

Assessment of Genetic Variations in Wild *Arum* Species from Jordan Using Amplified Fragment Length Polymorphism (AFLP) Markers

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ABSTRACT

Arum is a wild herbaceous tuberous perennial plant belonging to the Araceae family. So far, there is no report published in Jordan about the application of molecular markers to characterize wild *Arum* plant. This study was initiated to assess levels of genetic variation of this plant using molecular markers. Twenty one locations were surveyed to assess the genetic variation in this plant. Ten plants from each location were collected and measured directly in the field. In total, 210 plants were studied and three species were found in the 21 locations.

Amplified fragment length polymorphism (AFLP) technique was used to study the genetic variation among *Arum* species. Six combinations of selective primers were generating a total of 2882 reproducible and clearly scorable bands of different sizes; 98.6% of them were polymorphic. Genetic distance was constructed based on Nei dissimilarity coefficient. The overall genetic distance between *Arum* species was 25%. The results showed that *Arum* populations of the same species or having a common genome were grouped in the same cluster, regardless of the collection site. The wide range of genetic distance was represented by the high level of DNA polymorphism occurring among *Arum* species.

The high level of reproducibility indicates that AFLP method is an effective approach to detect genetic variation among *Arum* species. Results attained in this study will be of great importance for further work aiming at establishing a conservation strategy for preserving and maintaining the germplasm of this plant using *in situ* and *ex situ* gene banks.

Keywords: AFLP, *Arum*, DNA molecular marker, Genetic variation, Molecular techniques.

INTRODUCTION

The genus *Arum* is composed of 28 species, largely distributed in Europe, North Africa, the Middle East and

Central Asia (Boyce, 1993; Mayo et al., 1997). In Jordan, *Arum* grows naturally in mountains, red soils, alluvial soils, near water canal, rocky places, forests, fallow fields, among rocks and in the upper Jordan Valley in many regions including: Ajlun, Irbid, Jarash, Al-Balqa', Wadi Shua'ib and Amman (Al-Eisawi 1998). Al-Eisawi (1998) in his list of wild flora of Jordan recorded 3 species of *Arum*: *A. dioscoridis*, *A. hygrophilum* and *A. palaestinum*. The chromosome number of each of these species is $2n = 28$ (Bedalov, 1981). *Arum* is utilized as spices, cooked like leafy

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Received on 28/4/2009 and Accepted for Publication on 7/12/2009.

crops, and it was also used as a medicine against cancer, circulatory system, obesity problems, internal bacterial infections, diabetic symptoms and poisoning problems (Said et al., 2002). Due to increasing demand, consumption, rural development and continuous removal of plants from the natural habitat, *Arum* plants have become threatened, which necessitates establishing a certain strategy to conserve the germplasms of these plants (Oran and Al-Eisawi, 1998).

AFLP is a PCR-based DNA fingerprint technique and considered to be a reliable marker for the assessment of genetic variation among and within plant populations. For instance, Aggarwal et al. (2002) mentioned that AFLP analysis is an effective tool for the identification of Indian rice cultivars to estimate their genetic diversity, and they also indicated that AFLP-based clustering, in general, conforms to the putative pedigree of the improved genotypes.

Geographical patterns of genetic variation in the world collections of wild annual *Cicer* were characterized using AFLP markers (Shan et al., 2005). Another research was initiated to assess the intra-specific diversity in *Oryza longistaminata* and how the variation is partitioned within and between different geographic locations using molecular markers (Kiambi et al., 2005). Genetic parameters estimated from AFLP data indicated that there are high levels of genetic diversity in the wild populations of *Oryza longistaminata* studied, and this diversity is higher within a population than between populations (Kiambi et al., 2005).

The genetic variation among and within populations of *Achillea fragrantissima* from Jordan using AFLP analysis was assessed, and a high level of genetic variation was obtained among and within populations

collected from different locations (Rawashdeh, 2007).

An AFLP study utilizing eight pairs of AFLP primers was conducted to evaluate the level of genetic variation in 10 wild populations of *Phellodendrom amurense*, in order to initiate suitable conservation strategies (Zhifeng et al., 2006).

Coulibaly et al. (2001) found that wild annual Cowpea was more diverse than domesticated Cowpea and wild Cowpea was more diverse in eastern than in western Africa using AFLP markers, where they suggested an eastern African origin for the wild taxon. AFLP was successfully utilized to assess genetic diversity within and between populations of *Helianthus argophyllus* collected in the Maputo area, Mozambique, both for taxonomic and breeding purposes. Three primer combinations were used to study twelve populations from two areas. The results showed that most of the variation (71%) was within populations. The authors suggested that populations of *H. argophyllus* that possessed most of the observed variation can be the most promising material for crossing with cultivated sunflower (Qugliaro et al., 2001).

