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Full Length Research Paper

Molecular markers associated with genetic diversity of some medicinal plants in Sinai

F. M. Domyati[1,2], Rania A. A. Younis[1], S. Edris[1]*, A. Mansour[3], G. Sabir[2] and A. Bahieldin[1,2,4]

[1]Genetics Department, Faculty Agriculture, Ain Shams University, Cairo, Egypt.
[2]Biological Science Department, Faculty of Science, King Abdulaziz University, P. O. Box 9028, Jeddah-21413, Saudi Arabia.
[3]Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.
[4]Faculty Biotechnology, Misr University for Science and Technology (MUST), 6 October City, Egypt.

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The main objective of this work is to fingerprint some selected plant germplasm along the Western Red Sea coast of Sinai. Selection is based on the relative economic importance of these plants on the medicinal and pharmaceutical levels. Molecular markers such as RAPD, ISSR and AFLP technologies were used in this work to detect genetic diversity of the selected medicinal plants. The study showed that taxonomical locations can be distinguished for each subspecies (with as low as 0 to 1% polymorphism using AMOVA analysis) according to its molecular fingerprint but it cannot be recognized as a different subspecies.

Key words: Molecular markers, genetic diversity, RAPD, ISSR, AFLP, Sinai, medicinal plants.

INTRODUCTION

In developing countries, there is no reliable data available on the economy of the wild medicinal plants, which may be extinct within a few years. Referring to the general agreement on tariffs and trade (GATT), it is important to assess the value of these plants as important biological resources and to document the intellectual property rights. The production of herbal remedy drugs became an important task, especially with flooding the pharmaceutical Egyptian market with a number of synthetic drugs of questionable efficacy associated with the increasing cost of such drugs. Therefore, the demand of high-yield/high-quality medicinal plants will continue to increase in the future. Also, a growing concern with the awareness of the side effects of the drugs associated with regular exposure to synthetic chemicals has triggered a “back-to-nature” idea with an appeal of new discovery of natural products necessary to meet primary health care and veterinary needs. Genetically improved medicinal and aromatic plants, with a legal standard of purity, uniformity and high levels of the economically important active ingredients, could assure a constant supply of quality medicinal plants and individual natural compound for processing, marketing industry and biotechnology.

Genetic diversity among individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have limitations since they are influenced by environmental factors and the developmental stage of the plant. In contrast, molecular markers, based on DNA sequence polymorphisms, are independent of environmental conditions and show higher levels of polymorphism. The latter have proved their utility in fields like taxonomy, physiology, embryology, genetics, etc. Among the different types of molecular markers available, random amplified polymorphic DNA (RAPD) is useful for the assessment of genetic diversity among rare species (William et al., 1990), despite of the existing limitations (dominant mode of inheritance of RAPD loci), because of their simplicity, speed and relatively low costs as compared to other molecular markers. RAPD markers have been used extensively in analyzing genetic diversity (Garacia et al.,

*Corresponding author. E-mail: sedris@aucegypt.edu.

1998; Gwanama et al., 2000; Levi et al., 2001; Artyukova et al., 2004; Sureja et al., 2006; Guerra et al., 2010).

Inter simple sequence repeats (ISSRs) have been developed as an anonymous, RAPDs-like approach that accesses variation in the numerous microsatellite regions dispersed throughout the genome (Zietkiewicz et al., 1994). The ISSR marker is based on a PCR-amplification of 100-3000 base pair regions between inversely oriented SSRs or microsatellites (Bussell et al., 2005). The ISSR marker is simple and has reproducibility. It requires small amounts of DNA and does not require information on DNA sequence. ISSR primers are designed from SSR motifs and can be undertaken for any plant species containing a sufficient number and distribution of SSR motifs in the genome (Gupta et al., 1996; Buhulikar et al., 2004). Therefore, ISSR has been widely used in many respects such as the study of genetic diversity in barley (Brantestem et al., 2004) and cultivar identification in tobacco (Denduangboripant et al., 2010).

Microsatellites are very short stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species. They are characterized by mono-, di- or trinucleotide repeats that have 4 to 10 repeat unit side-by-side (Morgante and Olivieri, 1993).

