Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 12 (6-8): 271-278, 2013
ISSN 1682-3974 / DOI: 10.3923/ajps.2013.271.278

Identification of DNA Markers with the Intention of Discriminate between Seven *Eremophila* Species Cultivated at Makah Al Mukaramah Region, Saudi Arabia

[1,2]Sahar R. EL Hadad and [1]Abeer Atwa
[1]Department of Biological Science, Faculty of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[2]Research Center of Genetic Engineering and Bioinformatics, VACSERA, Cairo, Egypt

Abstract: The unambiguous identification of plant varieties is important in many areas of plant biology research. *Eremophila* (E) is a genus of plant characterized by a great economic aspects and distribution throughout the dry and warm climates. Saudi Arabia was succeeded to cultivate seven species of *Eremophila* (*E. bignoniiflora*, *E. divaricata*, *E. glabra*, *E. laanii*, *E. maculata*, *E. oppositifolia* and *E. pterocarpa*). This study employed Amplified Fragment Length Polymorphism (AFLP) to investigate the genetic variation in the selected *Eremophila* species introduced to Saudi Arabia. *Eremophila* DNA was isolated, purified and restricted using *Eco*RI and *Mse*I enzymes. Restricted DNA fragments were amplified using different sets of primer combination in order to illustrated differences between the amplified DNA fragments of each species. Our results verified 195 polymorphic DNA fragments including 14 unique markers between the seven cultivars. Phylogenetic tree was showed highest genetic interrelation (81%) between *E. bignoniiflora* and *E. oppositifolia* while the lowest interrelation (15%) was illustrated between *E. glabra* and *E. pterocarpa* in comparing to the other selected species. This study succeeded to verify unique DNA markers that can easily use to distinguish between the seven species of *Eremophila* genus cultivated in Makah Al-Mukaramah region and identified the interrelation between them.

Key words: DNA markers, *Eremophila*, AFLP markers, genetic, taxonomy

INTRODUCTION

Eremophila (E) is a genus of Australian plants derived from the Greek words Eremos (desert) and phileo (love) of the family Myoporaceae. It is the largest genus of 215 species, which vary between shrubs and woody trees up to a height of 1.5 m (Chinnock, 2007). These species were distributed throughout the dry and the warm climates as Australia, South Pacific Islands, South Africa and Asia (Gardner, 1934; White, 1944; Smith, 1956; Shaw, 1967; Dell, 1975). In addition to, they are known to produce diverse range of unusual secondary compounds, tolerant of drought, fire, frost, grazing and salinity and would be very suitable for re-vegetation programmer and rooted cuttings (Richmond and Ghisalberti, 1994).

Some *Eremophila* species have been listed as poisonous (Chinnock, 2007), while others have played an important role in treatment of colds, internal pain, septic wounds, fever, sores, boils and for insect repellent for Australian Aboriginal people (Della and Jeffries, 1961; Richmond and Ghisalberti, 1994, 1996). Due to the biological and pharmacological impact of *Eremophila*, much work has been performed on the genus *Eremophila* (Ghisalberti, 1994). Since, that time, a huge amount of both chemistry and biology data has been generated of *Eremophila* species. In an effort to better understand and present an overview of the *Eremophila* order (Singab *et al*., 2013).

Molecular phylogenetic has dramatically reshaped our views of organism's relationships and evolution. This impact has been manifested at all taxonomic levels of the hierarchy of life, from the species level (and below) to kingdoms (and above) (Woese, 1987; Woese *et al*., 1990). Researchers have used several DNA fingerprinting techniques for an accurate categorization of plant species and cultivars (Gawel *et al*., 1992; Bhat *et al*., 1995; Grapin *et al*., 1998; Ude *et al*., 2003, 2006; Toral Ibanez *et al*., 2009) including Amplified Fragment Length Polymorphism (AFLP) (Ude *et al*., 2003; Bhat *et al*., 2004). AFLP is the marker technology that rapidly generates hundreds of highly replicable DNA markers, thus allowing high resolution genotyping (Loh *et al*., 2000).

Corresponding Author: Sahar R. El Hadad, Department of Biological Science, Faculty of Science, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia Tel: 00966 533 450 633

Saudi Arabia succeeded to cultivate seven *Eremophila* species as *E. bignoniiflora*, *E. divaricata*, *E. glabra*, *E. laanii*, *E. maculata*, *E. oppositifolia* and *E. pterocarpa*. These species are as similar as to each other's in terms of morphological and anatomical characters so there is difficulty in identifying them where previous taxonomical studies performed on these species are very limited. This study aimed to assess the genetic diversity and relationships among *Eremophila* cultivars, to identify cultivar-specific markers and to determine phylogenetic relationships among *Eremophila* species introduced to Saudi Arabia using AFLP techniques.

