MORPHOLOGICAL AND MOLECULAR GENETICS CHARACTERIZATION OF HOLOPARASITIC PLANT, *Cistanche phelypea* L. IN SIWA OASIS, EGYPT

H. E. ELWAKIL[1], N. R. ABDELSALAM[1], REHAM M. ABD EL-AZEEM[2], A. A. HEMEIDA[3], NERMEEN Y. ABASS[1] AND A. NASSAR

1. *Department of Agricultural Botany, Faculty of agriculture-Saba Basha, Alexandria Univ., Egypt*
2. *Dept. of Environ. Biotechnology, Institute of Genet. Eng. and Biotechnology, Univ. of Minufiya*
3. *Dept. of Bioinformatics, Institute of Genetic Engineering and Biotechnology, Univ. of Minufiya*
4. *Plant Protection Dept., Faculty of Agricultural, Damnhour Univ.*

Egypt is famous for a vast desert spaces. About 4/5 the total area is desert. Only about one-fifth of the total area is cultivated land. Most of the soils of this desert are sand dunes, sand sheets, sand bed rock, sandstone, limestone and salt marshes. In the western desert of Egypt there are a series of oases which stretching longitudinally from the north to the far south in a line almost parallel to the Nile valley. This represents natural depressions rich in natural springs and wells which are used in agriculture. One of the advantages of this desert is the presence of wild flora species adapted to a biotic stresses such as drought, high temperature and salinity. Despite the rarity of wild plant cover in such region, this existing flora is a treasure of genetic resources. Most of these flora species are considered as the ancestors of domesticated plants, others have medicinal benefits either on the popular level or on the documented medicinal treatments.

One of these species is the Angiosperm Dicotyledonous holoparasitic plant; *Cistnche phelypeae*. The genus *Cistanche* is represented in Egypt by four species namely *C. phelypaea C. tubulosa, C. violacea* and *C. salsa* (Vivi Tackholm, 1974; Boulos 2002). In the observed and collected areas,*Cistanche phelypaea* restricted its host range in two families only, *Chenopodiaceae* and *Zygophyllaceae*. This holoparasitic species which parasitic on the roots of other plants lacks chlorophyll and therefore cannot performs photosynthesis. So it is an obligate parasite which totally depends on its host for water, minerals and organic nutrients (Boulos, 2002). *Cistanch philypea* L. grows in Egypt in low density but scattered on a large area of the country. It can be recognized in the Nile region including Faiyum, the Oases of the western desert including Siwa, the Red Sea coastal strip, and the entire Sinai Peninsula including the coastal of the Mediterranean. It grows in sandy and alluvial soil, edges of cultivated fields as a parasite on plant roots (Vivi Tackholm, 1974; Boulos, 2002).

Cistanche species have many medical benefits. Medical tests proved their beneficial effects as follows: exhibited

hypocholesterolemic activity in diet-induced hypercholesterolemia mice (Shimoda *et al.*, 2009), enhanced antibody production in human lymph node lymphocytes (Maruyama, 2008). *Cistanche* may delay aging, which may be related to antagonizing free radical injury and enhancing immunity of aging mice (Zhang *et al.*, 2008). Anti-fatigue properties have been shown in mice. *Cistanche* appeared to enhance the swimming capacity of mice by decreasing muscle damage, delaying the accumulation of lactic acid and by improving the energy storage and a recent clinical study, a memory was found to enhance properties in *Cistanche* (Choi *et al.*, 2011). The objective of this research is to describe the environmental condition, host range, morphological feature and determine the extent of genetic variation among individuals of this species in two different populations from two different localities by applying biochemical and molecular genetic analyses.

MATERIAL AND METHODS

A. Surveying localities

Cistanche phelypaea plants were surveyed and photographed combined with their natural hosts in the western desert of Egypt from two different localities; south of Siwa Oasis, in the sand sheets region and in uninhabited (neglected) Oasis (Sheiata) away from Siwa with about 80 kilometers in the north west direction (Fig. 1). Different morphological features of different developmental stages of the species were photographed in its natural habitats accompanied with its hosts to describe the nature of the ecological niches where it lives.