Amplified fragment length polymorphism (AFLP) utilizes fragments of DNA amplified using directed primers from restriction digested genomic DNA (Vos et al., 1995). AFLP provides high levels of resolution to allow delineation of complex genetic structures, to differentiate individuals in a population in gene flow experiments, and also to register plant varieties (Powell et al., 1996; Law et al., 1998; Barker et al., 1999; Aparajita and Rout, 2010; Misra et al., 2010). Considering the potentials of DNA-based genetic diversity analysis, the present study aims at the evaluation and the usefulness of molecular markers, that is, RAPD, ISSR and AFLP, in assessing and analyzing the nature and the extent of genetic diversity among genotypes of seven medicinal plants grown in Red Sea coastal region of Egypt.

MATERIALS AND METHODS

Plant material

Selection is based on the relative economic value of the collected medicinal and pharmaceutical plants.

Collection, gathering and surveying economically important medicinal plant species

Rules, guidelines and requirements for collecting medicinal plants were taken into consideration during this research activity. In addition, information and scientific background had to be gathered before the collection mission. *Proper storage*: Collected plant parts should be dried as soon as possible after harvesting and be stored in a dry, dark place and at a relatively low temperature, not exceeding 15°C, in airtight containers that help prevent their deterioration and contamination. Missions involved the area between Ras Ghareb – Hurghada – Safaga – Kuseir and the area between Marsa Alam and Edfo (Figure 1 and Table 1) in which plant germplasm was collected from 44 different locations in 21 valleys for seven species as the following:

(i) *Artemisia judaica* L.
(ii) *Arnebia hispidissima* (Lehm.) DC.
(iii) *Aerva javanica* (Burm. f.) Juss
(iv) *Balanites aegyptiaca* (L.) Del.
(v) *Cleome droserifolia* (Forssk.) Delile
(vi) *Zygophyllum simplex* L.
(vii) *Zilla spinosa* (L.) Prantl

Fifty accessions were gathered and surveyed and 19 were selected due to their economic importance (Table 1) for subsequent molecular characterization and computer analyses.

Genomic DNA extraction and purification

Extraction of total DNA was performed using methods for medicinal and aromatic plants according to Anna et al. (2001). To remove RNA contamination, RNase A (10 mg/ml, Sigma, USA) was added to the DNA solution and incubated at 37°C for 30 min. Estimation of the DNA concentration in different samples was done by measuring optical density at 260 nm according to the following equation:

Conc. (ug/ml) = OD_{260} × 50× dilution factor

Random amplified polymorphic DNAs (RAPDs)

A set of 58 random 10 *mer* primers (Operon Technology, USA) from groups A, B, C, O and Z was used in detecting polymorphism among different wild medicinal species but 20 primers only were successful in generating reproducible and reliable amplicons (Table 2). The amplification reaction was carried out in 25 µl reaction volume containing 1 × PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmole primer, 2 units Taq DNA polymerase and 25 ng template DNA. PCR amplification was performed in a Perkin Elmer 2400 thermocycler (Germany), programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 37°C for 2 min, and an extension step at 72°C for 2 min, following by extension cycle for 7 min at 72°C in the final cycle.

Inter simple sequence repeats (ISSRs)

Thirty primers for ISSR were used in the study but only 17 were successful in generating reproducible and reliable amplicons for different wild medicinal species. Names and sequences of the selected primers are shown in Table 3. PCR analysis was performed in 25 µl reaction and amplification was programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 40°C for 2 min, and an extension step at 72°C for 2 min, following by extension cycle for 7 min at 72°C in the final cycle.

Amplified fragment length polymorphisms (AFLPs)

AFLP analysis was performed using the AFLP Analysis System I (Invitrogen, cat. no. 10544-013) according to the manufacturer's protocol. Genomic DNA samples were digested with *Eco*RI and *Mse*I restriction enzymes in which *Eco*RI and *Mse*I adapters were

Figure 1. Map of western Red Sea coast showing the area (inside the box) in which collection missions have taken place.

Table 1. Information involving scientific names, valley names as well as location numbers of the germplasm collected from Sinai.

