World Applied Sciences Journal 18 (10): 1475-1480, 2012
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2012.18.10.2753

Genetic Variation in Seven Natural Populations of *Artemisia judaica* L. in South Sinai Using RAPD Markers

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Abstract: Genetic diversity among seven natural local populations of the aromatic shrubby herb; *Artemisia judaica* L. from different sites in South Sinai, Egypt has been investigated using random amplified polymorphic DNA (RAPD) markers. A total of 87 amplified bands, including 50 polymorphic, were scored using 10 selected RAPD primers, with an average of 8.7 amplified bands per primer and 57.47% polymorphism, indicating a marked genetic variation in the examined populations. The RAPD markers were used to calculate the similarity between the examined populations and construct a distance tree that illustrates the genetic distance between them. These data provide important baseline data for conservation and collection strategies for this species. Meanwhile more studies are recommended to determine the populations that should be sampled in ex-situation protection so as to retain as much genetic diversity as possible.

Key words: *Artemisia judaica* · Genetic diversity · DNA fingerprinting · RAPD

INTRODUCTION

Artemisia judaica L. is one of the important species in the genus *Artemisia* of the family Asteraceae, as a perennial bushy herb, strongly aromatic, with woody bases and strong spreading branches, covered by woolly hairs, leaves grayish, dissected, short, crowded, inflorescence rounded heads crowded and made of tubular yellow, all fertile florets; achenes narrowly obovoid, striate, glabrous, epappose [1]. In Egypt, *A. judaica* is recorded in wadi beds, terraces and stony plains particularly in Sinai [1]. Like other wild medicinal plants, *A. judaica* in Egypt is exposed to serious threats due to natural drought and heavy human impacts such as uncontrolled tourism, overgrazing and uncontrolled collection, mining and quarrying. The sustainable conservation of the threatened plants requires evaluation of the genetic diversity of different populations in different habitats to elucidate the genetic differences between populations. *A. judaica* (Wormwood) is used in folk medicine; leaves are inhaled to relieve congestion of colds, infusion of flowering branches of this species relieves gastro-intestinal cramps, stomach upset, abdominal disturbances and constipation and antispasmodic in case of intestinal colic and volatile oil of this species has antimicrobial activity [2, 3], also its volatile components have antioxidant activity [4] and the extracts of aerial parts induced antidiabetic activities [3]. Two compounds isolated from *A. judaica* namely, piperitone and trans-ethylecinnamate showed pronounced insecticidal and antifeedant activities against the third instar larvae of *Spodoptera littoralis* [5]. In addition, Abd-Elhady [6] found that aerial parts of *A. judaica* show a potential to be developed into biopesticide for controlling the cowpea weevil insects. Genetic diversity has been documented in different species of *Artemisia*. Morphological and molecular variability was addressed in three species; *A. vulgaris*, *A. roxburghiana* and *A. absinthium* from Jamou and Kashmir by Nazar and Mahmood [7]. High chemical polymorphism was also associated with intra-specific variability of *A. herba alba* from Southern Spain [8]. It has

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also been documented that geographical conditions affect the active constituents of *A. annua* [9]. In the Northern part of central Saudi Arabia, RAPD fingerprinting also indicated sharp polymorphism among populations of *A. judaica*, *A. monosperma* and *A. herba alba*; geographic and local ecological variations related to elevation of the sites of the examined populations may be regarded to have played a role in the genetic diversity of these populations in the studied area [10]. RAPD analysis confirmed the presence of genetic variations within *A. judaica* in Jordan [11]. Genetic diversity among *A. judaica* and *A. monosperma* populations in Egypt was addressed by Badr *et al.* [12]; the results showed that the populations of *A. judaica* growing in the mountains of Sinai were clearly distinguished from other populations growing at lower elevations in other parts of Egypt based on morphological differences. The mountains populations in Saint Catherine area were also distinguished by RAPD profiling supporting the recognition of some populations of *A. judaica* in South Sinai as a separate variety as proposed by Boulos [1]. In the present investigation RAPD as DNA marker was used to investigate genetic differentiation among *A. judaica* natural populations from different sites in South Sinai, to provide genetic data and a theoretical basis for protection of the species.

MATERIALS AND METHODS

Study Sites and Sampling Materials: Healthy leaf samples of *A. judaica* were collected, for RAPD analysis, from seven individuals representing seven populations; five from Saint Catherine area and two from Nuwieba-Dahab road, the sites are given in Table 1. The selected populations were visited over a period of 18 months (2010-2012). The identification and nomenclature of the studied plant was carried out with the help of Boulos [1]. The dried plant materials were preserved in the Herbarium of Botany and Microbiology Department, Faculty of Science, Al-Azhar University.

