

Molecular Variations of *Achillea fragrantissima* (Forssk.) SCH. BIP. Growing in Five Areas of South Sinai

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ABSTRACT

Five populations of *Achillea fragrantissima* (Forssk.) SCH. BIP., sampled from five locations in South Sinai; were characterized and identified on biochemical and molecular bases. The soils of the studied areas were derived from igneous and metamorphic rocks. Protein electrophoresis (SDS-PAGE) of the studied populations indicated the presence of about 16 bands for total protein. The maximum bands (7) were associated with *Achillea fragrantissima* from Taba, while the minimum bands (4) were from Nuwieba. Generally, the studied five populations have one monomorphic band with molecular weight of 48.36 KDa, which could be considered as a positive marker for all populations. Isozymes polymorphism using Native-PAGE for three isozyme systems revealed five bands for esterase, six for peroxidase and three for acid phosphatase. Generally, the isozyme system gave good discrimination for all individuals except for *Achillea* from Ras-Sudr and Nuwieba, which yielded high similarity (82.6%). The combined class pattern based on protein and isozyme analysis gave better resolution for discrimination of these populations. The dendrogram divided the five populations into two clusters, the first included *Achillea fragrantissima* from Nuwieba, Taba and Saint-Katherine, while the second included those from EL-Naqab and Ras-Sudr. The similarity matrix indicated that the highest similarity (73.1%) was between two populations from Taba and St. Katherine, while the lowest (3.3%) was between populations from Taba and Ras-Sudr. Randomly amplified polymorphic DNA (RAPD) technique was used to fingerprint the five populations. It revealed that differences in locations were particularly reflected on DNA fingerprint. The combined class pattern based on protein, isozyme and RAPD-PCR, allowed for the complete discrimination of all populations.

Key Words: *Achillea fragrantissima*; Genetic diversity; SDS-PAGE; Isozyme; Fingerprints

INTRODUCTION

Because plants can't move there can be a great deal of genetic differentiation in a small area especially within populations of self-fertilizing plants; in other words, since individuals in these populations don't get mixed up a lot, genetic differences may be preserved on a very small scale (Huenneke *et al.*, 1986). Within the same population, covering only a few tens of meters, there may be several distinct genotypes (individuals sharing the same genetic constitution) adapted to take advantage of different conditions (Filipiak, 1987). Some members, for instance, may be adapted to drought, while others will grow only under normal moisture conditions. Some of these genotypes may be common, others may be rare. In managing such a population, awareness of such within-population genetic diversity can make an important difference in assuring that population's long-term survival. For example, if a portion of such a population must be disturbed, which portion is chosen may make a big difference in how much genetic diversity the population loses (Tyser, 1991). Application of biochemical genetic techniques have an important potential to provide a new tool for the study of wild as well as domesticated species, as for example, in the investigation of evolution and migration of species from their gene pool centers. Electrophoresis methods are considered a rapid and

accurate test to identify and characterize species (Paradies & Ohms, 1987; Lioi *et al.*, 1999). Genetic variation is present at the level of individual genes and at the level of the organization of genes in chromosomal domains or whole chromosomes (Oldfield, 1989). Genetic variation not only affects the properties of the gene products, but also the regulation of genes. El-Shanshoury (2002) used seed protein by SDS-PAGE to study the genetic variability between 30 (*Lathyrus sativus* L.) samples collected from different countries. The resulted profiles showed different patterns indicating the variability among accessions of different conditions. Isozymes have been used in genetics for defining systematic phylogenetic relationships and to assess the genetic divergence between taxa (Tanksley, 1983; Bonnin *et al.*, 1996; Yang *et al.*, 1996). Similarly, polymorphisms scored across molecular techniques such as randomly amplified polymorphic DNA (RAPD) have allowed direct comparisons of variations in nucleotide sequences and have proved to be a potential tool in various type of genetic analysis (Monckton & Jeffreyes, 1993). The aim of this work is to establish biochemical and molecular fingerprints of five populations of *Achillea fragrantissima* growing in different areas of south Sinai, to elucidate the genetic relationships and to address the impact of the geographical location on their genetic fingerprint.

