http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.623.629

Research Article
Ecological Distribution and Genetic Variations of Some *Aloe* Species in Taif, KSA

[1,2]Attia O. Attia and [1,3]Yassin M. Al-Sodany

[1]Department of Biology, College of Science, Taif University, Taif, Kingdom of Saudi Arabia
[2]Department of Plant Genetic Transformation, Agricultural Genetic Engineering Research Institute, Agricultural Research Centre, 12619 Giza, Egypt
[3]Department of Botany, Faculty of Science, Kafrelsheikh University, Kafr El-Sheikh, Egypt

Abstract

Background and Objectives: *Aloe* is a medicinally and economically important genus. Many *Aloes* seem an endangered species because of over-collection, destruction of plants and destroyed of natural habitats. The objectives of current study was to survey, collect and identification of some *Aloe* species and to analyze genetic variations between the collected *Aloe* species. **Materials and Methods:** Four *Aloe* species (*A. armatissima*, *A. edentata, A. parvicoma* and *A. pseudorubroviolacea*) and *Agave americana* (Asperagaceae) were used as plant materials for ecological and genetic studies. In RAPD and ISSR analysis 23 and 16 primers, respectively were screened. **Results:** Ecological study showed that the 4 species are endemic: 2 are endangered (*A. edentata* and *A. parvicoma*) and the others are not-endangered (*A. armatissima* and *A. pseudorubroviolacea*), while *A. americana* was introduced as ornamental species. Concerning RAPD, a total of 134 reproducible bands of them 131 bands are polymorphic ~ 97.65% polymorphism were produced, which ranged from 9 bands (primer OPC-04) to 18 (primer OPA-03) bands, with an average 13.4 bands/ primer, ranging from ~300-2500 bp. According to ISSR, 113 reproducible bands were totally yielded with an average 12.6 bands/primer, from ~180-1500 bp, of which 107 poly-morphic bands number (PBN) ~94.96% polymorphism ranged from 10 bands (primer UBC-818 and primer UBC-819) to 14 (primer UBC-814) with an average of 11.9 PB/primer. **Conclusion:** The results revealed high genetic variations between 4 bands *Aloe* species and *A. americana* species, which will be in concern for improvement, breeding and conservation programs.

Key words: Genus *Aloe*, genetic variation, *Agave americana*, random amplified polymorphic-DNA (RAPD), inter simple sequence repeat (ISSR)

Citation: Attia O. Attia and Yassin M. Al-Sodany, 2019. Ecological distribution and genetic variations of some *Aloe* species in Taif, KSA. Pak. J. Biol. Sci., 22: 623-629.

Corresponding Author: Attia O. Attia, Department of Biology, College of Science, Taif University, Taif, Kingdom of Saudi Arabia Tel: (+966)592271096

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many *Aloes* seem an endangered species because of over-collection of plants, destruction of plants in harvesting leaf exudates and destroyed of natural habitats[1]. To develop the medicinal values and to remove the gap among supply of plant material and demand, for sustainable use in future, it is very urgent to conserve this species. To develop effective conservation, breeding program and management strategies, since morpho-chemical characters are dependent on age and environment, it is important to use genetic analysis to characterize this economically and medicinally important genus[1].

Genus *Aloe* is a flowering succulents consisting of over 500 known species including shrubs, perennials and trees[2]. The habitat of approximately 50 species is the Arabian Peninsula and almost 30 species are present in western and central African countries. Worldwide, *Aloe* genus plants such as *A. vera*, *A. perryi*, *A. arborescens* and *A. ferox* are using widely for their medicinal properties[3].

Since ancient times, treatment of inflammatory conditions, gastrointestinal disorders and microbial infections were carried out using the Aloes. It has different uses such as cosmetic, beverage and food industries[4].

Analysis of phytochemical showed that several of carbohydrate polymers (notably glucomannans) and other phenolic compounds with low molecular weight are contained in many *Aloe* species[4]. Diverse DNA based molecular characterizations have been evolved as methods for analysis of variation and to establish similarity among cultivars and species[5]. RAPD is widely used as it permits a fast and low cost assay with distinctive primers[6]. Although the speed and simplicity of RAPD technique, it's been efficiently used to evaluate genetic structure and similarity analysis[7] and effectively applied to research of genetic variability in some genus such as *Gossypium*[8], *Eucalyptus*[9], *Asparagus*[10] and *Mangifera*[11].

