ASSESSMENT OF GENETIC DIVERSITY IN SOME ENDANGERED ECOTYPES OF *Solenostemma argel sp.* IN EGYPT

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Solenostemma argel (Argel) in the Asclepiadaceae is a monotypic genus distributed widely in the Arabian Desert, North Africa and Palestine (Jafri *et al.*, 1977). It is the only species found in Egypt (El-Hadidi *et al.*, 1994). It is an erect shrub 60-100 cm high, with profuse branching especially in favoured places (Hanna *et al.*, 2001). It is a vulnerable species which has a limited distribution in Egypt being endangered because of its intensive overuse. *Solenostemma argel* is a wild perennial plant commonly growing in the eastern desert (Tackholm, 1974). It is used as medicinal herbalism within the family Dogbane. The leaves are used in herbal medicine herbal for the treatment of some diseases such as of liver and kidney and allergies. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica (Innocenti *et al.*, 2005). In addition, the plant is used in herbal mixtures for the treatment of viral B and C hepatitis, as an immunostimulant, and in the treatment of hypercholesterolemia (Shawkat, 1997)

Climatic changes over the years are usually responsible for drastic changes of some habitats, the western and eastern desert of Egypt, which enjoyed a savanna and grassland vegetation during the pluvial period up to some 6,000 years ago, are now is a part of the arid zone of the Sahara (Boulos and Barakat, 1998).

Genetic diversity in natural populations is a major concern of evolutionary biologists because the amount and distribution of genetic diversity are likely to affect the evolutionary potential of species and/or populations (Futuyma, 1986). Analysis of the genetic structure is necessary not only to fully evaluate the impact of the endangered status on genetic variation of the population, but also because knowledge on the genetic structure of the species can be applied to the preservation of the evolutionary potential of species, which is one of the conservation goals. Thus, an accurate estimate of the level and distribution of genetic diversity of threatened and endangered species is an important element in designing conservation programs (Kim *et al.*, 2005).

Molecular markers have been shown to be useful for diversity assessment in a number of plant species (Waugh and Powell, 1992). These markers, based on the polymerase chain reaction (PCR) technique, are the most commonly used

for these purposes. Several different PCR-based techniques have been developed during the last decade, each with specific advantages and disadvantages. The randomly amplified polymorphic DNA (RAPD) technique is quick, easy and requires no prior sequence information, which detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Williams *et al.*, 1994). Inter-simple sequence repeat (ISSR) marker permit detection of polymorphisms in inter -microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats. ISSR is a microsatellite-based technique, with the superiorities of simple, quick, reliable, and generating higher levels of DNA polymorphism, and being used as a new molecular marker for genetic study.

The objectives of the present study were to collect some Solenostemma argel ecotypes from their natural habitats in Egypt and elucidate specific markers using protein, RAPD and ISSR markers to assess genetic diversity among this endangered plant species, detecting the discriminating capacity and efficiency of those markers, and explore the cause of the endangered status in order to establish conservation recommendations based on the studied genetic data.

MATERIALS AND METHODS

1. Species and ecotypes sampling

Solenostemma argel, a wild medicinal plant, was used in this investigation. Plants of this species were collected in South Sinai in Egypt from different ecotypes by Desert Research Center from their natural habitat. According to the field survey information, five wild ecotypes of *Solenostemma argel* were sampled throughout its distribution range using geographic position system (GPS) (Table 1, Fig 1). Fresh tender leaves were collected randomly from four adult plants in each site. The distances between sampled plants ranged from 10 to 20 m, which depended on the site size. The sampled leaves were kept at 4°C in sealed bags and stored at -70°C until DNA extraction.

2. Molecular analyses

2. 1. *SDS-PAGE Protein profiles*

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was used to develop proteins profiles according to Laemmli (1970). PAGE of proteins was performed by standard cooled dual vertical slab units (Biometra). Aliquots (20-50 µg protein per lane) were loaded on 1.5 mm thick 12% denaturing gels and run at 4°C. The total soluble protein bands were visualized by staining the gel overnight in staining solution containing Coomassie Brilliant Blue R- 250. The molecular weight (MW) in kilo Dalton of protein corresponding to each band was estimated by reference to protein marker (Sigma).

2. 2. Total DNA extraction

Genomic DNA was extracted and purified using the DNeasy Plant Mini Kit

(QIAGEN, Chatsworth, CA). The concentration of DNA was determined at a wavelength of 260 nm (Biometra UV) and its quality was verified by electrophoresis on a 0.8% agarose gel.

2. 3. RAPD and ISSR analyses

PCR reactions for RAPD and ISSR were performed according to Williams *et al.* (1990) using seven preselected 10-mer primers (Operon Technology, USA), while for ISSR analysis reactions were conducted according to Sharma *et al.*, (1995) using 15 preselected primers which were synthesized by Metabion Germany as shown in Table (2). Agarose gel electrophoresis (1.2%) was used for resolving the PCR amplification products according to Sambrook *et al.* (1989).

