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Genetic Diversity Analysis in the Genus *Aloe vera* (L.) Using RAPD and ISSR Markers

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Abstract: *Aloe vera* L. (*Aloe barbadensis* Miller) is an ayurvedic, xerophytic, succulent, medicinal plant commonly known as *Aloe vera* and it is used worldwide in drug and cosmetic industry. In the present investigation, genetic diversity in 12 elite accessions of *Aloe vera*, collected from different geographical regions of India, were evaluated using Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers. Molecular polymorphism was 71.8% with 64 RAPD primers and 80.9% with 25 ISSR primers indicating high level of genetic variation among the accessions and the mantel test revealed positive correlation between the two marker systems. Dendrogram was constructed based on pair wise genetic similarities and two-dimensional principal coordinate analysis using data from RAPD and ISSR marker systems showed similar clustering pattern and separated accessions into two major groups. The accession IC111279 and IC111272 appeared to be more divergent with 14.3% similarity, while high similarity of 84.7% was recorded between IC111280 and IC111279. The study clearly indicates that RAPD and ISSR marker profiles were best-suitable for assessing genetic relationships among *Aloe vera* accessions.

Key words: Genetic diversity, RAPD markers, ISSR markers, cluster analysis, *Aloe vera*

INTRODUCTION

Aloe vera is an ancient, perennial plant found in tropical and subtropical areas, particularly in South Africa and Arabia. The name *Aloe vera* was derived from the Arabic word 'alloeh' meaning 'shining bitter substance'. Aloe species are generally recognized by their rosettes and propagated worldwide for pharmacological and cosmetic industries (Bhaludra *et al*., 2013). Although about 360 species of *A. vera* have been reported, only *Aloe barbadensis* Miller commonly referred as "Ghrith Kumari" in Hindi and has become naturalized almost in all regions of India (Klein and Penneys, 1988). *Aloe vera* is the most important among the *Aloe* species and it can be found growing in the tropical climates of the South America, Southern United States, Central America, India, Australia, the Caribbean and Africa. Traditionally, *A. vera* is grown as an ornamental plant and used in herbal medicine. However, the commercial use of the *Aloe vera* plant was in the production of a latex (yellowish sap) substance called aloin used for many years as a laxative and purgative ingredient (Boudreau and Beland, 2006). *Aloe vera* gel has been used in cosmetics like moisturizers, sunscreens, soaps and shampoos. Aloe juice has been shown to lower cholesterol and triglycerides while demonstrating anti diabetic activity. Various scientific studies on aloe gel have demonstrated for its anti-inflammatory, wound healing, immune modulating, analgesic, anti-tumor activities, anti-bacterial, antifungal and antiviral properties (Manvitha and Bidya, 2014). It accelerates, regulates the metabolism and purifies the toxins from human body. In order to improve the medicinal values of *Aloe vera* and also to fill the gap between demand and supply of elite plant material, there is a need to conserve this species for sustainable use in future. The success of any genetic conservation and breeding program depends largely on the identification of the amount and distribution of genetic diversity in the gene pool of the concerned plant. Knowledge on the genetic diversity and relationships among plant varieties is important to recognize gene pools, to identify gaps in germplasm collections and to develop effective

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conservation and management strategies. Since morpho-chemical characters are dependent on age and environment, it is essential to characterize this medicinally and economically important genus genetically (Nayanakantha *et al*., 2010). In recent years, DNA-based molecular markers have been used to assess the genetic diversity among the germplasm in many plant species. DNA based molecular markers have the advantage of being free from environmental modulations. In *A. vera*, RAPD and AFLP markers have been used to study the genetic diversity among different *Aloe* species (Darokar *et al*., 2003; Shioda *et al*., 2003) using RAPD markers identified three species of Aloe (*Aloe vera*, *A. arborescence*, *Aloe ferox*). Nejatzadeh-Barandozi *et al*. (2012) and Tripathi *et al*. (2011) has used RAPD and AFLP markers to assess genetic diversity among aloe accessions obtained from different geographical regions of Madhya Pradesh in India and Iran, respectively.

In this current investigation, genetic diversity was evaluated in *Aloe vera* germplasm obtained from different geographical regions of India using RAPD and ISSR primers keeping in view the low development and running costs per data point besides detection of genome-wide variation.