ISSR technique, PCR based method; it is an inexpensive technique, convenience of use and high stage of reliability in reproducing consequences[12,13]. ISSRs have excessive reproducibility probable compared to RAPD primers (10 mers) because of using longer primers (16-25 mers) with high annealing temperature (45-60°C) leading to higher stringency. ISSR is used extensively and is approved as a method in genetic researches of both cultivated and wild plants[13].

In *Aloe* species, RAPD, amplified fragment length polymorphism (AFLP) and ISSR markers have been applied to investigate genetic diversity between *Aloe* species. Genetic analysis between 3 species *A. vera*, *A. arborescence* and *A. ferox* were performed using RAPD marker[14]. Analysis of genetic diversity using RAPD and morphological markers in some *Aloe* species was performed by Nayanakantha *et al*.[15]. Diversity of genetic amongst accessions of *Aloe* collected from different ecological regions in India and Iran, was assessed using RAPD, ISSR and AFLP markers[16,17]. As mentioned above, the morpho-chemical characters and genetic analysis are very important tools for improvement, breeding and conservation program of economically and medicinally important *Aloe* species. Therefore the main objectives of the current research are (1) Surveying, collecting and identification of *Aloe* species from Taif region. (2) Analysis of the genetic variations between the collected *Aloe* species using RAPD and ISSR as molecular markers.

MATERIALS AND METHODS

Plant materials: This study was carried out, since May 2018-June 2019, in plant molecular genetic and tissue culture laboratory, Deanship of Scientific Research, Taif University. Four *Aloe* species (*A. armatissima*, *A. edentata*, *A. parvicoma* and *A. pseudorubroviolacea*) and *A. americana* (Asperagaceae) were collected from different localities 5 years ago at Taif highlands of Saudi Arabia, it were transplanted at Taif University Campus. The collected wild materials were identified according to Collentette[18] and Chaudhary[19] as shown in Table 1.

Extraction of DNA: For DNA extraction, 0.1 g of fresh leaves tissues was collected from *A. americana, A. armatissima*, *A. edentata, A. parvicoma* and *A. pseudorubroviolacea* (Table 1). DNA extraction, quantity and quality of DNA were performed according to Attia *et al*.[20].

Table 1: Identification of 4 *Aloe* species *A. armatissima*, *A. edentata*, *A. parvicoma* and *A. pseudorubroviolacea*

Species	Chorotypes	Local distribution	Status
Aloe armatissima	Endemic	Sarawat mountains	Not endangered
Aloe edentata	Endemic	Sarawat mountains	Endangered
Aloe parvicoma	Endemic	Sarawat mountains	Endangered
Aloe pseudorubroviolacea	Endemic	Hejaz and Sarawat mountains	Not endangered

Source: Collenette[18] and Chaudhary[19]

Random amplified polymorphic DNA (RAPD): Twenty three RAPD primers, which were synthesized by Macrogen Inc. Biotechnology Company-Seoul-South Korea, were screened. Preparation and PCR reactions for DNA amplification were applied according to Attia *et al.*[20].

Inter simple sequence repeat (ISSR): Sixteen ISSR primers, from the University of British Columbia (UBC) series which were synthesized by Macrogen Inc. Biotechnology Company-Seoul-South Korea, were used. Amplification of DNA and evaluation of RAPD and ISSR products were carried out as described by Attia *et al.*[21].

Statistical analysis: For RAPD and ISSR analysis, the manual scoring present (1) or absent (0) was used for high resolution band patterns, average linkage between species was used to produce cluster analysis and dendrogram for RAPD, ISSR analysis by Hierarchical Cluster Analysis using (IBM SPSS Statistics Version 20).