Another study was initiated to determine the level of genetic diversity among 255 taro (*Colocasia esculenta*) accessions from Vietnam, Thailand, Malaysia, Indonesia and the Philippines utilizing AFLP markers. The 255 accessions were grouped according to their country of origin, their ploidy level (diploid or triploid) and their habitat (cultivated or wild). Gene diversity within these groups and the genetic distance between these groups were computed. In each country, the gene diversity within the groups of wild genotypes was the highest compared to the diploid and triploid cultivar groups. The authors indicated that the presence of two gene pools for cultivated diploid taro has major implications for the breeding and

conservation of germplasms (Kreike et al., 2004).

AFLP markers were used to establish genetic variation among *Crocus* species collected from three locations in Jordan. Results indicated that a high level of dissimilarity existed between plants of the same species with percentages ranging from 78% to 92% for samples from Al-Khanasri, 71% to 95% for samples from Rohaba and 58% to 85% for samples from O'saim location (Nazzal, 2007).

So far, there is no report published in Jordan regarding the application of molecular markers to characterize wild *Arum* plants. This study aimed at assessing the genetic variation of wild *Arum* species and the relationships among and within wild *Arum* species using Amplified Fragment Length Polymorphism (AFLP) molecular technique.

MATERIALS AND METHODS

Collection of Plant Materials

Field collections of *Arum* samples were carried out throughout January – April 2006. The collection sites were

selected according to bibliographic information and herbarium samples of the National Center for Agricultural Research and Extension (NCARE), Baqa, Jordan. Eight provinces were chosen to cover most of the geographical range of *Arum* species (Figure 1 and Table 1). Twenty one Locations were surveyed. The distance between 2 successive locations was not less than 2 kilometers. Individuals were chosen randomly. The number of collected plants in each location was (10 plants), which were collected, studied and measured directly in the field. In total, 210 plants were studied and only 40 plants from three species (*A. palaestinum*, *A.dioscoroides* and *A. hygrophilum*) were used in the current study.

Names of collection sites were registered according to the system used at the Department of Land and Survey (DLS). The exact site location, latitude, longitude and altitude of each location were determined using the geographical positioning system (GPS) using MAGELLAN (model NAV 5000 DXTM, USA) and a Digital Barometer (model AIR HB- IL, Atmospheric Instrumentation Research, Inc., USA).

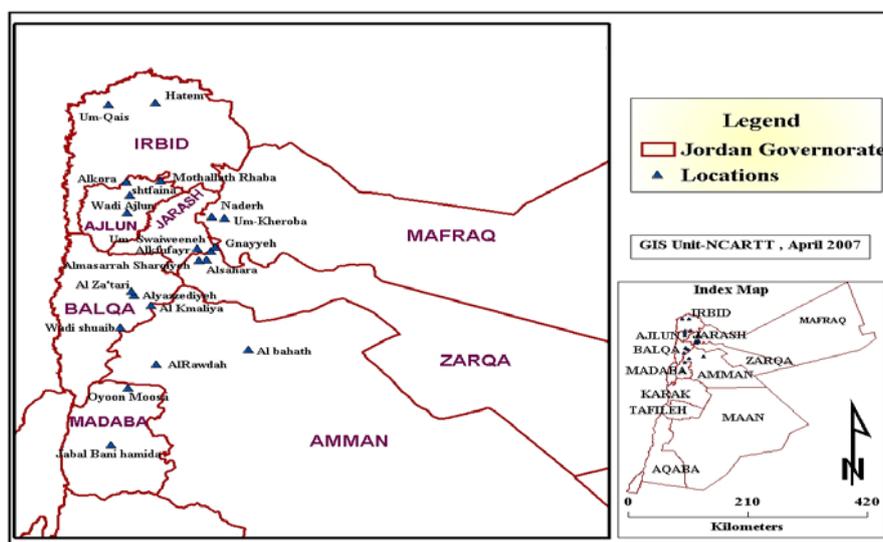


Figure 1: Jordan map showing the distribution of sampled *Arum* populations from twenty one locations in Jordan (GIS Unit).

Table 1: Eco-geographical parameters and description of the twenty one locations sampled of *Arum* species in this study.