DNA Extraction: Total genomic DNA was extracted from 2 g leaf materials from each collected sample of the seven populations of *A. judaica*, according to Sharma *et al*. [13] and Abd El-Tawab and Zahran [14]. Leaf samples were kept in a fixing solution (95% ethanol) for 60-90 min, dried and homogenized in a mortar and pestle in ice. The homogenized tissue was transferred to extraction buffer (2% w/v hexadecyl-trimethyl- ammonium bromide (CTAB), 100 mM Tris-HCl (PH 8.0), 1.4 M NaCl, 20 mM EDTA and mixed by gentle inversion. The samples were then incubated at 60°C for 1h, centrifuged for 5 min at 10 000 rpm. The supernatant was collected and a volume of chloroform: isoamyl alcohol (24: 1) was added and mixed by gentle inversion for 10 min, then centrifuged at 10 000 rpm for 10 min. The upper aqueous phase was transferred to another tube and this process was repeated until the chloroform-isoamyl alcohol became clear. Twice the volume of cold absolute ethanol was added to the aqueous solution and gently mixed, then centrifuged briefly to precipitate DNA. The DNA pellet was washed with 70% ethanol and mixed by gently inversion. Finally, DNA was left to dry at room temperature, then dissolved in 1x TBE buffer. RNA was digested with RNase at 37°C for 1h. The DNA was further purified by column filtration and quantified by taking the optical density at L260 nm with a spectrophotometer. Readings at L260 and L280 was taken to obtain the L260/L280 ratio as an indicator of DNA purity [15]. The purified DNA was observed on 1.5% Agarose gel after staining with ethidium bromide to ascertain its integrity.

Table 1: Collection sites of *Artemisia judaica* populations from South Sinai

Sample number	Collection sites
1	Nuwieba-Dahab Road
2	Wadi Eshgeraa, Nuwieba-Dahab Road
3	Wadi Ellojer, Saint Catherine
4	Wadi Elttorfa, Saint Catherine
5	Elnabi-Haroon, Saint Catherine
6	Wadi Elmarwa, Saint Catherine
7	Wadi Abo Zaitona, Saint Catherine

RAPD Analysis: RAPD analysis was conducted according to Abd El-Tawab and Zahran [14] and Huang *et al*. [16]. PCR amplification was done with 10 primers for detecting polymorphisms among the studied samples (Table 2), using 100 ng of genomic DNA. The 25 µL PCR mixture contained 2.5 µL of 10x buffer (*Taq* DNA polymerase complete high specificity reaction buffer); 2.5 µL dNTPs (2 mM stock), 3 µL primers (Operon Nippon EGT Co. Ltd.), 3 µL of *Taq* DNA polymerase, the DNA template (10 ng/µL), 2 µL $MgCl_2$ (1.5 mM) and 11.7 µL double distilled water. The amplification was performed in a DNA thermal cycle Gene Amp® PCR System 9700 (Perkin Elmer, England), programmed to 40 cycles after an initial cycle for 5 min at 94°C; each cycle consisted of a denaturation step at 94°C for 40 sec, annealing step at 36°C for 1 min, extension step at 72°C for 1 min, followed by a final step at 72°C for 7 min. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium

Table 2: Sequence of the 10 RAPD primers

Primer code	Nucleotide sequence 5' - 3'
B-06	TGCTCTGCCC
B-08	GTCCACACGG
E-04	GTGACATGCC
E-05	TCAGGGAGGT
G-04	AGCGTGTCTG
O-03	CTGTTGCTAC
O-17	GGCTTATGCC
F-06	GGGAATTCGG
W-18	TTCAGGGCAC
Z-18	AGGGTCTGTG

bromide (0.5 µg/ml) in 1x TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

Data Analysis: Fragment sizes were designated as amplified bands and the bands were shared as diallelic characters (present = 1, absent = 0). These bands amplifying in each instance were scored and included in the analyses. The number of multi-locus genotypes (unique arrays of amplified bands) was calculated for each population. The Jaccard's coefficient was employed to calculate pairwise bands similarities for all plant individuals and a distance tree was constructed by the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) using the NTSYS-pc 2.02 software package (Numerical Taxonomy System, Software) [17].