RESULTS AND DISCUSSION

Ecological distribution study: The genus *Aloe* in Saudi Arabia includes 24 species, 45.8% of them are endangered endemic species , 16.7% of them are endemic but non-endangered and 37.5% are near endemic which recorded in Yemen and Saudi Arabia, while one is belonged to tropical region (*A. niebuhriana*) and other once belonged to Sudano-Zambezian region (*A. vera* var. *officinalis*). All of these species were recorded in Sarawat mountains[18,22]. On the other hand, the 4 species in present study are endemic: 2 of them are endangered (*A. edentata* and *A. parvicoma*) and the 2 others are not-endangered (*A. armatissima* and *A. pseudorubroviolacea*)[18,19], while *A. americana* was introduced as ornamental species (Table 1).

Genetic variation analysis

RAPD analysis: Based on RAPD analysis, 10 primers out of 23 primers evaluated, were produced reproducible, distinct and polymorphic bands (Table 2). About 134 reproducible bands were produced with an average 13.4 bands/primer, ranging from ~300-2500 bp. The representative RAPD profiles using primers OPB-01, OPD-02 and OPD-03 are shown in Fig. 1a and b, respectively.

Polymorphic bands were produced in all RAPD primers showing ~97.65% polymorphism between 4 *Aloe* species and *A. americana*. Whereas, mono-morphic bands number (MBN) were 3 bands. As shown in Fig. 1b, the profile of *A. edentata* (lane 8) generated with primer OPD-03, showed 8 bands while *A. americana*, *A. parvicoma* and *A. pseudorubroviolacea* (lanes 6, 9 and 10, respectively) showed 4 bands were different from the other profiles. All RAPD primers produced unique bands with most of 4 *Aloe* species and *A. americana* in this study. Das *et al.*[23] reported that RAPD analysis between eleven *A. vera* populations, yielded 138 polymorphic bands (~87.34% polymorphism) of 158 total amplicons, which indicating a wide genetic variability between populations.

Bhaludra *et al.*[17] reported that analysis of genetic variability of *A. vera* collected from different regions of Hyderabad, RAPD and ISSR analysis yielded 71.8 and 80.9% of polymorphism with 4.34 and 4.47 polymorphic bands/primer, respectively.

Rathore *et al.*[24] checked genetic stability of sweet variety of *A. vera in vitro* propagated plantlets by RAPD and ISSR markers. Nayanakantha *et al.*[15] studied genetic variation between *A. vera* accessions using RAPD markers.

Mehetre *et al.*[25] demonstrated that bands that are produced in all individuals considered as mono-morphic bands. On the other hand, bands which are not resulted in all individuals (one or more) are poly-morphic and that which are recorded in at least one individual are unique bands. Three

Table 2: RAPD primers and amplified products by RAPD analysis of 4 *Aloe* species and *Agave americana*

Code	(5'-3')	NTB	RBS (bp)	MBN	PBN	PB (%)
OPA-03	AGTCAGCCAC	18.0	1500-350	0.0	18.0	100.0
OPA-04	AATCGGGCTG	12.0	1800-550	0.0	12.0	100.0
OPB-01	GTTTCGCTCC	15.0	2500-480	1.0	14.0	93.3
OPB-05	TGCGCCCTTC	16.0	1300-350	0.0	16.0	100.0
OPC-01	TTCGAGCCAG	14.0	2500-350	0.0	14.0	100.0
OPC-04	CCGCATCTAC	9.0	1750-500	0.0	9.0	100.0
OPC-05	GATCACCGCC	12.0	2000-600	0.0	12.0	100.0
OPD-02	GGACCCAACC	12.0	1500-400	1.0	11.0	91.6
OPD-03	GTCGCCGTCA	14.0	2000-350	0.0	14.0	100.0
OPF-04	GGTGATCAGG	12.0	1500-300	1.0	11.0	91.6
Total		134.0	2500-300	3.0	131.0	-
Average		13.4	-	0.3	13.1	97.65

NTB: Number of total bands, RBS (bp): Range of band size (bp), MBN: Mono-morphic bands number, PBN: Poly-morphic bands number, PB: Polymorphic bands

Table 3: Similarity matrix between 4 *Aloe* species and *Agave americana* based on RAPD analysis