MATERIALS AND METHODS

Plant materials and DNA extraction: Four replicate leaf samples from seven *Eremophila* species were randomly collected from Makah Al MuKaramah region, Saudi Arabia. Total genomic DNA was extracted from the collected leaf samples using DNeasy Plant System kit (Qiagen). DNA concentrations were quantified using Quant spectrophotometer and by visual inspection after electrophoresis in 1% agarose gels. The DNA samples were stored at -70°C until used (Doyle and Doyle, 1990).

AFLP analysis: AFLP analysis was performed using AFLP Analysis System I-Invitrogen (Cat. No. 10544-013) according to the manufacturer's protocol. Genomic DNA of *Eremophila* species were digested using 5 U of both *Eco*RI and *Mse*I restriction enzymes at 37°C for 3-4 h followed by incubation overnight at 4°C and 90 μL TE to generate restriction fragments. The restricted fragments were ligated to sets of *Eco*RI and *Mse*I adaptors that revealed in Table 1.

Pre-selective amplification of digested/ligated DNA was conducted using sets of primers of (*Eco*RI+3'A) and (*Mse*I+3'C) (Table 2). The amplification were carried out in a 20 μL reaction mixture consisting of 15 μL AFLP core mixtures, 1 μL AFLP pre-selective primers (5 μmol L^{-1} for each primer) and 4 μL diluted digested/ligated DNA. The cycling conditions were performed with an initial 2 min of preheating at 94°C followed by 20 cycles at 94°C for 20 sec, 56°C for 30 sec and 72°C for 2 min. Two dilutions were performed for each pre-selective PCR product. The first dilution included 1 μL product and 8.0 μL TE buffer (comprised of Tris and ethylene diaminetetraacetic acid (EDTA)) to protect DNA from degradation while the second dilution included 1 μL product in 4 μL TE buffer.

Selective amplification was performed using pre-selective PCR products as templates with 2 sets of combinations of *Eco*RI and *Mse*I primers (Table 2). The selective PCR had a total volume of 20 μL containing 3 μL diluted pre-selective amplified product, 1 μL *Mse*I primer (5 μmol L^{-1}), 1 μL *Eco*RI primer (5 μmol L^{-1}) and 15 μL AFLP core mixture. The PCR reaction profile was carried out with an initial 5 min of preheating at 94°C followed by 12 cycles at 94°C for 1 min, 65°C for 30 sec and 72°C for 2 min, then followed by 23 cycles at 94°C for 1 min, 56°C for 30 sec and 72°C for 30 sec and finally post extension cycle at 72°C for 10 min (Opara *et al*., 2010).

Table 1: *Eco*RI and *Mse*I adaptors

Primer name	Sequence
*Eco*RI adaptor I	5'-CTCGTAGACTGCGTACC-3'
*Eco*RI adaptor II	5'- AATTGGTACGCAGTCTAC-3'
*Mse*I adaptor I	5'-GACGATGAGTCCTGA-3'
*Mse*I adaptor II	5'-TACTCAGGACTCAT-3'

Table 2: Pre selective and selective primers sets utilized to amplify restricted DNA fragments

Primer name	Sequence
Pre selective primers	
*Eco*RI+1-A	5'- GACTGCGTACCAATTC+A-3'
*Mse*I+1-C	5'- GATGAGTCCTGAGTAA+A-3'
Selective primers combination I	
*Eco*RI-AAG	5'- GACTGCGTACCAATTCAAG-3'
*Mse*I-CAA	5'- GATGAGTCCTGAGTAACAA-3'
Selective primers combination II	
*Eco*RI-AAC	5'- GACTGCGTACCAATTCAAC-3'
*Mse*I-CAA	5'- GATGAGTCCTGAGTAACAA-3'

For gel analysis, samples PCR-reactions were denatured for 5 min at 95°C and snapped on ice. Five microliter of the samples mixture were loaded in 5% polyacrylamide gel and DNA fragment lengths were determined by comparisons with DNA ladders ranging from 194-1400 bp. The gels were silver stained and dried at room temperature for 24 h prior to visually scoring polymorphic bands (Vos *et al*., 1995).

