Australian Journal of Basic and Applied Sciences, 5(6): 1223-1234, 2011
ISSN 1991-8178

Genetic Diversity in *Artemisia Monosperma* and *Artemisia Judaica* Populations in Egypt Based on Morphological, Karyological and Molecular Variations

[1]Abdelfattah Badr, [2]Wafaa Morsy, [2]Sahar Abdel-Tawab, [2]Sahar Shams and [2]Amal Shehab

[1]Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt.
[2]Botany Department, Faculty College of Girls, Ain Shams University, Cairo, Egypt.

Abstract: The diversity of ten populations representing *Artemisia monosperma* and *A. judaica* has been analyzed, based on assessment of morphological, karyological and molecular variations. The analysis of morphological variation and molecular polymorphism as revealed by RAPD, using the software NTSYSpc-2.01, confirmed the differentiation of *A. monosperma* and *A. judaica* as two distinct species and showed wider variations among *A. judaica* populations compared to the *A. monosperma* populations. Karyotype analysis revealed that all *A. monosperma* populations are tetraploid with $2n$=36 and a basic number of x=9, while all samples of *A. judaica* are diploid with $2n$=16 and x=8. One B chromosomes was detected in three populations of *A. monosperma*. Like most other species of *Artemisia* both species have symmetric karyotype but the chromosomes of *A. monosperma* are generally shorter than those of *A. judaica*. 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 the Egypt based on morphological differences. However, these two populations differ considerably in chromosome length being 4.85±0.42 μm for the population growing in wadi beds and 3.81±0.28 μm for population growing on the terraces. The latter population is clearly distinguished by RAPD profilin from the other four populations supporting the recognition of some populations of *A. judaica* in South Sinai as a separate variety.

Key words: *Artemisia*, morphology, Karyotype, RAPD

INTRODUCTION

The genus *Artemisia* L. (*Asteraceae*, *Anthemideae*, *Artemisiinae*) comprises around 500 taxa at the specific or subspecific level (Bremer and Humphries, 1993). It has been divided into five large sections namely: *Absinthium* DC, *Artemisia* L., *Dracunculus* Besser, *Seriphidium* Besser and *Tridentatae* (Rybd.) (Torrell *et al.,* 1999). A general review of different systematic and evolutionary aspects of the genus, with special emphasis on cytogenetic and molecular data was given by Vallès and McArthur (2001). Most species of *Artemisia* are perennial plants with less than 10 annual species and the majority of species are widely dispersed across the Northern Hemisphere (Bremer and Humphries, 1993). The present infrageneric classification does not represent natural groups (Vallès and McArthur 2001) and there is still no agreement about the global treatment of the genus (Watson *et al*. 2002).

Some members of *Artemisia* are foraged by ungulates, rodents, birds, and insects despite the production of sesquiterpenes that afford a bitter taste to the herbage (Marco and and Barbera, 1990) and woody species increase dramatically under grazing pressure (Young *et al*., 1996). All *Artemisia* species produce aromatic oils, and several are culinary herbs or used as flavorings, hallucinogens, vermifuges, and pharmaceuticals; many species cause allergies to humans and some are toxic (Marco and Barbera, 1990; Burrows and Tyrl, 2001). *Artemisia annua* (annual wormwood) and *A. mexicana* produce anti-malarial drugs and artemisinin extracted from *A. annua* appears to selectively kill human breast cancer cells (Singh and Lai, 2001). Oral artemisinin was reported to prevent and delay the development of 7, 12-dimethylbenz[a] anthracene (DMBA)–induced breast cancer in the rat (Lai and Singh, 2006).

In Egypt, *Artemisia* is represented by four wild species (*A. monosperma, A. scoparia, A. judaica,* and *A. verlotiorum*) and one more species (*A. vulgaris*) is cultivated (Boulos, 2002). *Artemisia monosperma* is widespread in the desert plains and wadis, both inland and in the Mediterranean coastal region, often not too far from the coast in northern Sinai. On the other hand, *A. judaica* is recorded in wadi beds, terraces and stony plains. The two plants have economic importance, the basal woody parts of old plants from *A. monosperma*

Corresponding Author: Wafaa Morsy,Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt.
E-mail: satawab@yahoo.com

are used as firewood by the Bedouin and the leaves of *A. monosperma* and *A. judaica* are used in the folk medicine. An infusion prepared from the flowering branches of the latter species is used as stomachic, anthelmintic, expectorant, diaphoretic, analgesic, and antispasmodic in case of intestinal colic and the volatile oil of this species has antimicrobial activity (Batanouny, 1999) and its volatile components has antioxidant activity (El-Masry *et al.*, 2003). More recently, the extracts of *A. judaica* aerial parts induced anti-diabetic activities (Nofal *et al.,* 2009).

Comparative karyotype analysis of related species has traditionally been used to describe patterns and directions of chromosomal evolution within plant groups and show the nature of the chromosome differences between and within species (Stace, 2000; Levin, 2002). The karyotype is the phenotypic aspect of the chromosome complement as seen at mitotic metaphase (Darlington and LaCour, 1976). A description of the karyotype typically includes the 2*n* chromosome number, the absolute and/or relative length of chromosomes (reflecting genome size), the position of primary and secondary constrictions (Levan *et al*., 1964) and the degree of symmetry; a symmetrical karyotype is characterized by mainly metacentric and sub-metacentric chromosomes of approximately equal size. Karyotype symmetry is also measured by the total form percentage (TF%) as proposed by Huziwara (1962). Asymmetric karyotypes can arise by shifts in centromere position towards the telomere (intra-chromosomal) and/or by the addition or deletion of chromatin from some but not all chromosomes, leading to differences in size between chromosomes (inter-chromosomal). Zarco (1986) has proposed the two indices A_1 and A_2 for karyotype asymmetry based on these changes. In Asteraceae growing in Egypt, detailed karyotype features for 23 species of subfamily Asteroideae were described by Badr *et al.* (1997) and karyotypes of additional 36 species of Asteraceae were described by Kamel (1999; 2001) and Badr *et al*. (2009). Shams (2004) confirmed counts by Badr *et al.* (1997) that *A. monosperma* is tetraploid with 2*n*=36 and and *A. judaica* is diploid with 2*n*=16.