| No. | Province | Rainfall * (mm) | Location (sample numbers) | Code | Longitude (E) | Latitude (N) | Altitude (M) | Number of samples/ location | Number of species/ location | Number of samples used in AFLP | 260/280 DNA ratio |
|-----|-----------|--------------------|--|-------|------------------|-----------------|-----------------|--------------------------------------|--------------------------------------|--|-------------------------|
| 1 | Irbid | 463.52 | Alkora (1 and 2) | Alko | 32°24'30 | 35°43'64 | 793 | 10 | 2 | 2 | 1.5 |
| | | | Hatem (3) | Hat | 32°39'11 | 35°48'30 | 464 | 10 | 1 | 1 | 1.9 |
| | | | Um-Qais (4) | UMQ | 32°38'50 | 35°40'29 | 350 | 10 | 1 | 1 | 1.6 |
| 2 | Ajlun | 614.66 | Wadi Ajlun (5) | Waj | 32°18'49 | 35°43'57 | 631 | 10 | 1 | 1 | 1.5 |
| | | | Eshtafaina (6,7) | Shta | 32°22'03 | 35°44'19 | 837 | 10 | 2 | 2 | 1.6 |
| | | | Mothallath Rhaba (8,9) | Mrha | 32°24'47 | 35°49'34 | 1062 | 10 | 2 | 2 | 1.7 |
| 3 | Jarash | 315.0 | Algnayyeh (10,11) | Alg | 32°11'13 | 35°58'22 | 469 | 10 | 2 | 2 | 1.6 |
| | | | Um-Swaiweeneh (12) | Umsw | 32°11'54 | 35°58'21 | 382 | 10 | 1 | 1 | 1.8 |
| | | | Al Kufayr (13,14) | Alku | 32°12'22 | 35°56'01 | 540 | 10 | 2 | 2 | 1.8 |
| 4 | Zarq'a | 130.33 | Al sharah (15,16) | Alsh | 32°10'14 | 35°57'44 | 487 | 10 | 2 | 2 | 1.5 |
| | | | Al masarra Al Sharqiyeh (17, 18, 19) | Alsa | 32°10'03 | 35°56'26 | 567 | 10 | 2 | 3 | 1.5 |
| 5 | Mafraq | 146.54 | Um-Kheroba (20, 21) | Umkh | 32°17'55 | 36°00'38 | 729 | 10 | 2 | 2 | 1.5 |
| | | | Naderh (22, 23) | Nad | 32°18'12 | 35°58'27 | 778 | 10 | 2 | 2 | 1.5 |
| 6 | Al- Balqa | 513.89 | Wadi Shua'ib (24) | Wsh | 31°57'34 | 35°43'04 | 379 | 10 | 1 | 1 | 1.6 |
| | | | AlYazzediyyeh (25, 26, 27) | Alyaz | 32°03'32 | 35°45'24 | 893 | 10 | 2 | 3 | 1.7 |
| | | | Al Za'tari (28, 29, 30) | Alzat | 32°04'18 | 35°44'58 | 992 | 10 | 2 | 3 | 1.6 |
| 7 | Amman | 433.17 | Al Kmaliya (31, 32) | Alka | 32°01'44 | 35°48'17 | 962 | 10 | 2 | 2 | 1.7 |
| | | | Al Bahath (33, 34, 35) | Alba | 31°53'39 | 35°47'41 | 597 | 10 | 3 | 3 | 1.7 |
| | | | Al Rawdah (36) | ALR | 31°50'49 | 35°49'18 | 860 | 10 | 1 | 1 | 1.6 |
| 8 | Madaba | 311.92 | Oyoon-Moosa (37, 38) | Omus | 31°46'23 | 35°44'34 | 593 | 10 | 2 | 2 | 1.8 |
| | | | Jabal Bani Hamida (39,40) | JBH | 31°35'50 | 35°41'44 | 774 | 10 | 2 | 2 | 1.5 |

Source: Department of Meteorology/ Jordan.

*The rainfall was the long- term average (1997-2006) of the twenty-one locations included in this study.

Isolation of Genomic DNA

Genomic DNA was isolated from young leaves of each location using Cetyl Trimethyl Ammonium Bromide (CTAB) procedure (Doyle and Doyle,1990). The frozen leaves were ground to fine powder in liquid nitrogen, the powder was extracted in 50 ml pre-warmed (60°C) CTAB buffer [100 mM Tris HCl; pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% (W/V) CTAB; 1% PVP; 0.2% *B*-Mercaptoethanol; and 0.1% NaHSO₃]. The mixture was incubated in a shaker water bath for one hour at 65°C, then was mixed with an equal volume of chloroform – isoamylalcohol (24:1) followed by shaking for 10 minutes at room temperature. The samples were centrifuged for 15 minutes at 13000 rpm, whereafter the aqueous phase was transferred into a new tube. One volume of cold Isopropanol was added and mixed carefully until DNA showed precipitation. The tubes were centrifuged for 10 minutes at 13000 rpm, the aqueous phase was decanted and DNA was washed with 70% ethanol. After complete drying of the pellet, the crude DNA was suspended in 400 µl of TE buffer (100 mM Tris-HCl pH 8, 1 mM EDTA), 2 µl of RNase (10mg/ml) was added to each sample, and samples were kept at 37°C for 30 minutes to degrade the RNA.

DNA concentration was determined using spectrophotometer (Bio Wave, S2100 Diode Array Spectrophotometer). DNA was diluted to 50 ng/µl. DNA purity was estimated by the ratio of absorbance at 260 and 280 nm according to Johanson (1994). The ratio (260/280) of DNA concentration ranged between 1.5 and 1.9 (Table 1).