Data analysis: Clear, definite and reproducible bands were considered for each band was considered a single locus. Data were scored as (+) for the presence and (-) for the absence of a DNA band. DNA band size was estimated by comparing the DNA bands with DNA ladder (Bioron, Germany) using Total lab, TL120 1D v 2009 (nonlinear Dynamics Ltd, USA). The binary data matrices were entered into the NTSYS ver.2.1 and analyzed using qualitative routine to generate similarity coefficient used to construct a dendrogram using UPGMA (Unweighted pair group method with arithmetic average) and SAHN (Sequential hierarchical and nested clustering) routine. Similarity matrix produced by 2 selective AFLP primer combinations were compared based on the genetic distance of the NTSYS ver.2.1 the normalized Mantel statistic Z (Mantel, 1967; Sokal and Rohlf , 1995).

RESULTS

AFLP profiles of *Eremophila* cultivars: AFLP analysis was standardized to obtain DNA fingerprint profiles of seven *Eremophila* cultivars using two sets of *Eco*RI and

Table 3: Polymorphic DNA fragments sizes observed amongst the seven *Eremophila* species using selective primers of combination I and II

AFLP primers	Types of DNA markers	Size of DNA marker	*Eremophila* species
Combination I	-ve	AAG/CAA-280	*E. divaricata* and *E. Laanis*
		AAG/CAA-480	*E. divaricata*, *E. glabra* and *E. maculata*
		AAG/CAA-580	*E. maculata* and *E. pterocarpa*
		AAG/CAA-1380	*E. divaricata* and *E. pterocarpa*
	+ve	AAG/CAA-610	*E. bignoniiflora* and *E. oppsitifolia*
		AAG/CAA620	*E. divaricata*, *E. glabra* and *E. maculata*
Combination II	-ve	AAC/CAA-271	*E. glabra* and *E. Laanis*
		AAC/CAA-300	*E. divaricata* and *E. maculata*
	+ve	AAC/CAA-740	*E. divaricata*, *E. maculata* and *E. ptreocarpa*
		AAC/CAA-800	*E. divaricata* and *E. maculata*
		AAC/CAA-880	*E. divaricata*, *E. glabra* and *E. maculata*
		AAC/CAA-910	*E. Laanis*, *E. oppositifolia* and *E. ptreocarpa*
		AAC/CAA-950	*E. glabra*, *E. maculata* and *E. ptreocarpa*

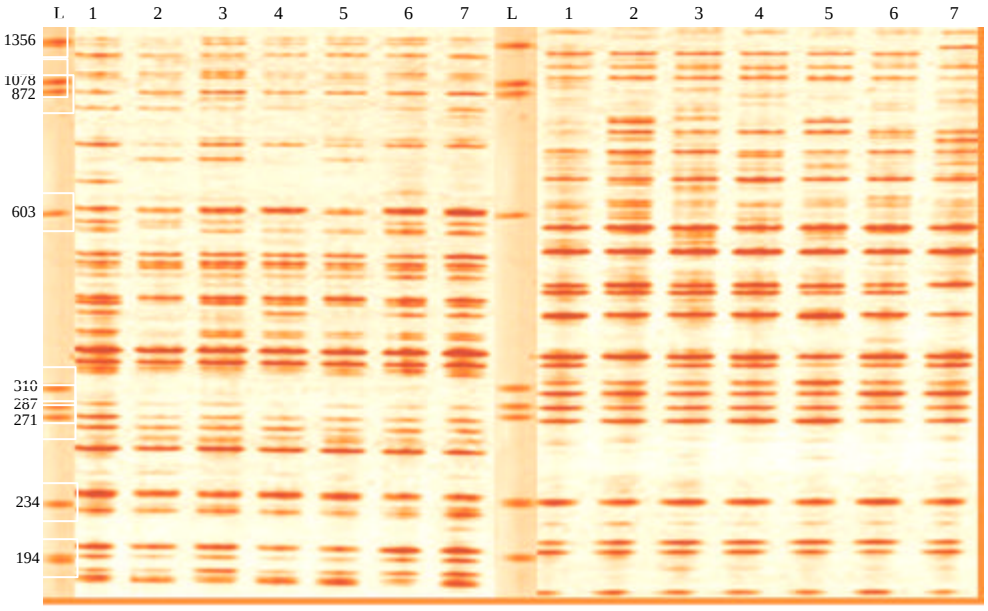

Fig. 1: DNA polymorphism of seven *Eremophila* wild plants species using both combination I and II of AFLP-PCR analysis, where (L) represent the DNA ladder that ranged as 194, 234, 271, 287, 310, 603, 872, 1078 and 1356 bp, lane 1, 2, 3, 4, 5, 6, 7 represent DNA fragments of *E. bignoniiflora*, *E. divaricata*, *E. glabra*, *E. laanis*, *E. maculata*, *E. oppositifolia* and *E. pterocarpa*, respectively

*Mse*I selective primers combinations. Both selective primers were identified 195 polymorphic bands out of total 413 bands (47% polymorphism). The size of the fragments obtained ranged from 170-1370 bp (Fig. 1).