In the last two decades, several PCR-based molecular markers have been made available for biological research. The simple sequence repeats (Tautz, 1989), random amplification of polymorphic DNA (Williams *et al.*, 1990), amplified fragment length polymorphism (Vos *et al.*, 1995) and the inter-simple sequence repeats (Zietkiewicz *et al.*, 1994) are the most common of these markers and have been widely used in genetic diversity analysis (Henry, 2001; Gostimsky *et al.,* 2005). The RAPD procedure works with anonymous genomic markers, requires only small amount of DNA, and is simple and less labor than the other DNA markers (Wolfe and Liston 1998). RAPD is highly suitable for quick fingerprinting and for analysis of genetic relationships among populations. The observed variations in the number of bands amplified by different random primers may be influenced by variable factors such as primer sequence, template quantity and less by the number of annealing sites in the genome (Kernodle *et al*. 1993).

Morphological and molecular variability was addressed in the species of *Artemisia*. The random amplified polymorphic DNA (RAPD) markers have provided a powerful tool for the investigation of genetic diversity in *A. vulgaris*, *A. roxburghiana* and *A. absinthium* from Jamou and Kashmir by Nazar and Mahmoud (2010). Similarly, polymorphism of *Artemisia capillaris* from different parts in Malaysia was assessed by Hasan *et al.* (2009; 2010). Infraspecific variation in *Artemisia herba alba* in Tunisia was addressed by Mohsen and Ali (2008) using ISSR markers; the results indicated patchy distribution of the genetic variability among different populations of this species revealing a contribution of local ecological and geographic conditions on its variability. RAPD analysis confirmed the presence of genetic variation within *A. judaica* in Jordan (Al-Rawashdeh, 2011). High chemical polymorphism was also associated with infra-specific variability of *Artemisia herba alba* from southern Spain (Solido *et al.* (2004). It has also been well documented that geographical condition affects the active constituents of the medicinal plants (Wallaart *et al.,* 2000).

In the present study, detailed karyological features and RAPD profiling have been applied to assess the genetic diversity among populations of two species of *Artemisia*; *A. monosperma* and *A. judaica* collected from ten different localities in Egypt. In addition to comparative karyotype analysis and RAPD profiling, selected morphological characters have been assessed to supplement karyotype variation and molecular polymorphism in measuring the genetic diversity among populations of the two species in Egypt.

MATERIALS AND METHODS

Plant material:

Morphological samples of ten populations were collected from different locations in Egypt as indicated in map 1. Detailed morphological measurements were made for at least five mature plants for each population. The morphological criteria include eight quantitative traits and the average value of each trait ± standard deviation was calculated for each trait. In addition, the state of 22 qualitative traits was determined (Table 2).

Vouchers for the ten populations have been deposited at the herbarium of the Botany Department, Women's College, Ain Shams University. The measured characters, their states and the codes given to each character state for data analysis are listed in Table 2.

Cytological Procedures:

For karyotype preparation, seeds gathered from ripe achenes of the ten populations of both *A. monosperma* and *A. judaica* were germinated on wet filter paper in Petri dishes in the dark at room temperature. Germinating roots were pretreated with 0.05% colchicine solution for 3-4 hours at 20-25 C, fixed in absolute ethanol and glacial acetic acid (3:1) for 24 hours and stored in 70 % ethanol at 4 C until use. Cytological preparations were made using the standard Feulgen's squash technique according to Darlington and La Cour (1976). A karyotype for each population was constructed as follows. Chromosome images from well-spread preparations were cut from photographic prints enlarged to a magnification of ×=3300, matched in pairs according to their length and arm ratio and arranged in order of decreasing length (Levan *et al.*, 1964).

Karyotype analysis, for the ten populations, was made based on mean chromosome length in µm ± standard error (MCL±SE); calculated by dividing the total length of all chromosomes on the number of chromosomes and mean arm ration ± SE. (MAR±SE); calculated by dividing the sum of long arms on the sum of short arms of the chromosomes. MAR and its SE usually reflect the symmetry of the karyotypey. However, karyotype asymmetry was calculated as the total form percentage (TF%) as proposed by Huziwara (1962). Karyotype asymmetry for each population was also estimated using the A_1 (intrachromosomal asymmetry index) & A_2 (interchromosomal asymmetry index) formulas as suggested by Zarco (1986). Both formulas are formulated to obtain lower values when chromosomes tend to be metacentric.

RAPD procedures:

RAPD profiling was performed as recommended by William *et al.*, (1990) with some modifications. It was carried out using oligonucleotide sequences of five 10-mer random primers; the sequence of these primers is illustrated in Table 3. The primers were synthesized at the Agricultural Genetic Engineering Research Institute (AGERI) in ARC, Giza, Egypt on an ABI 392 DNA/RNA synthesizer and used to screen the ten *Artemisia* populations. Polymerase chain reaction (PCR) was made in 50 µl reaction mixture composed of 2.5µl of the appropriate template DNA, 0.5 µl Taq polymerase, 0.5 µl primer, 6.0 µl $MgCl_2$, 5.0 µl of NTP's mix, 0.5 µl 10x reaction buffer and 35.0 µl distilled water. Amplification of DNA was carried out in thermal cycler (Perkin Elmer Cetus 480) as follows: 1 cycle at 94 C for 5 min. followed by 40 cycles each for 1 min at 94 C, 1 min at 36 C, 2 min at 72 C with the final extension phase of seven min at 72 C and storage at 4 C. Amplified DNA fragments were separated in 1.4% agarose gel. After electrophoresis, the RAPD profiles were visualized using UV trans-illuminator and photographed for analysis.