AFLP Procedure

The procedure was performed essentially as described by Vos et al. (1995). We used five µl of genomic DNA (50 ng/µl) with 2µl deionized sterile

water, 0.5 µl of reaction buffer, 0.063 µl *Tru91*enzyme (10 u/ 1 µl) and 0.03 µl *EcoRI* enzyme (20 u/1µl), "these quantities of enzymes were enough to digest the DNA because it was for one sample only" (in this case, we followed Vos 1995 protocol Genomic DNA in which (0.5 µg) was incubated for 1 h at 37°C with 5 U *EcoRI* and 5 U *MseI* in 40 µl 10 mM Tris-HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/µl BSA). In our study, 0.03 µl (100xBSA) was incubated for 3 hours at 37°C using MJ-Research, Model PTC 200 thermocycler. Ligation mix [1.8 deionized distilled water, 0.3 µl of an Adapter / Ligation solution (*EcoRI* adapters 5 pmol /µl / *Tru91*dapters 50 pmol / µl), 0.3 µl of reaction buffer of ligase and 0.3 µl T4 DNA Ligase (1u/µl)] was added to the digest and incubated at 20°C for 3 hours using MJ – Research Model, PTC 200 thermocycler. The digested – ligated product was then diluted ten times in TE buffer [10mM Tris – HCL (pH8.0), 0.1 mM EDTA] to be used in the pre- amplification step (Vos et al., 1995).

Pre-amplification was performed with two AFLP primers having a single selective nucleotide, one corresponding to the *EcoRI* ends and one corresponding to the *Tru91* ends. Five µl of the diluted digestion/ligation product was added to pre-amplification PCR mix [3.6 µl deionized sterile water, 0.5 µl 10x polymerase buffer [100 mM Tris HCL (pH 8.0), 15 m M MgCl₂, 500 m M KCL and 0.1% Difco Gelatin], 0.15 µl of (5 mM dNTPs), 0.15 µl of each primer *EcoRI* + C and *Tru91* + A, and 5u/ul *Taq* polymerase (Promega, Madison, USA); 9.85 µl total volume], and subjected to the pre-amplification thermocycle profile [94°C for 5 min followed by 21 cycles of 30 sec at 94°C, 40 sec at 56°C and 1 min at 72°C]. The pre-amplification product was diluted ten times in sterile deionized water.

Selective amplification was performed with AFLP primers having longer selective extensions. Two μl of the diluted pre-amplification product was added to selective amplification PCR mix [11.5 μl sterile deionized water, 2 μl of 10x polymerase buffer, 2 μl of 5 mM dNTPs, 1 μl of each primer (*EcoRI* + 3 nucleotides + *Tru91* + 3 nucleotides) (Table 2), 0.25 μl of 15 mM MgCl_2 and 5u/ μl *Taq* polymerase]. The selective

amplification PCR mix was subjected to the selective amplification thermocycle profile [30 sec at 94°C, 30 sec at 68°C and 60 sec at 72°C for one cycle followed by 12 cycles over which the annealing temperature is decreased 0.7°C per-cycle] followed by 23 cycles [94°C 30 sec, 56°C 30 sec and 72°C 1 min]. Oligonucleotide sequences used for adaptors and amplification primers are listed in (Table 2).

Table 2: Oligonucleotide sequences used in AFLP analysis.

| <i>Sequence</i> | |
|-----------------------|--|
| Adaptors | |
| <i>EcoRI</i> | Forward 5' -CTCGTAGACTGCGTACC Reverse 5' -AATTGGTACGCAGTC |
| <i>Tru91</i> | Forward 5' -GACGATGAGTCCTGAG Reverse 5' -TACTCAGGACTCAT |
| Primer sequences | |
| <i>Tru91</i> - primer | 5' -GATGAGTCCTGAGTAA+CAA-3' 5' -GATGAGTCCTGAGTAA+CAT-3' 5' -GATGAGTCCTGAGTAA+CAG-3' |
| <i>EcoRI</i> - primer | 5' -GACTGCGTACCAATTC+ACT-3' 5' -GACTGCGTACCAATTC+AAG-3' 5' -GACTGCGTACCAATTC+AAA-3' 5' -GACTGCGTACCAATTC+AAC-3' 5' -GACTGCGTACCAATTC+AAT-3' 5' -GACTGCGTACCAATTC+ATA-3' |
| Primer combinations | <i>Tru91</i> +CAA/ <i>EcoRI</i> +ACT, <i>Tru91</i> +CAT/ <i>EcoRI</i> +AAG <i>Tru91</i> +CAT/ <i>EcoRI</i> +AAA, <i>Tru91</i> +CAG/ <i>EcoRI</i> +AAC <i>Tru91</i> +CAG/ <i>EcoRI</i> +AAT, <i>Tru91</i> +CAT/ <i>EcoRI</i> +AAC |

Polyacrylamide Gel Electrophoresis

Apelex sequencing unit (vertical electrophoresis,

Apelex, MT 1002, France) was used in gel electrophoresis.

Four μL of the selective product was mixed with four μl of stop buffer (98% formamide, 10 mM EDTA, 0.15 Bromo Phenol Blue, 0.15 xylene cyanol). Then, this mixture was denatured at 94°C for 3 minutes, and chilled on ice. Samples were loaded in 0.4 mm thick 6% denaturing polyacrylamide gel resolved at constant power 1800 volts in 1X TBE running buffer for three and a half hours or until the last running dye reached the bottom of the gel.

Gel Visualization

AFLP bands were visualized by DNA silver staining system according to Promega silver staining kit protocol (Promega, Madison, USA).

