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Determination of Genetic Diversity among *Iris* species Using Random Amplified Polymorphic DNA Analysis

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Abstract: The genetic relationships among seven *Iris* species (*I. germanica*, *I. petrana*, *I. atrofusca*, *I. nigricans*, *I. vartanii*, *I. aucheri* and *I. edomensis*) were studied by using random amplified polymorphic DNA (RAPD). All the examined species were clearly identified using four primers (OPB-09, OPB-10, OPB-11 and OPB-12) out of twelve primers tested. The total number of amplification products produced with these primers was (125) and the fragment size ranged between 300 to 1500 bp. Primer OPB-10 produced the highest number of bands (38). The similarity among the seven *Iris* species ranged from 14% between *I. vartanii* and *I. petrana* to 85% between *I. germanica* and *I. atrofusca*; *I.germanica* and *I. aucheri*. The dendrogram of the seven species showed two main clusters. The first one included five species (*I. germanica*, *I. petrana*, *I. nigricans*, *I. vartanii* and *I. edomensis*); while the second cluster included two species (*I. atrofusca and I. aucheri*).

Key words: RAPD of *Iris*, *Iris* diversity, *Iris* species

INTRODUCTION

Iris grows naturally in the northern regions of Jordan including Mafraq, Wadi Shueib, Salt, Ajloun, Jordan Valley and the southern regions including Tafila, Karak and Shobak (Kareem and Qor'an, 1998). Ten species of *Iris* were confirmed to occur in Jordan, nine of which are perennial herbaceous plants growing in nature: *I. aucheri*, *I. regis-uzziae*, *I. edomensis*, *I. postii*, *I. vartanii*, *I. nigricans*, *I. petrana*, *I. atrofusca*, and *I. atropurpurrea*. The tenth species, *I. germanica*, is a cultivated one (Al-Eisawi, 1986; Al-Khader, 1997; Al-Eisawi *et al.*, 2000). Correct identification of *Iris* species is usually possible if the flowers are produced, but it is difficult before that because it is required phenotypic data that are often difficult to assess and some time variable due to environmental conditions.

Genetic diversity at morphological as well as biochemical levels has a limitations due to the influence of environment on the performance of the genotypes. Therefore, utilization of molecular markers for studying genetic diversity among genotypes is considering a good and valuable tool due to its stability and less effected by environment because the genetic material determines these variations.

Polymerase chain reaction (PCR)-based genetic markers has become a basic and essential tool in both research and analytical laboratories. The most widely used system being Random Amplified Polymorphic DNA (RAPD) that was developed by Williams *et al.* (1990). RAPD, also known as arbitrarily primed PCR, allows the detection of polymorphisms without prior knowledge of nucleotide sequence. The polymorphisms may be used as genetic markers and may also be used for the construction of genetic maps (Svitashev, 1997; Ko *et al.*, 1994; Vazquez *et al.*, 1996; Caetano-Anolles *et al.*, 1991).

RAPD technique is ideally suited for fingerprinting applications because it is fast (Gwanama *et al.*, 2000), requires little material (Lowe *et al.*, 1996) and technically easy (Fenwick and Ward, 2001). The wide availability of commercial primers makes this technique widespread (Gillies *et al.*, 1997), inexpensive and yields large numbers of markers (Martin and Bermejo, 2000).

The analysis of 56 random amplified polymorphic DNA (RAPD) markers in 12 Siberian *Iris* species showed the phylogenetic relationships among these species (Makarevitch *et al.*, 2003). Studies using RAPD have provided information regarding the genetic relationships between *I. haynei* and *I. atrofusca* (Arafeh *et al.*, 2002) and the genetic variability of *I. setosa* populations (Artyukova *et al.*, 2001).

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Analysis of seven *Iris* species-specific random amplified polymorphic DNA (RAPD) markers and two chloroplast DNA haplotypes are compared with the environmental gradients in a Louisiana *Iris* hybrid population. This study suggested that, at a very fine spatial scale, environment-dependent selection contributed to the genetic structuring of this hybrid zone (Jill *et al*., 2001).

