academicJournals

Vol. 11(4), pp. 92-98, April 2017
DOI: 10.5897/AJPS2017.1523
Article Number: 79543C463364
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Detection of genetic diversity among some species of *Anthemis* L. (Asteraceae) in Saudi Arabia by using RAPD-PCR analysis

Sameer Hasan M. Qari

Department of Biology, Aljamom University College, Umm Al-Qura University, KSA. P. O. Box 2203, Makkah, Saudi Arabia.

Received 29 January, 2017; Accepted March 3, 2017

The aim of the present study was to determine the unique molecular markers among, three species of the genus *Anthemis* and the construction of phylogenetic tree using the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. Genetic diversity was analyzed among 15 populations of three species of *Anthemis* (*Anthemis melampodina*, *Anthemis*, *pseudocotula* and *Anthemis*, *bornmuelleri*), collected from different locations at Saudi Arabia by using RAPD primers. Pairwise genetic distance was calculated based on Nei and Li coefficient. Unweighted pair group method with an average (UPGMA) was used for construction of dendrogram, based on the similarity matrix data. Results showed wide variations among *A. bornmuelleri* and other two species. A wide close genetic relation was observed between *A. melampodina* and *A. pseudocotula*. RAPD-PCR technique was shown to be an accurate tool, in other to ascertain plant relationships among species of genus *Anthemis*.

Key words: Genetic diversity, random amplified polymorphic DNA-polymerase chain reaction (RAPD- PCR), *A. melampodina*, *A. pseudocotula*, *A. bornmuelleri*.

INTRODUCTION

Anthemis L. is one of the largest genus of family Asteraceae, including more than 210 described species (Oberprieler et al., 2007). They are widely distributed across Europe extending into extreme southern Arabia and tropical east Africa and other parts of the world (Oberprieler, 2001).

The main center of biological diversity or species diversity is located in Mediterranean region (Lo Presti, 2010) and southwestern Asia with 150 to 210 species, including all of the presently accepted subgenera and sections (Kilica et al., 2011). Some species inhabit northern America and southern hemisphere as well (Oberprieler, 2001). *Anthemis* is a diverse group that can be easily distinguished by the paleaceous receptacle of

E-mail: sqarinet@gmail.com. Tel: +966555504086.

its mostly radiate capitula and the achene morphology. Some species are widely used in pharmaceutical, cosmetics, and food industry. They are also used as herbal tea for treatment of anxiety, flatulence, stomach disorders, insomnia and toothache (Vaverkova et al., 2001).

Saudi Arabia contains a large number of wild plants, both in arid and high lands. The greatest plant diversity was recorded in Asir, Hijaz and the western area, bordering the Red Sea (Collenette, 1998). Many previous studies showed that landscape of an area and the climatic influences are the main factors affecting the degree of species diversity (El-Kady et al., 1995; Shaltout et al., 1997). Some species of *Anthemis* are distributed depending on the chemical nature of bedrock and climate of geographic area. Four species of *Anthemis* were illustrated by Migahid (1996), 12 species by (Ghafoor and Al Turki, 1999) and 19 species by (Ghafoor, 2010).

Several studies on the genus *Anthemis* were focused on ecology and taxonomy (Ghafoor, 2010), biochemistry and toxicity (Uzel et al., 2004; Grace, 2002; Pavlović et al., 2010), therapeutic (Jafari et al., 2003; Darriet et al., 2009; Jaradat et al., 2016) and molecular studies (Oberprieler, 2001; Oberprieler, 2002; Lo Presti, 2010**)**. Reports dealing with diversity using molecular studies on species of *Anthimus* in Saudi Arabia, in particular *A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) are scanty.

Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Variable Number of Tandem Repeats (VNTR) and Restriction Fragment Length Polymorphism (RFLP) have been established as useful markers, for discovering genetic diversity of floras (Wang, et al., 1996). RAPD is a PCR-based technique developed by (Williams et al., 1990) and (Welsh and McClelland, 1990) which employs decamer random primers, for amplifying random DNA fragments from genomic DNA without any prior knowledge of the genomic sequence of any organism. Recently, several studies have used this tool to measure the levels and patterns of variations within different plants (Abd El-Ghani and El-Sawaf, 2004; Sarwat et al., 2008; Thendral et al., 2010; Hammadi and Qari, 2012; Ismail et al., 2016; Lu et al., 2016; Patil et al., 2016).