2. 4. Data analysis

Analysis for Protein, RAPD and ISSR were scored as present (1) or absent (0) and examined to estimate the relationships among the investigated ecotypes. The similarity matrix by SPSS computer package was estimated by pair wise comparisons of the ecotypes based on the percentage of common fragments. Each band was assumed to represent a unique genetic locus. To characterize the capacity of each primer to detect polymorphic loci, we used the number of polymorphic bands, percentage of polymorphic bands, polymorphic information content (PIC). To compare the efficiency of the three markers in *Solenostemma argel*, we estimated the data for each assay unit according to Anderson *et al.* (1993) and Demey *et al.* (2004).

- Number of non polymorphic bands (n_{np})
- Number of polymorphic bands (n_p)
- Average number of polymorphic bands per assay unit (n_p/U)
- Number of loci (L)
- % of polymorphism
- Total banding pattern (Bp)
- Range of polymorphism information content value (PIC)
- Total number of effective alleles (Ne)

Polymorphic information content (PIC) was calculated by applying the simplified phylogenetic analyses and analyzed visually with Biometra software from non-linear dynamics. A dendrogram was generated using the Un-weighted Pair-Group method using Arithmetic Averages (UPGMA) reported by Sokal and Michener (1985).

RESULTS AND DISCUSSION

1. Molecular genetic fingerprints

A comparison of the levels of polymorphism and the discriminating capacity of SDS-PAGE, RAPD and ISSR molecular markers among the five *Solenostemma argel* ecotypes as summarized in Table (3). Seven non polymorphic (n_{np}) and 11 polymorphic (n_p) distinct fragments (61% polymorphism) were obtained among the five tested ecotypes based on SDS-PAGE. Thirty five non

polymorphic (n_{np}) and 63 polymorphic (n_p) sharp fragments (66% polymorphism) based on RAPD markers were detected among the five studied ecotypes. While, Fifty six non polymorphic (n_{np}) and 102 polymorphic (n_p) sharp fragments (64% polymorphism) based on ISSR markers were detected among the five studied ecotypes. However, the overall results across SDS-PAGE, RAPD and ISSR markers showed 98 non polymorphic (n_{np}) and 176 polymorphic (n_p) fragments (65% polymorphism).

Higher Polymorphism Information Content values (PIC) were obtained by ISSR markers, with a value 0.99 of the PIC theoretical rank (0.75-0.99). Discriminating capacity, which is revealed through the probability of an identical match by chance, was also higher with ISSR markers. Also, the total banding pattern (*Bp*) and the highest number of effective alleles (*Ne*) were obtained with ISSR markers (101,349), respectively.

Thus, ISSR markers obtain more polymorphic information than SDS-PAGE and RAPD markers and have a better capacity for quantifying the genetic diversity especially for endangered plants such as *Solenostemma argel.*

In general, the results indicated that ISSR markers gave adequate distinctions among the five *Solenostemma argel* ecotypes. Wang and Fan Ming (1998) successfully used five ISSR primers to evaluate 90 accessions of pepper germplasm collected from 16 European countries. Pharmawati *et al.* (2005) evaluated the genetic variations among 30 Leucadendron cultivars using 64 ISSR primers and reported that ISSR profiling is a powerful method for the identification of these cultivars. Said (2005) tested the genetic variations among five individual plants from *Caper* and *Argel* species using five ISSR primers and reported that it was possible to differentiate among individual plants of the same species using ISSR markers. Abdel-Tawab *et al.* (2007) used ten ISSR primers to differentiate between four *Mentha* and three *Ocimum* sp and detected high polymorphism between them. Yao *et al.* (2008) used fourteen ISSR primers to detect the genetic diversity in wild populations of *Glycyrrhiza uralensis*, and reported that one ISSR marker was useful for further investigation. Zhou *et al.* (2008) tested seven ISSR markers to assess the genetic diversity and relationships of 28 cultivars of yam which is rare and famous Huai Chinese traditional medicine. The results suggested that ISSR markers was a powerful method for evaluating genetic diversity of Chinese yam and valuable information to assist parental selection in current and future yam breeding programs. Ding *et al.* (2009) used RAPD and ISSR primers to evaluation the genetic diversity within and among nine natural populations of *Dendrobium officinale* which is rare and endangered herb in China and reported that ISSR markers detected more diversity than RAPD markers.

2. Genetic relationships

Based on the combined data of SDS-PAGE, RAPD and ISSR markers,

similarity indices were developed by SPSS computer package as shown in Table (4). The analyses were based on the number of markers which were different between any given pair of ecotypes.