The RAPD technique was successfully used in studies of the systematic relationships between five of the Far Eastern *Iris* species. A set of arbitrary primers (OPD-08, OPD-11, OPD-13 and OPB-12) suitable for inter-and intraspecific polymorphism analysis in *Iris* was found; they gave stable, well reproducible, species-specific patterns for *Iris* species with no variation between individuals from the same populations (Zhuravlev *et al*., 1998).

The most recent phylogeny of Iridaceae was constructed by Reeves *et al*., (2001) using molecular data derived from four coding and noncoding plastid DNA regions.

Some plant species have also been studied by using RAPD techniques such as olive (Hdeib, 2004), *Achillea* (Kharma, 2004), chestnut (Galderisi *et al*., 1998), almond (Bartolozzi *et al*., 1998), *Brassica* species (Demeke , 1992; Ren *et al*., 1995) and tomato (Fooland *et al*., 1993). The objective of this study was to detect the genetic diversity among the *Iris* species grown in Jordan by using (RAPD) analysis.

MATERIALS AND METHODS

Plant material: Plants of seven *Iris* species (*I. germanica*, *I. petrana*, *I. atrofusca*, *I. nigricans*, *I. vartanii*, *I. aucheri* and *I. edomensis*) were collected from different areas of Jordan. Young leaves from each species were excited and stored at-70°C until being used for RAPD analysis.

Extraction of genomic DNA: Genomic DNA was isolated from the collected leaves according to the procedure described by Doyle and Doyle (1990) that modified by Maguire *et al*., 1994).

Polymerase chain reaction (PCR): After an initial screening of 12 single decamer primers from kit OPB (OPB-03, OPB-05, OPB-09, OPB-10, OPB-11, OPB-12, OPB-13, OPB-15 and OPB-17) and OPA (OPA-05, OPA-11 and OPA-13), four primers (OPB-09, OPB-10, OPB-11 and OPB-12) were selected for further analysis.

PCR was performed in a volume of 25 μL of reaction mixture containing: 2 μL of 20 mM $MgCl_2$, 0.5 μL DNA, 2.5 μL from 10X buffer [100 mM *Iris*-HCl (pH 8.0), 500 mM KCl, 15 mM $MgCl_2$, .0.1% Difco Gelatin], 1 μL dNTPs, 2 μL of the primer (Operon DNA Technologies, Alameda, USA), 0.2 μL Taq DNA polymerase (Promega, Madison, Wis). The final volume of the reaction was brought to 25 μL by adding 16.8 μL nuclease free water and one drop of mineral oil to prevent evaporation. Amplification reactions were performed in a thermal cycler (Gene, UK), which was programmed as follows:

- Initial strand separation step at 92 °C for 3 min.
- 30 cycles of:
  30 sec at 92°C (denaturation step).
  45 sec at 36°C (annealing step).
  1 min at 72°C (elongation step).
  Ramp rate between temperatures (0.3°C/sec).
- Five minutes at 72°C (final extension step).

The samples were then kept at 4°C until further analysis by gel electrophoresis.

Gel electrophoresis and documentation: The amplification products were separated by electrophoresis using 100 mL of 1.5% agarose gel in 0.5X TBE buffer (0.1 M *Tris*-base, 0.1 M Boric acid, 2 mM EDTA). To stain the DNA, 4 μL from ethidium bromide (10 mg mL^{-1}) was added to the agarose solution before pouring in the taped plate with casting comb. The agarose gel was allowed to solidify. A running buffer of 0.5X TBE was added to about 0.5 cm above the gel level. Three microliter of 10X agarose gel loading dye (40% sucrose, 0.17% Bromophenol Blue and 0.17% Xylene Cyanol) was added to 10 μL of each DNA sample, mixed and loaded into the wells. The gel chamber was connected to the power supply in a way that DNA runs towards the anode (+ ve pole). Electrophoresis was performed at 90 volts for about 2 h until the required separation was achieved. The gel was visualized under ultraviolet light at 254 nm and photographed using video Polaroid photograph camera (BioRAD. Gel DOC 2000). Molecular sizes of amplification products were estimated by using a 100 bp DNA ladder marker (Sigma Chemical Company, St. Louis).

