorized in group 4; 'Mazafati (Ma.Ba)' and 'Khorbak syah (Khs.Ba)' were placed in group 5, and finally 'Khosh kang (Khk.Ba)' was the only one listed in group 6 (Figure 6).

The dendrogram resulting from the ward's method clustering of jacquard's similarity matrix was presented in Figure 5. Similar types of grouping with PCA analysis were also obtained since we observe 2 members, 'Bazmani sefid' and 'Halilehi' from Jiroft in group 1 at 0.375 distance, and 5 other members, 4 of which being from group 2, 'Gordial', 'Kaluteh', 'Almehtari', 'Mahminai' from Jiroft, at 0.151 distance and finally there is 1 member being from group 3 from Bam district, 'Bazmani sefid'. A total of 14 band positions were identified in the 12 studied cultivars. These bands descriptions enabled the evaluation of wide protein polymorphism in cultivars. The frequency of individual bands was found to be variable. Groups of bands with very low occurrence in some cultivars were found (bands 10 and 13). In contrast to these bands, other bands with 100% frequency were also found (bands 1, 2 and 4) (Table 2). Therefore, the bands were evaluated with the 2-dimensional scatter gram of PCA in order to estimate their effectiveness in identifying cultivars (Figure 7 and Table 6).

DISCUSSION

Studying genetic variation has always been regarded essential to understanding gene pool, to guide in order to collect germplasm and to breed new fine cultivars. It is also necessary to identify affinities and similarity of plants for more comprehensive comparison of cultivars. Different methods such as protein and DNA molecular markers, or cytogenetical