The highest similarity values were observed between the two pairs of ecotype (4 & 5) and (1 & 3) with similarity value of 81%, followed by ecotype (3) and ecotype (4) (similarity value of 80%), and between ecotypes (1 & 2) and (2 & 3) (similarity value of 78 and 77%, respectively). While the moderate similarity value was scored between the ecotype (3) and ecotype (5) (similarity value of 74%). Whereas, the lowest similarity value was detected between ecotype (1) and ecotype (5) (similarity value of 70%).

The dendrogram classified the five ecotypes into two main clusters as shown in Fig. (2). The ecotype (4) and ecotype (5) were grouped together in the first cluster while ecotype (1), ecotype (3) and ecotype (2) were grouped together in the second cluster which separated into two sub-clusters. Within the first sub-cluster of the second cluster, ecotype (1) and ecotype (3) were grouped together.

In general, the relationships among the five Solenostemma argel ecotypes derived from SDS-PAGE, RAPD and ISSR analyses were in a partial agreement with the geographical distribution of these ecotypes. Lara *et al.* (2003) studied the genetic diversity among four populations of *Psychotria acuminate* and detected a high genetic diversity among these populations based on RAPD and ISSR analyses. Zhou *et al.* (2004) reported that RAPD and ISSR markers are suitable tools for the assessment of genetic diversity of *Rehmannia glutinosa* germplasm. Said (2005) used RAPD and ISSR markers to elucidate the genetic variations in *Caper* and *Argel* species for the conservation of its germplasm. Ramah (2006) suggested that RAPD and ISSR markers are good choices for the evaluation of diversity and assessing the genetic relationships between *Tortuosus* and *Fennel* genotypes with a high accuracy. Also, Abdel-Tawab *et al.* (2007) reported that constructed dendrogram based on RAPD and ISSR markers succeeded in separating two species of *Mentha* and *Ocimum* genera into two main clusters. Agostini *et al.* (2008) reported that constructed dendrogram based on ISSR markers succeeded in separating between 11 *Cunila* species used South Brazilian plants in popular medicine. Ding *et al.* (2009) examined the genetic diversity within and among nine natural populations of *Dendrobium officinale* which endangered and rare herb using ISSR and RAPD and detected a high genetic diversity among these populations. Finally, a relationship between genetic variability and geographic distribution has been observed in several species of aromatic plants, for instance, *Artemisia annua* (Sangwan *et al.*, 1999), *Tanacetum vulgare* (Keskitalo *et al.* 2001), and some plants of the Lamiaceae family (Fracaro and Echeverriga-

ray, 2006), (Liu *et al.* 2006) (Agostini *et al.*, 2008).

3. Ecotype-Specific markers

Specific markers for five Solenostemma argel ecotypes across SDS-PAGE, RAPD and ISSR analyses are listed in Table (5). Fifty three out of 275 SDS-PAGE, RAPD and ISSR markers were found to be ecotype–specific. These ecotype-specific markers represented 19% of the total markers and 58% of them were ISSR markers. These markers were scored for the presence of a unique band for a given ecotype. ALL five ecotypes had specific markers. The highest number of ecotype-specific markers was recorded for ecotype 2 (13 markers), followed by ecotype 5 (11 markers), ecotype 3 (10 markers). Whereas, the lowest number of ecotype-specific markers was recorded for ecotype 1 and ectype 4 (9 markers).

The results indicted that SDS-PAGE, RAPD and ISSR markers gave adequate distinctions among the five ecotypes of *Solenostemma argel.* Abdel-Tawab *et al.* (2004) characterized 13 out of 20 olive cultivars by detecting 20 cultivar-specific markers using RAPD and SSR markers. Obiadalla Ali *et al.* (2006) identified 81 cultivar-specific DNA markers for five Onion cultivars using RAPD analysis. Ding *et al.* (2009) examined nine natural populations of *Dendrobium officinale* using RAPD and ISSR markers and reported that both of them revealed high specific molecular markers. Abdel-Tawab *et al.* (2009) study the genetic diversity between twenty ecotypes of *Balanites aegyptiaca* which is considered one of the neglected medicinal plant species in Egypt and reported that fifteen is out of the 20 ecotypes had specific markers.

Exploration and evaluation of wild accessions not only provide enhanced information for *in situ* conservation but also supply sources of useful genes, such as those for disease or therapeutic drugs or insect resistance and physiological tolerance for crop improvement. In the present study, ISSR markers provided good insight into the genetic diversity available in *Solenostemma argel* accessions.

The fingerprints were useful for investigation or genetic variability and further characterizing the wild *Solenostemma argel* specie by detecting marker polymorphisms to construct dendrogram defining the genetic relationships within this species. Furthermore, unique markers were successfully identified indicating that PCR based techniques have great relevance for taxonomic studies. The results of this study will help to understand genetic variation and evolutionary dynamics of *Solenostemma argel* and to broaden the genetic base for *Solenostemma argel* breeding. Consequently, further selection of accessions in each species for evaluation of genetic diversity can lead to more insightful results.