Data scoring: The DNA profiles were scored visually from photographs of the gels. Reproducible bands were used in the analysis. For each species, the presence (+) or absence (-) of fragments, regardless of their intensity, was recorded.

Statistical analysis: Data produced from RAPD analysis were analyzed using the Nei similarity index (Nei and Li, 1979), which excludes common negative data. The following equation was used:

$$\text{Similarity} = 2Nxy / (Nx + Ny)$$

Where:

Nxy: Number of scored amplification fragments with the same molecular weight shared between species x and y.
Nx: Number of scored amplification fragments in species x.
Ny: Number of scored amplification fragments in species y.

Dendograms were constructed based on the similarity matrix data; by applying unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using the NTSYS pc program version 2.1 by Rohlf (Exeter, Software, New York).

RESULTS

Primer OPB-09: This primer successfully amplified the DNA for all species. The number of bands scored for OPB-09 varied from 1 to 8 (Fig. 1) and the fragment size ranged between 300 bp with species *I. vartanii* to 1500 bp with species *I. germanica*, *I. atrofusca* and *I. nigricans*. The total number of bands that produced with this primer was 37 (Table 1).

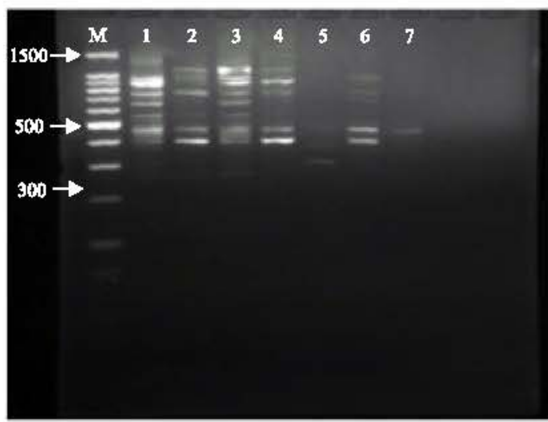

Fig. 1: RAPD patterns obtained from seven *Iris* species with the primer OPB-09. Species in the lanes are: M: 100 bp DNA ladder., 1: *Iris germanica*, 2: *Iris petrana*, 3: *Iris atrofusca*, 4: *Iris nigricans*, 5: *Iris vartanii*, 6: *Iris aucheri*, 7: *Iris edomensis*

Both species *I. germanica* and *I. atrofusca* produced the highest number (8) of bands and each of the three species *I. petrana*, *I. aucheri* and *I. nigricans* produced six bands. *I. edomensis* produced two bands (400 and 500 bp) and *I. vartanii* produced only one band (300 bp).

Primer OPB-10: Primer OPB-10 generated the highest number of amplified bands with the seven *Iris* species, compared with the other three primers. The total number of bands produced with this primer was 38 (Table 2). This primer was the only one that produced bands with molecular size of 200 bp.

The number of bands scored for this primer varied from 2 to 8 (Fig. 2) and the fragment size ranged between

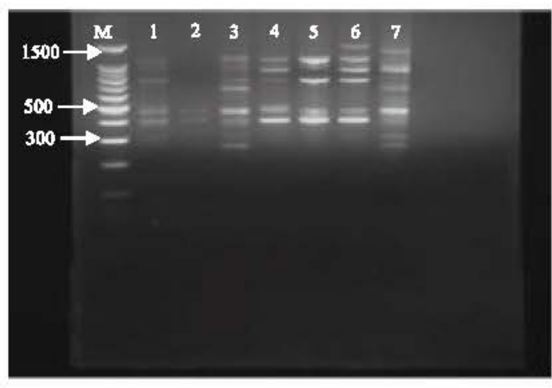

Fig. 2: RAPD patterns obtained from seven *Iris* species with the primer OPB-10. Species in the lanes are: M: 100 bp DNA ladder., 1: *Iris germanica*, 2: *Iris petrana*, 3: *Iris atrofusca*, 4: *Iris nigricans*, 5: *Iris vartanii*, 6: *Iris aucheri*, 7: *Iris edomensis*

