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Full Length Research Paper

Characterization of *Quercus* species distributed in Jordan using morphological and molecular markers

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Genetic diversity among 25 natural populations of three different species of *Quercus* in Jordan at morphological and molecular levels using random amplified polymorphic DNA (RAPD) primers was assessed. Significant morphological and molecular variations among and within 25 *Quercus* populations were estimated. Standardized canonical discrimination functions for the investigated morphological traits showed that the first function explains 72.44% of the total variability between populations and was strongly influenced by leaf and scale length. Twenty-seven polymorphic markers and 5917 scored bands were generated using six RAPD primers. Based on morphological and RAPD data, the populations were grouped together in the same cluster according to species regardless of local of collections. Result of cluster analysis based on RAPD showed significant correlation with morphological characters based on Mantel's test (r = 0.145). This study has emphasized the ability of the morphological and molecular markers in determining the genetic diversity among and within the populations of *Quercus* and that the resulted high genetic variability could be utilized in implications of improving conservation, restoration and reforestation strategies of *Quercus* in Jordan.**

Key words: *Quercus* spp., genetic diversity, random amplified polymorphic DNA (RAPD) markers, conservation, restoration.

INTRODUCTION

Quercus L. (oak) is one of the exceptionally important woody genera worldwide. It is a large genus in the family Fagaceae with about 600 species growing in a wide range of habitats and distributed in temperate and subtropical regions of the northern hemisphere (Shrestha, 2003). Members of the genus grow as shrubs and trees and form prominent deciduous forests or evergreen woodlands with a range of distribution extending from cold latitudes to tropical Asia and the Americas (Manos et al., 1999). The morphology of oak is strikingly diverse, reflecting a tremendous variation in the genetic makeup within and among *Quercus* populations (Bacilieri et al., 1996; Elena-rossello et al., 1993; Johnson et al., 2001; Kremer and Petit, 1993; Zanetto and Kremer, 1995; Bruschi et al., 2000; Dodd and Kashani, 2003). As a result, the genus has received considerable attention for research studies of genetic diversity, population genetic structure and management of forest ecosystems. The amount of genetic variation within and among populations has been proposed to be correlated with the life and migration history characteristics along with geo-climatic forces (Kremer et al., 2002; Petit et al., 2002; Pizzurro et al., 2010; Valero et al., 2011a, b). Degradation of *Quercus* biodiversity in the wild is now a matter of global concern. Increased land colonization, changes in land use, over-cutting, over-grazing and climatic factors are all contributing factors to destruction of *Quercus* vegetation and continuing threat of extinction to many species of the genus. Thus, efforts should be made in order to protect *Quercus* biodiversity mainly through restoration and rehabilitation programs. To achieve these tasks, investigating levels of genetic variation within species and

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among populations of *Quercus* is an essential first step.

In Jordan, the genus constitutes an important component of the forest ecosystems in the Mediterranean topographic zone. Three species of the genus are known to occur naturally in this region with a general range of distribution extending from Aum Qais in the North to Tafilah in the South. These species are *Quercus calliprinos* L., *Quercus ithaburensis* and *Quercus infectoria* (Kasapligil, 1956; Long, 1957; Zohary, 1961, 1962, 1973). *Q. calliprinos* is the most widespread and evergreen species; distributed throughout the Mediterranean region from Ajloun in the North through Salt and Fuhais to Tafilah and Shobak in the south. *Q. infectoria* is the least abundant and deciduous species; restricted largely to the northern parts of the country. *Q. ithaburensis* is intermediate in terms of distribution and abundance; it forms deciduous forests in the northern and middle parts of the country, mainly around cities of Irbid, Jarash, Salt and Fuheis. To date and despite published research work on *Quercus* from the west, bank information on levels of genetic diversity within and among populations of *Quercus* species in Jordan per se is lacking. Knowledge of the genetic variation of this important genus provides a robust framework for follow-up systematic studies and facilitates its use in genetic conservation and rehabilitation. In addition, this information will help understand the dynamics of the population genetics of *Quercus*, its evolutionary trends and its responses to changes in the environment.

In Israel, the *Quercus boissieri* Reut. is an associated species within the *Q. calliprinos–Pistacia palestine* association of the Mediterranean sclerophyllous broad-leaf forests (Schiller et al., 2004a). The small genetic distances among and within populations of *Q. boissieri* in Israel revealed the use of random amplified polymorphic DNA (RAPD) on one hand and the lack of aggregations or relatedness to geographic parameters on the other hand, indicating that all the populations analyzed are remnants of a larger population that existed during the last glaciations (Schiller et al., 2004b). Liphschitz and Waisel (1967) indicated that all populations examined in Israel, Cyprus or Turkey were very variable and in every stand, a few form was distinguished. Also, dendro-climatological studies of *Q. boissieri* revealed were high within population diversity in the response of the annual-ring growth parameters to temperature but not to rainfall variations. In this study, the classical technique of RAPD and resources of morphology were employed to investigate for the first time levels of genetic and morphological diversity of natural populations of the genus *Quercus* in Jordan.