Scientific name	Valley name	Location no.
Artemisia judaica	Hagool	2
	Om-Nfeia	8
	Abo El-Toyour	29
Arnebia hispidissima	Abo Halfaia	31
	El-Sebaae	24
	Abo Maryouh	36
	Sedi Salem	37
	El-Omra	38
Aerva javanica	El-Sebaae	20
	Abo Maryouh	37
	Sedi Salem K.38	42
	Marsa Alam – Edfo way K.38	42
	Marsa Alam – Edfo way K.70	44
Balanites aegyptiaca	Esley	15
	El-Sebaae	17
	Hegleg	32
	Abo Maryouh	37
	Marsa Alam – Edfo way K.47	43
	Abo Maryouh	42

Table 1. Continued.

Cleome droserifolia	Om-Nfeia	6
	El-Sakey	11
	El-Sebaae	18
	Abo Halfaia	31
	Abo Maryouh	36
Zygophyllum simplex	El-Sebaae	19
	Abo Maryouh	36
	Delta El-Sokary	41
	Sedi Salem K.38	42
Zilla spinosa	El-Sebaae	24
	Abo Maryouh	36
	El-Sebaae	19
	Delta El-Sokary	41

Table 2. List of random 10*mer* primers (groups A, B, C, O and Z) and their nucleotide sequences.

Primer	Sequence (5'–3')	Primer	Sequence (5'–3')
OPA-04	AATCGGGCTG	OPC-07	GTCCCGACGA
OPA-09	GGGTAACGCC	OPC-11	AAAGCTGCGG
OPA-13	CAGCACCCAC	OPC-14	TGCGTGCTTG
OPA-17	GACCGCTTGT	OPC-16	CACACTCCAG
OPB-01	GTTTCGCTCC	OPC-18	TGAGTGGGTG
OPB-02	TGATCCCTGG	OPO-01	GGCACGTAAG
OPB-03	CATCCCCCTG	OPO-02	ACACACGCTG
OPB-04	GGACTGGAGT	OPO-07	CAGCACTGAC
OPB-05	TGCGCCCTTC	OPO-09	TCCCACGCAA
OPB-07	GGTGACGCAG	OPZ-04	AGGCTGTGCT

ligated to the digested DNA fragments. One primer combination between *Eco*RI primer plus was used to selectively amplify the DNA fragments matching the primer-extension sequence. Pre-amplification was carried out using *Eco*RI primer plus three 3 extension bases (AAC) and *Mse*I primer plus three 3 extension bases (CAA) to amplify fragments that contain complementary sequences.

Detection of PCR products

The products of both RAPD- and ISSR-based PCR analyses were detected using agarose gel electrophoresis (1.2% in 1 × TBE buffer), then stained with ethidium bromide (0.3 ug/ml) and then visually examined with UV transilluminator and photographed using a CCD camera (UVP, UK). AFLP products were detected by electrophoresis on polyacrylamide denaturing sequencing gel. DNA silver staining system (Promega, CA, USA) was used for band detection then gels were photographed.

Data analysis

Clear, unambiguous and reproducible bands recovered through different techniques were considered for scoring. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. Band size was estimated by comparing the with 1-kb ladder (invitrogen, USA) using Gel Works 1D advanced gel documentation system (UVP, UK). The binary data matrices were entered into the TFPGA (Ver. 1.3) and analyzed using qualitative routine to generate similarity coefficient. Dissimilarity coefficients were used to construct a dendrogram using un-weighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine.

Matrix comparison

Similarity matrix produced by 20, out of 58, RAPD primers, 14, out of 17, ISSR primers and one selective AFLP primer combination were compared based on the genetic distance of the TFPGA, the normalized Mantel statistic (Mantel, 1967). The marker index (MI) was calculated by applying the following formula given by Powell et al. (1996)**;** Smith et al. (1997):

$$PIC = 1 - \sum_{i=1-n} f_i^2$$

Table 3. List of ISSR primers and their nucleotide sequences.