B. Chemical analysis

The concentration of macro and micro elements in the parasite and its host species were estimated by soil, water and plant analysis Unit, Department of Soil and Agricultural Chemistry, Faulty of Agriculture-Saba Basha, Alexandria University. Atomic absorption was used for estimating micro elements; zinc, iron, manganese and cupper. Macro elements; calcium, magnesium were estimated by titration method, while sodium and potassium were estimated by flame photometry.

C. Biochemical genetic analysis (isozymes analysis)

Twenty different plant stems of *Cistanche phelypaea* from each locality were examined individually for their isozyme patterns. A combination of agar-starch gel electrophoresis and enzyme activity attaining was used to screen for polymorphisms of peroxidase. The laboratory methods were performing according to Jonathan and Norman (1989). According to the electrophoretic pattern exhibited by each of peroxidase loci under investigation, genotypic frequencies were calculated and used to estimate gene frequencies of alleles segregating at each locus. A simple method of calculating gene frequency of S (allele coding for slow migrating band) and F (for fast) uses the following equations: $q(S) = m(S) + 1/2\ H$ and $q(F) = m(F) + 1/2\ H$, where q (S) and q (F) are the gene frequencies of S

and F alleles, respectively, m (S) and m (F) are the frequencies of SS and FF homozygotes, respectively, and H is the frequency of heterozygotes (EL-Metainy *et al*., 1977).

D. Molecular genetic analysis

Twelve individual plants were taken at random from two different localities of Siwa Oasis, six plants were taken from each locality. One locality is in the west of the Oasis (A), while the other is in the East (B). The two localities are far away from each other with about 80 Km (Fig. 1). Genomic-DNA from each population was isolated from plants stem using modified CTAB method (3% CTAB, 3% PVP and 3% β mercaptoethanol) according to Doyle and Doyle (1987).

The PCR operon primers used for RAPDs are listed in Table (1). These primers were selected from the Operon kits (Operon Technologies Inc., Alabameda CA). RAPD-PCR analysis was performed according to the method of Williams *et al.* (1990). The polymerase chain reaction mixture (25 µl) consisted of 0.8 U of Taq DNA polymerase; 25 pmol dNTPs; 25 pmol of primer and 50 ng of genomic DNA. PCR amplification was performed in a Biometra T1 gradient thermalcycler for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94°C for 1 min; annealing at 36°C for 1 min; extension at 72°C for 2 min and final extension at 72°C for 10 min (Soliman *et al.,* 2003). Amplification products were separated on 1.5% agarose gels at 100 volts for 1.30 hrs with 1 x TBE buffer. To detect ethidium bromide/DNA complex, agarose gels were examined on ultraviolet transilluminator (302 nm wavelength) and photographed. Using 100 bp DNA ladder (V-gene Biotechnology Limited, shiqao, P. R. China), the lengths of the different DNA fragments were determined. The reproducible DNA fragments from two runs were scored for their presence (1) or absence (0) for each genome.

E. Data analysis

Data matrices were entered into the NTSYS program (Numerical Taxonomic and Multivariate Analysis System) software package, version 2.1, Applied Biostatistics Inc. (Rohlf, 2000). Similarity coefficients were used to construct dendrograms using the UPGMA (unweighted pair group method with arithmetic average) and the SAHN (Sequential Agglomerative Hierarchical Nested clustering) routine in the NTSYS.

RESULT AND DISCUSSION

A. Morphological description

Figure (2) illustrates the morphological features of different developmental stages of the inflorescence of the parasitic plant *Cistanche phelypaea*, it has been surveyed in two different localities in the western desert of Egypt; Siwa and Sheiata Oases. The plant is mostly existed in dry sandy soil. It is a chlorophyll-free obligate parasite with stout fleshy flowering stems bearing bright yellow flowers and rising to 30 cm tall from a swollen base. The botan-

ical description of the species was described in details by Boulus (2002) as follow, it is herbaceous parasitic glabrous perennial 20-80 cm; stem fleshy, robust scales 1.8-2.5 x 0.3-0.8 cm, ovate-lanceolate, the margin scarious and denticulate; inflorescence a dense spike 10-20 cm; bract 1.5 x 0.3-0.4 cm, lanceolate; bracteoles 1-1.2 x 0.2 cm, narrowly lanceolate; flowers are pollinated by insects, sessile; calyx 1.4-1.6 cm, the lobes 3-4 mm, broadly ovate; corolla 3-5 cm, uniform bright yellow; corolla-tube slightly curved, the lobes broadly rounded; filaments sparsely hairy at the base; anthers pubescent; capsule 1.2-0.4 cm narrowly ovoid; seeds 0.4 mm. These are dispersed by the wind over long distances, which increase their chance of find a new host.