MATERIALS AND METHODS

Collection of plant material: Twelve accessions of *Aloe vera* germplasm (NBPGR, New Delhi, India vide voucher no: NBPGR/2011/1771 dated 11.07.2011) representing totally from different geographical regions of India were obtained from Rajasthan (IC111267, IC111269, IC111271, IC111272), Gujarat (IC111279), Haryana (IC111280), Delhi (IC471882, IC471883, IC471884 and IC471885) and Central Institute of Medicinal and Aromatic Plants (CIMAP) Research Center, Boduppal, Hyderabad, Telangana State, India (*Aloe* CIM-Sheetal and commonly grown wild *Aloe vera*-local) vide voucher No: CIMAP/63/6222 dated 20.07. 2011. All the plants utilized in the present study were about two years of age. In all cases, the predominantly grown accession was used. In case of some states, where variation has been reported different provenances were collected. Accordingly, the accessions from the different regions of collection from Rajasthan, Gujarat, Haryana, Delhi, Uttarpradesh and Telangana State; the germplasm was maintained at the research farm of Indian Immunologicals Ltd, Hyderabad, India and the details of the accessions are presented in Table 1.

DNA extraction: Total genomic DNA was extracted from younger leaves of 12 *Aloe vera* accessions by following the standard CTAB method with minor modifications (Doyle and Doyle, 1990). Two grams of leaves were ground in liquid nitrogen, then homogenized in 10 mL of extraction buffer (4% CTAB, 20 mM EDTA, 2% PVP, 1.4 M NaCl, 100 mM Tris-HCl pH 8.0 and 1% β mercapto ethanol) and incubated at 65°C for 30 min. The supernatant was twice extracted with chloroform: isoamylalcohol (24:1, v/v) and treated with 2 μL RNase A (100 mg/mL), incubated at 37°C for 1 h. The DNA was precipitated with isopropanol and washed twice with 70% ethanol. The pelleted DNA was air dried and re suspended in 500 μL of sterile Millipore water and finally all DNA samples were diluted to get 50 ng/μL and were stored at -20°C for use in RAPD and ISSR assay (Table 2).

RAPD analysis: A set of 64 decamer primers (Table 3) synthesized from Integrated and Technologies (IDT) were used for DNA amplification with minor modifications (Williams *et al*., 1990). The PCR amplification was performed in 10 μL reaction volume containing of 5 ng of genomic DNA, 1x PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 100 μM of each the 4 dNTPs, 0.4 μM of RAPD primer and 0.3 U of *Taq* DNA polymerase (Bangalore Genei). PCR amplifications were performed in Gene Amp 9700 Thermal Cycler (Perkin Elmer Applied Biosystems) with an initial denaturation at 94°C for 3 min followed by 45 cycles of 94°C for 45 sec, 36°C for 30 sec and 72°C for 2 min with a final extension of 72°C for 7 min. The PCR amplified products were separated on 1.5%

Table 1: Details of *Aloe vera* accessions used in diversity analysis

Species	Accession	Obtained from	Source
Aloe barbadensis Mill	IC 111267	Rajasthan	NBPGR, New Delhi
Aloe barbadensis Mill	IC 111269	Rajasthan	NBPGR, New Delhi
Aloe barbadensis Mill	IC 111271	Rajasthan	NBPGR, New Delhi
Aloe barbadensis Mill	IC 111272	Rajasthan	NBPGR, New Delhi
Aloe barbadensis Mill	IC 111279	Gujarat	NBPGR, New Delhi
Aloe barbadensis Mill	IC 111280	Haryana	NBPGR, New Delhi
Aloe barbadensis Mill	IC471882	Delhi	NBPGR
Aloe barbadensis Mill	IC471883	Delhi	NBPGR
Aloe barbadensis Mill	IC471884	Delhi	NBBPGR
Aloe barbadensis Mill	IC471885	Delhi	NBPGR
Aloe barbadensis Mill	*Aloe CIM-Sheetal* (CAL14)	Telangana state	CIMAP, Hyderabad
Aloe barbadensis Mill	Wild *Aloe vera* (local)	Telangana state	CIMAP, Hyderabad

Table 2: Jaccard's similarity coefficient values of 12 *Aloe vera* accessions based on pooled data of RAPD and ISSR primers

Parameters	IC111267	IC111269	IC111271	IC111272	IC111279	IC111280	IC471882	IC471883	IC471884	IC471885	CIM Sheetal	Wild *Aloe vera*
IC111267	1.000											
IC111269	0.199	1.000										
IC111271	0.483	0.159	1.000									
IC111272	0.462	0.156	0.489	1.000								
IC111279	0.166	0.701	0.162	0.143	1.000							
IC111280	0.176	0.661	0.188	0.164	0.847	1.000						
IC471882	0.260	0.536	0.263	0.236	0.595	0.651	1.000					
IC471883	0.346	0.379	0.361	0.312	0.425	0.562	0.669	1.000				
IC471884	0.307	0.433	0.320	0.247	0.500	0.550	0.710	0.754	1.000			
IC471885	0.195	0.615	0.169	0.145	0.745	0.726	0.574	0.449	0.524	1.000		
CIM-sheetal	0.192	0.675	0.183	0.158	0.794	0.772	0.559	0.403	0.488	0.775	1.000	
Wild-*Aloe vera*	0.197	0.630	0.170	0.145	0.757	0.737	0.627	0.453	0.545	0.765	0.824	1.000