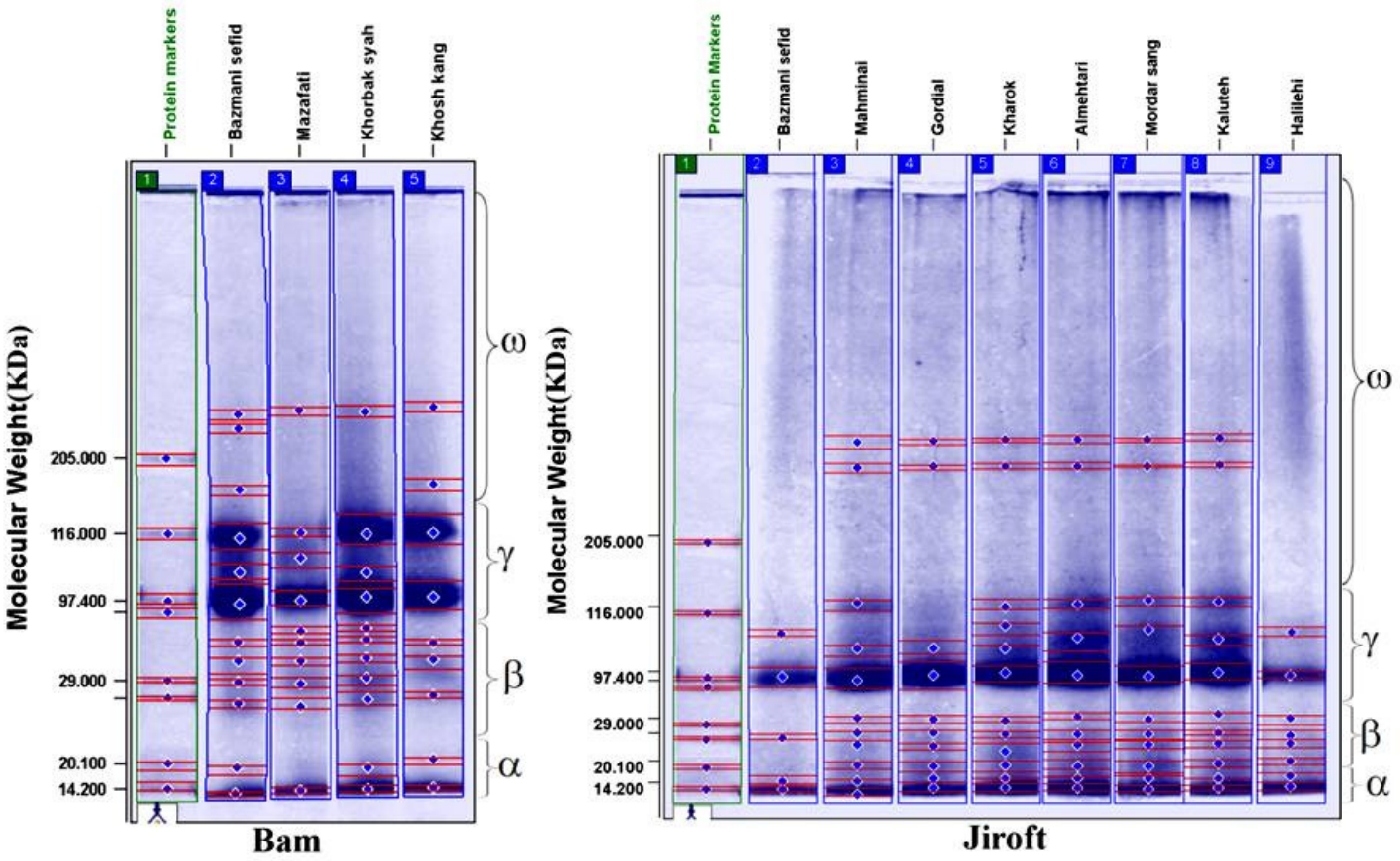

Figure 1. The electrophoresis profile of seed storage proteins of 4 cultivars from Bam (lane 2-5), 8 cultivars from Jiroft (lane 2-5) and protein markers (lane 1) (α-Lactalbumin =14.2, Trypsin inhibitor = 20.1, Trypsinogen = 24, Carbonic anhydrase = 29, Glyceraldehyde-3 phosphate dehydrogenase = 36, Phosphorylase B = 97.4, β-Galactosidase =116, Myosin Rabbit=205 KDa).

and biochemical, or morphological properties have been used for the analysis of genetic variations and cultivars identification (Collard and Mackill, 2008; Jaradat and Shahid, 2006; Malik et al., 2009; Miernyk and Hajduch, 2011; Pettengill and Neel, 2008; Tavaud-Pirra et al., 2009). As the major purpose of the present study, we have tried to deeply observe some Iranian date palm cultivars. This can assist the researchers in obtaining a better viewpoint about identification and classification of some Iranian date palm cultivars. In this way, we hope to resolve some of the existing arguments and confusion in the field. To fulfill this goal, we implemented SDS-PAGE analysis to study seed storage proteins of the investigated date palm cultivars.

As it was mentioned previously, 'Mazafati' and 'Bazmani sefid' cultivars are very important crops in Bam and 'Bazmani sefid' and 'Halilehi' in Jiroft. They have economic and commercial importance in the two regions of Bam and Jiroft. We came to the strong conclusion that the genetic variation among cultivars base on the seed storage proteins exhibit great divergence among cultivars. It was moreover concluded that cultivars of Bam bore the largest variation, although their Jaccard's similarity index was estimated to be only 0.571. Genetic variation within Jiroft cultivars was not so striking but distinguishable between cultivars. As a part of the study's achievement was the idea that SDS-PAGE analysis of seed storage proteins was a suitable procedure in order to identify plant genetic diversity and polymorphism (Caballero et al., 2009; Emre, 2009; Juan et al., 2007; Tamkoc and Arslan, 2010). Our results reveal that seed storage protein patterns were consistent with the cultivars and were also distinguishable among the studied cultivars. The difference among seed storage protein patterns demonstrated the differences that could be observed in the origin, evolution, and their genome (Takac et al., 2011). Hence, it can be argued that the differences earlier discussed lead to the gene expression products (Liu et al., 2010).

Despite wide variation in their detailed structures, all seed storage proteins have a number of common properties. First, they are synthesized at high levels in specific tissues and at certain stages of development. In fact, their synthesis is regulated by nutrition, and they act as a sink for surplus nitrogen. However, most also contain cysteine and methionine, and adequate sulfur is therefore also required for their synthesis. Many seeds contain separate groups of storage proteins, some of which are rich in sulfur amino acids and others of which are poor in them. The presence of