MATERIAL AND METHODS

The study areas. Sinai region may be divided into three sub-regions: southern, central and northern. Because of its high altitude, the southern section receives ample rainfall, which has produced wadis. Climatically, it can be divided into two main zones (Ayyad & Ghabbour, 1986): an arid zone, which includes the northern sub-regions, with hot summer and mild, rainy winter and hyperarid province with cool winter and hot summer located around the summits of the Sinai mountains. The study areas are shown in Fig. 1.

1- El-Naqab is an intermountainous area lies in the eastern boundary of Sinai. It is located between 29° 93' 58" of the northern Latitude and 34° 19' 28" of the eastern Longitude, where *Achillea fragrantissima* was recorded as a very rare associate in a community dominated by *Haloxylon salicornicum*.

2- Wadi-Sudr is one of the most developed wadis of south western Sinai, bounded by Gebel Raha (600 m) from the north and Sinn Bisher (618 m) from the south. *Achillea fragrantissima* was sampled from a main tributary flowing into W. Sudr, located between 29° 42' 09" N and 32° 59' 35" E. The plant cover was dominated by *Artemisia judaica*, *Artemisia herba-alba* and *Ochradenus baccatus*.

3- Coastal wadis dissecting the mountainous range in Nuwieba area are mostly short and shallow. The plant cover is often low, where *Acacia tortilis* dominates. Samples of *Achillea fragrantissima* were collected from one of its smallest populations existing at Lat 29° 17' 48" N and 34° 44' 05" E.

4- Saint-Katherine area is characterized by its peculiar vegetation, where *Alkanna orientalis* and *Stachys egyptiaca* were recorded in association with *Achillea fragrantissima* growing in the study site (28° 33' 48" N & 33° 57' 58" E).

5- Wadi Taba exists in the northern extremity of the Gulf of Aqaba dissecting the igneous mountainous area at Lat 29° 28' 26" N and Long 34° 49' 37". The vegetation of wadi Taba was dominated by *Pergularia tomentosa*, *Fagonia arabica*, and *Acacia tortilis* subsp. *tortilis*.

Soil characteristics. Owing to the rocky nature of the concerned areas, profiles were sampled at depth from 0 - 20 cm. Soil samples were collected and then air-dried, crushed, sieved through 2 mm sieve and subjected to analysis. Particle size distribution was carried out by the dry sieving method of Kilmer and Alexander (1949). Soil reaction was determined in the soil paste using a Beckman bench type pH-meter, (Richards, 1954). Total salinity (EC_e), cationic and anionic composition were determined following the methods described by Richards (1954).

Measurement of DNA and RNA. The root tips of 1 cm of the studied plants were fixed in 3 ethanol: 1 glacial acetic acid for 24 h then washed with distilled water and preserved in 70% ethanol. The quantity of DNA in the mitosis stages was measured using Fulgin stain. Concerning the three

types of RNA, their quantity in the mitosis stages was measured using Gallocyanine-Chromalum stain, (Dnyanagar, 1988). Then, the slides were already prepared for DNA and RNA measurements using the image processing and analysis system (Fukui, 1988).

Biochemical Analysis

Protein electrophoresis. SDS- polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total proteins of *Achillea fragrantissima* samples according to the method of Laemmli (1970), as modified by Studier (1973).

Isozymes electrophoresis. Native polyacrylamide gel electrophoresis (Native-PAGE) was used to identify isozymes variation among the five *Achillea fragrantissima* samples. Three isozymes (Esterase, Peroxidase & Acid phosphatase) were extracted from the plants. These isozymes were separated in 9% polyacrylamide gel electrophoresis according to Stegemann *et al.* (1985). After electrophoresis, the gels were stained (Jonathan & Wendel, 1990) according to their enzyme system with the appropriate substrate and chemical solution then incubated at 37°C in a dark room for complete staining.