The conservation of wild plants as genetic resources requires an understanding of the ways of genetic diversity maintained in their populations. Gene conservation strategies of this species could be designed using part of the information

obtained from this study. Conservationist may use the information of the present study to make effective decisions regarding the global protection and management of *Solenostemma argel* species. in Egypt.

SUMMARY

Solenostemma argel is one of endangered medicinal plant to South Sinai in Egypt and remains one of the neglected and rare plant species. The leaves are used in herbal medicine herbal for the treatment of some diseases such as of liver, kidney, allergies, treat neuralgia, sciatica, viral B and C hepatitis and an effective remedy for bronchitis. Genetic diversity among five ecotypes of *Solenostemma argel* which were collected from their natural habitat in South Sinai was investigated using Protein, RAPD and ISSR markers. Seven random, 15 ISSR primers and SDS-PAGE gave distinctive amplification products and were used to assess the polymorphism of these five ecotypes. The results indicated some variations in the banding patterns among these five ecotypes. Both molecular markers RAPD and ISSR revealed high percentages of polymorphic bands than SDS-PAGE, ISSR markers detected more diversity than RAPD markers. The genetic relationships based on SDS-PAGE, RAPD and ISSR markers were developed using SPSS computer program, and indicated that the relationships among these five ecotypes were closely related to their geographical distribution in Sinai. Fifty three out of 275 markers were found to be ecotype–specific markers. Moreover, these molecular markers are useful tools to assess the genetic diversity variations and conservation of *Solenostemma argel* germplasm. Exploration and evaluation of wild accessions provide enhanced information for *in situ* conservation and supply sources of useful genes, such as those for disease or therapeutic drugs or insect resistance and physiological tolerances for crop improvement.

REFERENCES

Abdel-Tawab, F. M., I. A. Hussein, A. H. Atta and M. H. Amar (2004). Development of Molecular fingerprints in twenty olive cultivars (*olea Europaea*). Egypt. J. Genet. Cytol., 33: 131-152.

Abdel-Tawab, F. M., Eman M. Fahmy, F. M. A. E. L. D. Huseen (2007). Molecular genetic studies on some irradiated medicinal plants. Egypt. J. Genet. Cytol., 37:

Abdel-Tawab, F. M., I. A. Hussein, A. H. Atta and M. H. Amar (2009). Assessment of genetic biodiversity in twenty ecotypes of Balanites aegyptiaca. Egypt. J. Genet. Cytol., 38: 387-403.

Agostini, G., S. Echeverrigaray and T. T. Souza-Chies (2008). Genetic relationships among South American species of Cunila D. Royen ex L. based on ISSR. Plant Systematic and Evolution, 274: 135-141.

Anderson, J. A., G. A. Churchill, J. E. Autrique, M. E. Sorells and S. D. Tanksley (1993). Optimizing parental selection for genetic-linkage maps. Genome, 36: 181-186.

Boulos, L. and H. Barakat (1998). Some aspects of the plant life in the Western Desert of Egypt. J. Union Arab Biol. Cairo, 5 (B) Botany, 79-94.

Demey, J. R., E. Gamez, S. Molina and I. Dogenes (2004). Comparative Study of the Discriminating Capacity of AFLP and ISTR Markers for Genetic Analysis of Agave fourcroydes. Plant Molecular Biology Reporter, 22: 29-35.

Ding, G., X. Li, X. Ding and L. Qian (2009). Genetic diversity across natural populations of Dendrobium officinale the endangered medicinal herb endemic to China, revealed by ISSR and RAPD markers. Russian Journal of Genetics, 2009, Vol. 45, 3: 327-334

El-Hadidi, M. N. and F. Abdel-Aziz (1994). Tackholmia, materials for Excursion Flora of Egypt (EFE), Vol. 15. El-Manar El-Arabi Printhouse, Cairo, 118, Feigl, F., 1956. Spot.

Fracaro, F. and S. Echeverrigaray (2006). Genetic variability in *Hesperozygis ringens* Benth. (*Lamiaceae*), an endangered aromatic and medicinal plant of Southern Brazil. Biochem. Genet., 44: 479-490.

Evolutionary Biology (2nd ed.), Sinauer, Sunderland, MA, USA.

Godt and Hamrick (1998). Allozyme diversity in the endangered pitcher plant *Sarracenia rubra* ssp. alabamensis (Sarraceniaceae) and its close relative *S. rubra* ssp. rubra, Am. J. Bot., 85: 802-810.

Hanaa, A. H., I. H. Arafa, A. Nasr, V. S., M. Hidemichi and M. Hiroaki (2001). Pregnene derivatives from Solenostemma argel leaves. Phytochemistry, 57: 507-511.