Table 3: List of RAPD primers

Primer code	Primer sequence 5'-3'	Primer code	Primer sequence 5'-3'
OPA-01	CAGGCCCTTC	OPD-15	CATCCGTGCT
OPA-02	TGCCGAGCTG	OPD-17	TTTCCCACGG
OPA-03	AGTCAGCCAC	OPE-07	AGATGCAGCC
OPA-04	AATCGGGCTG	OPE-08	TCACCACGGT
OPA-05	AGGGGTCTTG	OPK-04	CCGCCCAAAC
OPA-06	GGTCCCTGAC	OPK-08	GAACACTGGG
OPA-07	GAAACGGGTG	OPL-01	GGCATGACCT
OPA-08	GTGACGTAGG	OPL-05	ACGCAGGCAC
OPA-09	GGGTAACGCC	OPL-06	GAGGGAAGAG
OPA-10	GTGATCGCAG	OPL-10	TGGGAGATGG
OPA-13	CAGCACCCAC	OPL-11	ACGATGAGCC
OPA-14	TCTGTGCTGG	OPL-12	GGGCGGTACT
OPA-15	TTCCGAACCC	OPL-18	ACCACCCACC
OPA-16	AGCCAGCGAA	OPM-12	GGGACGTTGG
OPA-17	GACCGCTTGT	OPN-02	ACCAGGGGCA
OPA-18	AGGTGACCGT	OPN-03	GGTACTCCCC
OPA-20	GTTGCGATCC	OPN-04	GACCGACCCA
OPB-03	CATCCCCCTG	OPN-05	ACTGAACGCC
OPB-04	GGACTGGAGT	OPN-06	GAGACGCACA
OPB-16	TTTGCCCGGA	OPN-07	CAGCCCAGAC
OPB-17	AGGGAACGAG	OPN-09	TGCCGGCTTG
OPB-18	CCACAGCAGT	OPN-10	ACAACTGGGG
OPC-01	TTCGAGCCAG	OPN-11	TCGCCGCAAA
OPC-02	GTGAGGCGTC	OPN-12	CACAGACACC
OPC-03	GGGGGTCTTT	OPN-13	AGCGTCACTC
OPC-05	GATGACCGCC	OPO-20	ACACACGCTG
OPC-06	GAACGGACTC	OPP-09	GTGGTCCGCA
OPC-07	GTCCCGACGA	OPP-10	TCCCGCCTAC
OPC-08	TGGACCGGTG	OPP-11	AACGCGTCGG
OPC-09	CTCACCGTCC	OPAJ-02	TCGCACAGTC
OPC-11	AAAGCTGCGG	OPAJ-04	GAATGCGACC
OPC-13	AAGCCTCGTC	OPAK-03	GGTCCTACCA

agarose gel in 1x TAE buffer by electrophoresis at 100 V for 2 h and visualized with ethidium bromide staining and the gels were documented using an Alpha Image Gel Documentation System.

ISSR analysis: A total of twenty five ISSR primers (UBC primer set No. 9) were used for the analysis (Table 4). The PCR reaction mixture (10 μL) consisted of 5 ng of genomic DNA, 1x PCR buffer (10 mM Tris pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 200 μM of each the four dNTPs, 0.4 μM of ISSR primer and 0.3 U of Taq DNA polymerase (Bangalore Genei). PCR amplifications were performed using a GeneAmp 9700 Thermal Cycler (Perkin Elmer Applied Biosystems) with initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 30 sec, 1 min at the annealing temprature (Ta), 72°C for 2 min with a final extension of 72°C for 7 min. The amplified products were electrophoresed at 100 V on a 1.7% agarose gel using *Eco*RI and *Hind*III double digest as the molecular weight standard.