No.	Name	Sequence
1	814	$(CT)_8TG$
2	844A	$(CT)_8AC$
3	844B	$(CT)_8GC$
4	17898A	$(CA)_6AC$
5	17898B	$(CA)_6GT$
6	17899A	$(CA)_6AG$
7	17899B	$(CA)_6GG$
8	HB8	$(GA)_6GG$
9	HB9	$(GT)_6GG$
10	HB10	$(GA)_6CC$
11	HB11	$(GT)_6CC$
12	HB12	$(CAC)_3GC$
13	HB13	$(GAG)_3GC$
14	HB14	$(CTC)_3GC$
15	HB15	$(GTG)_3GC$
16	UBC-820	$(GT)_8C$
17	UBC-827	$(AC)_8G$

Where, *fi* is the frequency of the i^{th} amplicon. The number of amplicons refers to the number of scored bands. The frequency of an amplicon was obtained by dividing the number species, where it was found by the total number of species. The PIC value (polymorphism information content) provides an estimate of the discriminating power of a marker. Marker index was calculated for each primer as the product of PIC and the number of polymorphic bands.

Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) is a method of estimating population differentiation directly from molecular data and testing hypotheses about such differentiation. A variety of molecular data – molecular marker data (for example, RAPD, ISSR or AFLP), direct sequence data, or phylogenetic trees based on such molecular data – may be analyzed using this method, that is, a matrix of 1's and 0's, 1 indicating presence and 0 absence of a marker (Excoffier et al., 1992). AMOVA was performed using GENALEX 6 (genetic analysis in excel, Peakall and Smouse, 2006) in RAPD, ISSR and AFLP to partition the total molecular variance between and within populations.

RESULTS AND DISCUSSION

In this work, RAPD, ISSR and AFLP markers were utilized to analyze of germplasm of wild medicinal plants species (Figure 2). Generally, the optimal number of primers, required to discriminate among genomic DNAs of different plant genotypes, depends on the level of polymorphism obtained by type of molecular analysis (e.g., RAPD, ISSR, AFLP, etc.). Arguments about the value of genetic distance required to classify correlated plants accessions as distinct cultivars have been raised by several authors (Cabrita et al., 2001; Papadopoulou et al., 2002).

In the present study, primers (20 out of 58 for RAPD, 17 out of 30 for ISSR and one combination for AFLP) with informative patterns were selected on the basis of the number of amplicons recovered through PCR and the stability (reproducibility) of the patterns to characterize the 19 germplasm belonging to seven plants species. Less than 10% intra-plants polymorphism (within) was found for the plants at the same location across the three types of analyses (data provided upon request). As being dominant markers, however, pooling (bulk) strategy in RAPD, ISSR and AFLP analyses is ideal to saturate such an intra-plants polymorphism with no effects on the accuracy of the obtained results. Mengoni et al. (2000) indicated that this level of intra-plant polymorphism, following the procedure of analysis of molecular variance (AMOVA, Excoffier et al., 1992), is statistically insignificant.

Identification of RAPD markers

A high level of polymorphism was generated utilizing the 20 RAPD primers. A total number of 784 amplicons were separated on agarose gel electrophoresis across all plants. Of these, 591 bands were polymorphic (75%) and 193 were monomorphic (25%). The highest number of amplicons was generated from *B. aegyptiaca* (170 amplicons), while *Z. spinosa* generated the lowest (60 amplicons). The highest number of species-specific markers (49), due to the presence of a unique band or absence of a common band for a given plant species (positive or negative marker, respectively), was scored for *C. droserifolia*, while the lowest number of species-specific markers (20) was scored for *Z. spinosa*. In conclusion, the 20 RAPD primers used in the present study allowed for enough distinction among the seven wild plants species.

Identification of ISSR markers

ISSR, as a relatively more recent class of molecular markers, is based on inter tandem repeats of short DNA sequences. These repeats were proven to be highly polymorphic even among closely related germplasm due to the lack of functional constraints in these non-functioning DNA regions that results in the evolutionary changes in this kind of DNAs high enough to be scored. Similarly, a high level of polymorphism was generated utilizing the 17 ISSR primers. A total number of 914 amplicons were obtained, 610 of them were polymorphic (67%) and 304 were monomorphic (33%). The highest number of amplicons was generated from *B. aegyptiaca* (177 amplicons), while *Aerva javanica* plant generated the lowest (87 amplicons). Of these, 213 amplicons were useful species-specific markers. The highest number of species-specific markers (47) was scored for *Z. simplex*