The two host species which observed in the field of study (Fig. 3) were *Cornulaca monacantha* (fam. *Chenopodiaceae*) and *zygophyllum album* (fam. *Zygophyllaceae)*. Other species belonging to these two families and other families are also considered as natural hosts to such species (Fahmy *et al*., 1996; Fageer and Assubaie, 2006). *Cistanche phelypaea* parasite attached itself either on the apex or on the lateral of the host root (Fig. 4A and B). In the case of the terminal attachment, the host root resultantly ceased its growth in elongation, developed a dome-like shape at the apex (Fig. 4A). Ihsan *et al*. (2009) found that other parasite species; *C. tubulosa* attached itself on the lateral side of the host root which then continued its normal growth in case of *Capparis decidua* and *Tamarix indica* as host species. This association was established with the help of haustoria which then penetrated into the deeper layers of the host tissue. However, when the parasite occurred on *Calligonum polygonoides* and *Calotropis procera*, as *C. phelypaea*, it attached itself as on the apex of the root.

B. Macro and micro element concentrations in the parasite and host species

Table (2) illustrates the concentration of the nutrients as macro elements and micro elements in the parasite plant; *C. phelypaea* and in the two different hosts *C. monacantha* and *Z. album*. As shown in Table (2), it is evident that the parasite has a specific selective system for taking the required nutrients depending on the exactly what it needs for all biochemical pathways required for its physiological reactions during its life time. According to nitrogen, the parasite accumulates about double fold of such nutrient more than the two hosts. This may be due to the time of collection as it is in the flowering season and seed production. Bruce *et al*. (1992) found that late-season nitrogen (N) applications coupled with irrigation can lead to efficient fertilizer N uptake and partitioning to grain, and increased grain protein in winter wheat.

The same trend was observed for phosphorus, potassium, and calcium. It is well known that such three cations are the most essential macro elements for plant growth. So, the parasite overcomes the two hosts in accumulation of such required cations. On the contrary, the concentration of N^+ in the parasite significant-

ly decreased comparing with the two hosts. It depends on the genes which are maintaining K^+ or Na^+ homeostasis in higher plants. The K^+ Channels and transporters genes may regulate Na^+ transport. Such genes have the selective ability which control the required uptake of cations specially K^+, Na^+ and K^+/Na^+. So they can control the uptaking of Na^+ in the exact required amount from the host to the parasite (Tester and Davenport, 2003; Munnas, 2005).

As *C. phelypaea* is an obligate parasitic angiosperm plant. It is free of chlorophyll, so It is not in need to a big amount of cations related to chlorophyll synthesis and photosynthesis; Mn^{++}, Fe^{++}, $Zinc^{++}$, in addition to Mg^{++} (Marsh *et al.*, 1936; Ohki, 1976). It is evident from the results concerned with the microelements analysis (Table 2), the concentrations of such cations in the two host species are significantly exceeded what are existed in the parasite especially in the case of F^+.

C. Biochemical genetic analysis

Study the genetic polymorphism for peroxidase isozymes was carried out in stem tissue of *Cistanche phelypaea* populations. As conventional symbols in electrophoretic analysis, a pattern was first described in terms of Anodal (A) and Cathodal (C) zones according to their direction of mobility in the electrophoretic field. Each zone is assigned for a locus coding for a peroxidase isozyme. Figure (5) shows the peroxidase isozymes pattern of the stem tissue of the two population of *Cistanche phelypaea*. The banding patterns activity of peroxidase isozymes was pronounced in different loci (PER.C1 and PER.C2) to Cathodal direction, and one migrating to Anodal direction PER.A1). Regarding the frequency of allelic segregation in stem, Table (3) and Fig. (5) present gene frequency estimates for alleles segregating at different loci coding for peroxidase isozymes in stem of two populations. Data clearly indicated that, PER.A1, PER.C1 and PER.C2 were consistently monomorphic expressing, however was no alleles in PER.C1 in A population. It is important to note that, PER.A1 was 0.17 for A and 0.17 for B. PER.C1, however was 0 for A, 0.33 for B and PER.C2 was 1 for A and B.