MATERIALS AND METHODS

Sampling strategy

Twenty five natural populations of three different Jordanian *Quercus* species (Table 1) were surveyed during October, November and December of 2008. The locations were selected according to population trees density. Populations having more than fifty trees were selected and samples were collected. Fifteen trees (samples) were randomly selected from each population to represent the *Quercus* population. Samples were identified cautiously and taxa names were confirmed following Zohary (1962, 1966). Fresh leaf samples were stored at -20°C for DNA extraction and vouchers from each studied tree were prepared, preserved and kept in the herbarium of the Department of Biological Science, Yarmouk University.

Morphological characterization

The following characters were measured and recorded for the morphological characterization: Leaf length (cm), leaf width (cm), petiole length (cm), fruit length (cm), fruit width (cm), cupule length (cm), cupule width (cm), scale length (cm) and scale width (cm) using vernier scale and ruler.

Molecular characterization

For molecular study, leaves collected from each individual tree were manually ground in liquid nitrogen with a mortar and pestle, to a fine powder and 100 mg placed in a 1.5 ml micro centrifuge tube and suspended in 200 µl of TE buffer.

Genomic DNA was extracted following Genomic DNA Purification Kit (www.thermoscientific.com/fermentas) protocol. A 400 µl lysis buffer was added and the whole mixture was incubated at 65°C for 5 min, immediately 600 µl of chloroform was gently added to the tube, then the contents were mixed by inverting the tube 3 to 5 times, then centrifuged at 10,000 rpm for 5 min. The upper aqueous phase containing DNA was transferred to a new tube then 800 µl of precipitation solution was added and mixed gently by several inversions at room temperature for 1 to 2 min, then centrifuged at 10,000 rpm for 2 min. The supernatant was completely removed and the DNA pellet was dissolved in 100 µl of 1.2 M NaCl solution by gentle vortexing. 300 µl of cold ethanol was added to precipitate the DNA and for maximum precipitation, the samples were stored at -20°C for 10 min, then the samples were centrifuged at 10,000 rpm for 3 to 4 min. The pellet was washed once with 70% cold ethanol. The DNA was dissolved in 100 µl of TE buffer (10:1 Tris EDTA). The dissolved DNA was treated with RNase (10 µg/ml) at 37°C for 30 min and then the DNA samples were stored at -20°C until use.

Random amplified polymorphic DNA (RAPD)

Six DNA samples were tested using 60 primers from Operon kits A, B and D (Operon Technologies, Inc. Alameda, USA). Primers that amplified consistently reproducible polymorphisms were selected and used to analyze all the 25 *Quercus* populations. Only six RAPD primers were used to amplify the 25 populations (Table 2). RAPD reactions were performed in total volume of 15 µl containing 1.0 unit Taq-polymerase, 50 mM Tris-HCl pH 9.1, 3.5 mM $MgCl_2$, 200 µM dNTP (Sigma), 150 µg/ml BSA, 50 ng primer (Operon kits A, B, D) and 30 ng DNA. DNA amplification was performed using G-STROM thermal cycler programmed for initial denaturation at 95°C for 1 min, followed by 40 cycles each of 94°C denaturing for 1 min, at annealing temperature of 35°C for 1 min, then DNA elongation at 72°C for 1 min, and a final synthesis step at 72°C for 5 min, then the reaction tubes were kept at 4°C (Sambrook et al., 1989). The amplification products were loaded using 1.8% agarose gel electrophoresis at 100 V for 2 h using horizontal gel electrophoresis apparatus. The amplified products were visualized and documented by gel documentation system (Vilber Lourmat, IP-010-SD, France). 100 bp ladders were used as DNA marker to estimate the molecular weights of the amplified products.

Table 1. Latitude, longitude and elevation for the *Quercus* populations.