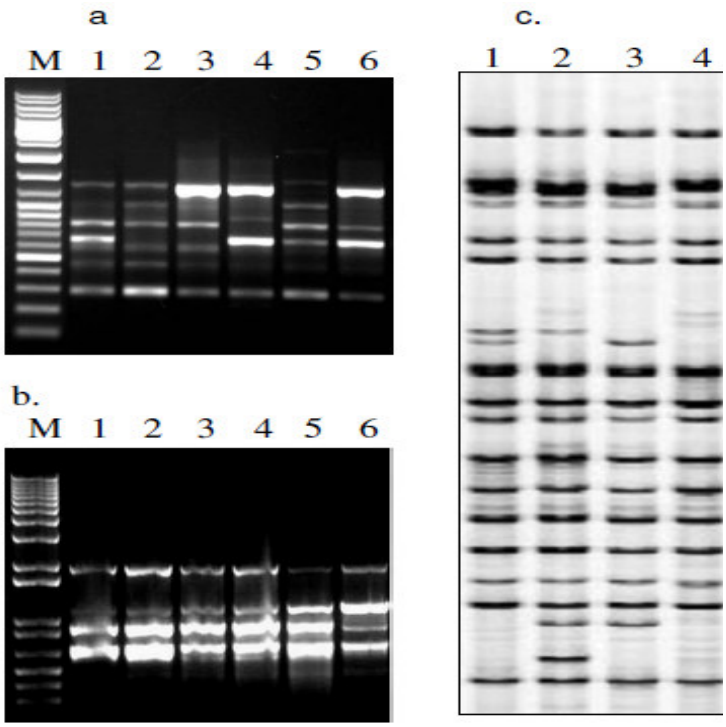

Figure 2. Models of different marker profiles (a. RAPD with primer OP-B02, b. ISSR with primer 844A and c. AFLP with one primer combination) of *Balanites aegyptiaca* (for RAPD and ISSR analyses) and *Zygophyllum simplex* (for AFLP analysis) in different locations (Table 2), refer to DNA standard 1 kb ladder, respectively).

while the lowest number was scored for *A. javanica*. In conclusion, the 17 ISSR primers used in the present study also allowed for enough distinction among the seven wild plants species

Identification of AFLP markers

AFLP analysis revealed 226 amplicons including 86 polymorphic amplicons (39%) among the seven wild plants species, separated by electrophoresis on acrylamide gel. The highest number of amplicons was generated from *B. aegyptiaca* (39 amplicons), while *C. droserifolia* generated the lowest (29 amplicons). The highest number of species-specific markers (10) was scored for *A. judaica*, while the lowest number of species-specific markers (3) was scored for *A. hispidissima*.

Genetic relationships and cluster analysis

In order to clarify the different hierarchical ranges, species were distributed in different groups according to their locations. The genetic similarity between locations for every species, based on Nei's method (Nei's, 1978), indicated that the distance coefficients were varied from 0.74 to 0.62 for *A. judaica,* from 0.76 to 0.58 for *A. hispidissima*, from 0.83 to 0.73 for *A. javanica*, from 0.79 to 0.61 for *B. aegyptiaca*, from 0.79 to 0.69 for *C. droserifolia*, from 0.79 to 0.63 for *Z. simplex* and from 0.87 to 0.75 for *Z. spinosa* (Table 4 and Figure 3) for *C. droserifolia*, while the lowest number of species-specific

Fingerprint evaluation and computer analysis of different molecular data

The partition of variation within species was studied with the analysis of the Dice's distance matrix by the analysis of molecular variance (AMOVA) approach. A hierarchical analysis of genetic diversity using a two-way nested AMOVA was performed. Results from AMOVA within species are shown at Table 5. Data for *A. judaica* and *Z. simplex* indicated that 1% of the genetic variation is attributed to differences among populations, while 99% of the genetic variation is attributed to differences within populations. Sum of squares for *A. judaica* were found to be 1.068 and 145.918, respectively, while 2.0 and 311.5, respectively for *Z. simplex*. Results for *A. hispidissima*, *A. javanica*, *C. droserifolia, Z. simplex* and *Z. spinosa* data indicated that 0% of the genetic variation in each species is attributed to differences among accessions groups, while 100% of the genetic variation is attributed to differences within accessions groups. Sum of squares among and within accessions groups in each species

Table 4. Similarity matrixes between locations based on molecular data for the seven species.