Gels were scored visually from a photo taken by a scanner with high resolution. The banding pattern of each primer was scored by choosing the clearest bands and coded by 0 and 1 for the absent and present bands, respectively. Data were first analyzed using NTSYS software package, version (2.1, 2000). Genetic distance analysis was run using Nei (1972) method. Nei (1972) genetic distance value was used to establish a

dendrogram using the un-weighted pair group method with arithmetic average (UPGMA). The polymorphism rates were estimated by dividing the number of polymorphic markers by the total number of markers (Hongtrakul et al., 1997).

RESULTS AND DISCUSSION

AFLP Marker Profile

A total of 2882 reproducible and clearly scorable bands, produced from six primer combinations, were assessed across the entire collection of 40 *Arum* samples. The six AFLP primer combinations revealed a total of 108 polymorphic markers and one monomorphic marker (Table 3). The number of polymorphic markers ranged from 11 for the primer combinations *Tru 91 (CAT) / EcoRI (AAC)* to 26 for *Tru 91(CAT) / EcoRI (AAG)* with an average of 18 markers per primer combinations. The molecular weights ranged from approximately 50 to 800 bp. The most informative primer combination was the *Tru91 (CAT) / EcoRI (AAG)* - which produced the largest number of bands (678). This accounted for 24.1% of the total polymorphic bands examined in this study.

Table 3: Total number of bands, average number of bands per species, total number of markers, number of polymorphic markers, number of monomorphic markers, polymorphism % and polymorphism rate for the Arum species studied.

| <i>Primer number</i> | <i>Primer combinations</i> | <i>Total number of polymorphic bands</i> | <i>Average number of poly. bands/population</i> | <i>Total number of markers</i> | <i>Number of polymorphic markers</i> | <i>Number of monomorphic markers</i> | <i>Polymorphism %</i> | <i>Polymorphism rate</i> |
|----------------------|--------------------------------|--|---|--------------------------------|--------------------------------------|--------------------------------------|-----------------------|--------------------------|
| 1 | <i>Tru9 + CAA/ EcoRI + ACT</i> | 571 | 14.3 | 20 | 20 | 0 | 100 | 0.185 |
| 2 | <i>Tru9 + CAT/ EcoRI + AAG</i> | 678 | 17.0 | 26 | 26 | 0 | 100 | 0.24 |
| 3 | <i>Tru9 + CAT/ EcoRI + AAA</i> | 439 | 11.0 | 19 | 19 | 0 | 100 | 0.176 |
| 4 | <i>Tru9 + CAG/ EcoRI + AAC</i> | 581 | 14.5 | 19 | 19 | 0 | 100 | 0.176 |
| 5 | <i>Tru9 + CAG/ EcoRI + AAT</i> | 375 | 9.4 | 13 | 13 | 0 | 100 | 0.120 |
| 6 | <i>Tru9 + CAT/ EcoRI + AAC</i> | 238 | 6.0 | 12 | 11 | 1 | 91.66 | 0.111 |
| Total | | 2882 | 72.2 | 109 | 108 | 1 | 591.7 | 1.008 |
| Average | | | 12.0 | | 18 | | 98.6% | |

AFLP markers display trans-specific polymorphisms, thus elevating interspecific genetic similarities as compared to within species similarities. Such trans-specific polymorphisms cause high levels of homoplasy, especially among distantly related species, attributed to either incomplete lineage sorting (Mallikarjuna et al., 2004) or retention of ancestral polymorphisms in the derived lineage.

The primer combinations generated a total of 2882

reproducible and clearly scorable bands of differently sized markers. The 2882 scorable bands of different size markers were produced by six- primer combinations. Counting of bands was carried out using computer. The score was checked, and only the total numbers of different bands were taken ; 98.6% of them were polymorphic and the remaining 1.4% were monomorphic (Table 3). AFLP banding patterns are shown in Figure 2.