Combination I related to the selective primers *Eco*RI-AAG and *Mse*I-CAA: AFLP analysis using pairs of primers namely *Eco*RI-AAG and *Mse*I-CAA (Combination I) was exhibited approximately 29 bands for each *Eremophila* cultivated species. These bands were including 17 monomorphic fragments and 12 polymorphic bands (41% polymorphism) (Fig. 2). Four negative polymorphic fragments AAG/AAC-280, AAG/AAC-480, AAG/AAC-580 and AAG/AAC-1340 were shared among some selected *Eremophila* species. Otherwise, two positive markers AAG/AAC-610 and AAG/AAC-620 were reported at different species of *Eremophila* (Table 3).

Unique seven polymorphic DNA markers (five negative and two positive markers) were observed among six *Eremophila* species. Three negative specific DNA markers were demonstrated in *E. bignoniiflora*, *E. lanais* and *E. maculata* at (AAG/CAA-240 bp), (AAG/CAA-271 bp) and (AAG/CAA-780 bp), respectively while two unique negative markers were verified in *E. divaricata* at (AAG/CAA-505 and AAG/CAA-450 bp). Both *E. glabra* and *E. pterocarpa* showed one positive marker with about (AAG/CAA-810 bp) and (AAG/CAA-180 bp), respectively. On the other hand,

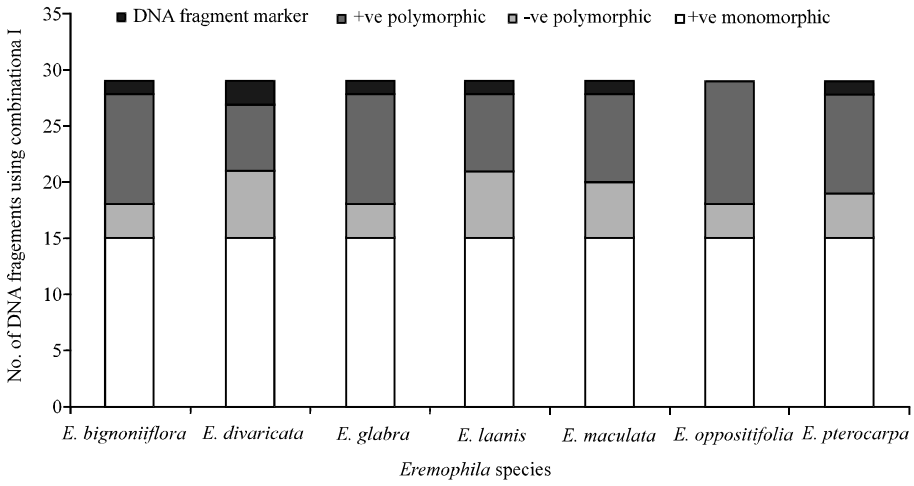

Fig. 2: DNA polymorphism of the seven *Eremophila* wild plants species using combination I

Table 4: DNA specific markers sizes observed in seven cultivars species using AFLP-PCR combination I and II primers

		AFLP DNA markers		
Species	No. of AFLP species specific marker	Marker types	Markers size (bp)	Combination
E. bignoniiflora	2	-ve	(AAG/CAA-240 bp)	I
		-ve	(AAC/CAA-850 bp)	II
E. divaricata	3	+ve	(AAG/CAA-610 bp)	II
		-ve	(AAC/CAA-505 bp)	I
		-ve	(AAC/CAA-450 bp)	I
E. glabra	4	+ve	(AAG/CAA-810 bp)	I
		+ve	(AAC/CAA-900 bp)	II
		-ve	(AAC/CAA-750 bp)	II
		+ve	(AAC/CAA-700 bp)	II
E. laanis	1	-ve	(AAG/CAA-271 bp)	I
E. maculata	1	-ve	(AAG/CAA-780 bp)	I
E. pterocarpa	3	+ve	(AAG/CAA-180 bp)	I
		+ve	(AAG/CAA-1390 bp)	II
		-ve	(AAG/CAA-1250 bp)	II

E. oppositifolia illustrated neither positive nor negative markers in comparing with the other selected species of *Eremophila* (Fig. 2, Table 4).