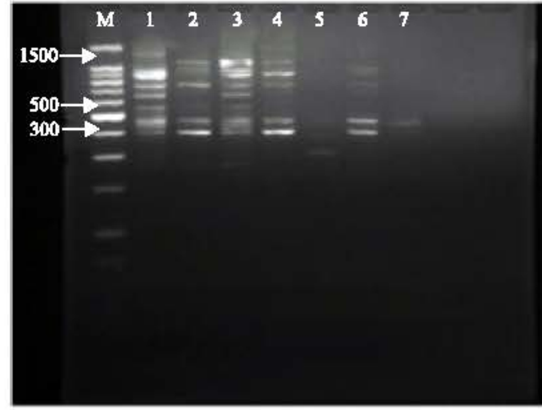

Fig. 3: RAPD patterns obtained from seven *Iris* species with the primer OPB-11. Species in the lanes are: M: 100 bp DNA ladder., 1: *Iris germanica*, 2: *Iris petrana*, 3: *Iris atrofusca*, 4: *Iris nigricans*, 5: *Iris vartanii*, 6: *Iris aucheri*, 7: *Iris edomensis*

Table 1: Survey of primer OPB-09 marker in 7 *Iris* species

	Size of band (bp)										
Species	1500	1000	900	800	700	600	500	400	300	200	100
I. germanica	+	+	+	+	+	+	+	+	-	-	-
I. petrana	-	+	+	+	-	+	+	+	-	-	-
I. atrofusca	+	+	+	+	+	+	+	+	-	-	-
I. nigricans	+	+	+	+	-	-	+	+	-	-	-
I. vartanii	-	-	-	-	-	-	-	-	+	-	-
I. aucheri	-	+	+	+	+	-	+	+	-	-	-
I. edomensis	-	-	-	-	-	-	+	+	-	-	-

Table 2: Survey of primer OPB-10 marker in 7 *Iris* species

	Size of band (bp)										
Species	1500	1000	900	800	700	600	500	400	300	200	100
I. germanica	-	+	+	+	-	-	+	+	-	-	-
I. petrana	-	-	-	-	-	-	+	+	-	-	-
I. atrofusca	-	+	+	+	+	-	+	+	+	-	-
I. nigricans	-	+	+	+	-	-	+	+	-	-	-
I. vartanii	-	+	+	+	-	-	+	+	-	-	-
I. aucheri	+	+	+	+	-	-	+	+	-	-	-
I. edomensis	+	-	+	+	+	-	+	+	+	+	-

200 bp with species *I. edomensis* to 1500 bp with *I. aucheri* and *I. edomensis*, *I. edomensis* produced the highest number (8) of bands, while *I. atrofusca* species produced seven bands and *I. aucheri* species produced six bands.

Three species *I. germanica*, *I. nigricans* and *I. vartanii* produced five bands each, while *I. petrana* produced the lowest number (2) of bands (400 and 500 bp).

Primer OPB-11: Primer OPB-11 generated the lowest number of amplified bands with the seven species of *Iris*. The total number of bands produced with primer OPB-11 was 25 (Table 3). The number of bands scored with this primer varied from 0 with *I. vartanii* and *I. nigricans* to 6 with *I. atrofusca* and *I. edomensis* (Fig. 3). The fragment size ranged between 400 bp with most of the species to 1500 bp with species *I. atrofusca* and *I. edomensis*.