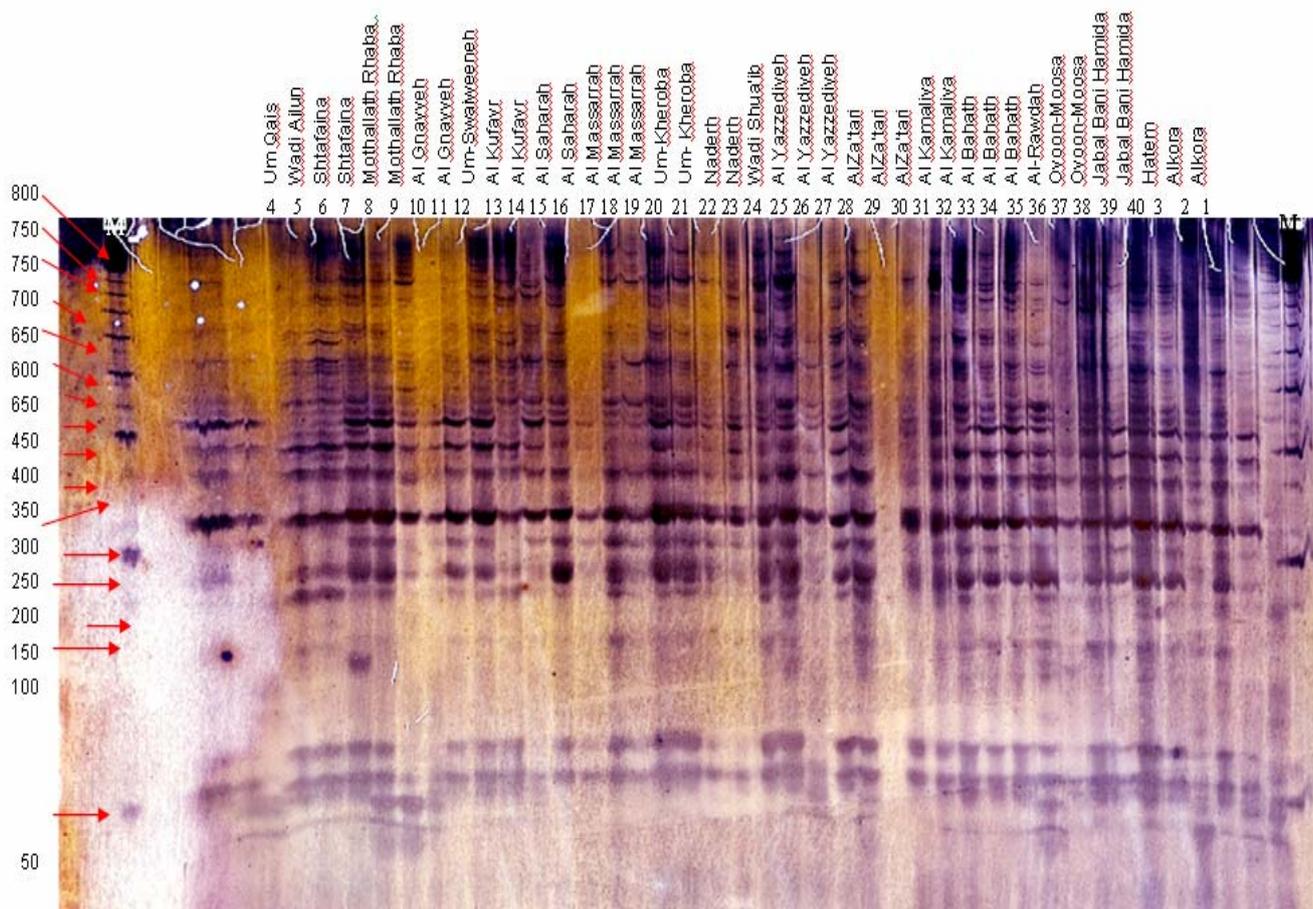


Figure 2: AFLP fingerprints produced by primer combinations *Tru9*+CAA and *EcoRI*+ACT markers for 40 of *Arum* plants from different locations in Jordan. M is a molecular weight marker (50 bp step DNA ladder) and sizes in bases are shown to the left.

Genetic Distance among *Arum* Species

Nei's coefficient of genetic distance (Nei, 1972) between 40 samples representing 3 different species of *Arum* collected from twenty one locations in Jordan ranged from 0.04 to 1.19 with an overall mean genetic distance of 0.25 (Table 4). This wide range of genetic distances indicated a high level of polymorphism at the DNA level among *Arum* samples. Therefore, there is a large amount of genetic variation among *Arum* species.

The results of the present study showed that species

from the same locality (Figure 3) tended to constitute the same sub-cluster, whereas accessions of the same species (Figure 3) (three species were used in this study) and those with common genomes (Figure 3) tended to form the same cluster, which is consistent with the classification previously determined by (Al-Lozi et al., 2008), based on only the morphological characters of *Arum* species in Jordan.

The genetic distance between any two accessions of the same species was generally quite low (Table 4).

Conversely, the genetic distances were high between two single plants from different species. For instance, among 17 accessions of *Arum* species (Table 4), the genetic distance ranged from 0.04 to 0.18, while the distances between accessions of other species (Table 4) were high and ranged from 0.19 to 1.19.

The highest genetic distance (1.19) was recorded for sample 28 (*A. dioscoridis*) from Alz'atari location and sample 3 (*A. palaestinum*) from Hatem location. The lowest genetic distance (0.04) was recorded for sample 32 (*A. palaestinum*) from Alkmaliya location and sample 33 (*A. palaestinum*) from Albahath location. Overall analysis of AFLP markers showed that clustering based on genetic distance basically showed clustering of the same species regardless of collection site, sample 16 creating a separate cluster within the three main groups (Figure 4). These findings are in agreement with (Nguyen et al., 2004; Turpeinen et al., 2003) who found similar results in different plant populations. Also, the degree of diversity is greater among *Arum* species than within species, which could be due to limited migration between species or due to low or rare cross-pollination within the species.

Here, in an out-crossing population, a high level of heterozygosity within individuals and variability between individuals and species and also between species is maintained (Hayward and Hamilton, 1997). Large numbers of alleles are kept and even minor recessive alleles can be maintained in the heterozygous state, contributing to a high level of genetic diversity within species.