Gel analysis. All gels resulted from protein and isozyme electrophoresis, were scanned using Gel Doc-2001 Bio-Rad system. The densitometric scanning of the bands was performed on three directions. Each band is recognized by its length, width and intensity. Accordingly, relative amount of each band could be quantified and scored.

RAPD-PCR. Randomly amplified polymorphic DNA (RAPD) technique has been used in many different applications involving the detection of DNA sequence polymorphisms (Carlson *et al.*, 1991; Reiter *et al.*, 1992). DNA was extracted according to El-Fiky *et al.* (2002). DNA quantification involves the use of UV-spectrophotometers. Spectrophotometric measurement indicates the amount of Ultraviolet irradiation absorbed by the bases of the nucleic acid (Sambrook *et al.*, 1989). Fifteen (10-mers) random primers were used in this study, however; only six primers gave reproducible results, (Table I). PCR reactions were conducted according to Williams *et al.* (1990).

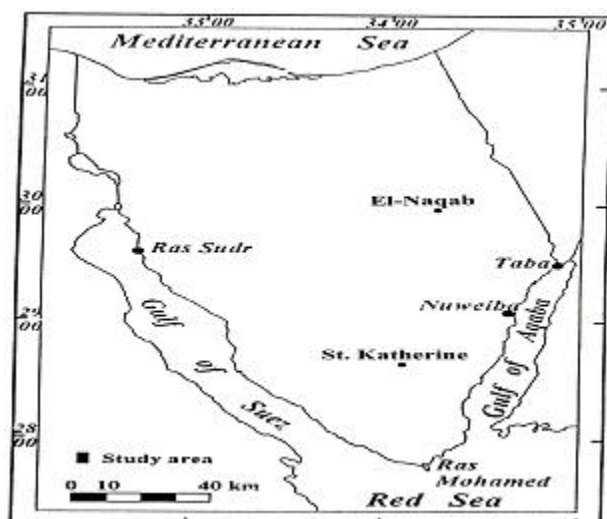
Each RAPD-PCR marker was named by the primer used and DNA fragment size in base pairs (bp). RAPD patterns were scored for each plant and genetic distances were calculated according to Sokal and Snetath (1963) by using RAPD distance software package, version 1.04 (Armstrong *et al.*, 1994).

RESULTS AND DISCUSSION

Ecological settings. Data presented in Table II and III indicated that soils were generally gravelly coarse-textured soils with no variation with depths. Soil reaction ranged between slightly alkaline and alkaline, where pH values varied from 7.5 to 8.3. EC fluctuates in a narrow range, where it ranged from 0.26 to 0.72 ds/m⁻¹. On the other hand, the soils of the collected studied plant were salt free. The cations were dominated by Ca²⁺ followed by Mg²⁺ and Na⁺, whereas the K⁺ ion was the least. The anions were

Table I. List of Primers code, sequences and the percentage of the (G+C) content

Primer code	Sequences 5,-----3,	% of (G+C)
OPA2	TGC CGA GCT G	70%
OPA4	AAT CGG GCT G	60%
OPA6	GGT CCC TGA C	70%
OPO7	CAG CAC TGA C	60%
OPO8	CCT CCA GTG T	60%
OPO10	TCA GAG CGC C	70%

Fig. 1. Location map of the studied areas

dominated by SO_4^{2-} and/or HCO_3^- followed by Cl^- ion. Therefore, the expected salt composition in these soils is CaSO_4 or $\text{Ca}(\text{HCO}_3)_2$. The discrimination of the salt composition and its variation in the soils is completely dependent on the parent rock from which these soils are formed. It is taken into consideration that the ecological setting of the studied sites is to some extent, comparable. However, minor variations were observed between them due to the differences in their geomorphologic characters.