Species	*A. americana*	*A. armatissima*	*A. edentata*	*A. parvicoma*	*A. pseudorubroviolacea*
A. americana	1.000				
A. armatissima	0.31	1.000			
A. edentata	0.30	0.61	1.000		
A. parvicoma	0.33	0.51	0.54	1.000	
A. pseudorubroviolacea	0.19	0.40	0.39	0.32	1.000

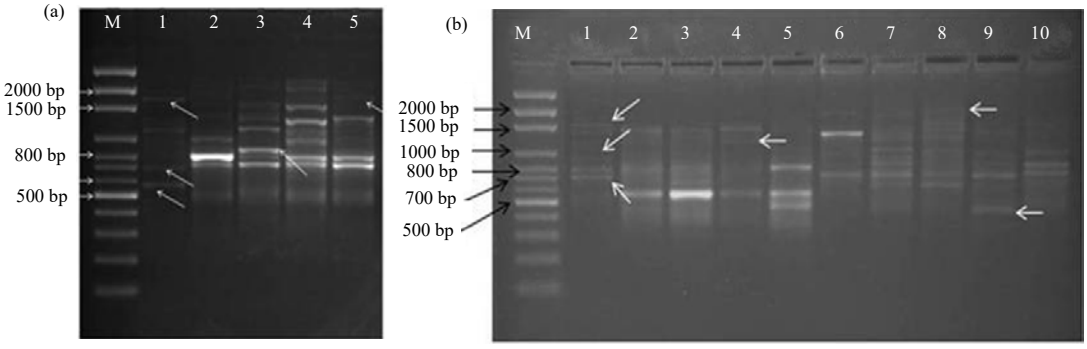

Fig. 1(a-b): RAPD analysis of 4 *Aloe* species and *Agave americana*, (a) Lanes 1-5 indicate to PAPD profiles of 4 *Aloe* species and *Agave americana* using primer OPB-01 and (b) Lanes 1-5 indicate to PAPD profiles of 4 *Aloe* species and *Agave americana* using primer OPD-02 and lanes 6-10 indicate to RAPD profiles of 4 *Aloe* species and *Agave americana* using primer OPD-03
M: DNA marker 100 bp

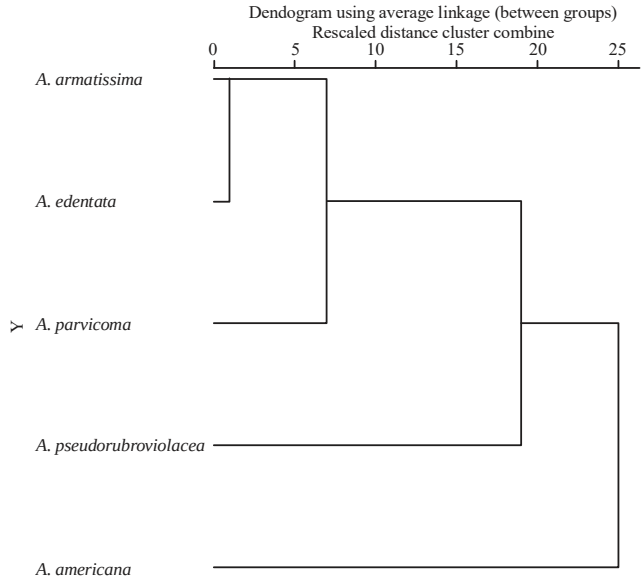

Fig. 2: Dendrogram showing genetic relationship between 4 *Aloe* species and *A. americana* based on RAPD analysis

unique bands, Fig. 1a, (1800, 750 and 550 bp) were resulted in *A. americana* species (lane1) generated with OPB-01 primer. However, the same primer generated 2 unique bands 900 and 1900 bp with species *A. edentata* (lane 3) and *A. pseudorubroviolacea* (lane 5) respectively. As shown in Fig. 1b, 3 unique bands (1500, 900 and 700 bp) were found in *A. americana* species (lane1) generated with OPD-02 primer. Whereas, one unique band 1200 bp was resulted in *A. parvicoma* species (lane 4) generated with the same primer. Two unique bands 2000 and 450 were recorded in *A. edentata* (lane 8) and *A. parvicoma* (lane 9) respectively, were generated with OPD-03 primer.