The present study aimed to use DNA (RAPD) markers to investigate genetic diversity, genetic relationships and polymorphism in natural populations of three species of *Anthemis* in Saudi Arabia.

MATERIALS AND METHODS

Plant materials

During the period of March to May 2015, 15 plant samples of three species of *Anthemis* were collected from different geographic regions in Saudi Arabia through Jizan, Makkah, Hail and Asir (Figure 1, Table 1). Samples comprise of 9 specimens of *A. melampodina,* 3 specimens of *A. pseudocotula* and 3 specimens of *A. bornmuelleri.* The plants were identified by the Plants Taxonomist at the Herbarium of the Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia. Young leaves were harvested and preserved in sealed bags with suitable label. Leaves were used immediately for DNA extraction, while excess leaves were stored in -80°C for subsequent use.

Chemicals

Reagents including Taq DNA polymerase (TaKaRa), dNTPs (Boehringer Mannheim), DNA extraction kit, and agarose gel (Qiagen), oligonucleotides as random primers (Operon technologies, USA), Egypt), DNA Marker for agarose gel electrophoresis (Gibco BRL), loading dye solution (Fermentas, Lithvuania) were used. All other chemicals were obtained from Sigma Aldrich (USA).

DNA extraction

Total genomic DNA extraction of plant leaves was performed using a modified CTAB method according to the protocol of (Doyle and Doyle, 1990). Quantitative estimation of total genomic DNA in each sample was confirmed spectrophotometericaly at 260 and 280 nm, Whereas, quality was checked by running samples on 1.2% agarose electrophoresis with DNA ladder and visualized under UV light in gel documentation system.

RAPD analysis

Polymorphic primers were identified by screening fifteen random decamer primers (Table 2) with DNA of *Anthemis* sp. Out of 15 primers, only 5 gave precise and stable PCR-production for RAPD analysis (Table 2). DNA amplification was performed in a Perkin ElmerCetus 480 DNA Thermal Cycler programmed for 45 cycles as follows: 1st cycle of 3.5 min at 92°C, 1 min at 35°C, 2 min at 72°C; followed by 44 cycles each of 1 min at 92°C, 1 min at 35°C, 2 min at 72°C followed by one final extension cycle of 7 min at 72°C.

The amplification products were resolved by agarose gels electrophoresis. Controls lacking template DNA were included. Amplified PCR products were resolved on 1% agarose gel electrophoresis, visualized under UV light and photographed with gel documentation system. Each band was considered as RAPD marker. All the reactions were repeated for at least twice.

Data analysis

A binary matrix was prepared by manually scoring of photographed bands on gels, where 1 or 0 represent. The data were used for similarity – based analysis using the software program NTSYS (2.20). RAPD analyses were analyzed using the Nei genetic similarity index (Nei and Li, 1979). On the basis of the similarity matrix data, a dendrogram was constructed by unweighted pair group method with average (UPGMA) cluster analysis (Figure 2).

RESULTS AND DISCUSSION

Different approaches in genetic diversity analyses, reveal the different level of polymorphism (Porter and Smith, 1982) and also, DNA markers are independence of environmental or localities factors which show a greater level of polymorphism (Heywood, 2002). Therefore, they