Population	Specie	Latitude	Longitude	Elevation (m)
Beraldbagat	*Q. calliprinos*	30°30'0.89"N	35°30'18.63"E	1345
Rashadia	*Q. calliprinos*	30°38'20.32"N	35°38'15.47"E	1332
Heisha	*Q. calliprinos*	30°30'44.07"N	35°30'29.59"E	1450
Dana	*Q. calliprinos*	30°38'20.09"N	35°36'55.17"E	1168
Achtifina	*Q. calliprinos*	32°21'38.17"N	35°46'32.34"E	1050
Ebien(1)	*Q. calliprinos*	32°21'59.81"N	35°49'20.39"E	1110
Enbah	*Q. calliprinos*	32°27'55.24"N	35°46'3.25"E	700
Anjara	*Q. calliprinos*	32°18'32.30"N	35°44'32.40"E	785
Rahaba	*Q. calliprinos*	32°24'33.98"N	35°49'15.74"E	1055
Fuhies	*Q. calliprinos*	31°59'3.31"N	35°47'24.96"E	990
Gelad	*Q. calliprinos*	32° 4'26.73"N	35°45'54.63"E	920
KufurHuda	*Q. calliprinos*	32° 6'54.56"N	35°42'6.59"E	590
Zobia	*Q. boissieri*	32°24'11.53"N	35°49'2.57"E	1045
Bargash	*Q. boissieri*	32°22'26.24"N	35°46'45.64"E	1010
Ebien (2)	*Q. boissieri*	32°21'59.81"N	35°49'20.39"E	1110
Ashah	*Q. ithaburensis*	32°43'22.81"N	35°45'44.68"E	350
Umm Qaiss	*Q. ithaburensis*	32°39'30.96"N	35°41'11.35"E	310
Aossra	*Q. ithaburensis*	32°22'57.39"N	35°41'36.36"E	438
JaninAssafa	*Q. ithaburensis*	32°31'30.04"N	35°42'36.62"E	404
Aqraba	*Q. ithaburensis*	32°43'33.50"N	35°49'5.70"E	350
Kufour Kifya	*Q. ithaburensis*	32°30'41.25"N	35°45'25.67"E	515
Makhraba	*Q. ithaburensis*	32°34'43.37"N	35°40'25.22"E	297
Alouk	*Q. ithaburensis*	32° 9'20.29"N	35°55'19.82"E	740
Jobbah	*Q. ithaburensis*	32°10'2.60"N	35°53'24.23"E	800

Table 2. The features of RAPD primers selected in *Quercus* genetic diversity.

Primer	Sequence (5'-3')	Total markers[a]	Average bands[b]	Polymorphic markers[c]	Size range (Bp)	Total number of bands[d]
OPA12	TCGGCGCTCG	4	43.4	4	280-700	1086
OPA17	GACCGCTTGT	5	42.0	5	250-750	1050
OPA19	CAAACGTCGG	4	35.5	4	300-790	887
OPA20	GTTGCGATCC	5	47.9	5	250-680	1197
OPB05	TGCGCCCTTC	4	35.5	4	400-780	887
OPD17	TTTCCCACGG	5	32.4	5	380-750	810
Total		27		27		5917
Average		4.5	39.4	4.5		986.2

[a]Total number of differently sized RAPD markers amplified across all 25 populations, [b]average number of RAPD bands scored per population, [c]total number of RAPD markers found to be polymorphic across the 25 populations, [d]total number of RAPD bands (data points) scored for all populations.

Statistical analysis

Analysis of variance (ANOVA) was first performed to test (population) the measured characters. For characters with significant F-values, means were separated using Student's least significance difference (LSD), mean separation test. ANOVA analysis and mean separation were analyzed using Statistical Package for the Social Sciences (SPSS 15.0) software (SPSS Inc., 2006, Chicago). Data were then subjected to discriminate multivariate analysis to investigate the separate ability of these populations based on their morphological characters. Discriminate analysis was performed using Statistical Package for the Social Sciences (SPSS 15.0) soft ware (SPSS Inc., 2006, Chicago).

Data generated from RAPD analysis were analyzed using Jaccard similarity coefficients (Jaccard, 1908). These similarity coefficients were used to construct dendrograms using the unweighted pair group method with arithmetic average (UPGMA) employing SAHN (sequential, agglomerative, hierarchical and nested clustering) using the NTSYSpc (ver.2.10) program (Rohlf, 2005). Then, a simple Mantel's test (Mantel, 1967) was computed to test the