RESULTS

RAPD analysis of *A. judaica* individuals revealed obvious genetic variation among the seven investigated populations as indicated by the polymorphic products illustrated in Figure 1. A total of 87 amplified fragments were scored of which 50 (57.47%) were polymorphic. The number of amplified DNA fragments generated by each primer varied from 3 (B-08) to 14 (O-17) with an average of 8.7 (Table 3). Primer W-18 produced the highest number of polymorphic bands (13); in contrast to primers B-08 and F-06, which were unable to produce polymorphic bands. On the other hand, primers W-18 and O-17 were found to be useful to generate specific bands. The RAPD product sizes ranged from 215 to 2556 bp.

DNA fingerprints generated by the tested primers were used to calculate Euclidean distance between the investigated samples of *A. judaica* (Table 4). Such distance ranged from 59.7 between population 1 (Nuwieba road) and population 6 (Wadi Elmarwa) to 83.6 that characterized the most closely related populations

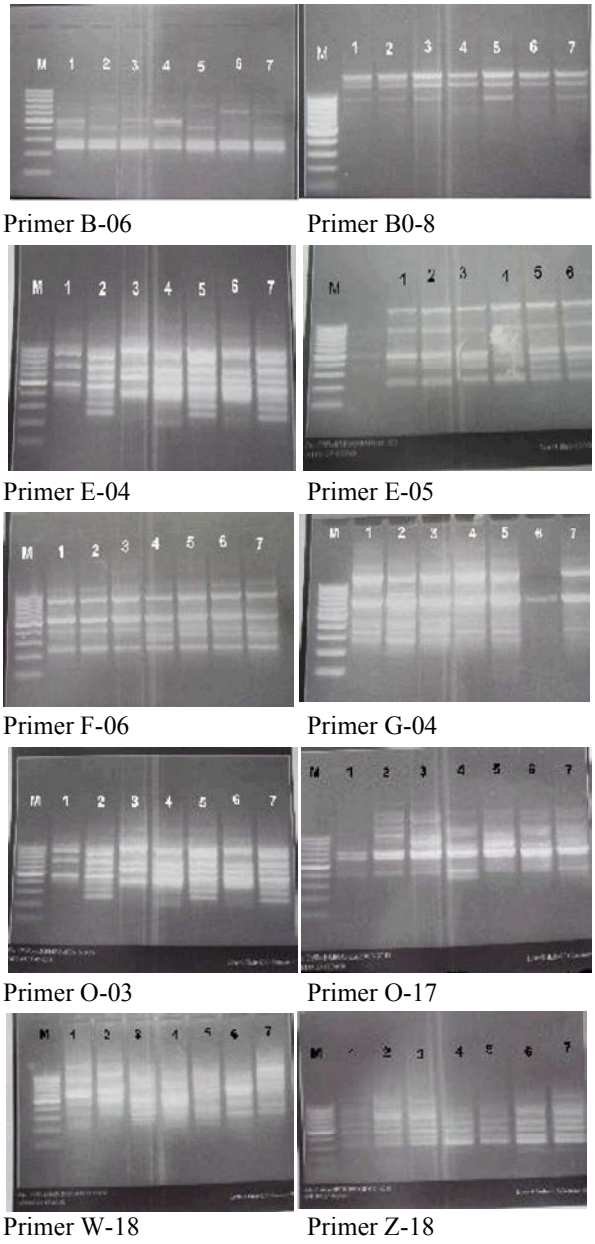

Fig. 1: RAPD profile of seven individuals of *A. judaica* from different sites *via* ten primers
M: 1kb DNA ladder Lane 1-7: sites of collection according to Table 1.

from Nuwieba road (2) and Wadi Abo Zaitona (7). The distance tree based on Jaccard's coefficient (Fig. 2) showed that the investigated populations were classified into two main groups and sample of population 6 (Wadi Elmarwa) was obviously separated. The first group contains populations 1 (Nuwieba road) and 4 (Wadi Elttorfa). While, the second one was clearly

Table 3: Polymorphic amplified bands detected with 10 RAPD primers for seven studied populations of *A. Judaica*.

Primer	Amplified fragment size (bp)	G-C content (%)	No. of amplified bands	No. of polymorphic bands	% of polymorphism
B-06	260-869	70	9	5	55.50
B-08	998-1578	70	3	0	0.00
E-04	389-1829	60	7	2	28.60
E-05	229-1489	60	9	6	66.60
G-04	246-1133	60	8	7	87.50
O-03	270-1761	60	10	5	50.00
O-17	215-1057	60	14	11	78.60
F-06	356-2556	60	6	0	0.00
W-18	277-1349	60	13	13	100.00
Z-18	311-1163	60	8	1	12.50
Total	--	--	87	50	57.47

Table 4: Euclidean distance among the studied populations of *A. judaica* based on RAPD data analysis

