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Genetic Variability in a Medicinal Plant *Artemisia judaica* using Random Amplified Polymorphic DNA (RAPD) Markers

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ABSTRACT

Random amplified polymorphic DNA (RAPD) markers were used to study the genetic variability in a wild medicinal plant species *Artemisia judaica*, which grows in desert areas in the south of Jordan, mainly at Al-mudawarah. Among forty tested RAPD primers, ten primers showing polymorphic bands were used for the construction of the dendrogram and similarity matrix. A total of 1073 bands were obtained, 165 of them were polymorphic. Similarity, values among the studied accessions ranged from 0.61 to 0.02. High similarity values were obtained between two sample number (23 & 24). No similarity (value = 0.000) was not detected between studied samples of *A. judaica* species. RAPD analysis confirmed the presence of genetic variation within tested *A. judaica* species, that has the capability to evaluate genetic variation for other species of *Artemisia* genus. The obtained data will help adopt conservation strategies to avoid the extinction of medicinal plants from their natural habitats in the future.

Key Words: *Artemisia judaica;* Ba´atharan; *A. herba-alba;* Jordan; Medicinal plant; Polymorphism

INTRODUCTION

Artemisia judaica perennial plant, known Al-Ba´atharan in Arabic, is one of the common species of the genus *Artemisia*, this genus has two basic chromosome numbers x=9 and x=8 (Torrell & Vallès, 2001) with 2n=16 of *A. judaica*. This species grows in the valley bottoms in the desert areas particularly at Southern part nearest Saudi-Jordan borders (Al-Mudawarah), where camels graze on it. El-Ghonemy *et al*. (1977) reported that *A. judaica*, reflect the nature of vegetation associated with high environmental aridity and in most cases siliceous sandy soils. According to Al-Esawi (1998), *A. judaica* described as perennial, bushy herbs, strongly aromatic, with woody bases and strong spreading branches, covered by woolly hairs, leaves grayish, dissected, short, crowded, heads are rounded, crowded and made of tubular florets, habitat. Subtropical conditions, Wadi-Yutom, Wadi rum and Eastern Desert of Ma´an. *A. judaica* widely used in folk medicine, in Egypt it was recommended as healer plant in traditional medicine by (Nofal *et al*., 2009) and used for treatment of gastrointestinal disorders (Liu *et al*., 2004). *A. judaica* has important medicinal constituents that could be used in pharmaceutical drug production. Al-Mustafa and Al-Thunibat (2008) reported that *A. judaica* was one amongst of medicinal plants which has potential of antioxidant activity and used by Jordanian population as a traditional anti-diabetic agent. Nofal *et al*. (2009) mentioned that the single and multiple doses of both water and alcoholic extracts of *A. judaica* showed significantly reduced the blood glucose level in experimentally diabetic rates. *A. judaica* was used by Palestinian population in traditional Arabic medicine to treat fever, menstruation regulator and nerve system (calming) (Said *et al*., 2002).

Zohary (1978) described the *A. judaica* as follow: Chamaephyte, 50-80 cm, aromatic, branched from base, densely tomentose, grayish to whitish. Branches ending in panicle-like inflorescence. Leaves of sterile shoots crowded, short, petiolate, round in outline. 1-2- pinnatified or –partite, ultimate lobes ovate to oblong. Leaves of flowering branches very small and clustered; heads 3-4 mm in diameter, hemispherical, many-flowered, on slender peduncles, often nodding. Involucral bracts hairy, nearly orbicular, broad hyaline-margined and florets pale yellow. Achens minute, obovoid and glabrous. Flowering time: March-April. Habitat: sandy soils and wadi beds in deserts. Area: E. Saharo-Arabian.

Variation in the essential oil of *A. judaica* was studied by Putievsky *et al*. (2006) who found two distinct chemotypes the first artemisyl-oil type was predominant in Negev samples, while apiperitone-oil type was predominant in samples collected from Southern Sinai. Two compounds isolated from *A. judaica* namely, piperitone and trans-ethyl cinnamate showed pronounced insecticidal and antifeedant activity against the third instar larvae of *Spodoptera littoralis* (Boisd) (Abdelgaleil *et al*., 2008). Seventeen flavonoid glycosides and 12 a glycones were isolated and identified from *A. judaica* (Saleh *et al*., 1987). Lately, using

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of DNA molecular techniques was commonly used for studying the genetic relationship among accessions and analysis of genetic diversity between and within the populations. The classical systematics of *Aretmisia* has been refined by molecular methods, such as chloroplast DNA restriction site and randomly amplified polymorphic DNA (Vallès *et al*., 2001). RAPD technique was used to study phylogenetic relationship among 10 species belonging to six genera (Asreaceae) in Egypt (Abd El-Twab & Zahran, 2010). Also, RAPD profiling was used to determine the differences amongst 45 genotypes of mulberry, which included 12 species and 4 varieties (Hafiz *et al*., 2000).