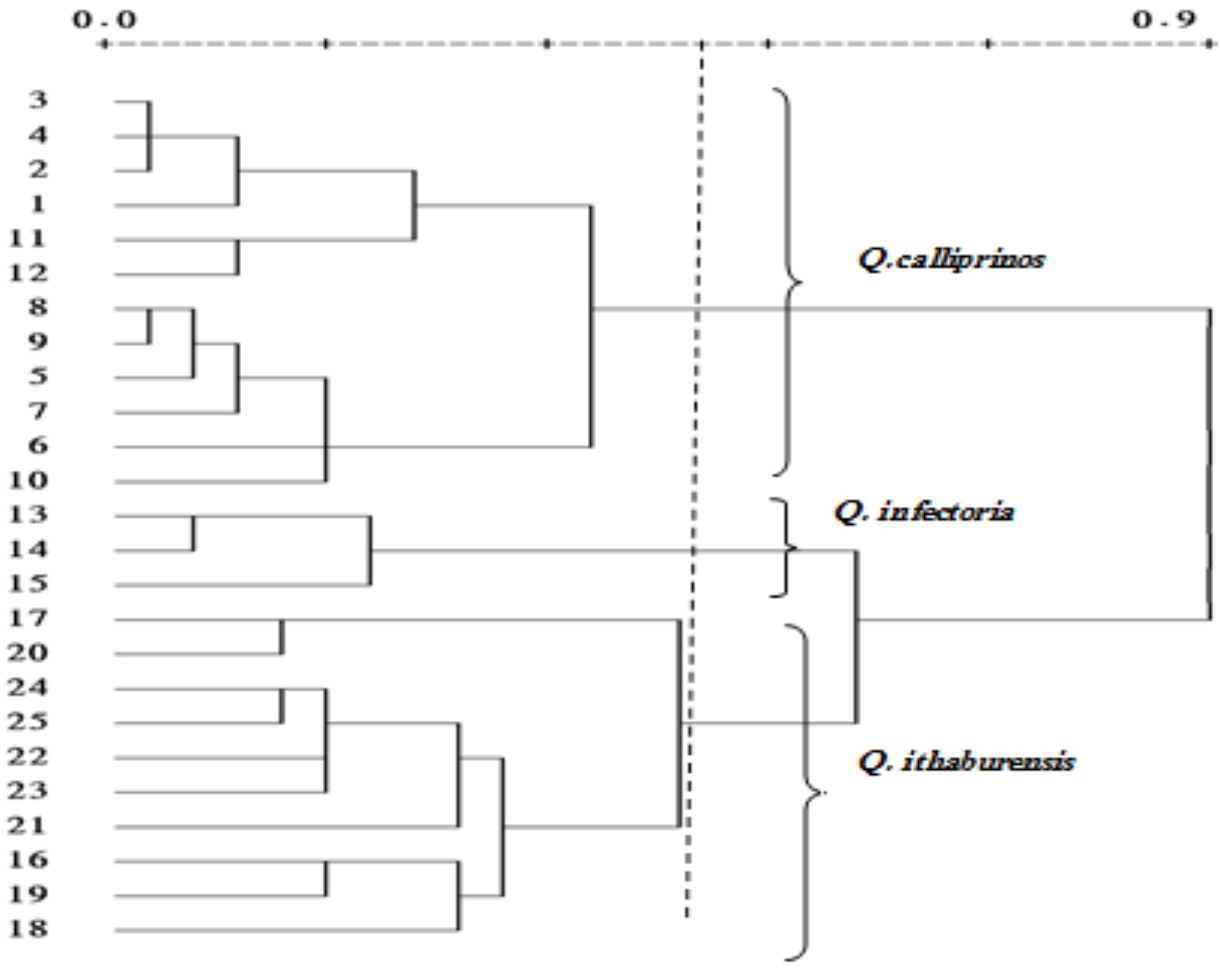

Figure 1. Hierarchical cluster of the twenty five populations of *Quercus* spp. in Jordan performed on the basis of morphological characters; 1 =Ber Adbagat, 2 = Al-Rashadia, 3 = Heisha, 4 = Dana,5 = Achtifina, 6 = Ebein, 7 = Enbeh,8 = Anjara, 9 = Rahaba,10 = Fuheis,11 = Gelad, 12 = Kufour Houda, 13 = Zobia, 14 = Bargesh 15 = Ebein, 16 = Ashah, 17 = Umm Qaiss, 18 = Aosra, 19 = Jeneen Safa, 20 = Aqraba, 21 = Kufour Kifya, 22 = Makhraba, 23 = Alouk, 24 = Jobbah, 25 = Gelad.

correlations between the morphological and genetic distance matrices. Mantel's test was computed to verify the correlation among the morphological and molecular data sets. Significance level (p) of matrix correlation (r) was tested by means of 1000 random permutations.

RESULTS

Morphological diversity of *Quercus* species in Jordan

The relationship between collected *Quercus* populations or measured characters was studied using cluster analysis that resulted in the grouping of variables into clusters. Distances between clusters were analyzed and reported as Dendrogram of Euclidean distance to assess the phenotypic relatedness among the 25 *Quercus* populations.

The hierarchical clustered based on morphological characters for the twenty five *Quercus* populations and the distance index are shown in Figure 1 revealing that populations are separated into three different species. At 40% dissimilarity index, samples are divided into three main clusters representing the three species. The first main cluster includes combination of the *Q. calliprinos* Webb collected across Jordan. This main cluster is further subdivided into three sub clusters representing southern, central and northern parts of Jordan. The second main cluster encompasses the *Q. infectoria* populations collected from northern part of Jordan. The third main cluster is grouped in the population of *Q. ithaburensis* Decne and further subdivided into subclusters compassed populations collected from central part of Jordan in one subcluster, northern populations in another subcluster and those from western part in a third subcluster. The canonical discriminant analysis was performed with the standardized canonical discriminant function coefficients for all morphological characters. The first five functions account for 95.28% of the total variation.