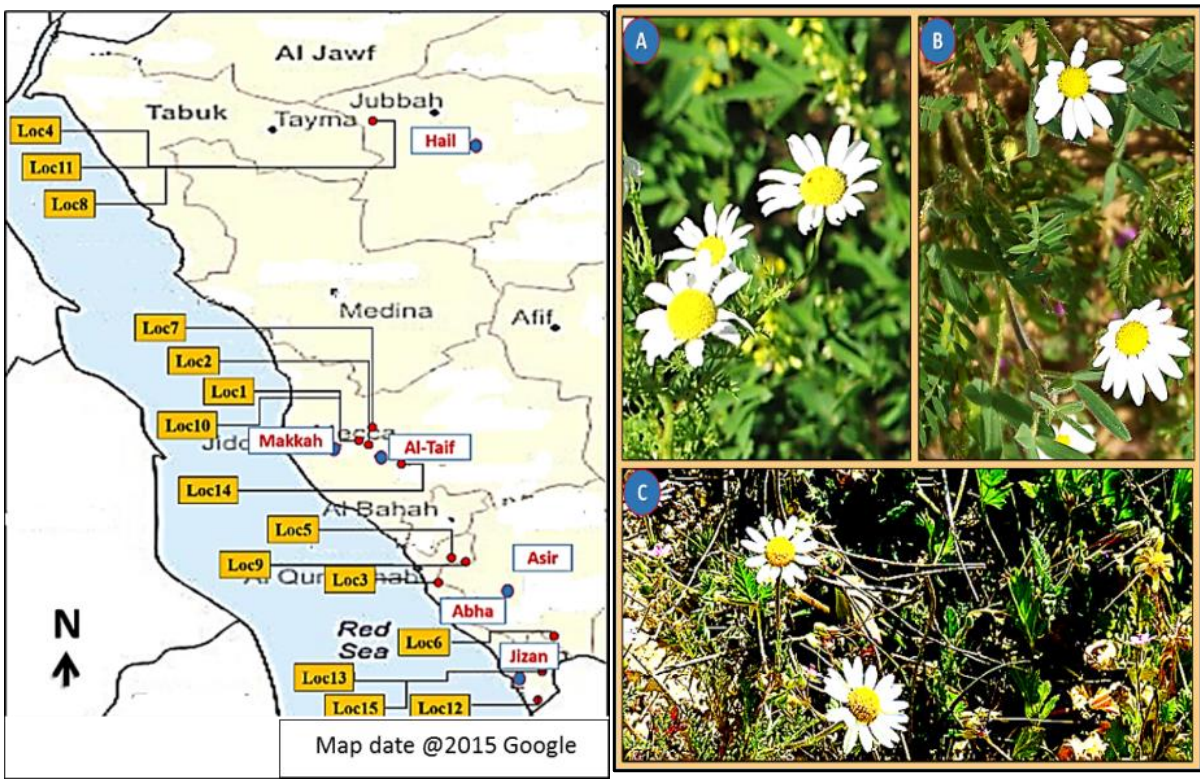

Figure 1. (Left said) map of Saudi Arabia indicating the localities where *Anthemis* populations of the three species were collected in March-May 2015, (Right side) Photos of *Anthemis* species (from field) as A: *Anthemis melampodina* (Loc6), B: *Anthemis pseudocotula* (Loc11) and C: *Anthemis bornmuelleri* (Loc15).

Table 1. Locations of plant collection.

Population ID	Scientific name	Location	
		Loci name	Description
Loc1	*A. melampodina*	Wadi Zimmah,	60km Makkah – Al-Taif road, Makkah Region.
Loc2	*A. melampodina*	Wadi Al-Madik	70km Makkah – Al-Taif road, Makkah Region.
Loc3	*A. melampodina*	Khawr 'Amiq	30 km northwest of Al-Birk, Asir Region.
Loc4	*A. melampodina*	Al-Odair valley	Hail in the Shammar Mountain, Hail region
Loc5	*A. melampodina*	Jabal Alswda	40 km west of Abha City, Asir Region.
Loc6	*A. melampodina*	Jabal Fayfa	90 Km Northest Jazan, Jizan region
Loc7	*A. melampodina*	Wadi Saulah	80 km Makkah – Al-Taif road
Loc8	*A. melampodina*	Al-Odair valley	Hail in the Shammar Mountain, Hail region
Loc9	*A. melampodina*	Jabal Alswda	30 km west of Abha City, Asir Region.
Loc10	*A. pseudocotula*	Wadi Zimmah,	60km Makkah – Al-Taif road
Loc11	*A. pseudocotula*	Al-Odair valley	Hail in the Shammar Mountain, Hail region
Loc 12	*A. pseudocotula*	Khawr Wahlan	35 km south of Jizan, Jizan Region.
Loc 13	*A. bornmuelleri*	Malaki Dam	15 km east of Abu Arish, Jizan Region.
Loc 14	*A. bornmuelleri*	Wadi Ze Ghazal	Al-Shafaa, 37Km southeast Al-Taif
Loc 15	*A. bornmuelleri*	Malaki Dam	15 km east of Abu Arish, Jizan Region

are considered as valuable tools for determining genetic relationships. Among various molecular markers, Random amplified polymorphic DNA (RAPD) markers have proved to be a very useful tool, providing a convenient and rapid

Table 2. Sequences of used (15) primers.