Das *et al*.[23] noticed that unique bands that present in population and were absent in others, could be used as population-specific diagnostic markers when it cloned, sequenced and converted into a locus-specific sequence characterized amplified region (SCAR), it could apply in future for identification of plants belonging to that population.

The similarity matrix Table 3, ranging from 19-62% indicate that there is a highly genetic variability between 4 *Aloe* species and *A. americana* investigated in current work. The cluster analysis based on the similarity showed that, 3 main clusters were resulted in the dendrogram according to the similarity matrix between 4 *Aloe* species and *A. americana*.

Cluster I consisted of 2 sub cluster, sub cluster 1 containing 2 species *A. armatissima* and *A. edentata* while sub cluster 2 containing *A. parvicoma*. Cluster 2 and 3 containing *A. pseudorubroviolacea* and *A. americana*, respectively (Fig. 2).

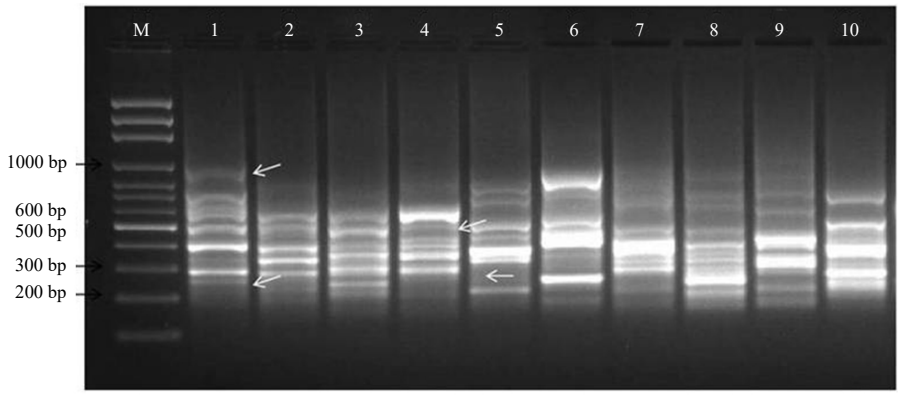

Fig. 3: ISSR analysis of *Agave americana* and 4 *Aloe* species

M: DNA marker 100 bp, lanes 1-5 and lanes 6-10 indicate to ISSR profiles of *A. americana* and 4 *Aloe* species using primers UBC-813and UBC-814, respectively

Table 4: ISSR primers and amplified products by ISSR analysis of 4 *Aloe* species and *Agave americana*

Codes	(5'-3')	NTB	RBS (bp)	MBN	PBN	PB (%)
UBC-805	$(TA)_8C$	13.0	1050-200	1.0	12.0	92.30
UBC-813	$(CT)_8T$	15.0	1000-220	2.0	13.0	86.70
UBC-814	$(CT)_8A$	14.0	1250-200	0.0	14.0	100.00
UBC-817	$(CA)_8A$	13.0	1100-300	0.0	13.0	100.00
UBC-818	$(CA)_8G$	12.0	1500-220	2.0	10.0	83.30
UBC-819	$(GT)_8A$	10.0	650-210	0.0	10.0	100.00
UBC-822	$(TC)_8A$	12.0	700-180	0.0	12.0	100.00
UBC-823	$(TC)_8C$	13.0	1250-220	1.0	12.0	92.30
UBC-826	$(AC)_8C$	11.0	900-220	0.0	11.0	100.00
Total		113.0	1500-180	6.0	107.0	-
Average		12.6	-	0.7	11.9	94.96

NTB: Number of total bands, RBS (bp): Range of band size (bp), MBN: Mono-morphic bands number, PBN: Poly-morphic bands number, PB: Polymorphic bands

According to the results of similarity matrix and the dendrogram, the highest similarity 62% was among *A. armatissima* and *A. edentata* followed by 54 and 51% similarity of *A. parvicoma* with *A. edentata* respectively, the lowest similarity 19% was between *A. americana* and *A. pseudorubroviolacea*. Within four *A.* species, lowest similarity 30% was resulted between *A. parvicoma* and *A. pseudorubroviolacea*.