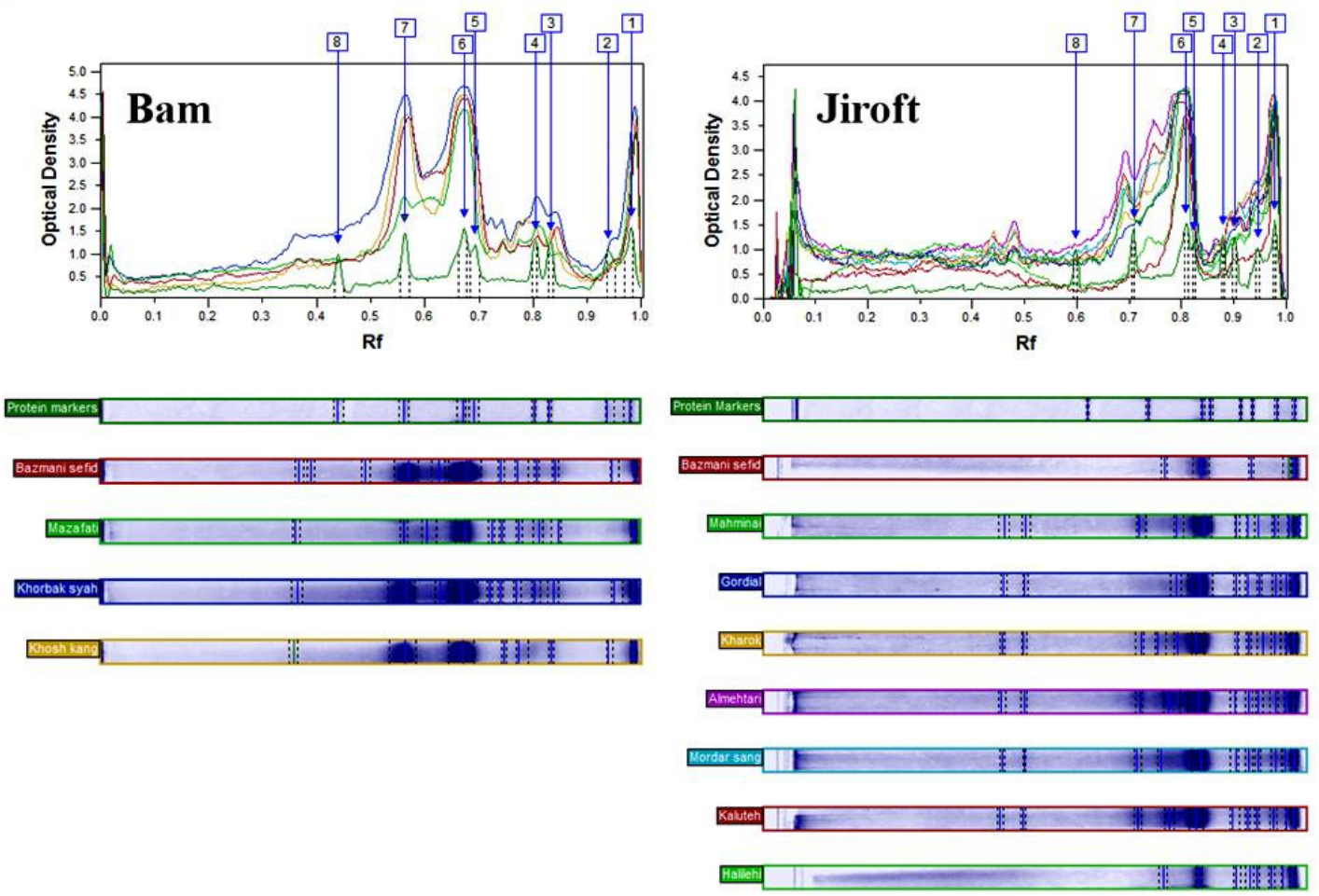

Figure 2. Scanning of SDS-PAGE gel of the total protein of date palm seeds from Bam and Jiroft.

these groups may allow the plant to maintain high levels of storage protein synthesis despite variations in sulfur availability. A second common property of seed storage proteins is their presence in the mature seed in discrete deposits called protein bodies, whose origin has been the subject of some dispute and may in fact vary both between and within species. Finally, all storage protein fractions are mixtures of components that exhibit polymorphism both within single genotypes and among genotypes of the same species. This polymorphism arises from the presence of multigene families and, in some cases, proteolytic processing and glycosylation (Miernyk and Hajduch, 2011; Shewry et al., 1995).