Combination II related to the selective primers *Eco*RI-AAC and *Mse*I-CAA: AFLP analysis with combination II using pairs of primers namely *Eco*RI-AAC and Mse I-CAA was exhibited total of 30 bands. These bands were included 16 monomorphic fragments and 14 polymorphic bands (about 47%) polymorphism with amplicons size ranging from 100-1410 bp (Fig. 3). Sixteen monomorphic amplicons fragments were observed in all selected *Eremophila* plants (Fig. 2). Two negative polymorphic fragments AAC/CAA-271, AAC/CAA-300 were shared among some selected *Eremophila* species. Otherwise, five positive markers AAC/CAA-740, AAC/CAA-800, AAC/CAA-880, AAC/CAA-910 and AAC/CAA-950 were mutual at different species of selected *Eremophila* as described in Table 3.

Four *Eremophila* species were demonstrated specific markers. Three negative markers were illustrated at *E. bignoniiflora* (AAC/CAA-850 bp), *E. glabra* (AAC/CAA-750 bp) and *E. pterocarpa* (AAC/CAA-1250 bp). Otherwise, *E. glabra* scored two positive specific markers (AAC/CAA-700 and AAC/CAA-900 bp). Both *E. divaricata* and *E. pterocarpa* verified only one specific positive marker (AAC/CAA-610 and AAC/CAA-1390 bp), respectively. Neither positive nor negative markers were illustrated in *E. laanii*, *E. maculata* and *E oppositifolia* with AFLP combination II (Fig. 3, Table 4).

Results of similarity matrix and dendrogram based on the data of AFLP-PCR: Similarity matrix verified that the closest relationship was scored between *E. bignoniiflora* and *E. oppositifolia* with identity percent equal 81% also *E. oppositifolia* and *E. laanii* with similarity identity equal 67%. On the other hand, the lowest similarity value

Table 5: Matrix of the genetic similarity based on AFLP banding patterns among seven *Eremophila* cultivates. The closest relation colored with green color while the lowest similarity colored with red color

Samples	*E. bignoniiflora*	*E. divaricata*	*E. glabra*	*E. laanis*	*E. maculata*	*E. oppositifolia*	*E. pterocarpa*
E. bignoniiflora	100	34	38	58	53	81	49
E. divaricata		100	27	31	62	38	27
E. glabra			100	46	49	27	15
E. laanis				100	49	67	56
E. maculata					100	49	38
E. oppositifolia						100	51
E. pterocarpa							100

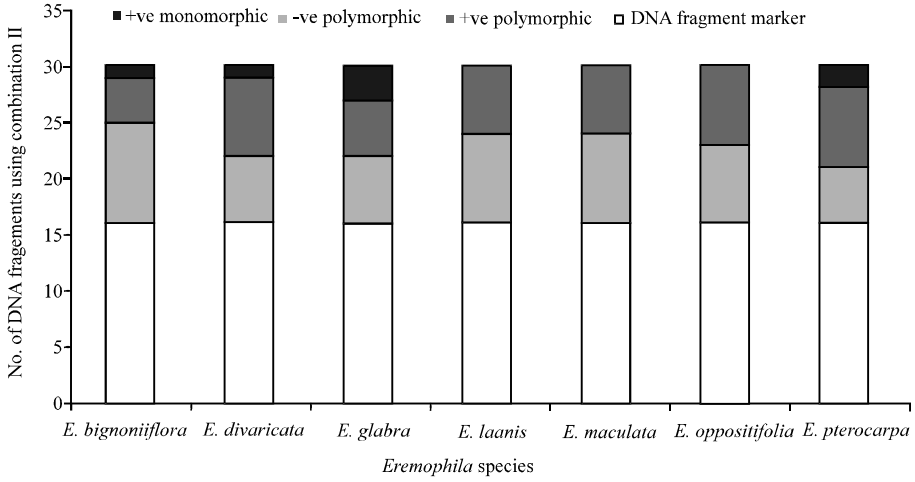

Fig. 3: DNA polymorphism of the seven *Eremophila* wild plants species using combination II

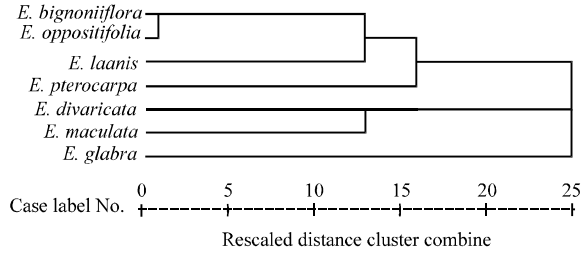

Fig. 4: Phylogenetic tree based on cluster analysis (UPGMA) of genetic similarity estimates for seven *Eremophila* species accessions from 195 polymorphic AFLP bands

revealed between *E. glabra* and *E. pterocarpa* with similarity percent equal 15% in addition to, *E. oppositifolia* rand *E. glabra* with percent equal 27% (Table 5).