Bhaludra *et al.*[17] found that RAPD analysis among twelve collected elite accessions of *A. vera* from different places of India showed 71.8% molecular polymorphism.

Rana and Kanwar[26] reported that similarity coefficient value ranged from 62-91% in RAPD analysis between 24 genotypes of *A. vera* L. collected from different provinces of Himachal Pradesh, India.

ISSR analysis: Hogbin and Peakall[27] reported that structure of species and phylogenetic considered as a results of interaction among different factors, such as evolution of species, geographical range, seed dispersal, mating method, gene flow and genetic drift.

In current work, out of 16 primers, 9 primers with high intensity and relatively high polymorphism bands were selected and used to amplify 4 *A.* species and *A. americana* (Table 4). According to analysis of ISSR, 113 reproducible bands were totally yielded with an average 12.6 bands/primer, from~180-1500 bp, of which poly-morphic bands number (PBN) 107 with an average of 11.9 PB/primer, on the other hand the mono-morphic bands number (MBN) were 6 bands, Fig. 3 showed representative ISSR profiles using UBC-813 and UBC-814 primers. The results indicated that the poly-morphic bands percentage (PB%) ranged from ~83.3% with an average 94.96%, indicating high genetic variability between four *Aloe* species and *A. americana* (Table 4).

According to ISSR analysis to evaluate genetic variation in twelve elite accessions of genus *A. vera*. Bhaludra *et al.*[17] they found that 85 bands, 81% polymorphism, of 105 total amplified bands. In the study to assess the genetic variation in *A. vera* L. genotypes from different provinces of Himachal Pradesh, India. ISSR analysis showed that 21 polymorphic with an average 87.5% polymorphism of 24 a total amplified bands[27].

Table 5: Similarity matrix between 4 *Aloe* species and *Agave americana* based on ISSR analysis

Species	*A. americana*	*A. armatissima*	*A. edentata*	*A. parvicoma*	*A. pseudorubroviolacea*
A. americana	1.000				
A. armatissima	0.44	1.000			
A. edentata	0.42	0.72	1.000		
A. parvicoma	0.41	0.69	0.65	1.000	
A. pseudorubroviolacea	0.35	0.45	0.58	0.49	1.000

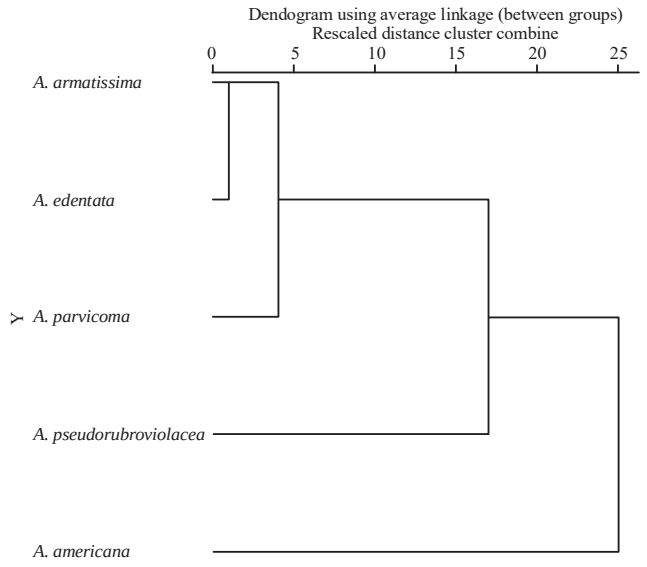

Fig. 4: Dendrogram showing genetic relationship between 4 *Aloe* species and *Agave americana* based on ISSR analysis