It was concluded that eight Jiroft cultivars used in this study were almost similar to each other; on the contrary, cultivars from Bam exhibited striking genetic polymorphism. Some cultivars could be distinguished from each other as well, example cultivar 'Bazmani sefid' (Jiroft) did not display four bands at β and no band at ω group compared to cultivar 'Halilehi', which did not display any bands at ω but had four bands at β group. 'Gordial' cultivar displayed two bands at ɣ but had two bands at ω group compared to cultivar 'Halilehi', which did not display any bands at ω group (Figures 1 and 2).

It is important to note that PCA indicated that different sets of cultivars displayed high loadings in clustering date palm cultivars in three components (Table 5). 'Bw.Ba', 'Khk.Ba', 'Mah.Ji', 'Go.Ji', 'Kha.Ji', ' Al.Ji', 'Mo.Ji' and 'Ka.Ji' had high loadings on the first component, 'Ma.Ba', 'Bw.Ji' and 'Hal.Ji' on the second and 'Khs.Ba' was the cultivar showing high loadings on the third component. When the bands were imposed on the 2-dimensional scatter gram (Figure 7), only the bands 4 and 11, which were highly loaded on the fifth component, put the least effect on the classification of these cultivars. We came to the result that the bands number 1, 2, 3, 5, 7, 8, 10, 12 and 14 belonging to the first component and the bands 9 and 13 belonging to the second component left their most effects on the classification of these date palm cultivars (Table 6).

Finally, it can be suggested that the cultivars with similar banding patterns are proposed to be studied for detailed biochemical and molecular analyses including DNA markers and 2-D electrophoresis. This can be

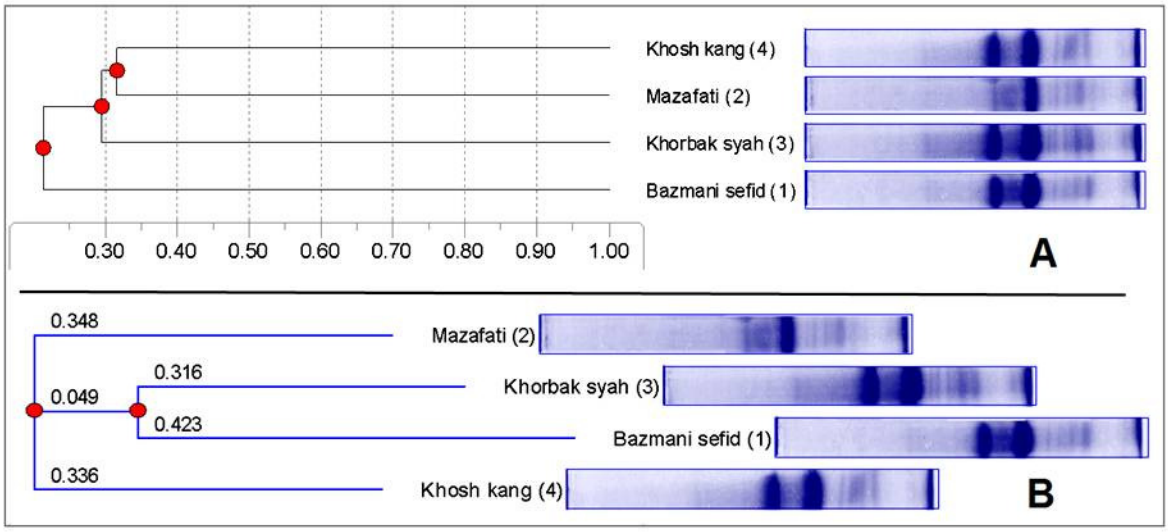

Figure 3. UPGMA (up) and NJ (down) dendrogram showing the genetic relationships of Bam cultivars obtained using electrophoresis pattern of seed storage proteins.