Most of *Arum* species have never been studied in depth, but data available from the literature indicate a high diversity of pollination strategy within this genus. Consequently, a general pollination model is not valid at the level of the whole genus. The origin of this diversity certainly results from the biogeographic history of the genus. The plants (i.e. species) have developed adaptations in response to climatic, ecological and biotic (i.e. entomofauna) constraints (i.e. selective pressures) according to the various habitats occupied in the different regions of Europe and the Middle East (Gibernau et al., 2004). This might be further causes that affect genetic diversity of the genus *Arum*.

positioned into species clusters (Population 16 creating a separate cluster within the three main groups) .

Evolutionary mechanisms determine the distribution of genetic diversity in time and space. However, drift and gene flow are important processes for genetic differentiation (Hayward and Hamilton, 1997). Genetic differentiation between populations is possible at any scale, but the potential for differentiation increases with the degree of isolation of the population (Hayward and Hamilton, 1997). Genetic variability is more likely to be found between populations from clearly different environmental conditions (Gustine and Huff, 1999).

The results in the dendrogram showed that clustering of different *Arum* species did not tend to be clustered according to sites of collection, but tend to cluster only according to genotypes, where AFLP markers proved to be very useful for assessing the genetic relationship among *Arum* samples that represented 3 species of the *Arum* genus. The ability to determine genetic variation among the accessions, species and species groups at the molecular level was directly related to the number of polymorphisms detected and their reproducibility. AFLP was considered to be a reliable marker for the assessment of genetic variation among and between plant populations (Aggrawal et al., 2002; Shan et al., 2005; Kiambi et al., 2005; Zhifeng et al., 2006; Kreike et al., 2004; Qugliaro et al., 2001; Coulibaly et al., 2001; Nguyen et al., 2004). AFLP markers were more informative than previously applied methods to study variation and genetic relationship in *Arum* such as isozymes (Echchgadda and Triest, 1999). The results of

the present study could be a useful parameter for better explanation of the variation in the three *Arum* species: *Arum dioscoridis*, *Arum palaestinum* and *Arum hygrophilum*.

CONCLUSION

The current study assessed the levels of genetic variation of *Arum* species in twenty one locations, in eight provinces in Jordan, to provide a baseline for further studies and conservation strategies. AFLP markers showed a significant separation between *Arum* populations reflecting differences among localities and climatic conditions. The differences here refer to collected samples from different environments. The wide range of genetic distance indicated high DNA polymorphism occurring among the *Arum* samples, which indicates high genetic variation among species. The studied species are now collected and conserved in NCARE gene bank. Based on this study, a chemodiversity study of these species is recommended, as well as the establishment of *in situ* and *ex situ* field gene banks to protect this plant and the development of legal measures to conserve this species and consider this plant as one of those that must be protected.

Acknowledgment

The authors would like to express their thanks to the Conservation of Medicinal and Herbal Plants Project (Ministry of Planning, Jordan) and the Deanship of Scientific Research at the Jordan University of Science and Technology, Irbid, Jordan, for funding this study.