Table 1: Localities from which the examined populations of *A. monosperma* and *A. judaica* were collected, for mapping these localities see map of Egypt shown in figure 1

Population	Species	Locality
01	*A. monosperma*	Al-Kattamia El-Ain El-Sokhna Road, 30 km East of Cairo
02	*A. monosperma*	Cairo-Suiz Road, 80 km East of Cairo
03	*A. monosperma*	Al-Arish North Sinai
04	*A. monosperma*	Burg El-Arab, 50 km South East of Cairo
05	*A. monosperma*	Wadi El-Natroun, 100 km North West of Cairo
06	*A. judaica*	Wadi Hajol, 50 km South West Suez
07	*A. judaica*	Al-Kattamia El-Ain El-Sokhna Road, 90 km East of Cairo
08	*A. judaica*	Wadi Feran, South Sinai
09	*A. judaica*	Wadi El-Sheikh, Saint Catherene, South Sinai
10	*A. judaica*	Wadi El-Deir, Saint Catherene, South Sinai

Table 2: Morphological characters and character status and codes used for the analysis of *A. monosperma* and *A. judaica* populations examined in this study.

	Character	Status	code
01	Stem color	green	0
		gray	1
02	Stem length	30-50	0
		50- 80	1
		> 90	2
03	Aromatic odor	weak	0
		strong	1
04	Surface	glabrous	0
		hairy	1

Table 2: Continue

05	Leaf polymorphism	absent	0
		present	1
06	Leaf margin	entire	0
		erosate	1
07	Leaf shape	round	0
		obtuse orbicular	1
08	Leaf apex	obtuse	0
		mucronate	1
09	Leaf surface	glabrous	0
		hairy	1
10	Leaf type	pinnatisect	0
		pinnatifid	1
11	Leaf shape	linear	0
		ovate-oblong	1
12	Number of lobes	2-3	0
		4-5	1
		>5	2
13	Leaf color	green	0
		golden yellow	1
14	No. of involucral bracts	one	0
		two	1
15	Shape of involucral bracts	round	0
		lanceolate ovate	1
16	Margin of involucral bracts	memberanceous	0
		hyaline	1
17	Apex of involucral bracts	apiculate	0
		ovate truncate	1
18	Length of involucral bracts	outer shorter than inner	0
		outer and inner are equal	1
19	Number of whorls of involucral bracts	2.0-3.0	0
		4.0-5.0	1
20	Texture of involucral bracts	glabrous	0
		hairy	1
21	Shape of head	ovate	0
		hemispherical	1
22	Length of head (mm)	2.5-3.5	0
		3.6- 4.6	1
		> 4.6	2
23	Number of female flower	1.0-3.0	0
		4.0-6.0	1
		7.0-9.0	2
24	Number of bisexual flower	4.0-9.0	0
		10.0-15.0	1
		16.0-20.0	2
25	Fertility of bisexual flower	sterile	0
		fertile	1
26	Anther rails	equaling the antheropodium	0
		half the antheropodium	1
27	Shape of achenes	obovoid	0
		oblong	1
28	Color of achenes	pale brown	0
		dark brawn	1
29	Number of seeds	1.0-9.0	0
		10.0-18.0	1
		>27	2
30	Length of Seed	< 0.50	0
		> 0.51	1

Table 3: The Euclidean similarity coefficients among the examined populations of *A. monosperma* (1-5) and *A. judaica* (6-10) based on the morphological variation.

Population	1	2	3	4	5	6	7	8	9	10
01	1.00									
02	1.00	1.00								
03	0.80	0.80	1.00							
04	0.78	0.78	0.80	1.00						
05	0.78	0.78	0.80	1.00	1.00					
06	0.07	0.07	0.14	0.07	0.07	1.00				
07	0.07	0.07	0.14	0.07	0.07	1.00	1.00			
08	0.07	0.07	0.14	0.07	0.07	1.00	1.00	1.00		
09	0.07	0.07	0.14	0.07	0.07	0.92	0.92	0.92	1.00	
10	0.07	0.07	0.14	0.07	0.07	0.92	0.92	0.92	1.00	1.00

Data analysis:

The relationship among the examined populations was estimated based on differences among them in both morphological traits and RAPD finger printing separately and in combination. The quantitative morphological traits were given codes ranging between 0 and 2 depending on the variation in the average value for the measured traits. The qualitative traits on the other hand were all two-state characters and were coded as 0 or 1. For the analysis of RAPD data, the presence or absence of unique and shared polymorphic as well as monomorphic PCR products was recorded as 1 for presence and 0 for absence. However, only polymorphic bands were used to calculate the genetic Euclidean similarity coefficient among the examined populations and were used to construct distance trees that illustrate the genetic diversity among populations based the UPGMA (un-weighted pair-group method with arithmetic average as defined by Sneath & Sokal (1973) and Dunn & Everitt (1982) using the software program NTSYS pc 2.01 (Rohlf, 2005).

RESULTS AND DISCUSSION

Genetic Diversity Based on Morphological Variation:

The Euclidean similarity coefficients, among the examined populations of *A. monosperma* (1-5) and *A. judaica* (6-10), based on the morphological variation are given in Table 3. A 100% similarity was scored between populations 1 & 2 and between 4 & 5 of *A. monosperma* and between populations 6, 7 & 8 and populations 9 & 10 of *A. judaica.* The relationships among the examined populations of *Artemisia*, based on morphological variation, are illustrated by the UPGMA distance tree shown in Fig. 2. In this tree, the examined populations are divided into two main groups at a total distance of 63 on the distance scale; group I comprises the populations of *A. monosperma* and group II had comprises the populations of *A. judaica*. The analysis of morphological criteria clearly reflects the specific differences among the two species especially in leaf characters (shape, margin, apex and the aromatic fragrance), the shape of capitulum, number of bisexual flower and number and length of seeds. The separation of the populations of the two species as two separate groups is also congruent with the taxonomic delimitation of *A. monosperma* in Subgenus *Dracunculus* and of *A. judaica* in subgenus *Artemisia* (Torrell and Vallès, 2001).