In view of its medical value, mainly as natural antioxidant plant, *in situ* and *ex situ* conservation strategies is needed through agriculture policies and legislations to protect this species which is threat to extinct from its native habitat. Weiguo and Yile (2004) reported that RAPDs offer a reliable and efficient tool for assessing genetic diversity and selection of parents for breeding purpose. To my knowledge, this is the first report on the use of RAPD technique to determine the genetic variability within *A. judaica* species growing in Jordan desert areas.

MATERIALS AND METHODS

Plant material: Seeds of *Artemisia judaica* species collected during spring period by the author from its natural habitat (Southern part nearest Saudi-Jordan borders, Al-Mudawarh). The seeds were planted in nursery trays filled with mixed peat moss and perlite (1:1). Germination took 6-11 days, after 45 days fresh leaves collected for DNA analysis.

DNA extraction: Total cellular DNA was extracted following the procedure as described by Doyle and Doyle (1987), with minor modifications. Approximately 18 to 20 mg of fresh leaves were taken and grounded in liquid nitrogen and mixed with 600 µL of freshly and preheated 2X CTAB solution with 0.8 g PVPP in 2 mL tubes then placed at 65ºC for 30 min. The mixture was added to 600 µL of chlorophorm/isoamyl alcohol (24:1), vortexed for few seconds, and then centrifuged at 13,000 × *g* for 10 min. The supernatant was placed in 2 mL tubes with 600 µL isopropanol and then shacked until the thread of DNA appeared, then centrifuged for 10 min at 13000 × g. The solution is poured in tubes and left to dry, then 600 µL of cooled 70% ethanol was added to the solution and placed in the refrigerator (–20ºC) overnight. Next day, ethanol was poured in the dried tubes and 150 µL of TE was added and the whole mixture which is placed at 65ºC for 60 min. Four µL of RNAase (10 mg/mL) was added per tube and left for 45 min at 37ºC. DNA quantity was measured using S2100 UV/VIS DIODE-Array-Spectrophotometer, machine Version 1.7.

Genetic relatedness between *A. judaica* and *A. herba-alba* species: Eight isolated DNA samples of a medicinal plant of *A. herba-alba* collected from Al-Baq´a site were preserved at -20ºC and used to estimate the genetic relatedness with eight samples of DNA of medicinal plant *A. judaica*.

PCR amplification: PCR reaction was performed as described by Williams *et al.* (1990) with 10-mer ologonucleotides synthesized by Operon technologies (Almeda, Calif.). The final volume of 25 µL contained 10 x buffer with MgCl2, 20 ng of total genomic DNA, 0.25 mM dNTPs (Promega), 100 µM of primers, 1.5 mM MgCl2 and 1 U of *Taq* polymerase. Amplification was carried out in thermocycler (MJ Research, USA, Model PCT-200), one cycle of 1 min at 94°C followed by 44 cycles, each consisting of a denaturation step for 1min at 94°C, followed by an annealing step for 1min at 36°C and an extension step for 2 min at 72°C, followed by a further extension step for 5 min at 72°C. After the final cycle the samples were cooled at 4°C. Samples of 10 µL RAPD-PCR product were analyzed by electrophoresis on 1.4% a garose gel and the amplified products were detected after staining by ethidium bromide. Forty 10-mer primers were initially applied to the whole group of genotypes (Table I). Ten RAPD primers showing polymorphism within *A. judaica* were used for construction the dendrogram (Table II). Four RAPD primers namely OPD14, OPT16, OPW04 and OPW17 were sued to assess the genetic relatedness between *A. judaica* and *A. herba-alba*.

Data analysis: RAPD bands were manually scored as present (1) or absent (0) for estimation the similarity among all tested samples. The matrix of similarity (Jaccard) and similarity of coefficients (Nei & Li, 1979) were calculated and the dendrogram obtained by clustering according to the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) using SPSS, V. (11.0), software. Polymorphism percentage was estimated by dividing the number of polymorphic bands over the total number of bands.