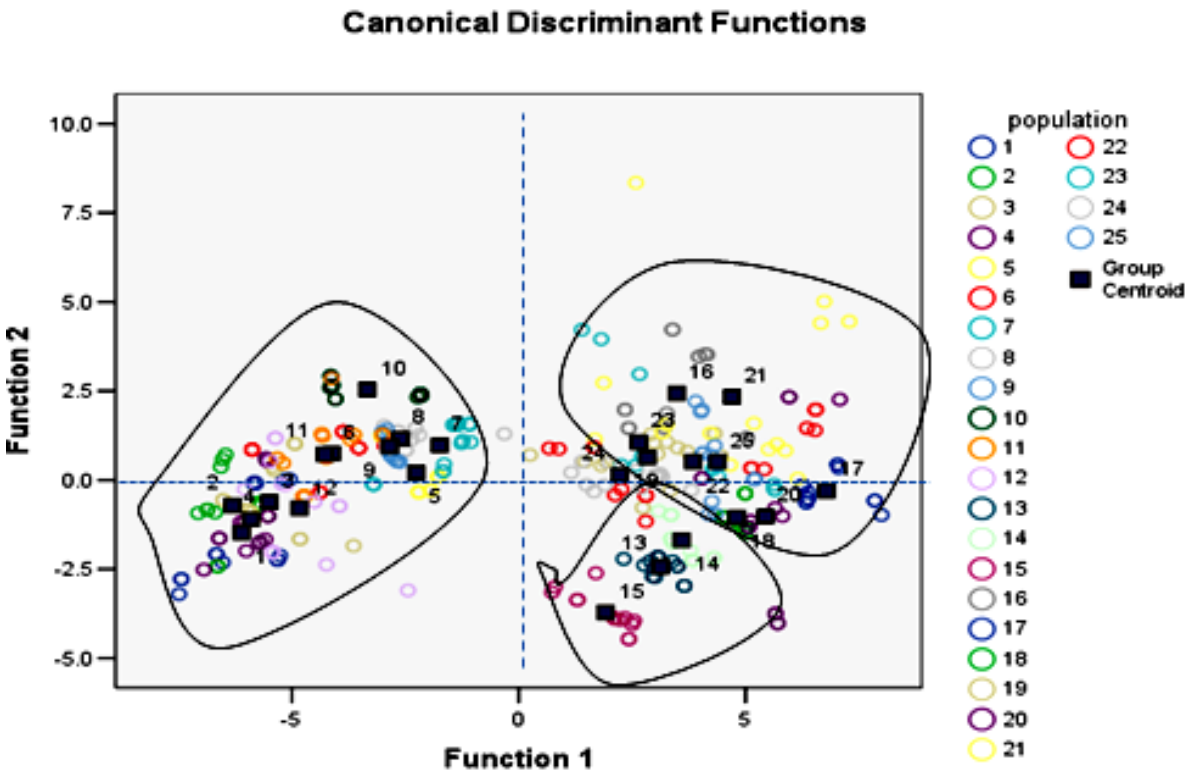

Figure 2. Canonical discriminant functions between the twenty five populations of *Quercus* spp. in Jordan (1 = Ber Adbagat, 2 = Al-Rashadia, 3 = Heisha, 4 = Dana, 5 = Achtifina, 6 =Ebein(1), 7 = Enbeh, 8 = Anjara, 9 = Rahaba,10 = Fuheis,11= Gelad, 12 = Kufour Houda, 13 = Zobia, 14 = Bargesh 15 = Ebein(2), 16 = Ashah, 17 = Umm Qaiss, 18 = Aosra, 19 = Jeneen Safa, 20 = Aqraba, 21 = Kufour Kifya, 22 = Makhraba, 23 = Alouk, 24 = Jobbah, 25 = Gelad) and the two main functions.

The first function account for 72.44% and the second function account for 8.71%, while the third account for 6.58% of the total variation and with the third function, the total variation account for 87.73%.

The standardized canonical discriminate functions coefficients for morphological traits under investigation revealed that the first function (which explains 72.44% of the total variability between populations) is strongly influenced by leaf and scale length, the second canonical discriminate function was found to be strongly influenced by cupules length and width and the third canonical discriminate function was found to be strongly influenced by cupules and scale width. The graphical representation of the distribution of *Quercus* in the space of the two discriminate functions is shown in Figure 2. Function 1 clearly discriminated the *Q. calliprinos* Webb collected across Jordan from populations of *Q. infectoria* and *Q.s ithaburensis* Decne and the second function discriminated *Q. infectoria* populations from *Q. ithaburensis* Decne.

Molecular diversity of *Quercus* species in Jordan

A total of 60 RAPD primers were evaluated for their ability to amplify polymorphic regions from six randomly selected populations. Of the 60 primers, 6 amplified consistently reproducible polymorphisms, and so these were used to analyze all the 25 *Quercus* populations. The features of the primers across the tested populations are summarized in Table 2. The 6 primer generated a total of 27 polymorphic markers (alleles). In total, 5917 data points (bands) could be scored with an average of 986.2 bands per primer pair across the genotypes, thereby confirming the high multiplex ratio expected for the RAPDs. The ability of different primer to generate RAPD markers varied from 4 to 5 markers with an average of 4.5 markers per primer pair across all genotypes. On a per-population basis, the number of markers generated by the primer pairs ranged from 32.4 for OPD 17 to 47.9 for OPA 20 with an average of 39.4 markers per primer. The percentage of polymorphism among the primers generated was 100%.