The tree illustrated in Fig. 2 clearly shows very close morphological resemblances among the populations of *A. monosperma.* This is demonstrated by the very small distance that separate the different populations of this species compared to the overall distance of 63 that separates the examined ten populations of the two species. The populations of *A. monosperma* are grouped in two clusters, one comprising populations 1 & 2 that were collected from dry parts of *A. monosperma* distribution range south east of Cairo and the other comprising 3, 4 & 5 that were collected from more wet localities close to the Mediterranean coast (Fig. 1). These results indicate that the five populations of *A. monosperma* show low level of morphological diversity. On the other hand, the populations of *A. judaica* showed more variation between each other, compared to those of *A. monosperma* as they have a distance value of 16 on the distance scale of the tree (Fig. 2). The five populations of *A. judaica* are also divided into two clusters; one comprising populations 6, 7 & 8 that were collected from localities in dry flat areas south of Suez and Wadi Feran in Sinai respectively and the other comprising populations 9 & 10 that were collected from the high mountains of Saint Catherene in South Sinai (see Fig. 1). The populations of *A. judaica* showed variation in shoot length, number of leaf lobes, shape and length of capitulum, number of female flowers, number of bisexual flowers and number of seeds. Wide morphological variation among different populations was also recorded in *A. vulgaris* populations in Canada (Barney and Ditommase, 2003) and in *A. vulgaris, A. roxburghiana* and *A. absinthium* in Iran (Nazar and Mohamed, 2010) indicating that morphological diversity may be widespread in *Artemisia*.

Genetic Diversity Based on Karyotype Features:

The karyotype features of the examined populations are summarized in Table 4; the karyotypes of *A. monosperma* populations are illustrated in Fig. 3 and the karyotypes of *A. judaica* populations are illustrated in Fig. 4. All populations of the former species are tetraploid with $2n$=36, based on x = 9 and the populations of *A. judaica* are all diploid with $2n$=16, based on x=8. Three populations of *A. monosperma* (2, 3, and 5) were found to exhibit one B chromosome each. The chromosome counts reported here for *A. monosperma* and *A. judaica* are in agreement with previous reports for material from Egypt (Badr *et al*. 1997). The chromosomes are rather small and the karyotypes rather symmetrical; features which are very typical of the genus *Artemisia* (Oliva and Vallès, 1994, Valès and Siljak-Yakovlev, 1997; Badr *et al.*, 1997; Vallès and McArthur, 2001). However, the presence of B chromosomes is reported for the first time in Egyptian material of *A. monosperma*.

Fig. 1: A map of Egypt on which the localities of A. *monosperma* and A. *judaica* populations used in this study are shown; serial numbers are as given in Table 1.

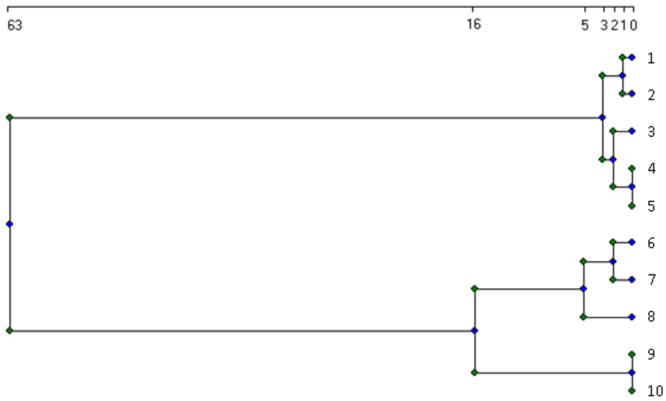

Fig. 2: UPGMA distance tree showing the relationships among the populations of A. *monosperma* (1-5) and A. *judaica* (6-10) based on the analysis of morphological variation; numbers refer to populations as given in Table 1.

The chromosomes of *A. monosperma* populations are shorter and their karyotypes are more asymmetric compared to that of *A. judaica* populations as indicated by higher values of MAR (1.52±0.07 - 1.65 ± 0.19), higher TF% (39.11-41.29) higher A_1 values (0.33-0.35), and lower A_2 values (0.13-0.16). Within *A. monosperma*, population 5 has the longest chromosomes (MCL=3.13±0.15 µm) and the most asymmetric karyotype while population 3 has the shortest chromosomes (MCL=1.97±0.10 µm) and most symmetric karyotype, However, all populations of this species have seven metacentric and two submetacentric chromosomes. Within *A. judaica*, population 6 has the largest chromosomes (MCL=4.30±035 µm), whereas population 10 has the shortest ones (MCL=3.81±0.28 µm); the latter population has the most symmetric karyotype as indicated by low MAR (1.32±0.13), high TF% (44.19) high A_1 value (0.31), and low A_2 value (0.21). The five populations of *A. judaica* are all comprised of seven metacentric chromosomes and one submetacentric chromosome (Table 4; Fig. 3). The karyotype symmetry calculations generally indicate little variation among populations of both *A. monosperma* and *A. judaica*. The similarities among populations in chromosome feature are thus congruent with the morphological similarities between populations of both species. However, the differences in chromosome length among *A. monosperma* are not correlated with morphological variations among them.