Primer OPB-12: Primer OPB-12 amplified the DNA of five *Iris* species only. The total number of bands scored for this primer varied from 0 to 6 (Fig. 4). The fragment size ranged between 400 bp with species *I. germanica*, *I. petrana*, *I. atrofusca* and *I. aucheri* to 1500 bp with *I. atrofusca*. The total number of bands produced with this primer was 25 (Table 4). The two bands (500 and 900 bp) produced by the five species *I. germanica*, *I. petrana*, *I. atrofusca*, *I. aucheri* and *I. edomensis*, while *I. atrofusca* produced only one band (1500 bp).

UPGMA dendrogram and similarity matrix: The genetic relationship among the seven *Iris* species was assessed on the basis of the Nei similarity index and unweighted pair group method with arithmetic averages (UPGMA).

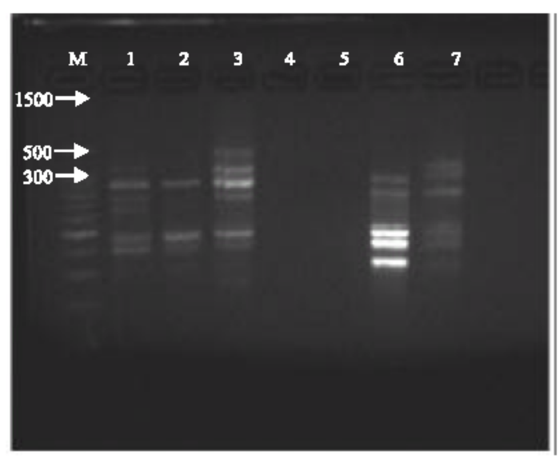

Fig. 4: RAPD patterns obtained from seven *Iris* species with the primer OPB-12. Species in the lanes are: M: 100 bp DNA ladder., 1: *Iris germanica*, 2: *Iris petrana* 3: *Iris atrofusca*, 4: *Iris nigricans*, 5: *Iris vartanii*, 6: *Iris aucheri*, 7: *Iris edomensis*

Pairwise comparison of all RAPD profiles revealed a similarity matrix (Table 5). The similarity among seven *Iris* species ranged from 0.14 between *I. vartanii* and *I. petrana* to 0.85 between *I. germanica* and *I. atrofusca*, *I. germanica* and *I. aucheri*. Within this range, high similarity (0.83) was found between *I. aucheri* and *I. petrana*, *I. aucheri* and *I. atrofusca*, while *I. edomensis* showed similarity value (0.73) with *I. atrofusca* and *I. aucheri*.

UPGMA dendrogram were constructed from the similarity values (Fig. 5). The dendrogram of the seven *Iris* species recognized two main clusters. The first one includes five species *I. germanica*, *I. petrana*, *I. vartanii*,

Table 3: Survey of primer OPB-11 marker in 7 *Iris* species

	Size of band (bp)										
Species	1500	1000	900	800	700	600	500	400	300	200	100
I. germanica	-	-	+	+	+	-	+	+	-	-	-
I. petrana	-	-	+	-	-	-	+	+	-	-	-
I. atrofusca	+	+	+	+	-	-	+	+	-	-	-
I. nigricans	-	-	-	-	-	-	-	-	-	-	-
I. vartanii	-	-	-	-	-	-	-	-	-	-	-
I. aucheri	-	+	+	+	-	-	+	+	-	-	-
I. edomensis	+	+	+	+	-	-	+	+	-	-	-

Table 4: Survey of primer OPB-12 marker in 7 *Iris* species

	Size of band (bp)										
Species	1500	1000	900	800	700	600	500	400	300	200	100
I. germanica	-	+	+	+	+	-	+	+	-	-	-
I. petrana	-	-	+	-	-	-	+	+	-	-	-
I. atrofusca	+	+	+	+	-	-	+	+	-	-	-
I. nigricans	-	-	-	-	-	-	-	-	-	-	-
I. vartanii	-	-	-	-	-	-	-	-	-	-	-
I. aucheri	-	+	+	-	-	+	+	+	-	-	-
I . edomensis	-	+	+	+	-	+	+	-	-	-	-