Measurement of DNA and RNA

DNA quantity in the cell division. Results in (Table IV)

revealed that DNA replication at interphase stage in mitotic division of *Achillea fragrantissima* populations differed among the study areas, where in Saint-Katherine was the highest. Nevertheless, it was noticed, to a certain extent, a similarity of DNA replication in each studied area. The differences in DNA replication in two areas could be render the synthesis and organization of substrate (RNA) and formation of enzymes (proteins) required for DNA synthesis and that took place in synthetic activities during this stage. The eco-impact of Saint-Katherine area, which lead to the increasing of the replication of DNA, decreasing of non-divided cells in the different stages. This behaviour will precisely affect the frequency of DNA synthesis processes. The nucleus, which has 2C value before DNA synthesis, assumes 4C value after its synthesis (Coming & Okada, 1972).

RNA quantity in the cell division. The results of the quantities of the three RNA types (rRNA, mRNA & tRNA) indicated that the transcription rates of RNA types were remarkably varied with regard to the selected areas and very coincide with those results already obtained from DNA (Table V). This correlation with the DNA replication may be attributed to the transcription of mRNA is complementary with DNA. The obtained results also revealed that the differences of the three types of RNA with variation of environment will affect cell synthesis processes. This finding is in agreement with those outlined by Dell Aquila and Spada (1992) and El-Saied (1998 & 2004).

Biochemical Fingerprint

Protein electrophoresis profiles. Polyacrylamide gel electrophoresis provides good markers for the identification and characterization of different species. Electrophoretic analysis of protein is assumed to provide information concerning the type and biosynthesis of different protein fractions. The studied five populations were fingerprinted by SDS-PAGE of total proteins. They exhibited a maximum number of 15 bands, which were not necessarily present in all populations. Bands number 7 and 11 were found only in populations from 1, 2 and 3, respectively while bands

Table II. Granulometric analysis of the soil profiles associated with the five populations of *Achillea fragrantissima*

Locations	2-1mm	1-0.5mm	0.5-0.25mm	0.25-0.125mm	0.125-0.063m	0.063mm <	Texture class
El-Naqab	19.1	15.9	18.2	0.18	48.0	4.34	Fine sand
Ras-Sudr	20.9	19.6	16.1	21.7	15.2	6.5	Coarse sand
Nuweiba	41.4	28.9	12.4	5.94	1.98	9.25	Coarse sand
Taba	51.6	30.9	13.8	2.93	0.36	0.28	Coarse sand
Saint-Katherine	21.3	29.7	18.5	9.76	4.72	5.99	Coarse sand

Table III. Some chemical analysis of the soil profiles associated with five populations of *Achillea fragrantissima*

Location	pH	EC _e ds/m ⁻¹	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	CO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻
							mg/100 g			
El-Naqab	7.9	0.72	1.17	0.24	5.0	3.0	2.4	Tr.	2.75	4.44
Ras-Sudr	8.3	0.60	2.90	0.33	3.5	1.5	2.4	Tr.	4.00	1.2
Nuweiba	7.8	0.60	0.87	0.42	5.0	2.5	3.6	Tr.	2.5	2.89
Taba	7.5	0.70	1.02	0.31	4.5	3.0	3.0	Tr.	2.5	3.33
Saint-Katherine	8.1	0.26	0.17	0.14	3.5	0.5	1.8	Tr.	1.25	1.26

Table IV. DNA replication (mitotic division) of *Achillea fragrantissima* populations (1-5)

Steps of division	1	2	3	4	5	
Less 2C	34.05	35.15	31.04	34.05	30.15	
2c	12.00	14.00	13.71	14.00	18.00	Non-divided cells
S-phase	38.14	37.23	40.19	35.14	40.25	DNA-synthesis
4C	8.50	7.62	8.06	11.50	4.60	
More than 4C	7.30	6.00	5.00	8.30	6.00	