Primers		Sequences of primers (5′ → 3′)	Primers		Sequences of primers (5′ → 3′)
No.	Name		No.	Name	
1	OPB-16	TTT GCC CGG A	9	OPE-05	TCA GGG AGG T
2	OPA-04	GTC GAA CGA G	10	OPG-18	GGC TCA TGT G
3	OPC-20	ACT TCG CCG A	11	OPZ-13	GGG TCT CGG T
4	OPE-03	CCA GAT GCA C	12	OPD-07	TTG GCA CGG G
5	OPA-02	AGC CTT CGC T	13	OPD-03	GTC GCC GTC A
6	OPB-03	CAT CCC CCT G	14	OPC-13	AAC CCT CGT C
7	OPA-01	CAG GCC CTT C	15	OPB-10	CTG CTG GGA C
8	OPB-08	GTC CAC ACG G			

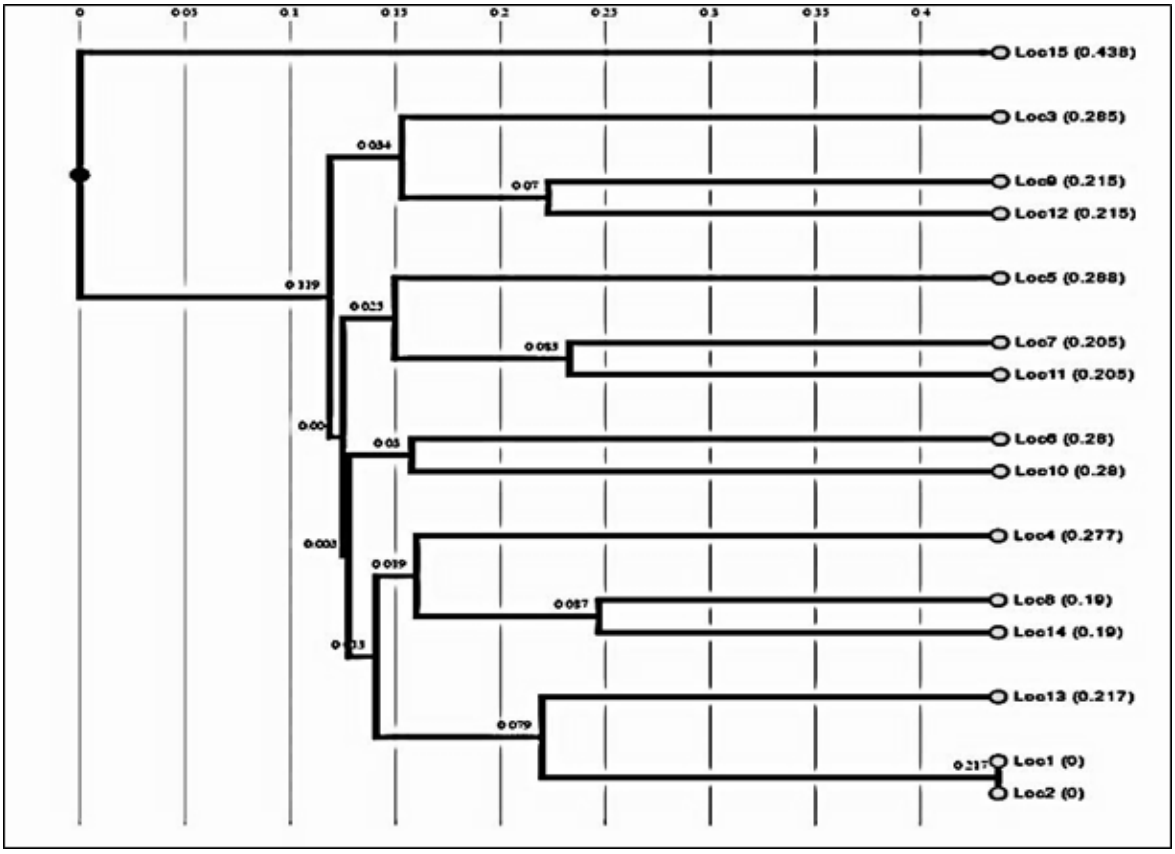

Figure 2. Dendrogram obtained from RAPD analysis using UPGMA.