Table 5: Similarity coefficient matrix for pairwise comparison of 7 *Iris* species

	I. germanica	*I. petrana*	*I. atrofusca*	*I. nigricans*	*I. vartanii*	*I. aucheri*	*I. edomensis*
I. germanica	1						
I. petrana	0.71	1					
I. atrofusca	0.85	0.66	1				
I. nigricans	0.47	0.35	0.42	1			
I. vartanii	0.25	0.14	0.21	0.25	1		
I. aucheri	0.85	0.83	0.83	0.45	0.23	1	
I. edomensis	0.62	0.52	0.73	0.36	0.23	0.73	1

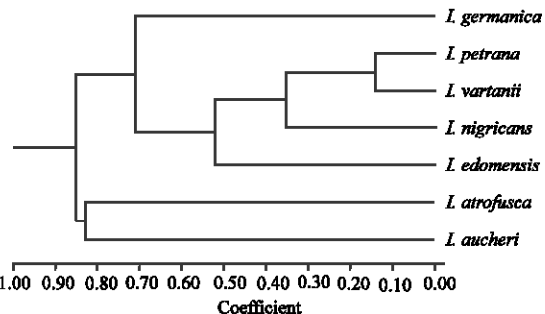

Fig. 5: Dendrogram of seven *Iris* species generated by UPGMA cluster analysis of the similarity values based on (Nei and Li, 1979) index

I. nigricans and *I. edomensis* with similarity of 71%. The second cluster, which has 83% similarity, includes to species *I. atrofusca* and *I. aucheri*.

DISCUSSION

Four primers were selected out of 12 primers used in this study. These primers generated polymorphic amplification fragments that were clear, conspicuous and highly reproducible but showed different intensity after illustrated under UV. Yang and Quiros (1993) have suggested that band intensity may reflect differences in the copy number of the amplified sequence among different species. These bands could be useful polymorphic markers for the seven *Iris* species.

The identification keys for the *Iris* species are presented in (Table 1-4). These keys could be of a practical use for *Iris* species identification and for distinguishing closely related species.

RAPD amplification of *Iris* DNA revealed a high degree of genetic variability among species investigated as revealed by the generation of unique sets of RAPD products, which indicates interspecific variations; i.e., RAPD markers can be used to differentiate some of *Iris* species grown in Jordan and are suitable to study their genetic diversity. This result provided further evidence that this technique offers a reliable and simple method for identification of closely related species and is in agreement with the findings of (Scovel *et al*., 1998; Shimizu *et al*., 1999; Artyukova *et al*., 2001).

In this study, the phylogenetic relationships of *Iris* species based on a molecular RAPD analysis were estimated; this agree with (Rodionenko, 1987; Mathew, 1989) who indicated that molecular data can supplement morphological studies and provide more reliable and powerful techniques for phylogenetic constructions.

As seen in the dendogram of *Iris* species, UPGMA separates the species into two main clusters. The species of the first cluster have diploid (2n = 20) number of chromosomes (Feinbrun, 1986) and growing in south (Karak and Tafila) of Jordan; except *I. vartanii* species, which grows in Wadi Shueib. The species of the second cluster includes *I. atrofusca* with (2n = 20) and *I. aucheri* with (2n = 22), (Darlington and Wylie, 1955). *I. atrofusca* is growing in Amman, while *I. aucheri* is growing in Karak. It seems that the high similarity (83%) between *I. atrofusca* and *I. aucheri* is not related to the chromosome number; it could be due to close origin of ancestry.

For PCR reactions, many studies were carried out using different concentrations of DNA. In this study, the optimum amount of template DNA for *Iris* was 0.5 µL. Additionally, it was found that adding more amount of $MgCl_2$ (2 µL) to the amount already exist in the buffer, may increase the relative intensities of the amplified DNA fragments. Magnesium is known to affect the primer-template interaction (Welsh and McCleland, 1990), polymerase activity and the melting temperature of the double-stranded DNA (Rolfs *et al*., 1992).