Table V. Three types of RNA (mitotic division) of *Achillea fragrantissima* populations

Type of RNA	1	2	3	4	5	
First peak	22.71	27.94	22.00	13.60	10.56	tRNA
Second peak	16.71	15.14	18.00	12.69	19.65	mRNA
Third peak	56.57	60.86	60.00	72.70	70.68	rRNA

Table VI. SDS-PAGE of total protein bands of five populations of *Achillea fragrantissima*

Bands No.	Mid. Marker	Mol. RF.	1	2	3	4	5	Mol. bands	M.
1	97.40	0.051	-	-	-	+	-	95.63	
2		0.090	+	-	-	-	-	71.25	
3	66.20	0.093	-	+	-	-	-	69.95	
4		0.168	-	+	-	-	-	53.56	
5	45.00	0.200	+	+	+	+	+	48.36	
6		0.268	-	-	-	+	+	38.20	
7	31.00	0.305	+	+	+	-	-	33.34	
8		0.380	-	-	-	+	+	26.60	
9	21.50	0.568	-	-	-	-	+	16.22	
10		0.598	-	-	-	+	-	15.05	
11	14.40	0.649	+	+	+	-	-	13.19	
12		0.661	-	-	-	+	+	12.76	
13		0.729	+	+	-	-	+	10.75	
14		0.744	-	-	+	-	-	10.33	
15		0.785	-	-	-	+	-	9.29	
Total			5	6	4	7	6		

Table VII. Number and types of bands as well as the percentage of the total polymorphism generated by protein fractions in five populations belonging to *Achillea fragrantissima*

Protein system	Monomorphic Bands	Polymorphic Unique non-unique	Total Bands	Polymorphic%	
Total protein	1	8	6	15	93%

Table VIII. The presence (+) and absence (-) of bands in Esterase isozyme among five populations of *Achillea fragrantissima*

Band Est	1	2	3	4	5
1	+	+	+	-	-
2	+	+	+	+	+
3	+	+	+	+	+
4	-	-	-	-	+
5	+	+	+	+	-
Total	4	4	4	3	3

number 6, 8 and 12 found in populations from 4 and 5, respectively. It can conclude that the latter bands can be considered as specific for these populations.

Generally, the studied five populations have one

monomorphic band with molecular weight of 48.36 KDa. (Table VI), which could be considered as a positive marker for all populations. The former results were quietly lined with those obtained from similar studies on *Acacia spp.* (Ahmed *et al.*, 2003), *Zygophyllum spp.* (Nour El-Din *et al.*, 2004) and some halophytic plants (El-Saied & Ahmed, 2004). The data of SDS-PAGE of total proteins were applied to the computer program SPSS version 10 and a dendrogram for genetic distances (Fig. 2a) was obtained. The results indicated that the highest similarity matrix (59.2%) was between *Achillea fragrantissima* from site (4) and (5) and the lowest (3.5%) was between (1) and (5). Therefore, it can point out that the genetic behaviour of *Achillea fragrantissima* populations was affected by environmental conditions.

Isozyme electrophoresis. Isozymes merely represent different structural configuration of the same polypeptide chain of an enzyme (Weeden, 1983). For this reason, three isozymes including esterase (Est), peroxidase (Px) and acid phosphatase (Acp) were used to study the effect of the different environmental conditions on the gene/genes expression for the five populations of *Achillea fragrantissima*. The occurrence of bands was expressed as (+) and absence as (-), (Tables VIII, IX & X). On the other hand, polymorphic and monomorphic bands for each enzyme were illustrated in (Table XI). The obtained results were as follows:

Table IX. The presence (+) and absence (-) of bands in Peroxidase isozyme among five populations of *Achillea fragrantissima*

Band prx.	1	2	3	4	5
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	-	-	-	+	+
5	+	+	+	+	+
6	+	+	+	+	-
Total	5	5	5	6	5