D. Molecular genetic analysis

The twelve genomic DNAs of *Cistanche phelypaea* (six from locality A and the other from locality B, Fig. 1) were assayed using the producible six RAPD primers. As shown in Table (4) and Fig. (6), the number of reproducible fragments per primer varied between 27 fragments for primer OPA-05 and nine fragments for primers OPD-19 and OPH-11. Fingerprinting revealed a total number of 100 unambiguous DNA fragments with an average of 16.7 fragment/primer. The number of polymorphic bands ranged from 8 to 7 per primer with an average of 41.75% polymorphic bands per primer. However, OPB-07 exhibited the lowest polymorphism (75%), while the highest bands number was 27 fragments for primer OPA-05 and the polymorphism percentage was100% in four primers (Table

4). The total number of polymorphic amplicons produced by the six primers was 95%, thus, representing a level of polymorphism in average 94% (Table 4).

These results indicated that the genetic variation among studied individuals of such species is clearly existed. These are in agreement with Zlatko *et al.* (2010), who indicated that *Cistanche phelypaea* populations retained the most of the variation within populations. It is well known that such species is an open pollinated plant in which it is a general phenomenon of its family.

E. Genetic similarity and dendogram

Based on RAPDs data, the genetic similarity among different individuals from the two localities range from 0.52 to 0.84 (Table 5). The lowest similarity value was observed between B3 and B6. In contrast, the highest genetic similarity was between B_1 and B_2. To reveal similarities between the 12 different individuals, a dendrogram based on similarity values from RAPDs data was constructed. The dendrogram was generated from the genetic distance matrix according to UPGMA clustering method using NTSYS-pc program. It illustrates two major clusters (Fig. 7). The first cluster was divided into two groups. The first group included locality A sample only (A1). The second group consisted of population samples from localities A and B. This group divided into two subgroups, the first one consisted of population samples from localities A and B (A2/A4, A3 and A5, A6, B1/B2). The second subgroup included locality B samples (B3 and B4). The second cluster included locality B samples only (B5 and B6).

In spite of the interference between the two localities in genetic similarity, each population from each locality has a specific genetic background differed from the other location. This might be due to the partially spatial isolation between the two localities in which the distance between them is about 80 Km. This distance might affect the gene flue between the two localities in which it can make them as two different populations with different gene frequencies. Zlatko *et al.* (2010) indicated that all the individuals belonging to different populations or subspecies of *C. phelypaea* (*lutea* ssp. and *phelypaea* ssp.) growing in Spain were grouped together. Also, the membership probabilities for all the individuals were above 99% for the subspecies it belonged. So, it can be concluded that our results demonstrated that RAPD markers can be of great value in management for the purposes of identification, measurement of variation, and establishment of genetic distance at inter- and intra-specific levels of different genetic back ground.

SUMMARY

Cistanche phelypea (Fam. *Orbanchaceae*) is a parasite plant on the root of the host plant. The main natural hosts are the woody species of family *chenopodiaceae* such as *Arthrocnemum macrostachyum* and family *Zygophyllacea* such as *Zygophyllum album*. It is an obligate parasite which totally depends on its

host for water, minerals and organic nutrients. Many species belonging to the same family have the same phenomenon such as *orbanche crenata* Forsk which attacks legume plants, specially, *Vicia faba*. *Cistanche phelypea* L., grows in Egypt in low density but scattered on a large area of the country. It can be recognized in the Nile region including Faiyum, the Oases of the western desert including Siwa, the Red Sea coastal strip, and the entire Sinai Peninsula including the coastal of the Mediterranean. It grows in sandy and alluvial soil, edges of cultivated fields as a parasite on plant roots. In this study the species was surveyed and photographed in Siwa Oasis and in different obliterated Oasis around Siwa Depression. For the first time in Egypt, morphological, chemical study, RAPD molecular markers and biochemical genetic analysis of Peroxidase isozymes were carried out in order to identify the genetical and morphological description of the species including the identification of genetic variation in the studied populations.