Genetic Diversity Based on RAPD Analysis:

A total of 63 RAPD bands have been revealed in the ten examined populations of *A. monosperma* and *A. judaica*, eight bands were monomorphoic and the other 55 bands were polymorphoic. The number of bands for each primer ranged from 10 for primer OPA-05 to 15 for primer OPA-02. The bands varied in size between 506 bp to 3072 bp. The percentage of polymorphism revealed by the different primers ranged from 53.8% for primer OPA-09 to100% for the two primers OPA-02 and OPA-05. The high proportion of total polymorphism (87.56%) indicates that the two examined species exhibit high level of molecular polymorphism. Table 5, and Fig. 5 illustrate that higher number of unique bands were revealed by primer OPA-02 in three populations of *A. monosperma* (2, 3, 5) and two populations of *A. judaica* (9, 10). In other species of *Artemisia*, 57 primers generated nearly 400 markers from the genomic DNA of five subspecies and hybrids of *A. tridentata* and supported the hypothesis that tetraploid *(4x) Artemisia tridentata* ssp. *vaseyana* derived *de novo* from diploid *(2x)* populations via autopolyploidy (McArthur *et al*., 1998). An average of 63% polymorphism was estimated among eight variants of *A. annua* from a single population of India illustrating the existence of high level of genetic variation (Sangwan *et al*. 1999). Similarly a total of 611 bands were produced by nine primers, of which 419 were polymorphic with an average of 68% polymorphism across 15 samples of *A. vulgaris*, *A. roxburghiana* and *A. absinthium* from Jamou and Kashmir (Nazar and Mahmoud, 2010). Meanwhile, 100% genetic polymorphism that was reported in Tunisian *A. herba-alba* accessions by Mohsen and Ali (2008) using ISSR markers.

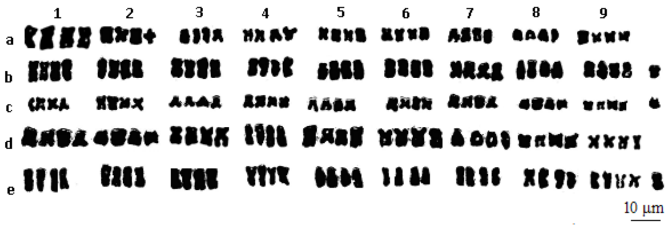

Fig. 3: Karyotypes of the five examined populations of A. *monosperma*: a = A. *monosperma* (1), b = A. *monosperma* (2), c = A. *monosperma* (3), d = A. *monosperma* (4), and e = A. *monosperma* (5); numbers refer to populations as given in Table 1.

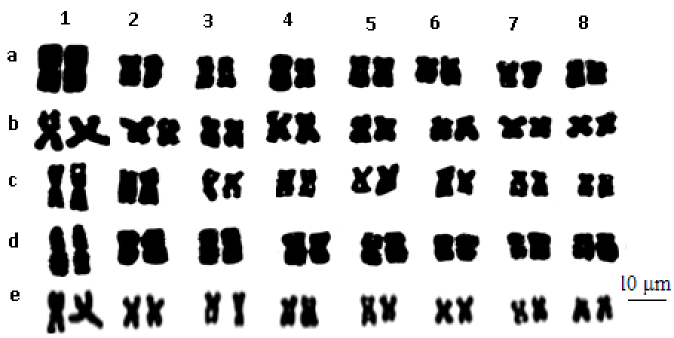

Fig. 4: The Karyotypes of the five examined populations of *Artemisia judaica*: a = A. *judaica* (6), b = A. *judaica* (7), c = A. *judaica* (8), d = A. *judaica* (9), e = A. *judaica* (10); numbers refer to populations as given in Table 1.

Table 4: The karyotype features of the studied populations of *A. monosperma* (1-5) and *A. judaica* (6-10); MCL = Mean chromosome length, MAR = Mean arm ratio, m = Metacentric chromosome, sm = Submetacentric chromosome, Chr = Chromosome, TF = Total form percentage, A_1 = Intrachromosomal asymmetry index, A_2 = Interchromosomal asymmetry index

Species and population	2*n*	MCL (µm) ±SE	MAR ±SE	TF %	A_1	A_2	Chr. Type	
							m	sm
A. monosperma (1)	36.0	2.46±0.11	1.60±0.11	39.11	0.35	0.13	07.0	02
A. monosperma (2)	36+1	2.62±0.14	1.63±0.25	39.91	0.30	0.15	7+B	02
A. monosperma (3)	36+1	1.97±0.10	1.52±0.07	39.86	0.33	0.16	7+B	02
A. monosperma (4)	36.0	2.58±0.09	1.65±0.19	41.29	0.34	0.10	7.0	02
A. monosperma (5)	36+1	3.13±0.15	1.64±0.20	39.41	0.33	0.16	7+B	02
A. judaica (6)	16	4.30±035	1.42±0.12	42.37	0.27	0.23	07	01
A. judaica (7)	16	3.94±0.29	1.54±0.20	41.28	0.29	0.21	07	01
A. judaica (8)	16	4.08±0.36	1.56±0.19	41.32	0.30	0.23	07	01
A. judaica (9)	16	4.85±0.42	1.44±0.18	42.88	0.25	0.27	07	01
A. judaica (10)	16	3.81±0.28	1.32±0.13	44.19	0.31	0.21	07	01