assessment of the genetic differences between genotypes (Williams et al., 1990). Meanwhile, polymorphism detected by RAPD markers has proven to be useful for discrimination of genetic diversity and relationships in several plant species.

The present work is a preliminary study, aim to use DNA (RAPD) markers to investigate genetic diversity, genetic relationships and polymorphism in natural populations of only three species of this genus (*A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) collected from Saudi Arabia. In this study, a total of 15 RAPD-PCR primers were used to test 15 samples (Table 1). Out of these, only 5 primers showed reproducible results and they were chosen to amplify the whole 15 samples (Figure 3). RAPD-PCR assay had been positively used in several taxonomical and genetic diversity studies (Hammadi and Qari, 2012; Alam et al., 2009).

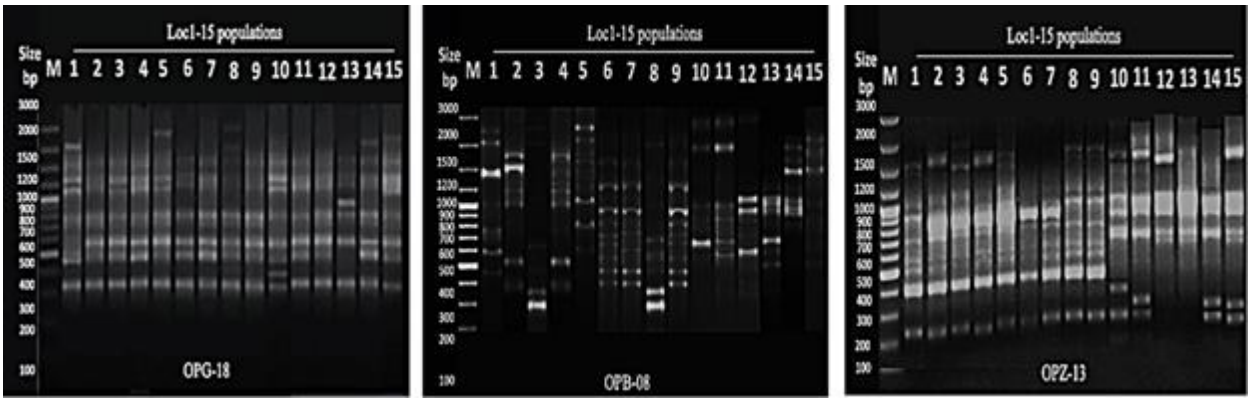

Figure 3. Polymorphic bands generated by some different RAPD primers, M: DNA stander; Numbers 1-15: Loc1- Loc15 population.

Table 3. RAPD primers data and the percentages of polymorphic bands.

Primer code	Size of fragments(bp)	Polymorphic bands	Monomorphism %	Polymorphism %
OPA-02	341-1670	11	14.2	85.8
OPB-08	310-2791	15	39.6	60.4
OPC-20	315-2105	8	31.8	68.2
OPG-18	292-2684	8	59.2	40.8
OPZ-13	263-1870	9	8.6	91.4

Table 4. Number of amplified fragments and specific markers of the three species of *Anthemis* (*A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) using RAPD analysis with five primers.

Primer	TFA	*A. melampodina*		*A. pseudocotula*		*A. bornmuelleri*		TSM
		AF	SM	AF	SM	AF	SM	
OPA-02	7	7	3	8	1	8	0	4
OPB-08	12	8	4	6	2	7	2	8
OPC-20	18	11	3	10	3	7	1	7
OPG-18	16	13	0	5	3	7	3	6
OPZ-13	10	6	3	8	1	5	0	4
Total	63	45	13	37	10	34	6	29

TFA, Total fragments amplified; AF, amplified fragments; SM, specific markers; TSM, total specific markers.