Artemisia judaica

	Hagool	Om-Nfeia	Abol Toyour
Hagool	1		
Om-Nfeia	0.62	1	
Abol-Toyour	0.62	0.74	1

Arnebia hispidissima

	Abo Halfaia	El-Sebaae	Abo Maryouh	Sedi Salem	El-Omra
Abo Halfaia	1				
El-Sebaae	0.75	1			
Abo Maryouh L36	0.63	0.67	1		
Sedi Salem	0.64	0.63	0.76	1	
El-Omra	0.58	0.56	0.70	0.70	1

Aerva javanica

	El-Sebaae	Abo Maryouh	Sedi Salem	Marsa Alam
El-Sebaae	1			
Abo Maryouh L37	0.83	1		
Sedi Salem	0.78	0.81	1	
Marsa Alam	0.73	0.77	0.80	1

Balanites aegyptiaca

	Esley	El-Sebaae	Hegleg	Abo Maryouh	Marsa Alam	Abo Maryouh
Esley	1					
El-Sebaae	0.79	1				
Hegleg	0.72	0.78	1			
Abo Maryouh L37	0.67	0.69	0.77	1		
Marsa Alam	0.67	0.70	0.73	0.72	1	
Abo Maryouh L42	0.61	0.63	0.66	0.65	0.72	1

Cleome droserifolia

	Om-Nfeia	El-Sakey	El-Sebaae	Abo Halfaia	Abo Maryouh
Om-Nfeia	1				
El-Sakey	0.78	1			
El-Sebaae	0.73	0.79	1		
Abo Halfaia	0.69	0.73	0.78	1	
Abo Maryouh L36	0.70	0.77	0.75	0.77	1

Zygophyllum simplex

	El-Sebaae	Abo Maryouh	Deltal-Sokary	Sedi Salem
El-Sebaae	1			
Abo Maryouh L36	0.79	1		
Deltal-Sokary	0.75	0.72	1	
Sedi Salem	0.63	0.64	0.75	1

Zilla spinosa

	El-Sebaae24	Abo Maryouh	El-Sebaae19	Deltal-Sokary
El-Sebaae24	1			
Abo Maryouh L37	0.75	1		
El-Sebaae19	0.79	0.81	1	
Deltal-Sokary	0.77	0.76	0.87	1