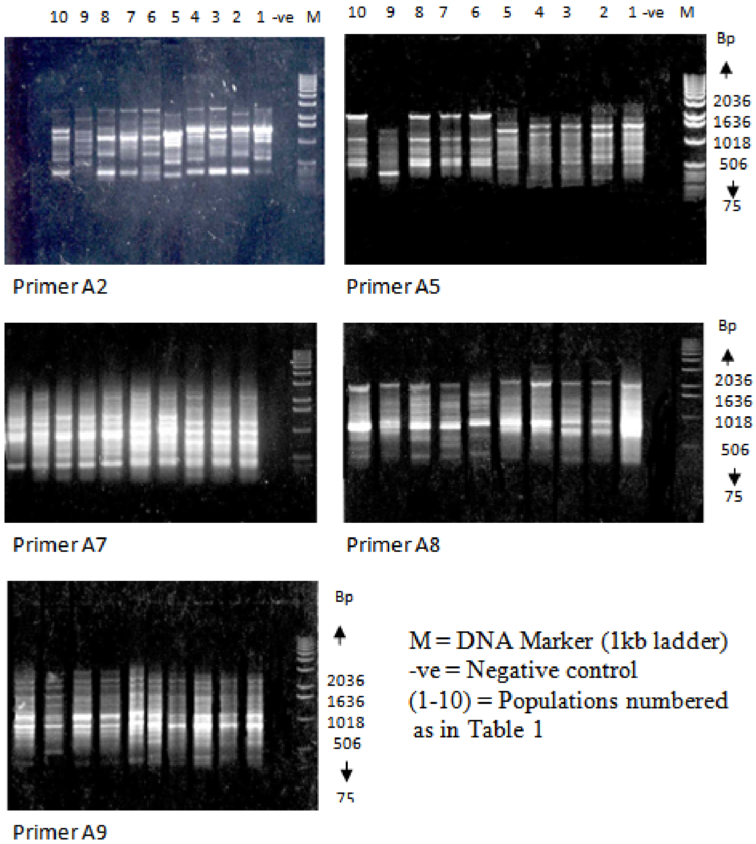

Fig. 5: RAPD profiling as revealed by five primers in A. monosperma (1-5), A. judaica (6-10) populations; Bp=Base pairs

The molecular similarity coefficients among the populations of *A. monosperma* (1-5) and *A. judaica* (6-10) based on Euclidean distance are given in Table 6 and the UPGMA tree illustrating their genetic distance are shown in Fig. 6. The average similarity coefficients among the populations of *A. monosperma* (0.60 to 0.76) are much higher compared to those of A. *judaica* (0.41 to 0.80) indicating more polymorphism among *A. judaica* populations compared to those of *A. monosperma* (Table 6). The UPGMA tree produced by the RAPD data also divided the populations of *A. monosperma* and *A .judaica* as two distinct groups. However, the analysis of molecular data revealed more diversity among the populations of both species compared to morphological criteria. The five populations of *A. monosperma* have an overall distance of 17 on the distance scale of the UPGMA tree and are delimited into two main clusters (Fig. 6); one comprising the populations 3, 4, and 5, that were collected from sites near the Mediterranean coast and the other comprises populations 1 and 2 that were collected from site 30-80 km south east of Cairo. In the group comprising the populations of *A. judaica*, population 10 was clearly distinguished as a separate identity at a distance of 28 on the UPGMA tree scale and the other populations were divided as two clusters at a distance of 18; one cluster comprising populations 6 & 7 that are differentiated from each others at a distance of 9 and the other cluster comprises populations 8 & 9 that are differentiated at a distance of 11. In *A. judaica* group, the distinction of population 10 from population 9 indicates molecular genetic variability due to contribution of local ecological and geographic conditions; the former was collected from wadi beds and the latter from terraces in the Saint Catherene area.

Genetic Diversity Based on Morphological Variation and RAPD Analysis:

The analysis of morphological variation and molecular polymorphism, as one set of data, also divided the populations of *A. monosperma* and *A. judaica* as two groups at a distance of 40 (Fig. 7). The genetic distances illustrated in this tree reflect the morphological differences and molecular polymorphism among the two species and confirm their identity as separate taxonomic units. The populations of *A. monosperma* are delimited in two clusters similar to that illustrated in the tree produced by the analysis of morphological data (Fig. 2) but the distance values among the populations are much higher (Fig. 7). In the *A. judaica* group, the two populations collected from sites in the Saint Catherene area (population 9 from Wadi El-Sheikh and population 10 from Wadi El-Deir) are more related to each other reflecting their morphological similarities in spite of the molecular diversity between them as indicated in the tree produced by the analysis of RAPD profiling (Fig. 6). These two populations also differ considerably in chromosome length (4.85±0.42 μm) for the former and (3.81±0.28 μm) for the latter. The evidence indicated by karyotype features and molecular diversity between population 10 of *A. judaica* and the other four populations may provide some support to the recognition of some populations of *A. judaica* in Saint Catherene as a separate variety as proposed by Boulos (2002).

Table 5: Sequence of arbitrary (10-mer) RAPD primers used to generate polymorphism among the examined populations of *A. monosperma* and *A. judaica*.

Ser.	Primer	Sequence (5' to 3')	GC %	Number of bands				
				Mono	Poly	Unique	Total	% Polymorphism
01	OPA-02	TGCCGAGCTG	70	--	15	2.0	15	100.0
02	OPA-05	AGGGGTCTTG	60	--	10	1.0	10	100.0
03	OPA-07	GAAACGGGTG	60	1.0	11	--	12	91.6
04	OPA-08	GTGACGTAGG	60	1.0	12	1.0	13	92.3
05	OPA-09	GGGTAACGCC	70	6.0	07	1.0	13	53.8
Total			8.0	55	5.0	63	87.5	

Table 6: Molecular similarity coefficients among the populations of *A. monosperma* (1-5) and *A. judaica* (6-10) based on Euclidean distance.