REFERENCES

Kareem, F.M. and S.A. Qor'an, 1998. Wild Flowers in Jordan. 1st Edn. Yarmouk University.

Al-Eisawi, D.M., 1986. Studies on the Flora of Jordan. Monocotyledon new to Jordan, with notes on some interesting species. Kew Bull., 41: 349-357.

Al-Khader, I., 1997. Systematic revision of the genus S. *Iris* (Iridaceae) in Jordan. M.Sc. Thesis, University of Jordan, Amman-Jordan.

Al-Eisawi, D. M., A. El-Oqlah, S. Oran and J. Lahham, 2000. Jordan country study on biological diversity: Plant Biodiversity and Taxonomy. United Nations Environment Program. Jordan, pp: 72.

Williams, J.G.K., A.R. Kubelike, K.J. Livak, J.A. Rafalaski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 8: 6531-6535.

Svitashev, S., 1997. Analysis of Triticeae genomes using RAPD markers. Swedish University of Agricultural. Agraia 61. Ph.D. Thesis Svalov, Sweden.

Ko, H.L., D.C., Cowan, R.J. Henry, G.C. Graham, A.B. Blakeney and L.G. Lewin, 1994. Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa L.*) varieties. Euphytica, 80: 179-189.

Vazquez, F.J., J.M.C. Jimenez and F.R. Vico, 1996. RAPD fingerprinting of Pepper (*Capsicum annum* L.) breeding lines. Capsicum and Eggplant Newslett., 15: 37-40.

Caetano-Anolles, G., B.J. Bassam and P.M. Gresshoff, 1991. High-resolution DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio, Technology, 9: 553-557.

Gwanama, C., M.T. Labuschagne and A.M. Botha, 2000. Analysis of genetic variation in *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. Euphytica, 113: 19-24.

Lowe, A.J., O. Hanotte and L. Guarino, 1996. Standardization of molecular genetic techniques for the characterization of germplasm collections: The case for Random Amplified Polymorphic DNA (RAPD). Plant Genetic Res. Newslett., 107: 50-54.

Fenwick, A.L. and S.M. Ward, 2001. Use of random amplified polymorphic DNA markers for cultivar identification in mint. Hortic Sci., 36: 761-764.

Gillies, A. C.M., J.P. Cornelius, A.C. Newton, C. Navarro, M. Hernandez and J. Wilson, 1997. Genetic variation in Costa Rican populations of the tropical timber species *Cedrela odorata* L. assessed using RAPDs. Mol. Ecol., 6: 1133-1146.

Martin, J.P. and J.E.H. Bermejo, 2000. Genetic variation in the endemic and endangered *Rosmarinus tomentosus* Huber-Morath and Maire (Labiatae) using RAPD markers. J. Hered., 85: 434-443.

Makarevitch, I., K. Golovnina, S. Scherbik and A. Blinov, 2003. Phylogenetic relationships of the Siberian species inferred from noncoding chloroplast DNA sequences. Intl. J. Plant Sci., 164: 229-237.

Arafeh, R.M.H., Y. Sapir, A. Shmida, N. Iraki, O. Fragman and H.P. Comes, 2002. Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. Onocyclus = the royal irises) along an ecogeographical gradient in Israel and the west Bank. Mol. Ecol., 11: 39-53.

Artyukova, E.V., M.M. Kozyrenko, M.V. Ilyshko, Y.N. Zhuravlev and G.D. Reunova., 2001. Genetic variability of *Iris* setosa. Mol. Biol., 35: 134-138.

Jill, A., A. Renate, C. Amy, A. Lisa and L. Michael, 2001. Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana *Iris* hybrid population. Mol. Ecol., 10: 673-681.

Zhuravlev, Y.N., M.M. Kozyrenko, E.V. Artyukova, G.D. Reunova and M.V. Ilyushko, 1998. Fingerprinting genomes of the Far Eastern species of the genus *Iris* L. by RAPD PCR. Russ. J. Genet., 34: 368-372.