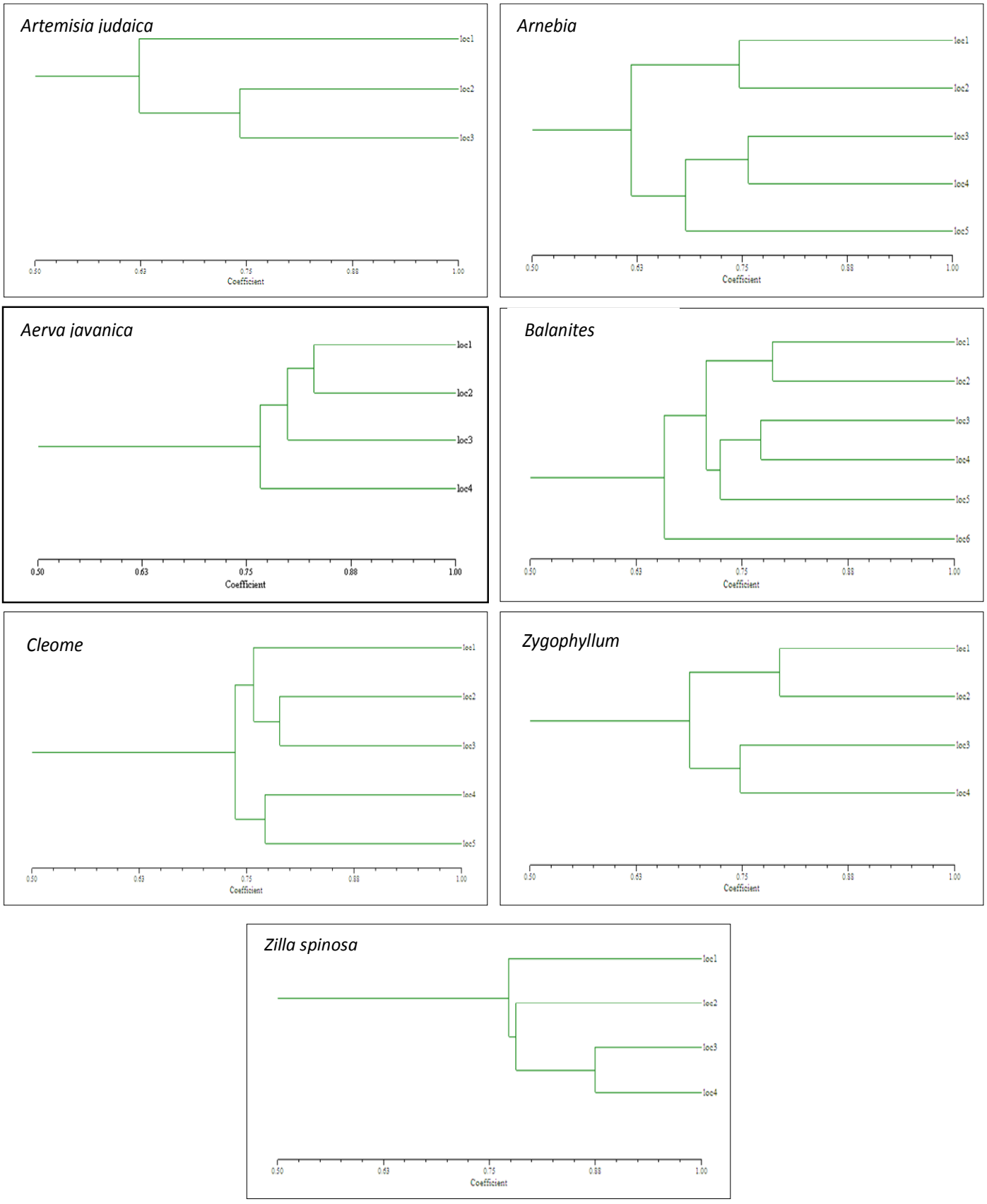

Figure 3. Dendrograms based on algorithm of unweighted pair group (UPGMA) method with arithmetic averages between locations within each species.

Table 5. Analysis of molecular variance (AMOVA) of the seven collected species.

Specie	Source	*d.f.**	*S.S.***	*M.S.****	*Est. Var.*	%
	Among Pops	2	1.068	0.534	0.001	1
Artemisia judaica	Within Pops	654	145.918	0.223	0.223	99
	Total	656	146.986	0.757	0.225	
	Among Pops	4	1.658	0.415	0.001	0
Arnebia hispidissima	Within Pops	1265	311.173	0.246	0.246	100
	Total	1269	312.831	0.661	0.247	
	Among Pops	3	0.420	0.140	0.000	0
Aerva javanica	Within Pops	708	147.230	0.208	0.208	100
	Total	711	147.650	0.348	0.208	
	Among Pops	5	0.739	0.148	0.000	0
Balanites aegyptiaca	Within Pops	2310	564.580	0.244	0.244	100
	Total	2315	565.320	0.392	0.244	
	Among Pops	4	1.322	0.331	0.000	0
Cleome droserifolia	Within Pops	1765	401.503	0.227	0.227	100
	Total	1769	402.825	0.558	0.228	
	Among Pops	3	2.072	0.691	0.001	1
Zygophyllum simplex	Within Pops	1364	311.558	0.228	0.228	99
	Total	1367	313.631	0.919	0.230	
	Among Pops	3	0.603	0.201	0.000	0
Zilla spinosa	Within Pops	752	151.947	0.202	0.202	100
	Total	755	152.550	0.403	0.202	