Population	1	2	3	4	5	6	7	8	9	10
1	1.0									
2	0.71	1.0								
3	0.68	0.74	1.0							
4	0.74	0.67	0.73	1.0						
5	0.76	0.60	0.62	0.76	1.0					
6	0.45	0.37	0.44	0.51	0.56	1.0				
7	0.48	0.41	0.47	0.55	0.53	0.71	1.0			
8	0.40	0.31	0.42	0.43	0.45	0.80	0.75	1.0		
9	0.40	0.33	0.36	0.50	0.49	0.44	0.43	0.41	1.0	
10	0.49	0.39	0.42	0.56	0.58	0.62	0.72	0.60	0.59	1.0

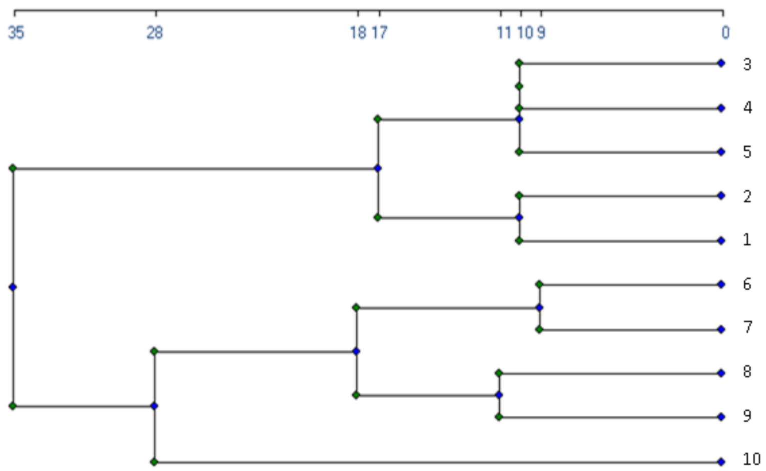

Fig. 6: UPGMA tree illustrating the relationship among the populations of A. *monosperma* (1-5) and A. *judaica* (6-10), based on RAPD polymorphism among the examined populations.

In conclusion, the analysis of morphological variation and RAPD polymorphism showed wider diversity among the populations of *A. judaica* compared to the populations of *A. monosperma*. Populations of *A. monosperma*, growing in the wet sites near the Mediterranean coast of Egypt in north Sinai and south west of Alexandria are differentiated from populations growing in drier sites east of Cairo and west of Sues. In addition, the populations of *A. judaica* growing in the mountains of South Sinai are clearly differentiated from other populations in support of the view that populations of this species from mountains of South of Sinai may be considered as a sub-specific identity.

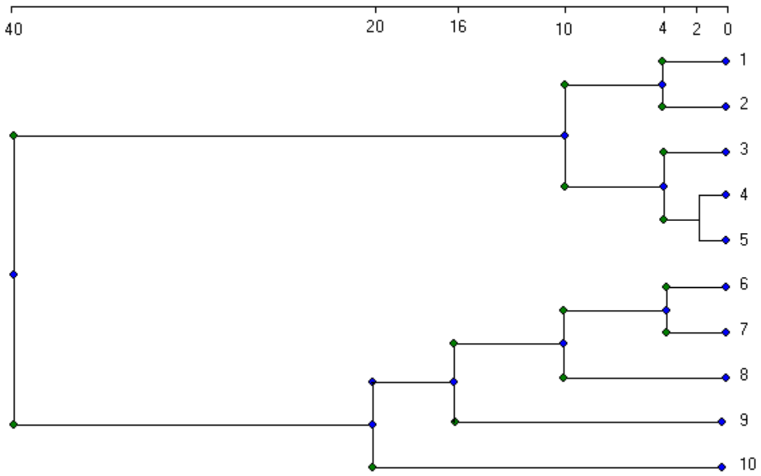

Fig. 7: UPGMA tree illustrating the relationship among the populations of A. *monosperma* (1-5) and A. *judaica* (6-10), based on morphological variation and RAPD polymorphism among the examined populations

REFERENCES

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.

Badr, A., E.A. Kamel and N. Gararcia-Jaceas, 1997. Chromosomal studies in the Egyptian flora: VI, karyotype features of some species in subfamily Asteroideae (Astraceae) Compositae. Newslet, 30: 15-28.

Badr, A., H.H. El-Shazly and E.A. Kamel, 2009. Chromosomal Studies in the Egyptian Flora. VII. Karyotype analysis of species in the two tribes Chicorieae and Cardueae of Asteraceae. Egypt. J. Bot., 49: 71-86.

Barney, J.N. and A. Ditommase, 2003. The biology of Canadian weeds. 118. *Artemisia vulgaris* L. Can. J. Plant Sci., 83: 205-215.

Batanouny, K.H., 1999. *Wild Medicinal Plants in Egypt; An inventory to support conservation and sustainable use*. With contributions by E. Aboutabl, M. Shabana and F. Soliman. Academy of Scientific Research and Technology, Egypt and IUCN, Switzerland., pp: 208.

Boulos, L., 2002. *Flora of Egypt*. Al-Hadara Publications, Cairo, Egypt., 3: 258-260.

Bremer, K., C.J. Humphries, 1993. Generic monograph of the Asteraceae – Anthemideae. Bull. Nat. Hist. Mus. Lond. (Bot.) 23: 71-177.

Burrows, G. and R. Tyrl, 2001. *Toxic Plants of North America*. Ames, Iowa State Univ. Press.

Darlington, C.D. and L.F. La-Cour, 1976. *The handling of chromosomes*, 6^{th} ed., George Allen and Unwin Ltd., London, UK.

El-Masry, K.F., A.H. El-Ghorab and A. Farouk, 2002. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chemistry, 79: 331-336.

Gostimsky, SA., Z.G. Kokaeva, F.A. Konovalov, 2005. Studying plant genome variation using molecular markers. Russ J Genet., 41: 378-388.

Hasan, S.M.Z., M.S.B. Shafie and R.M. Shah, 2009. Analysis of Random Amplified Polymorphic DNA (RAPD) of *Artemisia capillaris* (Wormwood capillary) in East Coast of Peninsular Malaysia. World Appl. Sci. J. 6: 976-986.

Hasan, S.M.Z., M.S.B. Shafie and R.M. Shah, 2010. Genetic variability of *Artemisia capillaris* (Wormwood capillary) by random amplified polymorphic DNA (RAPD) in Terengganu State, Malaysia. Afr. J. Biotech., 8: 1810-1814.