REFERENCES

Boulos, L. (2002). Flora of Egypt. vol 3, Elhadara Publishing, Cairo, Egypt.

Bruce, A., C. I. Ken, C. L. Linquist Cassman, E. Allan, P. Fulton Lee and F. Jackson (1992). Late-season nitrogen may be efficient way to increase winter wheat protein. California Agriculture, March-April-13.

Choi, J. G., M. Moon, H. U. Jeong, M. C. Kim, S. Y. Kim, M. S. Oh (2011). *Cistanches Herba* enhances learning and memory by inducing nerve growth factor. Behavioural Brain Research, 216: 652-658.

Doyle, J. J. and J. L. Doyle (1987). A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bul., 19: 11-15.

EL-Metainy, A. Y., A.Y. Abou-Youssef and M. El-Hadad (1977). Starch Degrading isozymes in *Triticumnaestivum*, *Triticumn pyramidal*, and their interspecific hybrid. Egypt. J. Genet. Cytol., 6: 375-379.

Fageer, Farah A. and F. N. Assubaie (2006). Ecological studies on thanoun (*Cistanche phelypaea* L) cout. (*Orobanchaceae*) in Al-Ahsa Oasis, Saudi Arabia. Scientific Journal of King Faisal University (Basic and Applied Sciences), 1: 14-27

Fahmy, G. M., H. El-Tantawy and M. M. Abd El-Ghani (1996). Distribution, host range and biomass of two species of *Cistanche* and *Orobanche cernua* parasitizing the roots of some Egyptian xerophytes. Journal of Arid Environment, 3: 263-276.

Jonathan, F. W. and F. W. Norman (1989). Isozymes in plant biology:

Visualization and interpretation of plant isozymes. Chapter, 1: 5-45.

Ihsan, I., S. Rehman and Z. Iqbal (2009). Some studies on the mode of parasitization of *cistanche tubulosa* on various host plants. Pak. J. Pl. Sci., 145-152.

Marsh, H. V., Jr. H. J. Evans and G. Matrone (1963). Investigations of the role of iron in chlorophyll metabolism. II. Effect of iron deficiency on chlorophyll synthesis. Plant Physiol., 38: 638-642.

Maruyama, S., S. Hashizume, T. Tanji, K. Yamada and H. Tachibana (2008). *Cistanche salsa* extract enhanced antibody production in human lymph node lymphocytes Pharmacologyonline, 2: 341-348.

Munns, R. (2005). Genes and salt tolerance: bring them together. Tansley Review, New Phytologist, 167: 645-663.

Ohki, K. (1976). Effect of zinc nutrition on photosynthesis and carbonic anhydrase activity in cotton. Physiologia Plantarum, 38: 300-304.

Rohlf, F. J. (2000). On the use of shape spaces to compare morphometric methods. Hystrix, Italian J. Mammology, 1: 8-24.

Shimoda, H., J. Tanaka, Y. Takahara, K. Takemoto, S. J. Shan and M. H. Su (2009). The hypochole-sterolemic effects of *Cistanche tubulosa* extract, a chinese traditional crude medicine, in mice American Journal of Chinese Medicine, 37: 1125-1138.

Soliman, S. S, A. Bahy and M. M. Mohamed (2003). Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. African Journal of Biotechnology, 2: 86-86.

Tester, M. and R. Davenport (2003). Na^+ Tolerance and Na^+ Transport in Higher Plants. Ann. Bot., 91: 503-527.

Vivi, Tackholm (1974). Students flora of Egypt 2nd edition, published by Cairo University.

Williames, J. K., A. Kubelik, K. Livak, J. Rafalski and S. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Zhang, Z., S. X. Wang, Y. Zhang, J. P. Chen and X. M. Liang. (2008). *In vitro* estrogenic activities of Chinese medicinal plants traditionally used for the management of menopausal symptoms. Journal of Ethnopharmacology, 98: 295-300.