RESULTS

From 40 initially applied primers, only 10 showed reproducible fragments with easily recordable bands. Total number of bands, number of polymorphic bands and percent of polymorphism shown in Table II. A total of 1073 RAPD fragments were consistently recognized and 165 (15.3%) of them were polymorphic for all samples (Table II). Representative polymorphic bands were appeared in the (Fig. 1). OPD11, OPD14, OPT03, OPT10, OPT16, OPT19 and OPT20, OPW04, OPW17 and OPZ12 primers used for differentiation among studied samples. The number of bands varied in different samples. Levels of similarity between samples ranged between 0.61 and 0.02 (Fig. 2). The highest average similarity index value of 0.61 was observed between two samples (23 & 24). The dendrogram was produced for *A. judaica* samples that show two main clusters (Fig. 2). The first cluster consisted of individuals' number from 1- 35, 36 and 38. Where this cluster can be subdivided into three sub-clusters, the first included sample

Table I: Primers names and their sequences used in this study

Primer name		Sequence 5'-3'	Primer name	Sequence 5'-3'
1.	OPA16	AGCCAGCGAA	21. OPD06	ACCTGAACGG
2.	OPA18	AGGTGACCGT	22. OPD10	GGTTCACACC
3.	OPA20	GTTGCGATCC	23. OPD11	AGCGCCATTG
4.	OPB01	GTTTCGCTCC	24. OPD14	CTTCCCCAAG
5.	OPB02	TGATCCCTGG	25. OPD16	AGGGCGTAAG
6.	OPB05	TGCGCCCTTC	26. OPD18	GAGAGCCAAC
7.	OPB06	TGCTCTGCCC	27. OPD20	ACCCGGTCAC
8.	OPB08	GTCCACACGG	28. OPT03	TCCACTCCTG
9.	OPB09	TGGGGGACTC	29. OPT05	GGGTTTGGCA
10.	OPB10	CTGCTGGGAC	30. OPT10	CCTTCGGAAG
11.	OPB12	CCTTGACGCA	31. OPT13	AGGACTGCCA
12.	OPB13	TTCCCCCGCT	32. OPT15	GGATGCCACT
13.	OPB14	TCCGCTCTGG	33. OPT16	GGTGAACGCT
14.	OPB17	AGGGAACGAG	34. OPT19	GTCCGTATGG
15.	OPB19	ACCCCCGAAG	35. OPT20	GACCAATGCC
16.	OPC09	CTCACCGTCC	36. OPW04	CAGAAGCGGA
17.	OPC10	TGTCTGGGTG	37. OPW17	CTCCTGGGTT
18.	OPC12	TGTCATCCCC	38. OPZ12	TCAACGGGAC
19.	OPC20	ACTTCGCCAC	39. OPZ15	CAGGGCTTTC
20.	OPD04	TCTGGTGAGG	40. OPZ16	TCCCCATCAC

Table II: Total bands, number of polymorphic bands and percent of polymorphism per primer of most polymorphic RAPD primers used in this study

Primer name	Total bands/primer	Number of polymorphic bands	% of polymorphism
OPD11	62	7	11
OPD14	44	9	20
OPT03	102	14	14
OPT10	79	16	20
OPT16	87	18	21
OPT19	117	18	15
OPT20	113	19	17
OPW04	144	22	15
OPW17	150	25	17
OPZ12	75	17	23
Total bands	1073	Average: 16.5	Mean: 16.3

numbers from 20 to 35, the second sub-cluster included sample numbers from 1-19, while the third sub-cluster had sample number 37 and 38. The second cluster was consisted of individual number of 36.

The level of similarity between *A. judaica* and *A. herba-alba* species ranged from 0.06 to 0.00 (No similarity) (Fig. 3). The dendrogram obtained by using four RAPD primers to assess the genetic relatedness between *A. judaica* and *A. herba-alba* species showed two main clusters the first included eight samples of *A. judaica* and the second cluster contained eight samples of *A. herba-alba* (Fig. 3).

DISCUSSION

Understanding of the genetic variation within and between populations is essential for the establishment of effective and efficient conservation for plants (Shafie *et al*., 2009). RAPD molecular marker technique indicated its potential for studying the genetic variability within *Artemisia* species. Higher similarity indices suggest that the

Fig. 1: RAPD-PCR polymorphism DNA of 19 (Lane1-19) samples of *Artemisia judaica* species based on OPW17 primer. M: 50 bp marker