After ISSR the results showed that a lot of unique bands were generated with most of ISSR primers used in current study, as shown in Fig. 3, Two unique bands 900 and 290 bp were found in *A. americana* species (lane 1) generated with UBC-813 primer. Whereas, two unique bands 550 (positive band) and 300 pb (negative band) were recorded in *A. parvicoma* (lane 4) and *A. pseudorubroviolacea* (lane 5) respectively, were generated with the same primer. Bhaludra *et al*.[17] they reported that several unique bands have been resulted by ISSR analysis to evaluate genetic variation in *A. vera* germplasm collected from different geographical places of India. The cluster analysis (Fig. 4) based on the similarity showed that; 3 main clusters were resulted in the dendrogram according to the similarity matrix between *A. americana* and four *A.* species, cluster I consisted of 2 sub clusters, sub cluster one containing 2 species *A. armatissima* and *A. edentata* while sub cluster 2 containing *A. parvicoma*. Cluster 2 and 3 containing *A. pseudorubroviolacea* and *A. americana*, respectively. According to the results of similarity matrix (Table 5) and the dendrogram (Fig. 4), the highest similarity 72% was among *A. armatissima* and *A. edentata* followed by 69 and 65% similarity of *A. parvicoma* with *A. armatissima* and *A. edentata*, respectively, the lowest similarity 35% was noticed between *A. pseudorubroviolacea* and *A. americana*. Within 4 *A.* species, lowest similarity 45% was resulted between *A. parvicoma* and *A. pseudorubroviolacea*. Rana and Kanwar[26] demonstrated that similarity coefficient values ranged from 0.38-1 with ISSR primers that were used to assess the *A. vera* L. genotypes collected from different regions of Himachal Pradesh, India, these results are consistent with current results that showed that RAPD and ISSR analysis revealed high genetic variations between 4 *Aloe* species and *A. americana* species.

CONCLUSION

In conclusion, the results showed that RAPD and ISSR analysis revealed high genetic variations between 4 *Aloe* species and *A. Americana* species. These results will be applied for improvement, breeding and conservation programs of *Aloe* species in future.

SIGNIFICANCE STATEMENTS

The present study proved that it is important to use genetic analysis along with morphological characters to study and characterize *Aloe* species. These results will help us and other researchers to study the endangered and threatened medicinally and economically important wild plants especially in high altitude regions like Taif province.

ACKNOWLEDGMENT

Authors are very gratefully to "Vice-Presidency of Graduate Studies and Academic Research, University of Taif" for the financial support of this project (Ecological distribution and genetic variations of some *Aloe* species in Taif, KSA), the project number (1-438-5901).

REFERENCES

1. Oldfield, S., 1997. Cactus and Succulent Plants-Status Survey and Conservation Action Plan. IUCN/SSC Cactus and Succulent Specialist Group/IUCN, Gland, Switzerland, ISBN: 2-8317-0390-5, Pages: 212.