Table X. The presence (+) and absence (-) of bands in Acid phosphatase isozyme among five populations of *Achillea fragrantissima*

Band Acp.	1	2	3	4	5
1	+	+	+	+	+
2	+	+	-	-	-
3	+	+	+	+	+

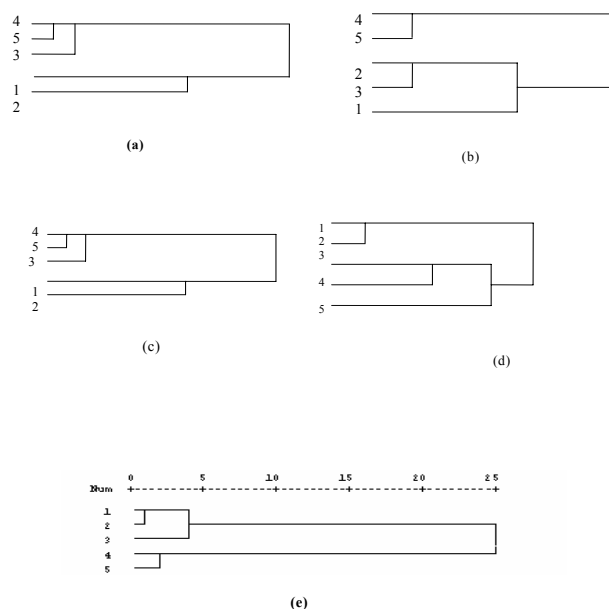
Table XI. Number and types of bands as well as the percentage of the total polymorphism generated by three enzymes (Esterase, Peroxidase and Acid phosphatase) of five populations of *Achillea fragrantissima*

Isozymes	Monomorphic Bands	Polymorphic Unique non-unique	Total bands	Polymorphic	
Est.	2	1	2	5	60.0 %
Px.	4	-	2	6	33.0 %
Acp.	1	-	1	3	33.0 %

Table XII. Number and types of the amplified DNA bands as well as the percentage of the total polymorphism generated by six primers in the four species belonging to five populations of *Achillea fragrantissima*

Primer code	Monomorphic Bands	Polymorphic Unique non-unique	Total bands	Polymorphic %
OPA2	1	3	6	83.33
OPA4	4	2	6	33.33
OPA6	2	-	4	66.66
OPO7	2	-	4	80.00
OPO8	2	1	5	60.00
OPO10	2	1	3	66.66

Fig. 2. (a) Dendrogram demonstrated relationship among five populations belonging to *Achillea fragrantissima* based on protein banding patterns, (b) Dendrogram demonstrated relationship among five populations of *Achillea*, (c) Dendrogram demonstrated relationship among five populations of *Achillea fragrantissima* based on protein and isozyme analysis, (d) Dendrogram demonstrated relationship among five populations of *Achillea fragrantissima* based on RAPD-PCR, (e) Dendrogram demonstrated relationship among five populations of *Achillea fragrantissima* based on protein, isozymes and RAPD-PCR



Esterase. Banding patterns of esterase for the five *Achillea fragrantissima* populations were illustrated in (Table VIII). The results indicated that five bands were noticed from the electrophoresis banding patterns, where two bands (2 & 3) were monomorphic and the remainder bands polymorphic with percentage (60%); (Table XI). The population from (5) was distinguished by band number (4) and absence of band number (5).

Peroxidase. Peroxidase electrophoretic patterns for the studied *Achillea fragrantissima* populations were analyzed as

shown in Table IX. Six bands were present, but they were not necessarily exhibited in all populations. However, their presence are different with respect to their densities and intensities. The results were also indicated that the four bands were monomorphic and the two remainder bands polymorphic with percentage (33%); (Table XI). The highest gene/genes expression associated with the population from site (4), while the population from site (5) was distinguished by absence of band number (5). On the other hand, the same trend of gene/genes expression was associated with the populations from (1 - 2 - 3).