Phylogenetic is perhaps the most traditional method used for analyzing genotype data. Phylogenetic dendrogram divided the seven *Eremophila* species plants into three main clusters, the first one divided into two sub-clusters; the first sub cluster included *E. bignoniiflora*, *E. oppositifolia* and *E. laanii*, while the second sub cluster comprised only one species *E. pterocarpa*. The second cluster comprised two species *E. divaricata* and *E. maculata*. Finally, the third cluster included only one species *E. glabra* (Fig. 4).

DISCUSSION

The obvious classification of plant varieties is important in many areas of agriculture and plant biology research. Techniques based on DNA profiling provide novel approaches to varietals identification which offer advantages over traditional morphological comparisons (Moller *et al*., 2007). The AFLP technique has the capacity to detect a higher number of polymorphic loci in a single assay than RFLPs or RAPDs (Powell *et al*., 1996). In the present study, AFLP technique was apply to perform detailed studies of polymorphism in seven species of *Eremophila* plant species cultivated in Saudi Arabia and developed a scientific strategy for taxonomy plant and conservation, because of their effectiveness and reliability, for studies on diversity, phylogeny, genomic linkage mapping and identification of varieties of living organisms (Becker *et al*., 1995; Maughan *et al*., 1996).

In the present study, AFLP results of both combinations I and II were demonstrated total of 419 DNA

bands including 14 specific DNA markers among the selected six *Eremophila* cultivated species. These specific DNA markers can be used to distinguish between all the seven *Eremophila* cultivated species. The specific DNA markers included 6 positive specific markers in three *Eremophila* species (*E. divaricate*, *E. glabra* and *E. pterocarpa*) and 8 negative specific markers of the six *Eremophila* species (*E. bignoniiflora*, *E. divaricate*, *E. glabra*, *E. laanii*, *E. maculata* and *E. pterocarpa*). Otherwise neither positive nor negative marker was verified at *E. oppositifolia* cultivar species. The results indicated that the AFLP markers gave adequate distinctions among the seven *Eremophila* species cultivated in Saudi Arabia. AFLP method allows the specific co-amplification of high numbers of restriction fragments. The AFLP technique provides a novel and very powerful DNA fingerprinting technique for DNAs of any origin or complexity (Vos *et al*., 1995) and represents an efficient method to certify plant materials (Passinho-Soares *et al*., 2006). Confirming to our results, Negi *et al*. (2000) used AFLP markers to study inter and intraspecific genetic variations using 35 individuals of *Withania somnifera* and five individuals of *Withania coagulans* which is an important medicinal plant. They suggested that AFLP marker is an efficient tool for estimating genetic similarity in plant species and effective management of genetic resources. Moreover, Adawy *et al*. (2004) reported that AFLP markers are the best choice for detecting polymorphism and estimating genetic diversity among date palm cultivars. In agree with, Du *et al*. (2006) studied the genetic relationships among ten wide populations of *Pinellia tornata* which collected from ten major production areas. The results showed a potential application of AFLP fingerprinting for identification of *P. ternate*.

A dendogram was constructed to study the interrelation between the seven *Eremophila* species. Phylogenetic tree divided the selected species into three main clusters, the first one divided into two sub-clusters; the first sub cluster included *E. bignoniiflora*, *E. oppositifolia* and *E. laanii* while the second sub cluster comprised only one species *E. pterocarpa*. The second cluster comprised two species *E. divaricata* and *E. maculate*, while the third cluster contained only one species *E. glabra*. We suggested some possibilities for the interpretation of cultivated *Eremophila* species origin, (a) *Eremophila* species which are grouped in three clusters might have originated from hybrid populations and then diverged into groups by domestication. This result in agree with the number of either positive or negative polymorphic DNA markers that are shared between the selected seven *Eremophila* species. Many *Eremophila* specie's germination occurs in response to heavy rain in autumn and winter especially with milder temperatures (Richmond and Chinnock, 1994) or due to transfer of the pollen grain by birds (Chinnock, 2007). (b) The ancestors of *E. oppositifolia* might have originated from hybrid between *E. bignoniiflora* and *E. laanii*. Confirmed to this suggestion, the highest similarity matrix was recorded 81% between the *E. bignoniiflora* and *E. oppositifolia* and 67% between *E. laanii* and *E. oppositifolia*, while the similarity matrix between *E. bignoniiflora* and *E. laanii* showed 58%. Although, *E. oppositifolia* showed neither positive nor negative specific markers using both combination primers, it shared with both *E. bignoniiflora* and *E. laanii* with one polymorphic positive marker using combination I and II primers, respectively. (c) *E. glabra* is clustered alone in an individual branch where the closest similarity between *E. glabra* and E. maculate have been demonstrated with similarity percent equal 49% while the lowest similarity was reported with *E. pterocarpa* with similarity percent equal 15%. Also, *E. glabra* was showed the highest no of the specific DNA markers (3 positive and 1 negative) comparing to the other selected *Eremophila* species. The present result related to *E. glabra* agree with Hutton *et al*. (2010) who recommended that *E. glabra* has good potential for antioxidant activity and can be used in lipid containing foods, also it has a strong antimicrobial activity. Otherwise, no morphological characterization was observed in *E. glabra* among the other specific species (Chinnock, 2007).