Polymorphic bands ranged in size from 250 to 790 bp. The size out of this range was not considered in the analysis. The densely stained markers were considered in scoring. The total bands for each primer ranged from 810 for primer OPD-17 to 1197 bands for OPA-20 using 389 plants representing 25 *Quercus* populations. Figure 3 shows an example of amplification using RAPD primer with amplified DNA patterns representing three populations.

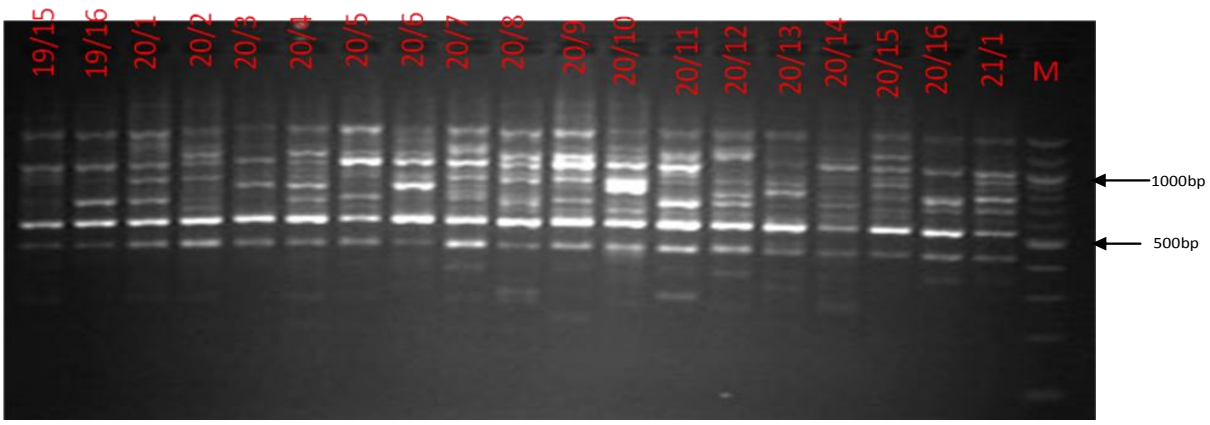

Figure 3. RAPD patterns of nineteen *Quercus* samples from populations number 19, 20, and 21 with primer OPB05. M = 100 bp DNA ladders.

Based on the Jaccard coefficients index (Jaccard, 1908), a genetic similarity matrix was constructed using the RAPD data to assess the genetic relatedness among the 25 *Quercus* populations. The within population means were used to construct the similarity matrix table. The mean similarity indices ranged from 0.24 between population 4 and population 11 to 0.84 within the population number 8 and 0.48 for all populations.

The results showed based on RAPD product data that populations from the different locality represent species grouped together in the same cluster (Figure 4). The species of the 25 *Quercus* populations clustered into two main clusters: the first cluster consists of the populations belonging to *Q. ithaburensis* and the second cluster consists of the populations belonging to the *Q. infectoria* and *Q. calliprinos* species. Mantel test (r = 0.145**) indicated significant correlation between the matrix based on morphological characters and that derived from the molecular analysis using RAPD marker. The clustering of populations based on morphological characters is consistent with that derived from the RAPD markers analysis according to the Mantel test.

DISCUSSION

Characterization of genetic diversity can be done using morphological traits or molecular markers. The 25 *Quercus* populations analyzed in this study represented species from a wide range of geographical areas in Jordan. In this work, we followed the nomenclature used by the previous workers (Kasapligil, 1956; Long, 1957; Zohary, 1962, 1973). Our results showed that Jordan has at least three *Quercus* species and each has its morphological characters. The significant morphological variations among 25 *Quercus* populations estimated were consistent with most previous studies of *Quercus* species where high genetic diversity was found even within individual populations. High variation leads to development of subspecies and varieties (Zohary, 1962).

The analysis of morphological characters of the *Q. calliprinos* species showed that the species is grouped into three sub clusters according to the geographical distribution, northern group, middle group and the southern group. This result is new to the genus *Quercus* in Jordan. In the same time, the population collected from Fuhies showed separate divergence in the middle area group, which is an indication that this population is a hybrid between *Q. ithaburensis* and *Q. calliprinos* because they are growing together in the same geographic area and gene flow could take place. *Q. infectoria* specie showed two main groups encompassing the Zobia and Ebein in one group and the Bargash in the second. The separation of Bargash could be due to elevation where Bargash has lower (800 m) altitude than Zobia and Ebein elevation which ranged from 1000 to 1200 m. It was found also that specimen collected from Bargash is mixed with the other two oak species, while the Zobia and Ebein group is just mixed with *Q. calliprinos* which is a new finding for the *Q. infectoria*. The analysis of morphological characters of *Q. ithaburensis* species showed that the populations of this species are grouped into three main clusters according to the geographical distribution: Northern, middle and southern part of Jordan. But the population of Aosra and Kufour Kifya (middle part of Jordan) formed separate clad, these two populations showed high variation in morphological character that made the previous taxonomist to create subsp. and varieties.