Zlatko, S., J. P. Antonio, A. Carmen, R. Diego, I. C. José and R. Belén (2010). Population structure of the

parasitic plant *Cistanche phelypaea* (L.) Cout. in Spain. 3rd Croatian Botanical Congress. 24th -26th September. Press reported: 4 Molecular Botany and Plant Physiology poster.

Table (1): Op-primers used for RAPD-PCR analysis.

Primer code	Sequence (5'-3')
1- OPA-05	AGGGGTCTTG
2- OPA-15	TTCCGAACCC
3- OPB-07	GGTGACGCAG
4- OPC-08	TGGACCGGTG
5- OPD-19	CTGGGGACTT
6- OPH-11	CTTCCGCAGT

Table (2): The concentrations of macro and micro elements in the parasitic plant, *C. phelypae* in relation to the two host plants, *Cornulaca monacantha* and *Zygophyllum album.*

Total nutrients	Parasite: *C. philypaea*	Host 2 *Cornulaca monacantha*	Fold P/H	Parasite: *C. philypae*	Host 2 *Zygophyllum album*	Fold P/H
Nitrogen (N)	1.50%	0.62%	2.42	1.80%	0.80%	2.25
Phosphorus (P)	0.16%	0.03%	5.33	0.20%	0.05%	4.00
Potassium (K)	2.80%	1.30%	2.15	2.00%	0.80%	2.5
Calcium (Ca)	3.00%	1.72%	1.74	2.80%	1.38%	2.17
Sodium (Na)	1.15%	2.70%	0.42	1.13%	1.90%	0.59
Magnesium (Mg)	0.55%	2.08%	0.26	0.45%	1.15%	0.39
Iron (Fe)	8.04 mg/kg	516.0 mg/kg	0.02	8.15 mg/kg	557.20 mg/kg	0.01
Manganese (Mn)	2.00 mg/kg	97.60 mg/kg	0.02	2.14 mg/kg	101.20 mg/kg	0.02
Zinc (Zn)	25.60 mg/kg	85.60 mg/kg	0.30	35.60 mg/kg	324.40 mg/kg	0.11

Table (3): Gene frequency estimates for alleles segregating at different loci coding for peroxidase isozymes in stem of the two *Cistanche ephelypaea* populations.

Loci	A	B
PER.A1	0.17	0.17
PER.C1	0.00	0.33
PER.C2	1.00	1.00

Table (4): RAPD analysis for different genomic-DNA of *Cistanche phelypaea* populations collected from two locations of Siwa depression.

Primer code	Amplified fragments		
	Total number	Length (bp)	Polymorphic Fragment
OPA-05	27	2440-190	27 (100%)
OPA-15	24	2690-40	24 (100%)
OPB-07	16	7960-250	12 (75%)
OPC-08	15	2850-190	15 (100%)
OPD-19	9	1770-180	9 (100%)
OPH-11	9	2400-140	8 (89%)
Total	100	7960-40	100-75 (Avg. 94%)

Table (5): Similarity indices calculated by NTSYS program among the *Cistanche phelypaea* population based on RAPDs data.

	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	B5
A2	0.77										
A3	0.70	0.76									
A4	0.70	0.80	0.77								
A5	0.67	0.69	0.74	0.76							
A6	0.64	0.72	0.79	0.77	0.82						
B1	0.65	0.75	0.74	0.78	0.79	0.82					
B2	0.66	0.70	0.73	0.75	0.82	0.81	0.84				
B3	0.55	0.61	0.65	0.67	0.66	0.73	0.72	0.77			
B4	0.60	0.65	0.74	0.68	0.67	0.74	0.69	0.76	0.78		
B5	0.59	0.57	0.63	0.65	0.57	0.63	0.59	0.63	0.58	0.70	
B6	0.59	0.57	0.60	0.58	0.59	0.65	0.57	0.58	0.52	0.64	0.73
	A					B					

Fig. (1): Surveying localities of *Cistanche phelypaea* plants.

Fig. (2): Developmental stages of the inflorescence of *C. phelypaea*.