**d.f.* = Degrees of freedom, ***S.S.* = Sum of squares, ****M.S.* = Mean square
*****Est. Var.* = Estimated variation.

were found to be 1.6 and 311.1, respectively, for *A. hispidissima*, 0.4 and 147.2, respectively, for *A. javanica*, 0.7 and 564.6, respectively, for *B. aegyptiaca,* 1.3 and 401.5, respectively, for *C. droserifolia* and finally 0.6 and 151.9, respectively, for *Z. spinosa.* The general grouping clearly established the separation of samples according to the taxonomical variety and the geographical origin of each population. Although, this study showed that taxonomical locations can be distinguished according to the molecular genotyping but it cannot be distinguished as different subspecies because the variation within species belonging to different locations is extremely low (0 to 1%).

Comparison among different markers for their efficiency in genome analysis

The average of heterozygosity (H_e), the effective multiplex ratio (E), and the marker index (MI) were computed for each assay based on the experimental data (Table 5). RAPD analysis revealed the highest expected heterozygosity H_e (0.46 for *A. judaica,* 0.50 for *A. hispidissima,* 0.48 for *A. javanica,* 0.49 for *C.* droserifolia, and 0.47 for *Z. spinosa*) as compared with ISSR analysis (0.50 for *B. aegyptiaca* and 0.47 for *Z. simplex*) and AFLP analysis (0.43 for *A. judaica*) (Table 6). The obtained results in the present investigation are in agreement with those of Powell et al. (1996); Muzher (2005) who found that the expected hetrozygosity H_e of RAPD analysis is more than that of AFLP analysis. As for marker index (MI), AFLP analysis revealed low values (2.6 to 6.07) as compared with RAPD analysis (20.64 for *A. judaica*, 35.58 for *A. hispidissima*, 16.35 for *A. javanica,* 47.09 for *B. aegyptiaca*, 44.79 for *C. droserifolia,* 34.71 for *Z. simplex* and 15.88 for *Z. spinosa*) and ISSR analysis (24.04 for *A. judaica*, 32.14 for *A. hispidissima*, 11.30 for *A. javanica*, 53.34 for *B. aegyptiaca*, 34.81 for *C. droserifolia*, 41.15 for *Z. simplex* and 16.40 for *Z. spinosa*) due to the lower effective multiplex ratio of AFLP analysis as compared to RAPD and ISSR analyses.

Table 6. Expected heterozygosity for polymorphic products (He), effective multiplex ratio (E) and the marker index (MI) of each marker type used.

		PIC	He	E	MI
Artemisia judaica	RAPD	0.36	0.46	58	20.64
	ISSR	0.34	0.44	70	24.04
	AFLP	0.34	0.43	18	6.07
Arnebia hispidissima	RAPD	0.37	0.50	95	35.58
	ISSR	0.37	0.49	87	32.14
	AFLP	0.29	0.35	14	4.02
Aerva javanica	RAPD	0.36	0.48	45	16.35
	ISSR	0.30	0.36	38	11.30
	AFLP	0.30	0.38	14	4.27
Balanites aegyptiaca	RAPD	0.37	0.49	127	47.09
	ISSR	0.37	0.50	143	53.34
	AFLP	0.29	0.35	15	4.29
Cleome droserifolia	RAPD	0.37	0.49	121	44.79
	ISSR	0.33	0.42	105	34.81
	AFLP	0.26	0.30	8	2.06
Zygophyllum simplex	RAPD	0.35	0.45	99	34.71
	ISSR	0.36	0.47	114	41.15
	AFLP	0.30	0.38	13	3.96
Zilla spinosa	RAPD	0.36	0.47	44	15.88
	ISSR	0.32	0.39	52	16.40
	AFLP	0.30	0.38	7	2.13

It can be concluded that markers differ in their ability to differentiate among individuals, in the mechanism of detecting polymorphism, in genome coverage, and in the ease of application. Therefore, they could complement one another depending on technical availability. Up to our knowledge, this is the first report dealing with the pattern of variation in red sea populations of wild species. As a result, the distribution of species is only related to its ability to disperse their pollen and/or seeds since no artificial acts are involved during the movement of wild seeds from one place to the other. The distribution of variation in this type of population should indicate more ancient events that those provided by parasites grow on crops.

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