Statistical Cluster Analysis	Euclidean distance						
Variables	1	2	3	4	5	6	7
1	100						
2	69.4	100					
3	67.6	71.4	100				
4	73.2	70.1	77.3	100			
5	72.6	78.7	81.3	73.1	100		
6	59.7	68.5	68.9	67.6	62.8	100	
7	70.3	83.6	74.4	68.8	79.2	71.6	100

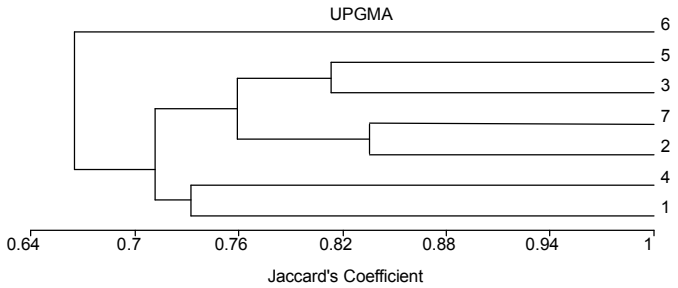

Fig. 2: UPGMA dendrogram based on data generated from 10 tested RAPD primers, showing the genetic distance (linkage distance) among the studied populations (1-7). Numbers are representing the site of the collected samples listed in Table 1.

separated into two subgroups, populations 5 and 3 (Wadi Elnabi Haroon and Wadi Ellojer, respectively) and populations 7 and 2 (Wadi Abo Zaitona and Wadi Eshgeraa, respectively).

DISCUSSION

The genetic variation observed in the present study among the tested samples of *A. judaica* may be due to variation of locations along environmental heterogeneity as well as reproductive system. The role of geographic conditions on morphological and molecular variability was proposed in three *Artemisia* species; *A. vulgaris*, *A. roxburghiana* and *A. absinthium* from Jammu and Kashmir [7]. Similarly, polymorphism of *A. capillaries* from different parts in Malaysia was observed by Hasan *et al*. [18, 19]; they also indicated that the geographical condition affects the active constituents of the medicinal plants. Geographic and local ecological variations related to elevation of the sites of populations were regarded to have played a role in the genetic diversity of populations in three species of *Artemisia*; *A. herba alba, A. monosperma* and *A. judaica* in the northern part of middle of Saudi Arabia [10, 12]. The data presented here support the view proposed by Badr *et al.* [10] that RAPD markers were particularly useful in differentiating populations of *A. judaica* growing in the bed of valleys from populations growing at terraces,

indicating the role of local geographic conditions in genetic diversity, in addition, the populations of *A. judaica* growing in the mountains of South Sinai are clearly distinguished from other populations, supporting the point of view said that populations of this species from mountains of South Sinai may be considered as a sub-specific identity. The populations of *A. judaica* growing in the mountains of Sinai were clearly distinguished from other populations growing at lower elevations in other parts of Egypt based on morphological differences. RAPD markers were also useful in differentiating *A. judaica* populations in Jordan. Al-Rawashdeh [11] reported that genetic differences within studied samples might reflect differences in the chemical composition of the essential oil.

On the basis of previous report of Sun *et al*. [20]; geographically close habitats can be ecologically being different and conversely, habitats that are geographically distant from one another can be very similar in their environmental conditions. Additional factors affecting genetic diversity assayed by different marker techniques are the number of probes used in the analysis, protocol for extracting DNA and methods of sample collection and storage. Kumar *et al*. [21] came to the conclusion that the method of storage after leaf sample collection and the type of the DNA extraction reagents as well as the secondary compounds had a major influence on the DNA quality and on the success of the molecular studies. RAPD can detect variation in both coding and non coding regions. Small repeated random sequence mutations would be accumulated in non-coding sequences and the diversity can be revealed by RAPD [20]. Another factor which needs to be considered for RAPD analysis as that band of identical mobility may occasionally correspond to nonhomologous fragments [20]. To ascertain the obtained data and observation, further conformation must be carried out using larger number of RAPD primers as well as other molecular approaches may be necessary. In addition, chemotype fingerprinting and karyotype, karyology *etc*. may add more information on the genetic differentiation among more individuals and populations of this species in order to identify areas of maximum diversity and to estimate genetic variability in natural populations.