Innocenti, G., S. Dall'Acqua, S. Sosa, G. Altinier and R. D. Loggia (2005). Topical anti-inflammatory activity of *Solenostemma argel* leaves. J. Ethnopharmacology, 102: 307-310.

Jafri, S. M. H. and A. El-Gadi (1977). Flora of Libya. Asclepiadaceae, Vol. 9. Al-Fateh University, Tripoli.

Keskitalo, M., E. Pehu and J. E. Simon (2001). Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes. Biochem. Syst. Ecol., 29: 267-285.

Kim, S. C., C. Lee and A. Santos-Guerra (2005). Genetic analysis and conservation of the endangered Canary Island woody sow-thistle, *Sonchus gandogeri* (*Asteraceae*), J. Plant Res., 118: 147-153.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Lara, A., R. Valverde, O. Rocha and L. Gomez (2003). Genetic diversity and differentiation of four populations of the medicinal plant Psychotria acuminata in Costa Rica. Agronomia Costarricense, 27: 29-42.

Li, F. and N. Xia (2005). Population structure and genetic diversity of an endandered species, *Glyptostrobus pensilis* (Cupressaceae). Botany Bulletin Sinica, 46: 155-162.

Liu, J., L. Wang, Y. Geng, Q. Wang, L. Luo and Y. Zhong (2006). Genetic diversity and population structure of *Lamiophlomis rotate* (*Lamiaceae*), an endemic species of Qinghai-Tibet Plateau. Genetica. 128: 385-394.

Obiadalla, H. A., A. A. Hemeida, and K. H. A. Abdelrehim (2006). Assessment of genetic relationships among five onion (*Allium cepa* L.) cultivars using morphological traits and RAPD markers Proceed. 2nd Inter. Conf. Genet. Eng. & Its Appl., Nov., 14-17, 2006: 11-24.

Pharmawati, M., G. Yan and P. M. Finnegan (2005). Molecular variation and fingerprinting of Leucadendron cultivars (proteaceae) by ISSR markers. Ann. Bot. (Lond), 7: 1163-1170.

Ramah, F. A., E. F. (2006). Genetic studies on some aromatic plants. Msc. Thesis, Ain Shams Univ., Fac. of Agric.

Said, O. A. M. (2005). Genetic characterization of some medicinal plants M.Sc. unpublished Thesis, Ain Shams Univ., Fac. of Agric.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). Molecular cloning. A laboratory manual, Second edition, 6: 3-9.

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

Sharma, P. C., B. Huttel, P. Winter, G. Kahl, R. C. Gardner and K. Weising (1995). The potential of microsatellites for hybridization and polymerase chain reaction based DNA fingerprinting of chickpea (*Cicer arietinum* L.) and related species. Electrophoresis, 16: 1755-1761.

Shawkat, T. (1997). Patent fast alert USA-05648089, July 15.

Sokal, R. R. and C. D. Michener (1985). A statistical method for evaluating

systematic relationships. Univ. Kansas Sci. Bull., 38: 1409-1438.

Tackholm, V. (1974). In Students ora of Egypt (p. 410). Cairo: Cairo University Press.

Wang, J. and Fan Ming (1998). Comparison on microsatellite DNA and random amplified polymorphic DNA markers for germplasm identification of Capsicum annuum. Journal of Agricultural Research of China, 47: 267-282.

Waugh, R., Powell W. (1992). Using RAPD markers for crop improvement. Trends Biotech., 10: 186-191.

Williams, J. G. K., M. K. Hanafey, J. A. Rafalski and S. V. Tingey (1994). Genetic analysis using random amplified polymorphic DNA markers. Meth. Enzymol., 218: 704-740.

Yao, H., Y. Zhao, D. F. Chen, J. K. Chen and T. S. Zhou (2008). ISSR primer screening and preliminary evaluation of genetic diversity in wild populations of *Glycyrrhiza uralensis*. Biologia Plantarum, 52: 117-120.

Zhou, Y. Q., J. JianZhou, L. ZhenYong, Z. BaoHua and J. JingFen (2004). Assessment of genetic diversity of *Rehmannia glutinosa* germplasm detected by RAPDs and ISSRs. Hereditas Beijing, 26: 922-928.

Zhou, Y., C. Zhou, H. Yao, Y. Liu and R. Tu (2008). Application of ISSR markers in detection of genetic variation among Chinese yam (Dioscorea opposita Thunb) cultivars. Life Science Journal, 5: 6-12.