Fig. (3): The two host species; *C. Monacantha* (left) and *Z. album* (right) with the parasite *C. phelypaea*.

Fig. (4): (A) dome-like shape of the apex of the host root at the attached point with the parasite *C. phelypaea.* (B) The two types of attachment of two parasitic plants at the same host root; one at the apex and the other is laterally attached.

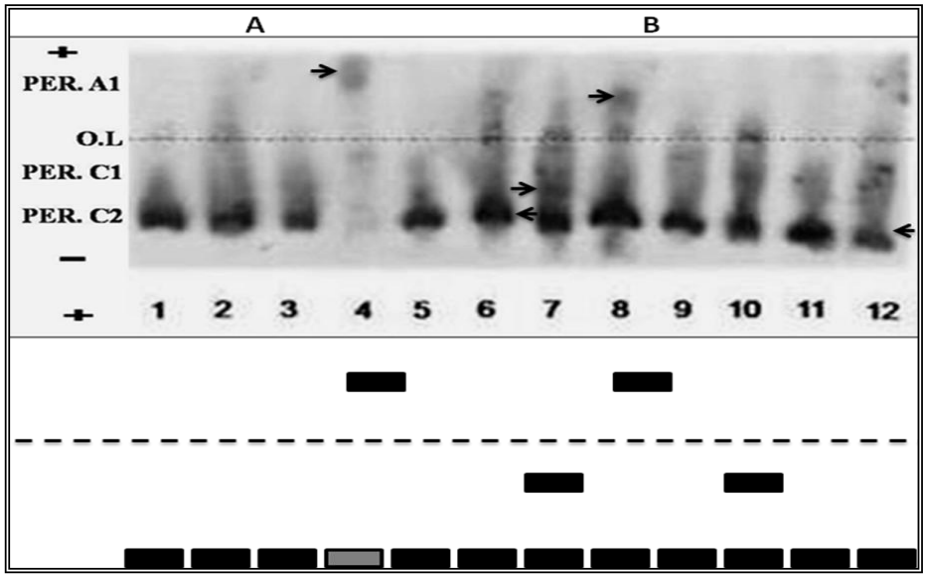

Fig. (5): Profiles of peroxidase electrophoresis in 12 stems of the two different populations (A and B).