Reeves, G., M.W. Chase, P. Goldblatt, P. Rudall, M.F. Fay, A.V. Cox, B. Lejeune and T. Souza-Chies, 2001. Molecular systematic of *Iridaceae*: Evidence from four plastid DNA regions. Am. J. Bot., 88: 2074-2087.

Hdeib, T.M., 2004. Genetic analysis of some olive genotypes (*Olea europaea* L.) grown in Jordan using random amplified polymorphic DNA (RAPD) markers. M. Sc. Thesis, Al-Balqa' Applied University, Al-Salt, Jordan.

Kharma, A.M., 2004. The genetic relationship between some medicinal plants and the antimicrobial activity of their crude extracts. M.Sc. Thesis, Al-Balqa' Applied University, Al-Salt, Jordan.

Galderisi, U., M. Cipollaro, G. Di Bernardo, L. Di Masi, G. Galano and A. Cascino, 1998. Molecular typing of Italian sweet chestnut genotypes by random amplified polymorphic DNA analysis. J. Hortic. Sci. Biotechnol., 73: 259-263.

Bartolozzi, F., M.L. Warburton, S. Arulsekar and T.M. Gradziel, 1998. Genetic characterization and relatedness among California almond cultivars and breeding lines detected by randomly amplified polymorphic DNA (RAPD) analysis. J. Am. Soc. Hortic. Sci., 123: 381-387.

Demeke, T., R.P. Adams and R. Chibbar, 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): A case study in *Beassica*. Theoretical and Applied Genetics., 84: 991-994.

Ren, J., J.R. Mcferson, R. Li, S. Kressovich and W.F. Lamboy, 1995. Identities and relationships among Chinese vegetable Brassica as determined by random amplified polymorphic DNA markers. J. Am. Soc. Hortic. Sci., 120: 548-555.

Fooland, M.R., R.A. Jones and R.L. Rodriguez, 1993. RAPD markers for constructing intraspecific Tomato genetic maps. Plant Cell Report, 12: 293-297.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Maguire, T.L., G. Collins and M. Sedgley, 1994. A modified CTAB DNA extraction procedure for plants belonging to family *Proteaceae*. Plant Molecular Biology Reporter, 12: 106-109.

Nei, M. and W.H. Li., 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc. Natl. Acad. Soc. USA., 25:803-814.

Welsh, J. and M. McCleland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Rolfs, A., I. Schuller, U. Finckh and I.I. Weber-Rolfs, 1992. PCR: Clinical Diagnostics and Research, Springer-verlag, Ny.

Yang, X. and C. Quiros, 1993. Identification and classification of celery cultivars with RAPD markers. Theoretical and Applied Genetics, 86: 205-212.

Scovel, G., H. Benmeir, M. Ovadis, H. Itzhaki and Vainstein, 1998. RAPD and RFLP markers tightly linked to the locus controlling Carnation (*Dianthus caryophyllus*) flower type. Theoretical and Applied Genetics, 96: 117-122.

Shimizu, K., Y. Miyabe, H. Nagaike, T. Yabuya and T. Adachi, 1999. Production of somatic hybrid plants between *Iris ensata* Thumb. and *I. germanica* L. Euphytica, 107: 105-113.

Rodionenko, G.I., 1987. The Genus *Iris* L.: Questions of Morphology, Biology, Evolution and Systematic. British Society, London: pp: 222.

Mathew, B., 1989. The *Iris*. Oregon Timber, Portland, Oreg: pp: 326.

Darlington, C. and A. Wylie, 1955. Chromosome Atlas of Flowering Plants. 2nd Edn., The University Press, Aberdeen, UK.,c pp. 63-70.

Feinbrun-Dothan, N., 1986. Iridaceae, In: Flora Palaestina, Part 4, (Eds., Zohari, M. and Feinbrun-dothan), Israel Academy of Sciences and Humanities, Jerusalem, pp: 112-127.