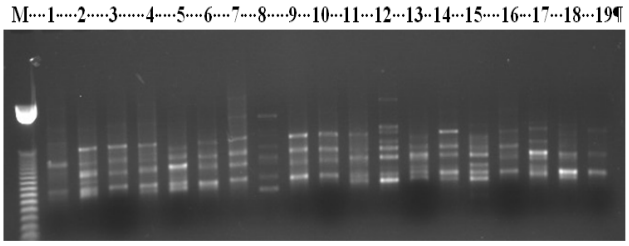
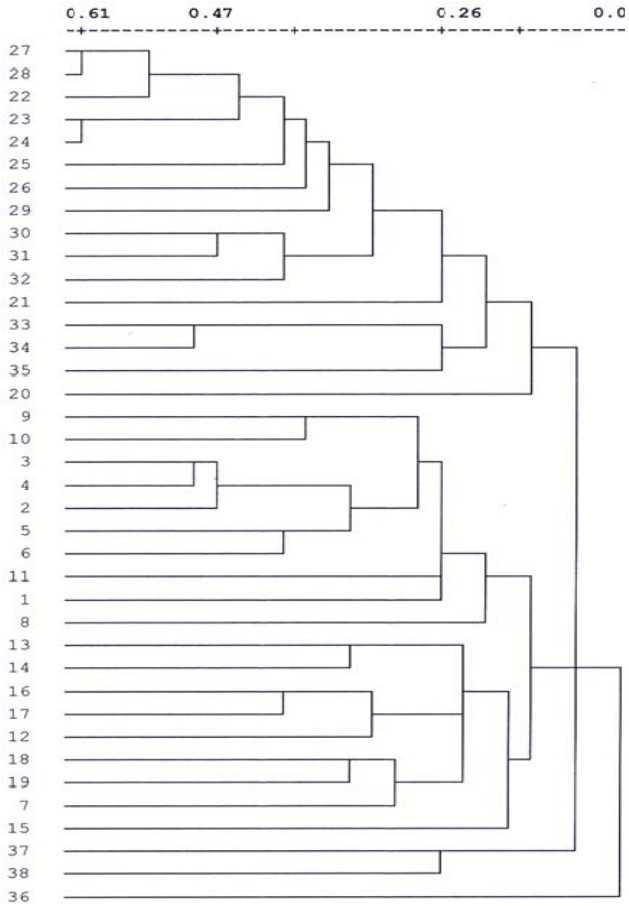
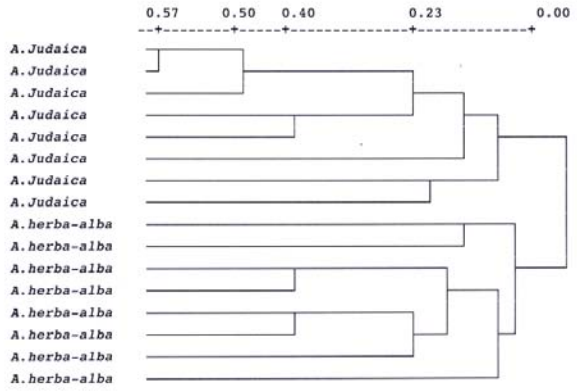


Fig. 2: A dendrogram within *Artemisia judaica* samples using ten polymorphic RAPD primers, based on Jaccard coefficient of similarity



tested individuals have closer genetic relation and slightly correlated with their close geographic location of studied area. Also, seeds collected from the plants in field could come from plants were naturally grown by sheded seed in the ground. Moreover, the samples with same banding profiles by using RAPD markers implying that those had genotypic similarity and could have originated from the same ancestor. Shafie *et al*. (2009) reported that the samples had the same patterns, which mean genotypic similarity. Genetic differences within studied samples might reflect

Fig. 3: A dendrogram between *Artemisia Judaica* and *Artemisia herba-alba* species using four polymorphic RAPD primers based on Jaccards´ coefficient of similarity



differences in the chemical composition of the essential oils from this species. Putievsky *et al*. (2006) reported that the differences found in the essential oil content and composition of *A. judaica* reflects the genetic differences between the studied populations. Genetic variability obtained within *A. judaica* in this study may provide an important source of genetic material for selection and improvement of this species. Very low similarity values found between *A. judaica* and *A. herba-alba* revealed that both species are genetically and phytochemically different. This indicated that RAPD markers can helpful in molecular taxonomy to differentiate between species within the same genus.

Rawashdeh *et al*. (2009 a & b) reported that more efficient results will be obtained if all findings correlated with ecogeographical information and if it is possible to compare them with other species grown within neighbor countries of Jordan. However, further study should be taken into consideration using a developing molecular markers techniques such as ISSR, SSR and AFLP as well *in vitro* production systems for investigation of bioactivity and germplasm conservation of *A. judaica* and studying the chemical composition of the essential oil from this species and compared to the other native species of *Artemisia* genus. The use of micropropagation techniques is recommended firstly to produce homogenous true types especially for plants with high percentages of specific essential oils and other active substances needed for drug industry and secondly to conserve and multiply this target species.

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