REFERENCES

1. Boulos, L., 2002. Flora of Egypt. Al-Hadara Publications, Cairo, Egypt, 3: 258-260.
2. Batanouny, K.H., 1999. Wild Medicinal Plants in Egypt: An Inventory to Support Conservation and Sustainable Use. With contributions by Aboutabl E, Shabana M and Soliman F. Academy of Scientific Research and Technology, Egypt and IUCN, Switzerland.
3. Nofal, S.M., S.S. Mahmoud, A. Ramadan, G.A. Soliman and R. Fawzy, 2009. Anti- diabetic effect of *Artemisia judaica* extracts. Res. J. Med. Sci., 4: 42-48.
4. El-Masry, K.F., A.H. El-Ghorab and A. Farouk, 2003. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chem., 79: 331-336.
5. Abdelgaleil, S.A.M., M.A. Abbassy, A.S.H. Belal and M.A.A. Abdel Rasoul, 2008. Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L. Bioresour. Technol., 99: 5947-5950.
6. Abd-Elhady, H.K., 2012. Insecticidal activity and chemical composition of essential oil from *Artemisia judaica* L. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Plant Prot. Res., 52: 3.
7. Nazar, N. and T. Mahmood, 2011. Morphological and molecular characterization of selected *Artemisia* species from Rawalakot, Azad Jammu and Kashmir. Acta Physiol. Plant., 33: 625-633.
8. Salido, S., L.R. Valenzuela, J. Altarejos, M. Nogueras, A. Sanchez and E. Cano, 2004. Composition and infraspecific variability of *Artemisia herba alba* from southern Spain. Biochem. Syst. Ecol., 32: 265-277.
9. Wallaart, T.E., N. Pras, A.C. Beekman and W.J. Quax, 2000. Seasonal variation of artemisinin and its biosynthetic precursors in plants of *Artemisia annua* of different geographical origin: proof for the existence of chemotypes. Planta Med., 66: 57-62.
10. Badr, A., W. Morsy, S. Abdelfattah, S. Shams and A. Shehab, 2012a. Genetic diversity in *Artemisia monosperma* and *Artemisia judaica* populations in Egypt based on morphological, karyological and molecular variations. J. Med. Plant Res., 6: 66-78.
11. Al-Rawashdeh, I.M., 2011. Genetic variability in a medicinal plant; *Artemisia judaica* using random amplified polymorphic DNA (RAPD) markers. Int. J. Agric. Biol., 13: 279-282.
12. Badr, A., H.H. El-Shazly, N.S. Helail and W. El Ghanim, 2012b. Genetic diversity of *Artemisia* populations in central and north Saudi Arabia based on morphological variation and RAPD polymorphism. Plant Syst. Evol., 298: 871-886.

13. Sharma, R., H.R. Mahla, T. Mohapatra, S.C. Bhargava and M.M. Sharma, 2003. Isolating plant genomic DNA without liquid nitrogen. Plant Mol. Biol. Rep., 21: 43-50.
14. Abd El-Twab, M.H. and F.A. Zahran, 2008. Extracting total genomic DNA of *Chrysanthemum sensulato* by CTAB and SDS Without Liquid Nitrogen and Phenol. Chromos. Bot., 3: 83-88.
15. Sambrook, J., E. Fritsch and T. Maniatis, 1989. Molecular is Cloning A Laboratory Manual, 2nd Edition, Vol. 3. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
16. Huang, S.C., C.C. Tsai and C.S. Sheu, 2000. Genetic analysis of *Chrysanthemum hybrids* based on RAPD molecular markers. Botanical Bull. Acad. Sinica, 41: 257-262.
17. Rolf, F.J., 2000. Numerical Taxonomy and Multivariate Analysis System, version 2.02. Setauket (New York), Exeter Publishing.
18. Hasan, S.M.Z., M.S.B. Shafie and R.M. Shah, 2009a. Analysis of random amplified polymorphic DNA (RAPD) of *Artemisia capillaries* (Worm Wood Capillary) in east coast of peninsular Malaysia. World Appl. Sci. J., 6(7): 976-986.
19. Hasan, S.M.Z., M.S.B. Shafie and R.M. Shah, 2009b. Genetic variability of *Artemisia capillaris* (Wormwood capillary) by random amplified polymorphic DNA (RAPD) in Terengganu State Malaysia. Afr. J. Biotechnol., 8: 1810-1814.
20. Sun, G.L., O. Diaz, B. Salomon and R. Von Bothmer, 1999. Genetic diversity in *Elymus caninus* as revealed by isozyme, RAPD and microsatellite markers. Genome, 42: 420-431.
21. Kumar, J., G.P. Mishra, K.P. Naik, A.A. Murkute and R.B. Srivastava, 2011. Genomic DNA isolation from *Artemisia* species grown in cold desert high latitude of India. Afr. J. Biotechnol., 10(37): 7303-7307.