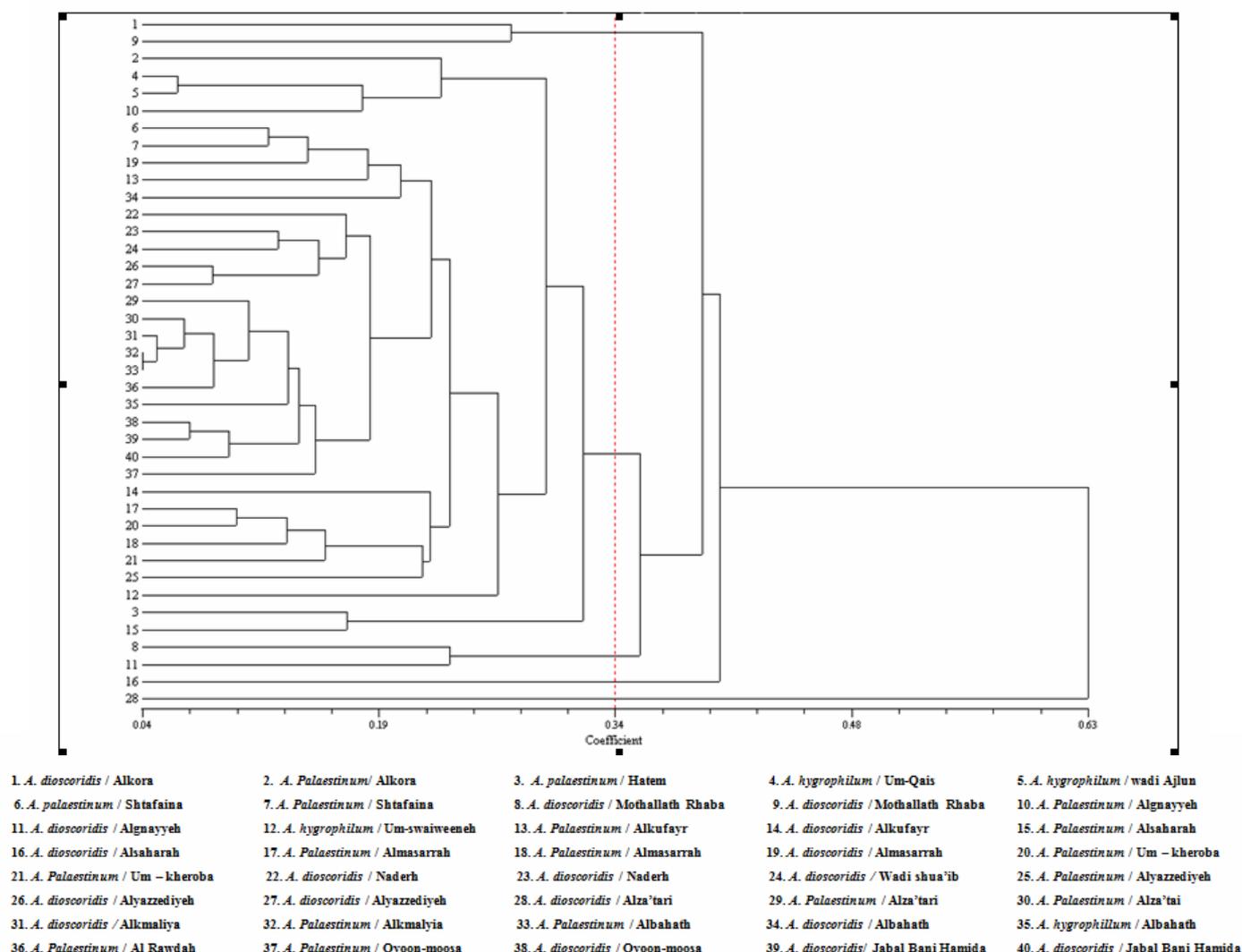


Figure 3: Hierarchical cluster of the 40 *Arum* samples collected from twenty one locations performed on the basis of genetic characters using Nei (1972) coefficient based on the unweighted pair group method with arithmetic Average (UPGMA).

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تقييم التباين الوراثي لأنواع اللوف في الأردن

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ملخص

اللوف (*Arum spp.*) نبات عشبي معمر، ينتمي للعائلة اللوفية. ولغاية الآن، لا تتوفر دراسات منشورة في الأردن تتعلق بتقييم التباين الوراثي لنبات اللوف البري. تم إجراء هذا البحث لتقييم مستويات التباين الوراثي لهذا النبات اعتماداً على المعلومات الوراثية. وقد تم تحديد إحدى وعشرين موقعاً تمت فيها دراسة هذا النبات، وتوزعت المواقع في (8) محافظات في الأردن. بلغ عدد العينات التي تم جمعها من المواقع كلها (210 نباتات)، ولكن استخدم 40 نباتاً فقط في الدراسة الجزيئية، وبلغ عدد الأنواع التي تتبع لها هذه العينات ثلاثة أنواع (اللوف الفلسطيني، واللوف المبرقش، واللوف المائي). تم استخدام تقنية مكائثة قطع DNA متباينة الأطوال (AFLP) لدراسة التباين الوراثي في مجتمعات نباتات اللوف المختلفة. وقد تم استخدام ستة أزواج من البادئات الانتقائية لتوليد ما مجموعه 2882 من المعلومات الوراثية ذات الحزم الواضحة والمختلفة في الحجم، منها 98.6% كانت متباينة. تم استخدام معامل ناي (Nei) لإنشاء جداول قيم البعد الوراثي وإنشاء مخططات القرابة. أما عن درجات التباين لأنواع اللوف المختلفة، فقد بلغ معدل التباين لهذه الأنواع 25%. وقد تبين أن نباتات اللوف التابعة للنوع نفسه قد تجمعت في مجموعة واحدة بغض النظر عن المواقع التي تم الجمع منها. إن هذا المدى الواسع لمعامل التباين في نباتات اللوف يدل على عظم التباين الوراثي بين هذه الأنواع. تعتبر تقنية مكائثة قطع DNA متباينة الأطوال (AFLP) طريقة فعالة ومفيدة في دراسات التباين الوراثي لنبات اللوف. وتعتبر هذه الدراسة الأولى من نوعها في الأردن التي تستخدم البصمة الوراثية (DNA) لتوصيف التنوع الوراثي لنبات اللوف وتغطيته في الأردن. النتائج التي تم تحقيقها من هذه الدراسة سوف تكون لها أهمية كبيرة، وهي تشكل أساساً لدراسات أخرى تهدف إلى وضع استراتيجيات لحفظ المادة الوراثية لهذا النبات وإدامتها عن طريق تأسيس بنوك وراثية من أجل عمليات الحفظ داخل الموقع وخارج الموقع لهذا النبات.

الكلمات الدالة: التباين الوراثي، نبات اللوف، المعلومات الوراثية، مكائثة قطع DNA متباينة الأطوال (AFLP).

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تاريخ استلام البحث 2009/4/28 وتاريخ قبوله 2009/12/7.