Table (1): Distribution of the five *Solenostemma argel* ecotypes included in the study.

ecotypes Number	Names of the sites	Latitude	longitude	Altitude (m)
E1	Nuweiba Dahab road	028° : 44° : 47.3° N	34° : 27° : 95.6° E	482 m
E2	Zaka valley	028° : 37° : 74.8° N	34° : 15° : 13.6° E	763 m
E3	Zaka valley	028° : 38° : 53.2° N	34° : 22° : 91.4° E	394 m
E4	Zaka valley	028° : 34° : 94.0° N	34° : 27° : 34.5° E	191 m
E5	Geny valley	028° : 26° : 76.2° N	34° : 27° : 57.7° E	31 m

Table (2): List of RAPD and ISSR primers and their nucleotide sequences.

Marker type	Primer code No.	Sequence (5'-3')	Primer code No.	Sequence (5'-3')
RAPD	OPA01	5-CAGGCCCTTC-3	OPA15	5-TTCCGAACCC-3
	OPA04	5-AATCGGGCTG-3	OPB01	5-GTTTCGCTCC-3
	OPA05	5-AGGGGTCTTGC-3	OPB07	5-GGTGACGCAG-3
	OPA07	5-GAAACGGGTG-3		
ISSR	HB1	$(CAA)_5$	844A	$(CT)_8$ AC
	HB2	$(CAG)_5$	844B	$(CT)_8$ GC
	HB4	$(GACA)_4$	814	$(CT)_8$TG
	HB9	$(GT)_6$ GG	17898A	$(CA)_6$ AC
	HB10	$(GA)_6$ CC	17898B	$(CA)_6$ GT
	HB11	$(GT)_6$ CC	17899A	$(CA)_6$ AG
	HB12	$(CAC)_3$GC	17899B	$(CA)_6$ GG
	HB13	$(GAG)_3$GC		

Table (3): Comparison of information obtained with a discriminating capacity of RAPD and ISSR markers.

Index with their abbreviations		Marker system		
		Protein	RAPD	ISSR
Number of markers	U	1.00	7.00	15.00
Number of non polymorphic bands	n_{np}	7.00	35.00	56.00
Number of polymorphic bands	n_p	11.00	63.00	102.00
Average number of polymorphic bands/assay unit	n_p/U	11.00	9.00	6.80
Number of loci	L	18.00	98.00	158.00
Number of loci/assay unit	n_u	18.00	14.00	10.53
% of polymorphism	%	61.00	66.00	64.00
Total Banding pattern	Bp	10.00	52.00	101.00
Minimum Range of PIC value	Min.	0.97	0.89	0.75
Maximum Range of PIC value	Max.	0.97	0.98	0.99
Total number of effective alleles	N_e	31.15	221.53	349.56

Table (4): Similarity indices among the five *Solenostemma argel* ecotypes based on SDS-PAGE, RAPD and ISSR analyses.

	ecotype1	ecotype2	ecotype3	ecotype4
ecotype2	0.78			
ecotype3	0.81	0.77		
ecotype4	0.71	0.72	0.80	
ecotype5	0.70	0.72	0.74	0.81

Table (5): Ecotype-specific markers in some of the *Solenostemma argel* ecotypes as revealed by SDS-PAGE, RAPD and ISSR analyses.

Ecotype number	SDS-PAGE markers and their fragment size in Kilo Dalton (KDa)	Primers code	RAPD markers and their fragment size in base pair (bp)	Primers names	ISSR markers and their fragment size in base pair (bp)
1	63	A07	1610	HB1	1370
		B01	1510	HB2	830
			790	844B	645
		B07	715		570
					440
2		B01	640	17899A	791
			415		450
		B07	410	HB2	1180
			240	HB9	925
				HB10	510
				17898B	1100
				844A	460
				814	1690
					890
3		A07	1740	HB12	1300
			1460	844A	640
			550		560
		B01	410	844B	470
		B07	1540		
			1320		
4		A15	1360	HB10	260
		A04	690	17898B	1485
					650
					490
				844A	940
					790
				814	860
5		A01	1650	17899A	1775
		A15	1230		1095
		A04	1410	HB9	870
		A07	1800	HB10	800
			950	HB11	510
				844B	790
Total markers for all ecotypes	1		21		31
	1%		41%		58%

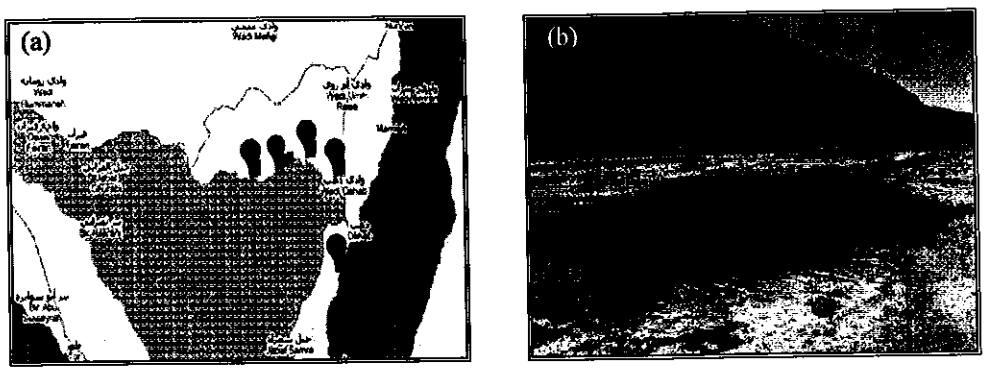

Fig. (1): Geographic distribution (a) and morphology (b) plant of five *Solenostemma argel* ecotypes.