The percentages of polymorphism and monomorphism of the obtained bands are presented in (Table 3). A total of 63 bands were produced for 15 samples, 51 bands of them were polymorphic and present one or more but not all of them. Mehetre et al., (2004) reported that monomorphic bands should present in all producible bands, and the unique ones should present in at least one producible band, not in any others. The mean percentage of polymorphic bands was 69.32% with molecular sizes ranged from 263 up to 2791 bp; approximately, 18 bands of the 63 were commonly detected in all the samples, so it could be the recognized genus bands of *Anthemis*.

The results of the total fragments amplified (TFA), amplified fragment (AF) specific markers (SM) for each species of *Anthemis*: (*A. melampodina*, *A. pseudocotula* and *A. bornmuelleri*) are presented in Table 4 and the genetic distance matrix for indices of the studied samples are presented in Table 5. Welsh and McClelland (1990) found that, reproducible fingerprints of genomes could be generated using arbitrary primers with PCR technique. Species-specific markers varied between three types of *Anthemis* species (TSM=29 markers) and are clearly shown in (Table 4). *A. melampodina* revealed 13 specific bands, while *A. pseudocotula* and *A. bornmuelleri* showed 10 and 6 specific bands (SM), respectively.

Table 5. Genetic distance matrix for indices of 15 samples of the three species of *Anthemis*.

Locations	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8	Loc9	Loc10	Loc11	Loc12	Loc13	Loc14	Loc15
Loc1	0	0.81	0.75	0.74	0.72	0.70	0.60	0.59	0.64	0.58	0.58	0.55	0.51	0.51	0.58
loc2		0	0.76	0.75	0.74	0.67	0.57	0.58	0.55	0.57	0.55	0.36	0.36	0.50	0.57
loc3			0	0.78	0.79	0.7.5	0.56	0.56	0.56	0.60	0.60	0.58	0.57	0.57	0.98
loc4				0	0.75	0.71	0.70	0.61	0.62	0.59	0.53	0.51	0.50	0.50	0.76
loc5					0	0.68	0.58	0.62	0.63	0.59	0.57	0.53	0.53	0.55	0.82
loc6						0	0.74	0.62	0.66	0.56	0.60	0.57	0.56	0.53	0.78
loc7							0	0.73	0.72	0.44	0.41	0.56	0.52	0.52	0.92
loc8								0	0.68	0.43	0.43	0.41	0.41	0.38	1.00
loc9									0	0.55	0.43	0.43	0.68	0.59	0.78
loc10										0	0.69	0.73	0.70	0.70	0.79
loc11											0	0.69	0.70	0.76	0.86
loc12												0	0.79	0.70	0.95
Loc13													0	0.76	0.92
Loc14														0	1.03
Loc15															0

These bands may be possible specific markers after verifying that, individuals of each species appear the same description (Roman et al., 2003; Manen et al., 2004).

Results of monomorphism and polymorphism of *Anthemis* using RAPD-analysis might be precise findings for the genetic diversity of genus *Anthemis,* to specify each species with the appearance of specific markers and definitive bands. Many studies conducted on other genus revealed similar results with insufficient molecular studies of Anthemideae (Matousek et al., 2007; Garcia et al., 2010; Riggins and Seigler, 2012). Similar results were obtained by other authors (Riggins and Seigler, 2012; Patil et al., 2016; Higgins et al., 2016; Kumar et al., 2016).

Conclusion

A high level of polymorphism and establishment of genetic diversity among three species of *Anthemis* was detected by using RAPD markers that could be a suitable tool for understanding the aspects of divergence, to solve a lot of taxonomical problems.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

ACKNOWLEDGEMENT

The author would like to acknowledge Dr. Hagen, head of molecular genetics laboratory, (KFR), for his technical support all over the research work and the graduate students Mohammed Al-Maliki and Afaf Khurami for their contribution in sample collection.

REFERENCES

Abd El-Ghani MM, El-Sawaf N (2004). Diversity and distribution of plant species in the agro-ecosystem of Egypt. Syst. Geogr. 74:319-336.