In conclusion the conservation of wild species plants as genetic resources were required an understanding the ways of genetic diversity maintained in their different natural habitats. Gene conservation strategies of this species should be designed using part of the information obtained from this study. The fingerprints were useful not only for investigation genetic variability but also for further characterizing the wild species by detecting marker polymorphisms to construct dendrogram that defining the genetic relationships within the seven selected *Eremophila* species. Furthermore, unique markers between the selected species were successfully identified indicating that AFLP techniques have great relevance for taxonomic studies. Conservationist may use the information of the present study to make effective decisions regarding the global protection and management of these species plants in Saudi Arabia.

ACKNOWLEDGMENTS

The authors would like to thanks Prof. Dr. Nahed Wally, Botany Department, Cairo University, Egypt, for her support and technical assistance.

REFERENCES

Adawy, S.S., E.H.A. Hussein, D. El-Khishin, M.M. Saker, A.A. Mohamed and H.A. El-Itriby, 2004. Genotyping Egyptian date palm cultivars using RAPD, ISSR, AFLP markers and estimation of genetic stability among tissue culture derived plants. Arab J. Biotech., 7: 53-64.

Becker, J., P. Vos, M. Kuiper, F. Salamini and M. Heun, 1995. Combined mapping of AFLP and RFLP markers in Barley. Mol. Gen. Genet., 249: 65-73.

Bhat, K.V., R.L. Jarret and R.S. Rana, 1995. DNA profiling of banana and plantain cultivars using Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) markers. Electrophoresis, 16: 1736-1745.

Bhat, K.V., Y. Amaravathi, P.L. Gautam and K.C. Velayudhan, 2004. AFLP characterization and genetic diversity analysis of Indian banana and plantain cultivars *Musa* spp. Plant Genet. Resour., 2: 121-130.

Chinnock, R.J., 2007. *Eremophila* and Allied Genera, A Monograph of the Myoporaceae. Rosenberg Publishing Ltd., Australia, Pages: 672.

Dell, B., 1975. *Eremophila ramiflora* (Myoporaceae), a new species from Western Australia. J. Royal Soc. West. Aust., 58: 93-95.

Della, E.W. and P.R. Jeffries, 1961. The essential oil of *Eremophila longifolia* F. muell. Aust. J. Chem., 14: 663-664.

Doyle, J.J. and J.L. Doyle, 1990. A rapid total DNA preparation procedure for fresh plant tissue. Focus, 12: 13-15.

Du, J., X.J. Max and X.D. Li, 2006. AFLP fingerprinting of *Pinellia ternate* and its application. Zhongguo Zhong Yao Za Zhi, 31: 30-35.

Gardner, C.A., 1934. Contributions florae australiae occidentials No VIII. J. Roy. Soc. West. Aust., 19: 79-93.

Gawel, N.J., R.L. Jarret and A.P. Whittemore, 1992. Restriction Fragment Length Polymorphism (RFLP) based phylogenetic analysis of *Musa*. Theor. Applied Genet., 84: 286-290.

Ghisalberti, E.L., 1994. The ethnopharmacology and phytochemistry of *Eremophila* species (Myoporaceae). J. Ethnopharmacol., 44: 1-9.

Grapin, A., J.L. Noyer, F. Carreel, D. Dambier, F.C. Baurens, C. Lanaud and P.J. Lagoda, 1998. Diploid *Musa acuminata* genetic diversity assayed with sequence-tagged microsatellite sites. Electrophoresis, 19: 1374-1380.