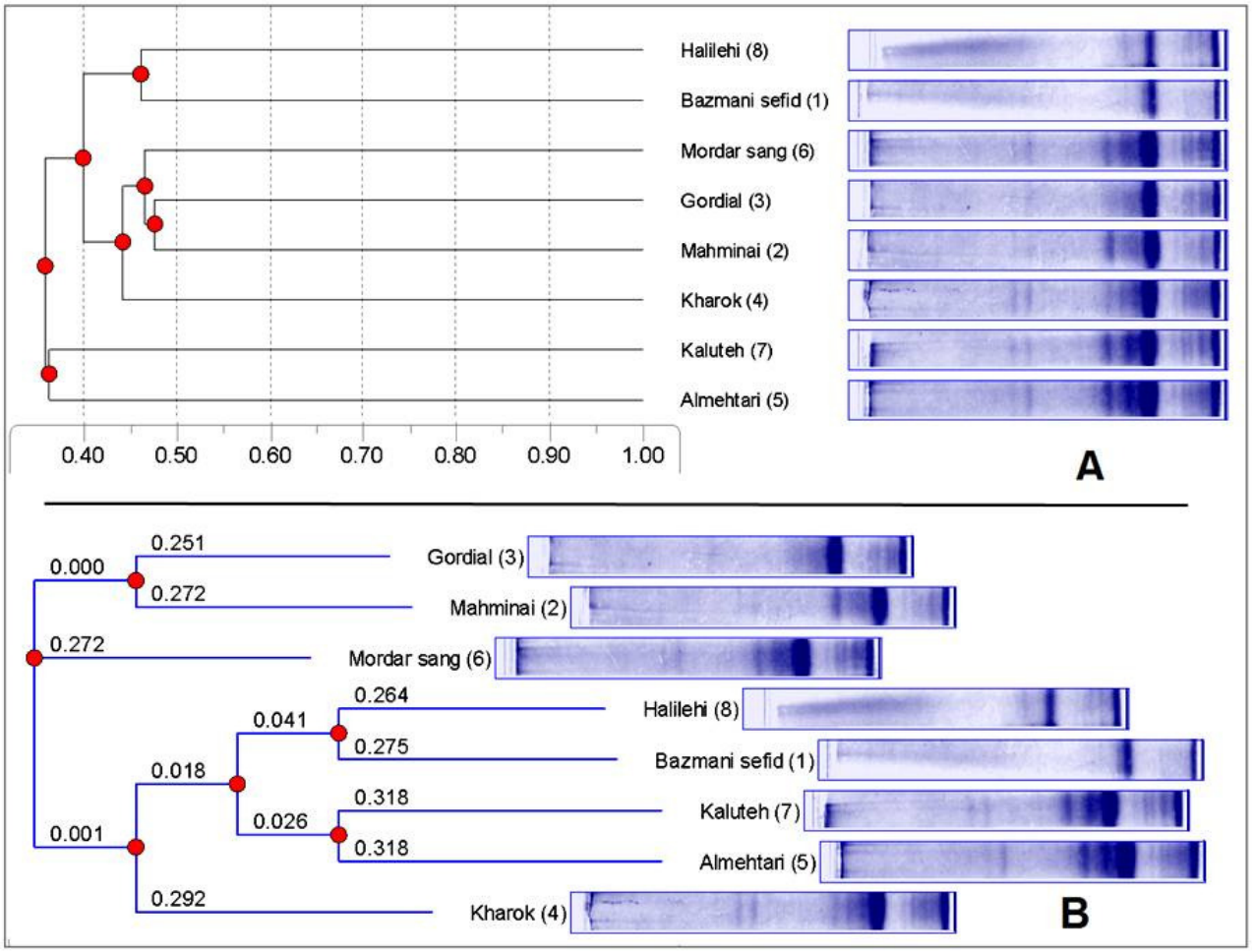

Figure 4. UPGMA (up) and NJ (down) dendrogram showing the genetic relationships of Jiroft cultivars obtained using electrophoresis pattern of seed storage proteins.