Table XIII. Molecular weight base pairs of amplified DNA fragment that were produced by 6 primers with five populations belonging to *Achillea fragrantissima*

DNA marker	Size (bp)	Populations				
		1	2	3	4	5
OPA-2						
AF01	1350	0	0	0	1	1
AF02	980	0	0	0	0	1
AF03	750	1	1	0	1	1
AF04	575	1	1	1	1	1
AF05	350	0	0	0	0	1
AF06	200	0	0	0	0	1
Total		2	2	1	3	6
OPA-4						
AF07	1390	0	1	0	0	0
AF08	1215	1	1	1	1	1
AF09	1000	1	1	1	1	1
AF10	955	1	1	1	1	1
AF11	650	1	1	1	1	1
AF12	580	0	0	1	0	0
Total		4	5	5	4	4
OPA-6						
AF13	1320	0	1	1	1	1
AF14	1000	0	1	0	1	1
AF15	550	0	0	0	1	1
AF16	500	0	1	1	1	1
AF17	450	1	1	1	1	1
AF18	340	1	1	1	1	1
Total		2	5	4	6	6
OPO-7						
AF19	1415	0	1	0	1	1
AF20	1020	1	1	1	1	1
AF21	850	1	1	1	1	1
AF22	650	0	0	0	1	1
AF23	625	1	1	1	0	0
Total		3	3	3	4	4
OPO-8						
AF24	1650	1	0	0	0	0
AF25	1285	1	1	1	1	1
AF26	1080	1	0	0	1	1
AF27	880	1	1	1	1	1
AF28	500	0	1	1	1	1
Total		4	3	3	4	4
OPO-10						
AF29	1250	1	1	0	1	1
AF30	1080	1	0	0	0	0
AF31	850	1	1	0	0	1
AF32	700	1	1	0	0	1
AF33	480	1	1	1	1	1
AF34	410	1	1	1	1	1
Total		6	5	2	3	5

AF =Amplified fragments

Acid Phosphatase. Acid phosphatase electrophoretic patterns, was summarized in Table X. Three bands were found with highly polymorphic with percentage 33% (Table XI). The results indicated that no differences of gene/genes expression were observed between all populations. Therefore, it can be assumed that acid phosphatase isozyme patterns did not give clear-cut markers for the discrimination between five populations of *Achillea fragrantissima*.

Based on the available data of the isozyme patterns, a dendrogram (Fig. 2b) was demonstrated. At the first cut level, the five populations were distinguished into two main clusters: the first included *Achillea fragrantissima* from sites (4) and (5), while the second included those from sites (1), (2) and (3). The highest similarity (82.6%) was between populations (2) and (3), while the lowest (17.5%) was between populations (1) and (4). Isozymes were dressed here to clarify the interspecific genetic relationships.