The geographical distribution of the genus as we found matches the distribution given by the previous workers (Kasapligil, 1956, Long, 1957; Zohary, 1962). This is due to the protection of the oak forest by the government since early twenties. The three *Quercus* species are clearly separated from each other; *Q. calliprinos* appears to be the most diverged species. While *Q. infectoria* and

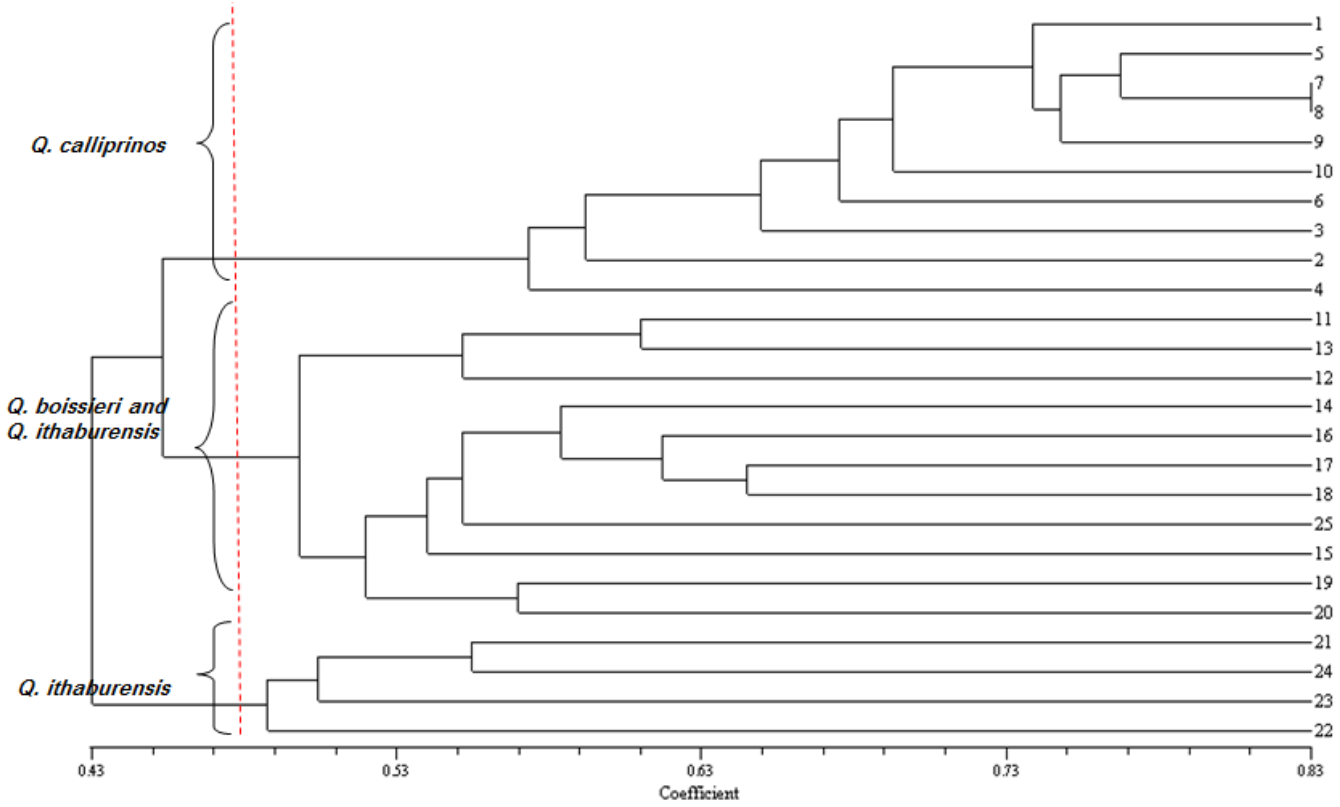

Figure 4. Dendrogram of 25 *Quercus* populations (1 = Ber Adbagat, 2 = Al-Rashadia, 3 = Heisha, 4 = Dana,5 = Achtifina, 6 = Ebein(1), 7 = Enbeh,8 = Anjara, 9 = Rahaba, 10 = Fuheis, 11 = Gelad, 12 = Kufour Houda, 13 = Zobia, 14 = Bargesh 15 = Ebein(2), 16 = Ashah, 17 = Umm Qaiss, 18 = Aosra, 19 = Jeneen Safa, 20 = Aqraba, 21 = Kufour Kifya, 22 = Makhraba, 23 = Alouk, 24 = Jobbah, 25 = Gelad) generated by UPGMA cluster analysis of the genetic similarity values.