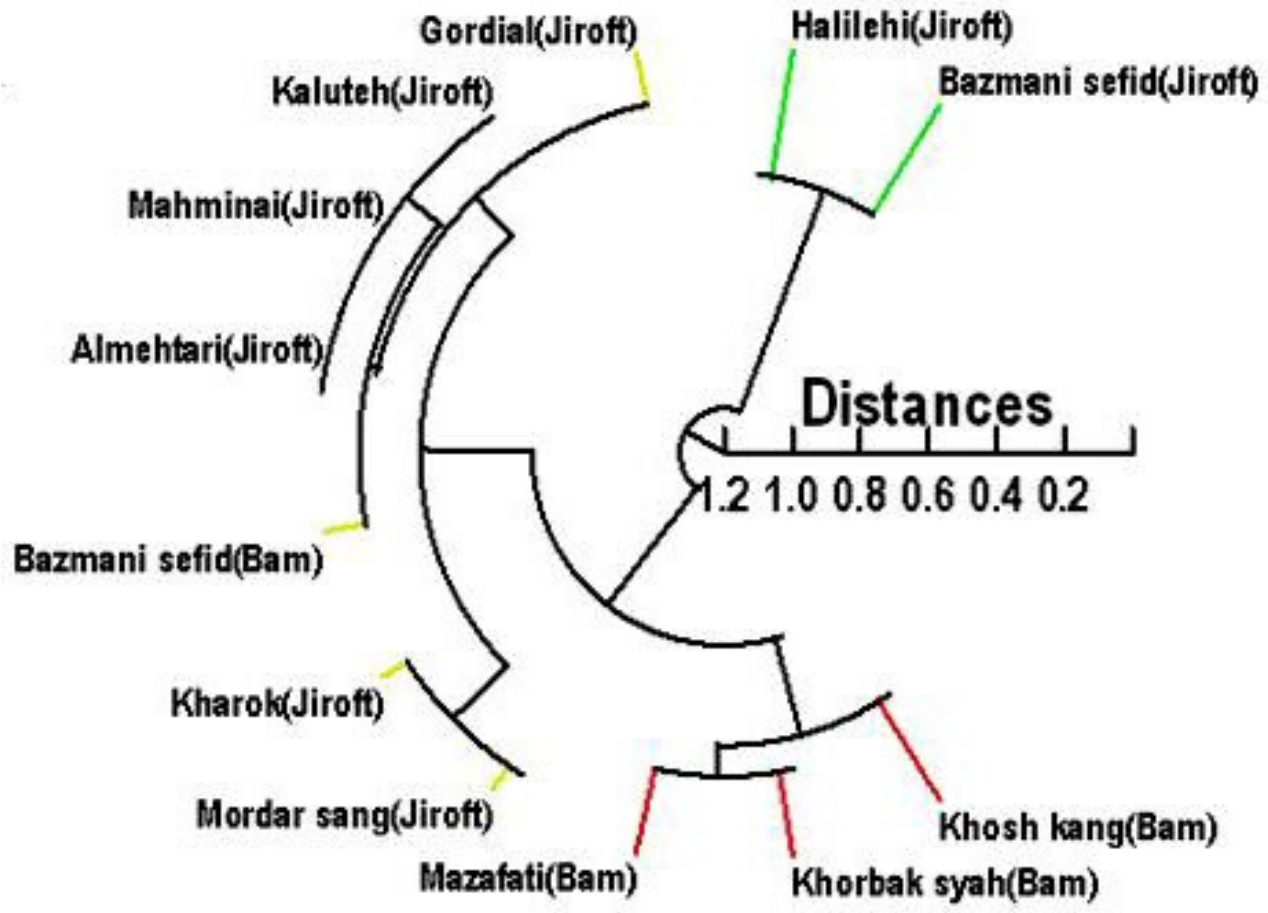

Figure 5. The ward minimum variance dendrogram according to distance of Jaccard's similarity coefficient for binary data.

Table 4. Total eigenvalues of 12 components, percent of variance and cumulative percent variation explaining each component and the first three components of the PCA base on SDS-PAGE seed protein patterns of 8 cultivars from Jiroft and 4 from Bam.

Component	Initial Eigen values			*Extraction sums of square loadings		
	Total	% of Variance	Cumulative %	Total	% of variance	Cumulative %
1	6.386	53.216	53.216	6.386	53.216	53.216
2	2.247	18.729	71.945	2.247	18.729	71.945
3	1.268	10.570	82.514	1.268	10.570	82.514
4	0.765	6.372	88.886			
5	0.564	4.700	93.586			
6	0.374	3.113	96.699			
7	0.160	1.334	98.033			
8	0.134	1.114	99.146			
9	0.081	0.676	99.823			
10	0.021	0.177	100.000			
11	$1.754E^{-16}$	$1.461E^{-15}$	100.000			
12	$2.468E^{-17}$	$2.056E^{-16}$	100.000			

* Extraction method: Principal component analysis.