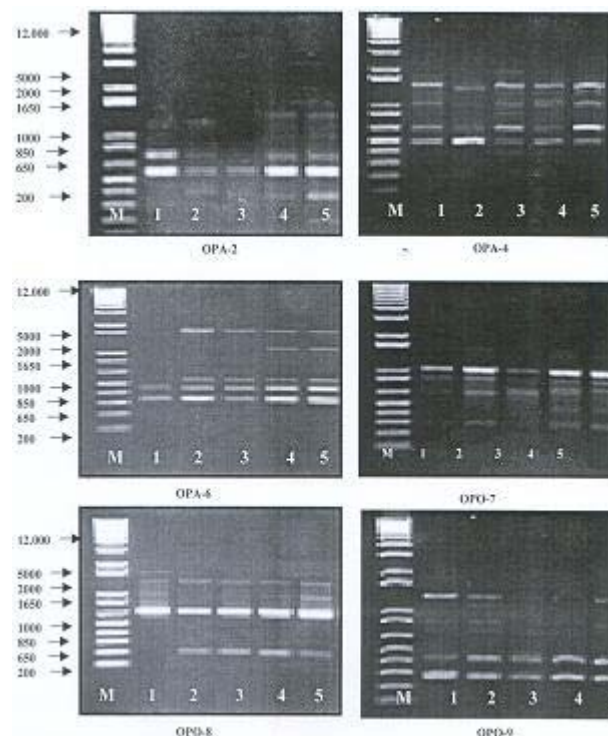
Combined class pattern based on protein and isozyme analysis. The single system was not able to differentiate between the populations in this study; it was required to apply the combined class pattern system to obtain better resolution (Abdelsalam *et al.*, 1998). The dendrogram, (Fig. 2c), divided the five populations into two clusters the first cluster included *Achillea fragrantissima* from Nuwieba, Taba and Saint- Katharine, while the second included those from El-Naqab and Ras-Sudr. However, within the first group, each population was the closest to each other under the different conditions. Also, the results revealed that in the second group the populations under the different conditions were closest. The similarity matrix indicated that the highest similarity was (73.1%) between populations (4) and (5), while the lowest similarity was (3.3%) between populations from (4) and (2). This combined system could discriminate between the five populations.

Randomly amplified polymorphic DNA (RAPD). In the present study, forty 10-per random primers were used to differentiate between the five populations of *Achillea fragrantissima*. However, only six primers gave reproducible results (Fig. 3; Table XIII).

The dendrogram based on all markers developed by all the primers of this study, (Fig. 2d) separated the five populations into two clusters the first cluster included *Achillea fragrantissima* from Nuwieba, Taba and Saint-Katharine, while the second cluster included the *Achillea fragrantissima* from EL- Naqab and Ras-Sudr. The similarity matrix between the two populations (1) and (2) related with similarity value (64.3%), while populations (1) and (5) were quite different with a low similarity value (1.5%). This analysis seems to be one of the powerful tools for discrimination between all the five populations. The results of this analysis revealed that differences in locality were particularly reflected on RAPD-PCR.

Over all combined class patterns. The dendrogram (Fig. 2e) resulting from pooling the obtained data of the three analytical systems; protein, isozymes and RAPD; could

Fig. 3. RAPD-PCR polymorphism of DNA of ten populations (Lane 1-5). 1 *Achillea fragrantissima* from EL- Naqab, 2 *Achillea fragrantissima* from, Ras-Sudr, 3 *Achillea fragrantissima* from Nuwieba, 4 *Achillea fragrantissima* from Taba, 5 *Achillea fragrantissima* from St.- Katherine using OPA-2, OPA-4, OPA-6, OPO-7, OPO-8, and OPO-9 primers. M: 1Kb DNA ladder



discriminate between all the five populations of *Achillea fragrantissima*. The similarity indices were either of moderate or low values, which allowed for the complete discrimination of each population. Combining protein, isozymes and RAPD systems offered the highest and most dependable resolution between the five populations. The five populations can be separated into three classes, the populations from El- Naqab and Ras-Sudr in a class and the populations from Nuwieba and Taba in a class except for the population from Saint-Katharine, which was placed in another class. These results were in consistence of finding of Hassan *et al.* (2002), who investigated genetic polymorphism in ten accessions representing five *Populus* species from different locations using isozymes and RAPD analyses. Also, El-Sherbeny (2004) reported that the combined class pattern for protein, isozymes and RAPD allowed for the complete discrimination of all populations of four species of family Labiatae. It is evident from the aforementioned observations that the superiority of fingerprints based on combined analysis of several molecular systems over fingerprints based on one system only.

CONCLUSION

In summary, the present study demonstrates that the single system (protein, isozyme) was not able to differentiate between the different populations. Meanwhile the combined system could discriminate between them. RAPD analysis was used for constructing relationships among the five populations. The results of this analysis revealed that differences in habitats conditions were particularly reflected on RAPD-PCR.

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(Received 04 August 2006; Accepted 20 October 2006)