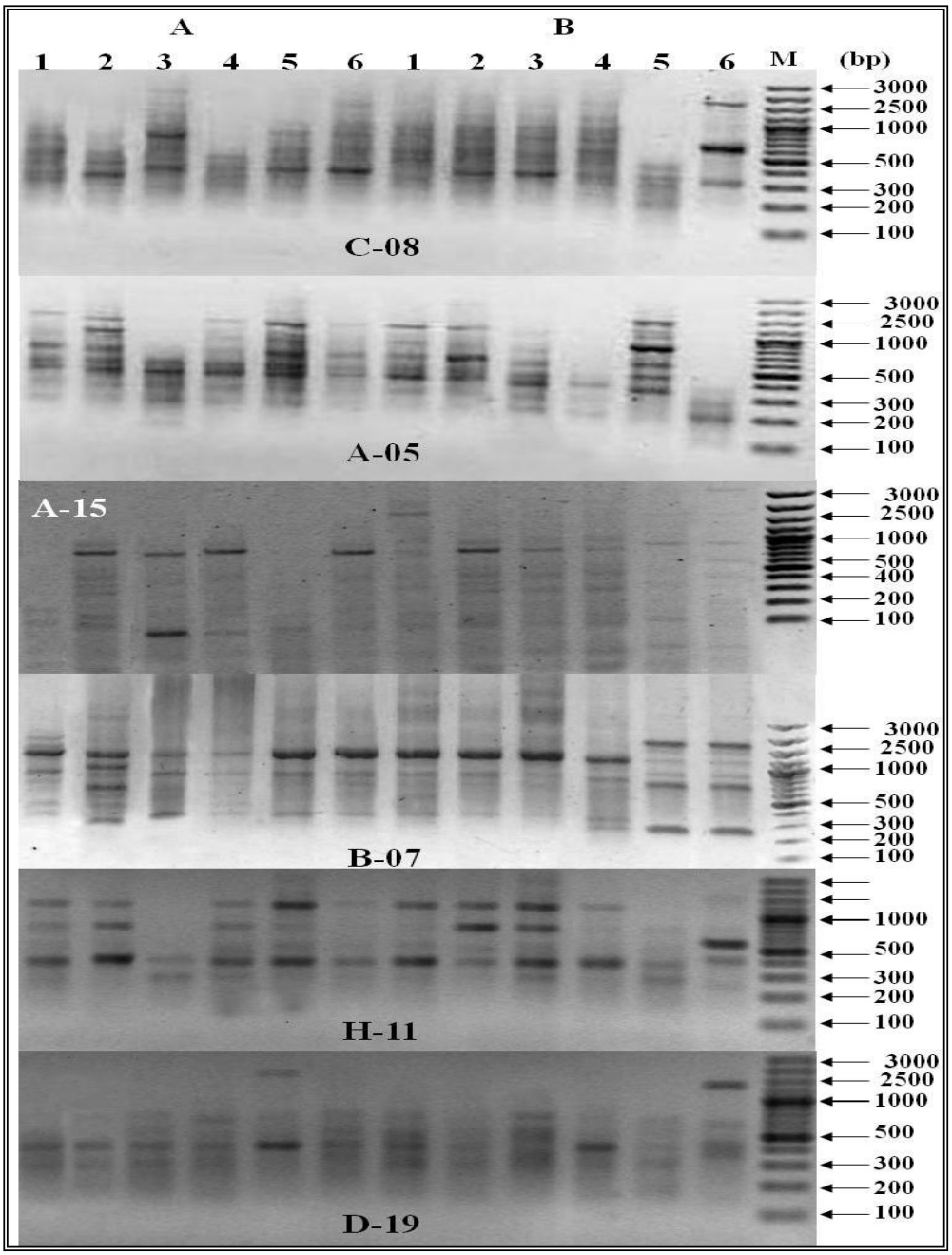

Fig. (6): Photographs showing RAPD patters from the *Cistanche phelypaea* population analyzed using six primers.

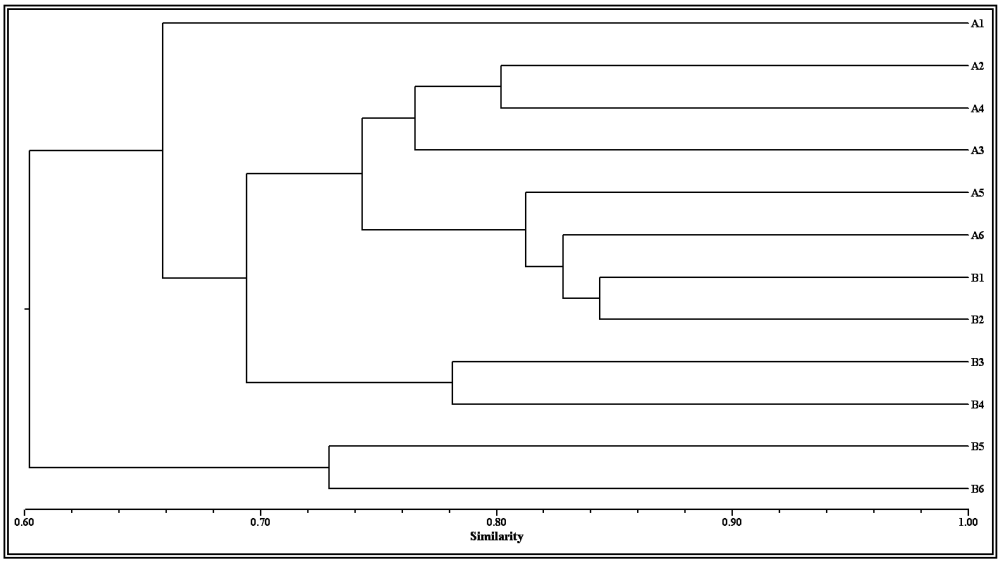

Fig. (7): Dendrograms of *Cistanche phelypaea* population at different locations of Siwa depression using RAPD markers.