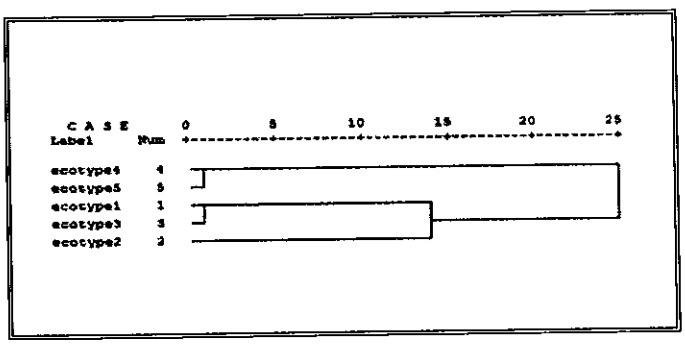

Fig. (2): The genetic distances among the five *Solenostemma argel* ecotypes based on SDS-PAGE, RAPD and ISSR analyses.

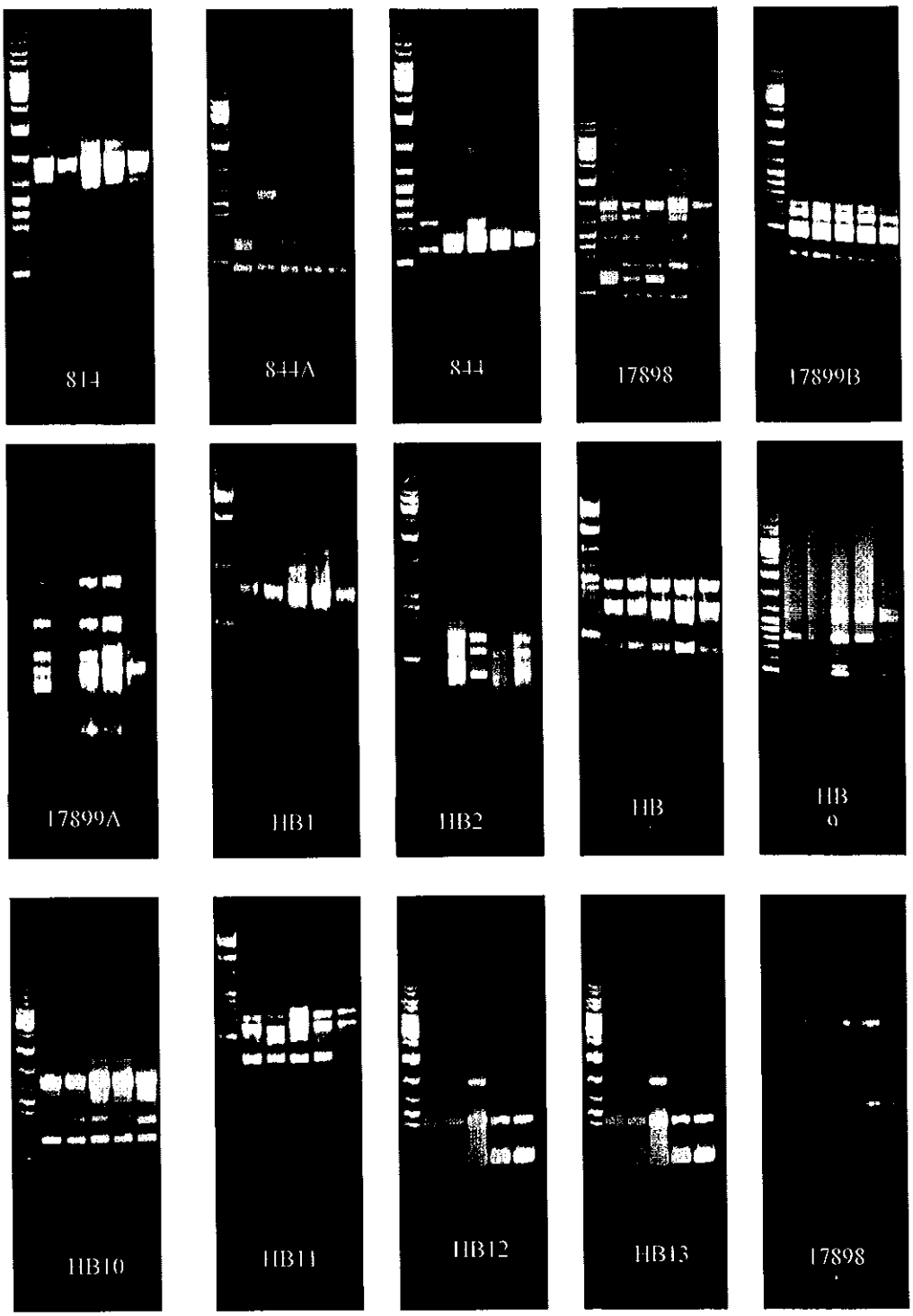

Fig. (3): (a) ISSR profiles of five *Solenostemma argel* ecotypes.