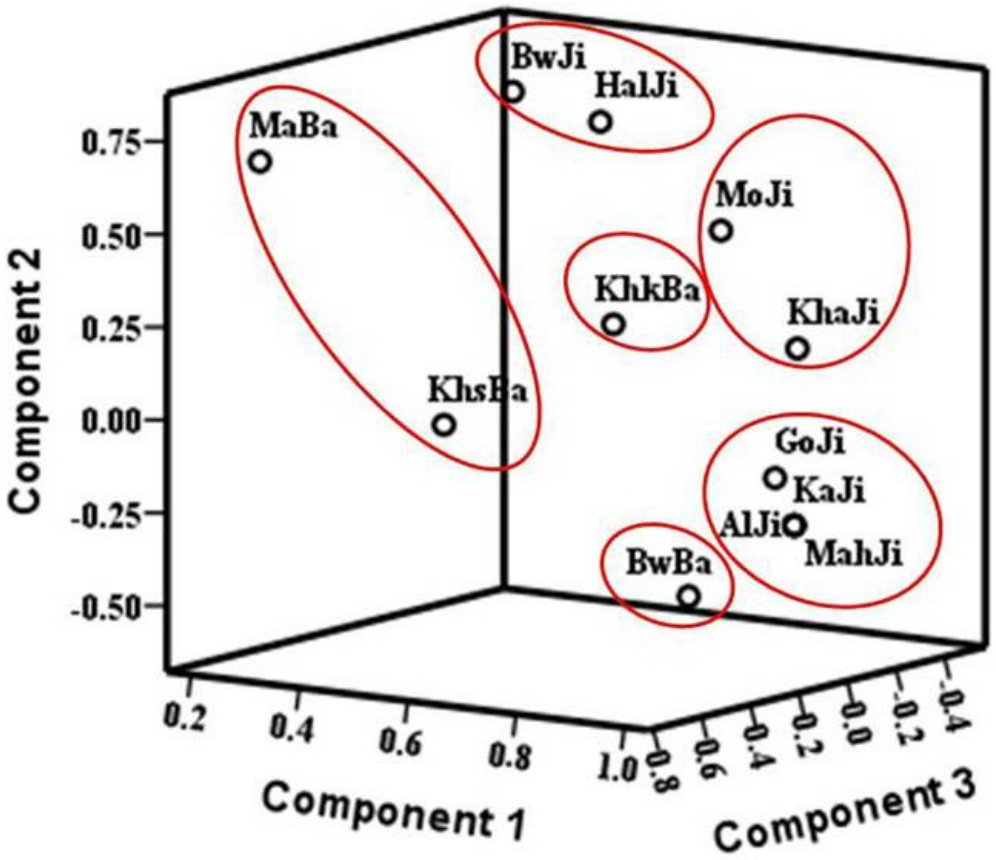

Figure 6. Plot of 12 cultivars date-palm genotypes according to the three first components of the PCA based on SDS-PAGE seed protein patterns (Table 2).

Table 5. The variation provided by the first three components of the PCA base on SDS-PAGE seed protein patterns of 12 cultivars.

Parameter	Component 1	Component 2	Component 3
Bw.Ba	**0.495***	0.279	0.011
Ma.Ba	0.051	**0.451***	0.351
Khs.Ba	0.361	0.002	**0.451***
Khk.Ba	**0.543***	0.074	0.077
Bw.Ji	0.055	**0.483***	0.185
Mah.Ji	**0.912***	0.074	0.000
Go.Ji	**0.688***	0.040	0.033
Kha.Ji	**0.697***	0.019	0.069
Al.Ji	**0.912***	0.074	0.000
Mo.Ji	**0.521***	0.198	0.039
Ka.Ji	**0.912***	0.074	0.000
Hal.Ji	0.239	**0.480***	0.050

* Values in bold correspond for each variable to the component for which the squared cosine is the largest.

performed for better management of preserving genetic material. Another option could be the use of gradient gels or larger scale gels, for finer analysis of genetic diversity.

ACKNOWLEDGEMENT

This work was done in International Center for Science,

Table 6. Squared cosines of the observations (bands) (Table 2) of 5 components explained by each component of the PCA.