Alam MA, Pallavi G, Aswini KG, Gyan PM, Pradeep KN (2009) Assessment of genetic diversity among *Podophyllum hexandrum* genotypes of assessed by random amplified polymorphic DNA. Afr. J. Biotechnol. 7(24):4407-4414.

Collenette S (1998) Checklist of botanical species in Saudi Arabia. International Asclepiad Society. 1:80.

Darriet F, Desjobert JM, Costa J, Muselli A (2009). Identification of chrysanthenyl esters from the essential oil of *Anthemis maritima* L. investigated by GC/RI, GC-MS (EI and CI) and 13C-NMR spectroscopy: chemical composition and variability. Phytochem. Anal. 20(4):279-292.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13-15.

El-Kady HF, Ayyad M, Born Karnum R (1995). Vegetation and recent land-use history in the desert of Maktala, Egypt. Adv. Geoecol. 28:109-123.

Garcia S, Panero JL, Siroky J, Kovarik A (2010). Repeated reunions and splits feature the highly dynamic evolution of 5S and 35S ribosomal RNA genes (rDNA) in the Asteraceae family. BMC Plant Biol. 16(10):176.

Ghafoor A, Al-Turki TA (1999). A new Anthemis (Asteraceae)

from Saudi Arabia. Edinb. J. Bot. 56(1):55-59.
Ghafoor A (2010). The Genus Anthemis L. (Compositae-Anthemideae) In Arabian Peninsula: A Taxonomic Study. Pak. J. Bot. 42:79-98.
Grace MH (2002). Chemical composition and biological activity of the volatiles of *Anthemis melampodina* and *Pluchea dioscoridis*. Phytother. Res. 16(2):183-185.
Hammadi I, Qari SH (2010). Genetic Diversity Among *Zygophyllum* Populations based on RAPD analysis. Genet. Mol. Res. 9(4):2412-2420.
Heywood VH (2002). The Conservation of Genetic and Chemical Diversity in Medicinal and Aromatic Plants. In. Biodiversity: Biomolecular Aspects of Biodiversity and Innovative Utilization. Sener, B. (Ed.). Springer, Berlin, Heidelberg, pp. 13-22.
Higgins CM, Chang WL, Khan S, Tang J, Elliott C, Dietzgen RG (2016). Diversity and evolutionary history of lettuce necrotic yellows virus in Australia and New Zealand. Arch. Virol. 161(2):269-277.
Ismail NA, Rafii MY, Mahmud TM, Hanafi MM, Miah G (2016). Molecular markers: a potential resource for ginger genetic diversity studies. Int. J. Mol. Sci. 17(8):120-128.
Jafari S, Massoud A, Katayoun B, Hassan F (2003). Comparartive Study of Zataria Multiflora and *Anthemis Nobelis* Extracts with Myrthus Communis Preparation in The Treatment of Recurrent Aphthous Stomatitis. DARU J. Pharm. Sci. 11(1):23-27.
Jaradat NA, Ayesh OI, Anderson CJ (2016). Ethno-pharmacological survey about medicinal plants utilized by herbalists and traditional practitioner healers for treatments of diarrhea in the West Bank/Palestine. J. Ethnopharmacol. 182:57-66.
Kilica O, Alpaslan K, Eyup B (2011). Composition of the volatile oils of two Anthemis L. taxa from Turkey. Z. Naturforsch. C 66(11-12):535-540.
Kumar H, Priya P, Singh N, Kumar M, Choudhary BK, Kumar L (2016) RAPD and ISSR Marker-Based Comparative Evaluation of Genetic Diversity Among Indian Germplasms of uryale ferox: An Aquatic Food Plant. Appl. Biochem. Biotechnol. 30:280-294.
Lo Presti RM (2010). Geological vs. Climatological Diversification in the Mediterranean Area: Micro- and Macro-evolutionary Approaches in Anthemis L. (Compositae, Anthemideae). Logos Verlag: Berlin: GmbH. P.186.
Lu PL, Yorkson M, Morden CW (2016). Population Genetics of the Endemic Hawaiian Species Chrysodracon hawaiiensis and Chrysodracon auwahiensis (Asparagaceae): Insights from RAPD and ISSR Variation. Int. J. Mol. Sci. 17(8):129-137.