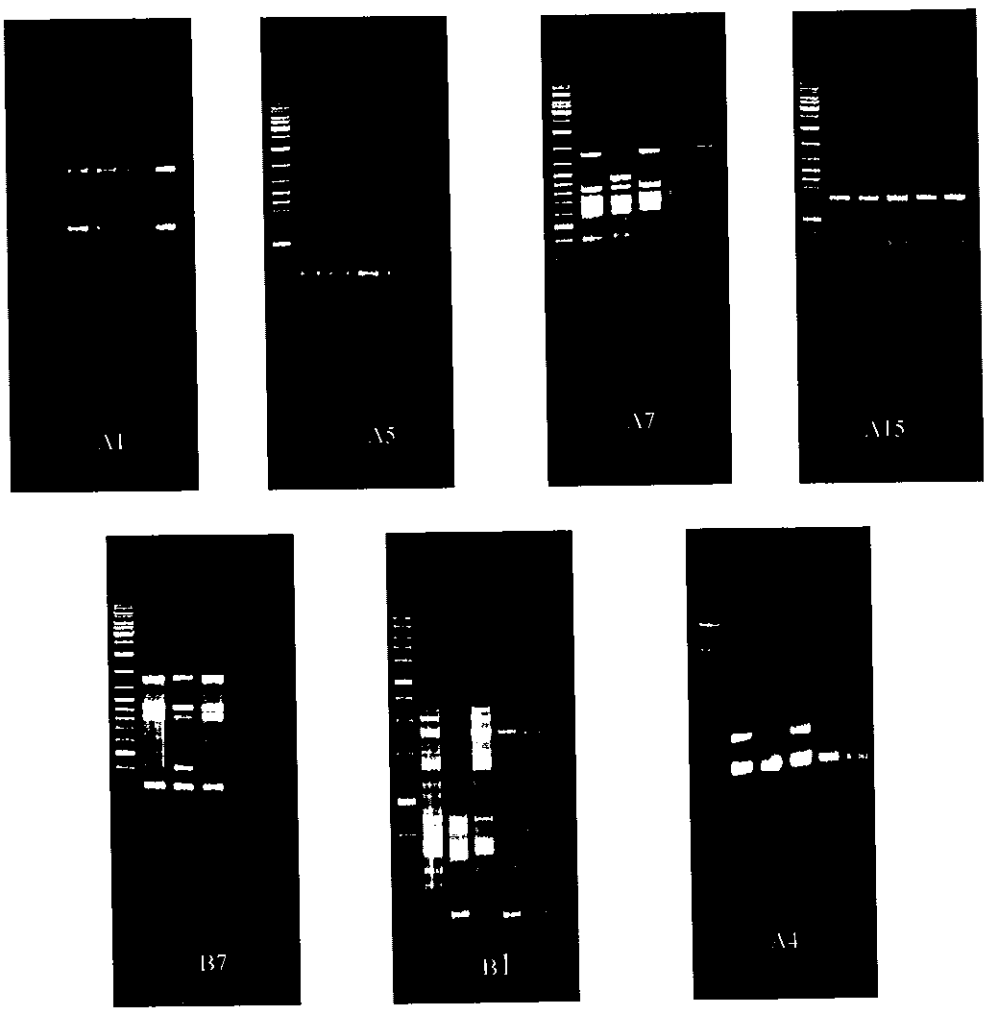

Fig. (3): (b) RAPD profiles of five *Solenostemma argel* ecotypes.

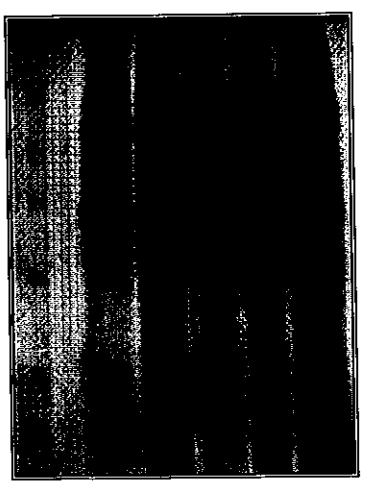

Fig. (3): (c) SDS-PAGE profiles of five *Solenostemma argel* ecotypes.