Band	Component 1	Component 2	Component 3	Component 4	Component 5
Band 1	**0.637***	0.232	0.003	0.087	0.005
Band 2	**0.377***	0.004	0.211	0.264	0.017
Band 3	**0.657***	0.008	0.099	0.018	0.004
Band 4	0.253	0.006	0.006	0.097	**0.395***
Band 5	**0.657***	0.008	0.099	0.018	0.004
Band 6	**0.637***	0.232	0.003	0.087	0.005
Band 7	**0.811***	0.008	0.142	0.010	0.015
Band 8	**0.637***	0.232	0.003	0.087	0.005
Band 9	0.002	**0.712***	0.002	0.049	0.124
Band 10	**0.447***	0.402	0.089	0.045	0.004
Band 11	0.052	0.028	0.282	0.186	**0.284***
Band 12	**0.813***	0.072	0.016	0.019	0.041
Band 13	0.000	**0.420***	0.373	0.165	0.000
Band 14	**0.349***	0.108	0.223	0.105	0.042

*Values in bold correspond for each case to the component for which the squared cosine is the largest.

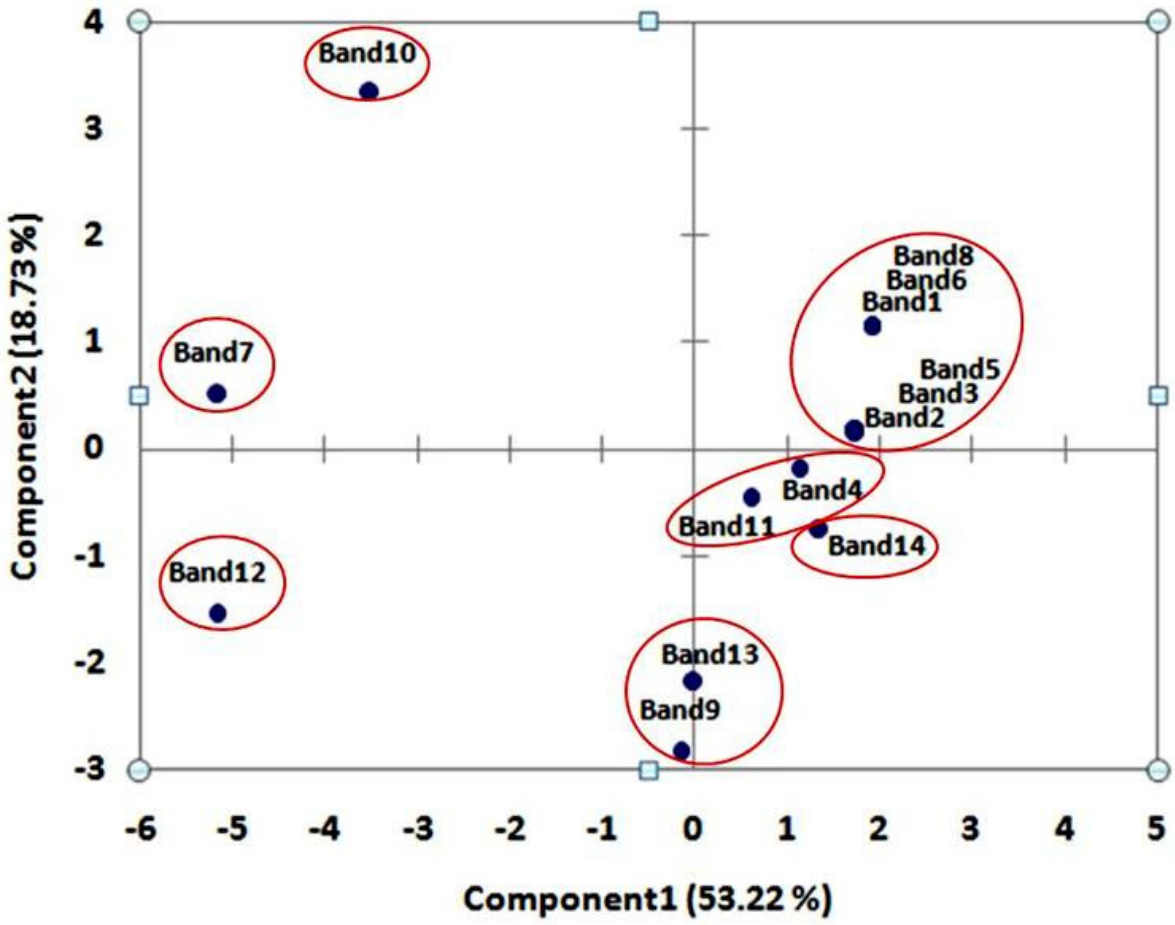

Figure 7. Scatter diagram of 14 bands according to the two first components of the PCA based on SDS-PAGE seed protein patterns (Table 2).

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