Q. ithaburensis forming big group divided into two sub groups. These results are matching with previous classical taxonomical studies (Zohary, 1962; El-Oqlah and Lahham, 1985). The current study uses the RAPD-PCR based protocol to assess genetic variability of the *Quercus* species in Jordan. Genetic diversity determines the adaptive potential of a species and is an essential component of the stability of ecosystems. Analysis of within- and among-population genetic diversity is a fundamental step in the development of strategies for conservation of genetic resources and, consequently, of their adaptability. With its oak forests that comprise *Q. ithaburensis*, *Q. boissieri* and *Q. calliprinos*, is in a geographically peripheral position to the main area of distribution of these species in the Mediterranean basin (Awishai, 1967). According to Safriel et al. (1994), unlike core populations, peripheral ones may be tolerant to environmental extremes and changes because of their higher genetic variability, which has resulted from fluctuating selection. It is also likely that peripheral populations evolve resistance to extreme conditions; therefore, they should be treated as biogenetic resources, to be used for rehabilitation and restoration of damaged ecosystems. Owing to their long life cycle, forest trees are among the species that cannot migrate or adapt quickly enough to cope with the rapid changes imposed on the environment by human activity, and this could create ecological and forest management problems. Thus, attention should be given to *in situ* and *ex situ* conservation of the varieties of *Q. ithaburensis* genetic material represented by the three main assemblages of its distribution in this region.

In this study, it was possible to show that the amplification products from six random primer (RAPD) assay were sufficient to discriminate among and within populations of *Quercus* species for each location. Also, the assay was useful in discriminating among plants of the same location. The ability to distinguish between closely related individuals was simply a function of the observed number of RAPD bands. The results of RAPD markers were compared in a genetic diversity of *Quercus* species, the differences in the level of polymorphism detected by the markers and evaluating the potential of these markers in assessing the genetic variation in 25 populations of *Quercus* to three species *Q. ithaburensis*, *Q. infectora* and *Q. calliprinos* matches the morphological result. The classes of molecular markers adopted in this study deserve additional discussion. The key of the success of multilocus PCR-based markers has to be found in their high multiplex ratio. In fact, owing to their own genetic nature, RAPD assays detect simultaneously many loci

randomly distributed in the genome. Moreover, as compared to SSRs, these marker systems allow a more precise estimate of marker allele frequencies at single loci and faster estimate of population polymorphisms over several loci.

From the similarity matrix, the highest values of similarity between populations was found between the populations Aqraba, Makhraba, Ashah and Umm Qiass, these population cluster together in the hierarchical cluster constructed on the base of the genetic similarity values. These populations belong to the same species *Q. ithaburensis* and the population are found in the same region and located at the same elevation. The results obtain confirm once again the great versatility, reliability and precision of the techniques based on molecular markers, which can be used to aid the classical evaluation of the differentiation between population based on the observation of morphological characteristics. Our molecular results is also in agreement with those of Cottrell et al. (2003) who used six microsatellite markers and found high expected heterozygosity values in *Quercus* robur and *Quercus petraea* populations ranging from 0.87 to 0.92 and from 0.76 to 0.82 in *Quercus crispula* populations (Ohsawa et al., 2007a, b). And with those of Schiller et al. (2003) who found that *Quercus aegilops* L. ssp. *Ithaburensis* populations were aggregated according to main geographic regions. The presence of consistent clustering of populations based on morphological characters with that derived from the RAPD analysis according to the Mantel test is likely due to the fact that, in this study, while the morphological analysis is based on allelic variation (presumably) in a small number of genes and here 9 traits, the molecular analysis based on 27 alleles assumed to be distributed across the genome. Many studies showed that clustering of genotypes based on morphological diversity does not conform to genetic dividing (Tahernezhad et al., 2010; Vojdani and Meybodi, 1993; Nevo, 1988; Semagn, 2002; Ebrahimi et al., 2009). While Taamalli et al. (2006) reported on Tunisian olive cultivars with only a significant but low correlation between some of morphological and genetic distance matrices obtained with SSR (r = 0.185) and AFLP markers (r = 0.156). On the other hand, a high correlation between the morphological and molecular data was found using Mantel's test in *Olea europaea* (Dimperio et al., 2011).

In conclusion, the variations among *Quercus* species studied at both morphological and molecular levels indicated that there is a high variation among these populations and the RAPD technique was useful for studying genetic variability of *Quercus*. The wide geographical distribution of *Quercus* populations across different environments means that this species has good genetic resources to fill the gap between northern natural distribution sites and the southern natural distribution sites. *In situ* as well as *ex situ* conservation, restoration and reforestation should be done in the nearest populations within the same geographic region.

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