2. Viljoen, A.M. and B.E. van Wyk, 2001. A chemotaxonomic and morphological appraisal of *Aloe* series Purpurascentes, *Aloe* section Anguialoe and their hybrid, *Aloe broomii*. Biochem. Syst. Ecol., 29: 621-631.
3. Reynolds, T., 2004. Geographical Distribution. In: Aloes: The Genus *Aloe*, Reynolds, T. (Ed.)., CRC Press, Boca Raton, ISBN: 9780203476345, pp: 39-74.
4. Cork, I.E., 2015. The Genus *Aloe*: Phytochemistry and Therapeutic Uses Including Treatments for Gastrointestinal Conditions and Chronic Inflammation. In: Novel Natural Products: Therapeutic Effects in Pain, Arthritis and Gastro-intestinal Diseases, Rainsford, K., M. Powanda and M. Whitehouse (Eds.)., Springer, Basel, ISBN: 978-3-0348-0926-9, pp: 179-235.
5. Khanam, S., A. Sham, J.L. Bennetgen and A.M.A. Mohammed, 2012. Analysis of molecular marker-based characterization and genetic variation in date palm (*Phoenix dactylifera* L.). Aust. J. Crop Sci., 6: 1236-1244.
6. Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.
7. Gepts, P., 1993. The Use of Molecular and Biochemical Markers in Crop Evolution Studies. In: Evolutionary Biology, Hecht, M.K. (Edn.). Plenum Press, New York, pp: 51-94.
8. Multani, D.S. and B.R. Lyon, 1995. Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome, 38: 1005-1008.
9. Keil, M. and R.A. Griffin, 1994. Use of random amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. Theoret. Applied Genet., 89: 442-450.
10. Shasany, A.K., M.P. Darokar, D. Saika, S. Rajkumar, V. Sindaresan and S.P.S. Khanuja, 2003. Genetic diversity and species relationship in *Asparagus* spp. using RAPD analysis. J. Med. Aromatic Plant Sci., 25: 698-704.
11. Karihaloo, J.L., Y.K. Dwivedi, S. Archak and A.B. Gaikwad, 2003. Analysis of genetic diversity of Indian mango cultivars using RAPD markers. J. Hortic. Sci. Biotechnol., 78: 285-289.
12. Pence, V.C., 1999. The Application of Biotechnology for the Conservation of Endangered Plant Species. In: Plant Conservation Biotechnology, Benson, E.E. (Ed.). University of London, London, pp: 227-242.
13. Gaara, A.H., M.I. Nassar, M. Younis, G.A. Elmegeed, T.J. Mabry and P.W. Pare, 2008. Biologically active polyphenolic compounds from *Acacia ehrenbergiana*. Rev. Latinoamer. Quim., 36: 52-59.
14. Darokar, M.P., R. Rai, A.K. Gupta, A.K. Shasany, S. Rajkumar, V. Sundaresan and S.P.S. Khanuja, 2003. Molecular assessment of germplasm diversity in *Aloe* species using RAPD and AFLP analysis. J. Med. Arom. Plant Sci., 25: 354-361.
15. Nayanakantha, N.M.C., B.R. Singh and A.K. Gupta, 2010. Assessment of genetic diversity in *Aloe* germplasm accessions from India using RAPD and morphological markers. Ceylon J. Sci. Biol. Sci., 39: 1-9.
16. Tripathi, N., N. Saini and S. Tiwari, 2011. Assessment of genetic diversity among *Aloe vera* accessions using amplified fragment length polymorphism. Int. J. Med. Arom. Plants, 1: 115-121.
17. Bhaludra, C.S.S., H. Yadla, F.S. Cyprian, R.R. Bethapudi, S.D. Basha and R.R. Anupalli, 2014. Genetic diversity analysis in the genus *Aloe vera* (L.) using RAPD and ISSR markers. Int. J. Pharmacol., 10: 479-486.
18. Collenette, S., 1999. Wild Flowers of Saudi Arabia. National Commission for Wildlife Conservation and Development (NCWCD), Riyadh, Pages: 799.
19. Chaudhary, S.A., 2001. Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and Water, Riyadh, Saudi Arabia, Pages: 542.
20. Attia, A.O., E.D.S. Dessoky, Y.M. Al-Sodany and I.A. Ismail, 2017. *Ex situ* preservation for some endemic and rare medicinal plants in Taif, KSA. Biotechnol. Biotechnol. Equip., 31: 912-920.
21. Attia, A.O., E.D.S. Dessoky and Y.M. Al-Sodany, 2017. Genetic diversity and *in vitro* propagation of some *Acacia* spp. trees grown in Taif governorate. Biosci. Res., 14: 924-933.
22. Al-Nafie, A., 2004. Plant Geography of Saudi Arabia. Riyadh, pp: 659 (In Arabic).
23. Das, A., K. Nandagopal and T.B. Jha, 2017. Molecular characterization of some Indian *Aloe vera* populations through RAPD and ITS markers. Plant Biosyst: Int. J. Deal. Aspects Plant Biol., 151: 695-703.
24. Rathore, M.S., J. Chikara and N.S. Shekhawat, 2011. Plantlet regeneration from callus cultures of selected genotype of *Aloe vera* L.-An ancient plant for modern herbal industries. Applied Biochem. Biotechnol., 163: 860-868.
25. Mehetre, S.S., M. Gomes and S. Eapen, 2004. RAPD analysis of hybrid nature of the offspring of *Gossypium hirsutum* x *G. raimondii*. Curr. Sci., 87: 25-28.
26. Rana, S. and K. Kanwar, 2017. Assessment of genetic diversity in *Aloe vera* L. among different provinces of H.P. J. Med. Plants Stud., 5: 348-354.
27. Hogbin, P.M. and R. Peakall, 1999. Evaluation of the contribution of genetic research to the management of the endangered plant *Zieria prostrata*. Conserv. Biol., 13: 514-522.