Hutton, P.G., Z. Durmic and P.E. Vercoe, 2010. Investigating *Eremophila glabra* as a bioactive agent for preventing lactic acidosis in sheep. Anim. Prod. Sci., 50: 449-453.

Loh, J.P., R. Kiew, O. Set, L.H. Gan and Y.Y. Gan, 2000. Amplified fragment length polymorphism fingerprinting of 16 banana cultivars (*Musa* cvs.). Mol. Phylogene. Evol., 17: 360-366.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.

Maughan, P.J., M.A. Saghai Maroof, G.R. Buss and G.M. Huestis, 1996. Amplified fragment length polymorphism AFLP in soy-bean, species diversity, inheritance and near- isogenetic line analysis. Theor. Applied Genet., 93: 392-401.

Moller, A.P., T.A. Mousseau, F. de Lope and N. Saino, 2007. Elevated frequency of abnormalities in barn swallows from Chernobyl. Biol. Lett., 3: 414-417.

Negi, M.S., A. Singh and M. Lakshmikumaran, 2000. Genetic variation and relationship among and within *Withania* species as revealed by AFLP markers. Genome, 43: 975-980.

Opara, U.L., D. Jacobson and N.A. Al-Saady, 2010. Analysis of genetic diversity in banana cultivars (*Musa* cvs.) from the South of Oman using AFLP markers and classification by phylogenetic, hierarchical clustering and principal component analyses. J. Zhejiang Univ. Sci. B., 11: 332-341.

Passinho-Soares, H., D. Felix, M.A. Kaplan, M. Margis-Pinheiro and R. Margis, 2006. Authentication of medicinal plant botanical identity by amplified fragmented length polymorphism dominant DNA marker: Inferences from the *Plectranthus* genus. Planta Med., 72: 929-931.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski, 1996. The comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) markers for germplasm analysis. Mol. Breed., 2: 225-238.

Richmond, G.S. and E.L. Ghisalberti, 1994. The Australian desert shrub *Eremophila* (myoporaceae): Mmedicinal, cultural, horticultural and phytochemical uses. Econ. Bot., 48: 35-59.

Richmond, G.S. and E.L. Ghisalberti, 1996. Population and plant growth studies of six species of *Eremophila* (Myoporaceae) from central Western Australia. J. Royal Soc. West. Aust., 79: 175-181.

Richmond, G.S. and R.J. Chinnock, 1994. Seed germination of the Australian desert shrub *Eremophila* (Myoporaceae). Bot. Rev., 60: 483-503.

Shaw, E.A., 1967. A new species of eremophila from south Australia eremophila-hillii-D new species. Trans. Royal Soc. S. Aust., 91: 111-114.

Singab, A.N., F.S. Youssef, M.L. Ashour and M. Wink, 2013. The genus *Eremophila* (Scrophulariaceae): An ethnobotanical, biological and phytochemical review. J. Pharm. Pharmacol., 65: 1239-1279.

Smith, L.S., 1956. New species and notes on Queensland plants. Proc. Roy. Soc. Qld., 67: 29-40.

Sokal, R.R. and F.J. Rohlf, 1995. Biometry. 3rd Edn., Freeman Press, New York, pp: 813-819.

Toral Ibanez, M., M. Caru, M.A. Herrera, L. Gonzalez, L.M. Martin, J. Miranda and R.M. Navarro-Cerrillo, 2009. Clones identification of *Sequoia sempervirens* (D. Don) Endl. in Chile by using PCR-RAPDs technique. J. Zhejiang Univ. Sci. B, 10: 112-119.

Ude, G., M. Pillay, E. Ogundiwin and A. Tenkouano, 2003. Genetic diversity in an African Plantain core collection using AFLP and RAPD markers. Theor. Applied Genet., 107: 248-255.

Ude, G.N., C.O. Dimkpa, P.O. Anegbeh, A.A. Shaibu, A. Tenkouano, M. Pillay and Z. Tchoundjeu, 2006. Analysis of genetic diversity in accessions of *Irvingia gabonensis* (Aubry- lecomte ex O Rorke) Baill. Afr. J. Biotechnol., 5: 219-223.

Vos, P., R. Hogers, M. Bleeker, M. Reijans and T.V.D. Lee *et al*., 1995. AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res., 23: 4407-4414.

White, C.T., 1944. Contributions to the Queensland flora No 8. Proc. Roy. Soc. Qld., 55: 73-73.

Woese, C.R., 1987. Bacterial evolution. Microbiol. Rev., 51: 221-271.

Woese, C.R., O. Kandler and M.L. Wheelis, 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria and Eucarya. Proc. Natl. Acad. Sci. USA., 87: 4576-4579.