Heinrich, M., M. Robles, J. West, Ortiz de B. Montellano, E. Rodriguez, 1998. Ethnopharmacology of Mexican Asteraceae (Compositae). *Ann Rev Pharmacol Toxicol*, 38: 539-565.

Henry, R.J., (ed.) 2001. *Plant Genotyping: The DNA Fingerprinting of Plants* CABI Publishing, Oxford, pp: 325.

Huziwara, Y., 1962. Karyotype analysis is some genera of Compositae VIII. Further studies on the chromosomes of *Aster*. *American Journal Botany,* 49: 116-119.

Kamel E.A., 2001. Chromosome count and Karyological studies on six taxa of the Egyptian Asteraceae. Compositae Newsletter., 36: 81-94.

Kamel, E.A., 1999. Karyological studies on some taxa of the Asteraceae in Egypt. Compositae Newsletter., 33: 1-18.

Kawatani, T. and T. Ohno, 1964. Chromosome numbers in *Artemisia*. Bull. Natl. Inst. Hyg. Sci., (Tokyo) 82: 183-193.

Kernodle, S.P., R.E. Cannon, J.G. Scandalios, 1993. Concentration of primer and template qualitatively affects product in random amplified polymorphic DNA-PCR. Biotechniques., 14: 362-364.

Levan, A., K. Fredga and A.A. Sanders, 1964. Nomenclature for centromeric position on chromosomes. Hereditas., 52: 201-220.

Levin, D.A., 2002. The role of chromosomal changes in plant evolution. Oxford University Press, Oxford.

Marco, J. and O. Barbera, 1990. Natural products from the genus *Artemisia*. In X Atta-ur-Rahman (Ed.)*: Studies in Natural Products,* Elsevier, Amsterdam.

McArthur, E.D., J. Mudge1, Van R. Buren, W.R. Andersen, S.C. Sanderson and D.G. Babbcl, 1998. Randomly amplified polymorphic DNA analysis (RAPD) of *Artemisia* subgenus *tridentatae* species and hybrids. Great Basin Naturalist., 58: 12-27.

Mohsen, H. and F. Ali, 2008. Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers. Afr J Biotechnol., 7: 44-50.

Nazar, N. and T. Mahmood, 2010. Morphological and molecular characterization of selected *Artemisia* species from Rawalakot, Azad Jammu and Kashmir. Acta Physiol Plant. Published Online 23 June 2010, DOI 10.1007/s11738-010-0545-3.

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. Med. Sci., 4: 42-48.

Oliva, M. and J. Valle`s, 1994. Karyological studies in some taxa of the genus *Artemisia* (Asteraceae). Can. J. Bot., 72 :1126-1135.

Rohlf, F.J., 2005. NTSYS-pc: *Numerical taxonomy and multivariate analysis system*, version 2.01. Applied Biostatistics, New York.

Sangwan, R.S., N.S. Sangwan, D.C. Jain, S. Kumar, S.A. Ranade, 1999. RAPD profile based genetic characterization of chemotypic variants of *Artemisia annua* L. Biochem. Mol. Biol. Int., 47: 935-944.

Salido, S., L.R. Valenzuela, J. Altarejos, M. Nogueras, A. Sanchez, E. Cano, 2004. Composition and infraspecific variability of *Artemisia herbaalba* from southern Spain. Biochem. Syst. Ecol., 32: 265-277.

Shams, S., 2004. *Diversity of Artemisia species in the Egyptian flora*. M.Sc. thesis, Ain Shams University, Cairo, Egypt.

Stace, C.A., 2000. Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. Taxon, 49: 451-477.

Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Res., 17: 6463-6471.

Torrell, M., N. Garcia-Jacas, A. Susanna, J. Vallès, 1999. Phylogeny in *Artemisia* (*Asteraceae*, *Anthemideae*) inferred from nuclear ribosomal DNA (ITS) sequences. Taxon., 48: 721-736.

Torrell, M. and J. Vallès, 2001. Genome size in 21 *Artemisia* L. species (Asteraceae, Anthemideae): Systematic, evolutionary, and ecological implications. Genome., 44: 231-238.

Vallès, J. and E.D. McArthur, 2001. *Artemisia* systematics and phylogeny: cytogenetic and molecular insights. In: McArthur E.D., and Fairbanks D.J. (eds.) Shrubland Ecosystem Genetics and Biodiversity. US Department of Agriculture Forest Service, Rocky Mountain Research Station, Ogden, pp: 67-74.

Vallès, J., S. Siljak-yakovlev, 1997. Cytogenetic studies in the genus *Artemisia* L.: fluorochrome banded karyotypes of five taxa, including the Iberian endemic species *Artemisia* barrelieri Besser. Can. J. Bot., 75: 595-606.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, Van T. Der Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res., 23: 4407-4414.

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.

Williams, J.G.K., A.R. Kubelik, K.T. Livak, J.A. Rafalski, S.V. Tingey, 1990. DNA polymorphisms amplified by random primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6539.

Young, J., W. Longland, R. Blank, 1996. Management of woody species of *Artemisia* in western North America. *In: Compositae: Biology and Utilization. Proceedings of the International Compositae Conference 1994 (Edited by: P Caligari, D Hind) Royal Botanic Gardens, Kew*, 2: 271-290.

Watson, L.E., P.L. Bates, T.M. Evans, M.M. Unwin, J.R. Estes, 2002. Molecular Phylogeny of subtribe Artemisiinae (Asteraceae), including *Artemisia* and its allied and segregate genera. BMC Evol. Biol., 2: 17-29. DOI: 10.1186/1471-2148-2-17 URL:

Wolfe, A.D. and A. Liston, 1998. Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis DE, Soltis PS and Doyle JJ (Eds.) *Plant Molecular Systematics* II. Kluwer, Boston, pp: 43-86.

Zarco, R.C., 1986. A new method for estimating karyotype asymmetry. *Taxon*., 35: 526-530.

Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics., 20: 176-183.