Manen JF, Habashi C, Jeanmonod D, Park JM, Schneeweiss GM (2004). Phylogeny and intraspecific variability of holoparasitic Orobanche (Orobanchaceae) inferred from plastid rbcL sequences. Mol. Phylogenet Evol. 33(2):482-500.
Matousek J, Orctová L, Ptácek J, Patzak J, Dedic P, Steger G (2007). Experimental transmission of pospiviroid populations to weed species characteristic of potato and hop fields. J. Virol. 81(21):11891-11899.
Mehetre S, Gomes M, Eapen S (2004). RAPD analysis of hybrid nature of the offspring of Gossypium hirsutum x G. raimondii. Curr. Sci. 84:24-28.
Migahid AM (1996). Flora of Saudi Arabia. 4th ed. Vol. 2, Riyadh: King Saud University Press. Saudia Arabia. pp. 1-150.
Nei M, Li WH (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 74:5267-5273.
Oberprieler C, Himmelreich S, Vogt R (2007). A new subtribal classification of the tribe Anthemideae (Compositae). Willdenowia 37:89-114.
Oberprieler C (2002). A phylogenetic analysis of *Chamaemelum* Mill. (Compositae: Anthemideae) and related genera based upon nrDNA ITS and cpDNA*trn*L/*trn*F IGS sequence variation. Bot. J. Linn. Soc. 138(3):255-273.
Oberprieler CH (2001). Phylogenetic relationships in Anthemis L. (Compositae, Anthemideae) based on nrDNA ITS sequence variation. Taxon 50:745-762.
Patil RR, Pawar KD, Rane MR, Yadav SR, Bapat VA, Jadhav JP (2016). Assessment of genetic diversity in Mucuna species of India using randomly amplified polymorphic DNA and inter simple sequence repeat markers. Physiol. Mol. Biol. Plants 22(2):207-217.
Pavlović M, Lakusić D, Kovacević N, Tzakou O, Couladis M (2010). Comparative analysis of essential oils of six Anthemis taxa from Serbia and Montenegro. Chem. Biodivers. 7(5):1231-44.
Porter WM, Smith DH (1982). Detection of identification errors in germplasm collections. Crop Sci. 22:701-703.
Riggins CW, Seigler DS (2012). The genus Artemisia (Asteraceae: Anthemideae) at a continental crossroads: molecular insights into migrations, disjunctions, and reticulations among Old and New World species from a beringian perspective. Mol. Phylogenet. Evol. 64(3):471-490.
Roman B, Alfaro C, Torres AM, Moreno MT, Satovic Z, Pujadas A (2003). Genetic relationships among Orobanche species as revealed by RAPD analysis. Ann. Bot. 91(6):637-642.
Sarwat M, Das E S, Srivastava EP (2008). Analysis of genetic diversity through AFLP, AMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. Plant Cell Rep. 27:519-528.
Shaltout KH, El-Halawany EF, El-Garawany MM (1997). Coastal low land vegetation of eastern Saudi Arabia. Biodivers. Conserv. 6:1027-1040.
Thendral HB, Premalakshmi V, Sekar T (2010). Genetic diversity in *Azima tetracantha* (Lam) assessed through RAPD analysis. Indian J. Sci. Technol. 3(2):300-309.
Uzel A, Guvensen A, Cetin E (2004). Chemical composition and antimicrobial activity of the essential oils of *Anthemis xylopoda* O. Schwarz from Turkey. J. Ethnopharmacol. 95(2-3):151-154.
Vaverkova S, Haban M, Eerna K (2001). Qualitative properties of *Anthemis tinctoria* and *Anthemis nobilis* (Chamaemelum nobile) under different environmental conditions. Ecophysiology of plant production processes in stress conditions. Abstracts of the Fourth International Conference, Račkova dolina, Slovakia. 2: 1-2.
Wang XP, Zou YP, Zhang DM (1996). Problems in the use of RAPD to the study of genetic diversity and systematic. Acta Bot. Sin. 38(12):954-962.
Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.
Williams JG, Kubelik AR, Livak KJ, Rafalski JA (1990). Tingey SVDNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.