Statistical analysis: For each RAPD and ISSR primer, the presence or absence of bands in each accession was visually scored and set in a binary matrix. The number of polymorphic and monomorphic fragments for each primer pair was scored and the monomorphic markers were

Table 4: List of ISSR primers

Primer code	Primer sequence 5'-3'
UBC-807	AGAGAGAGAGAGAGAGT
UBC-808	AGAGAGAGAGAGAGAGC
UBC-809	AGAGAGAGAGAGAGAGG
UBC-810	GAGAGAGAGAGAGAGAT
UBC-811	GAGAGAGAGAGAGAGAC
UBC-813	CTCTCTCTCTCTCTCTT
UBC-816	CACACACACACACACAT
UBC-817	CACACACACACACACAA
UBC-818	CACACACACACACACAG
UBC-819	GTGTGTGTGTGTGTGTA
UBC-820	GTGTGTGTGTGTGTGTC
UBC-825	ACACACACACACACACT
UBC-827	ACACACACACACACACG
UBC-830	TGTGTGTGTGTGTGTGG
UBC-840	GAGAGAGAGAGAGAGAYT
UBC-847	CACACACACACACACARC
UBC-848	CACACACACACACACARG
UBC-860	TGTGTGTGTGTGTGTGRA
UBC-861	ACCACCACCACCACCACC
UBC-862	AGCAGCAGCAGCAGCAGC
UBC-867	GGCGGCGGCGGCGGCGGC
UBC-873	GACAGACAGACAGACA
UBC-880	GGAGAGGAGAGGAGA
UBC-892	TAGATCTGATATCTGAATTCCC
UBC-894	TGGTAGCTCTTGATCANNNNN

excluded from the analysis. The binary matrices were read by NTSYS-pc version 2.02i (Rohlf, 1997) with Jaccard's similarity coefficients and estimates of genetic distances for all pair wise comparisons between accessions were determined using Similarity for Qualitative Data (SIMQUAL). Dendrograms were created independently for both the marker systems and also based on pooled marker data using Unweighted Pair Group Method with Arithmetical Averages (UPGMA). The correlation between matrices was determined using Mantel test. Principal coordinate analysis was also performedand the ordination displayed in two dimensions.

RESULTS

Both the marker systems being employed to assess the genetic diversity in *Aloe vera* accessions were quite informative and were able to generate adequate polymorphism (Fig. 1a, b) among the *Aloe vera* accessions tested.

RAPD analysis: Out of 64 RAPD primers tested, 61 primers produced amplification products, of which 58 revealed polymorphic fingerprint patterns. The number of bands amplified per primer varied from 4 (OPA-5) to 17 (OPA-3). A total of 351 bands were amplified, of which 252 were observed to be polymorphic resulting in a polymorphism frequency of 71.8% and average of 4.34 polymorphic bands/primer. The molecular size of the

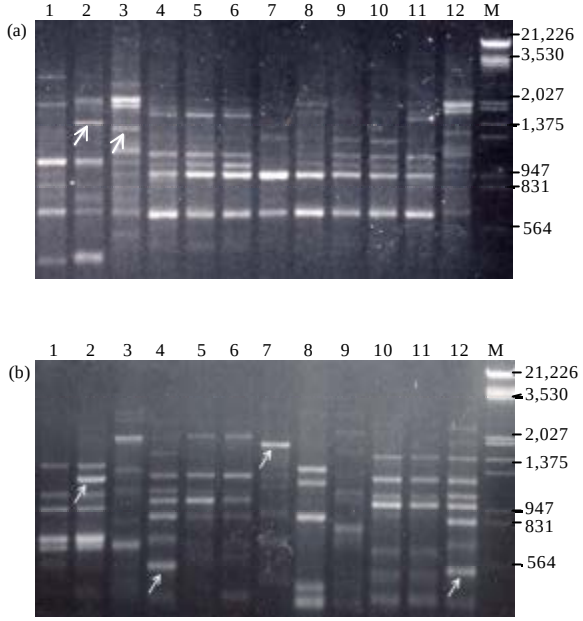

Fig. 1(a-b): (a) RAPD and (b) ISSR profile of 12 *Aloe vera* accession amplified with OPA-09 and UBC-861 Primer. Lanes 1-12 the samples used in the study as listed in Table 1 and lane M represents λ DNA double digest with *Eco*RI and *Hind* III restriction enzymes. Arrows indicates the accession specific bands

bands varied between 100-2600 bp and several accession specific bands have been identified (Fig. 1a). The extent of polymorphism per primer ranged from with ten alleles per primer OPA-9 (0.98%) to OPA-17 (0.87%) with three alleles per primer. The similarity matrix values using Jaccard's coefficient based on RAPD markers ranged from 0.082 between IC111272 and IC111269 to 0.890 between accessions IC111280 and IC111279. At 17% similarity, the accessions separated out into two major clusters and clustering of accessions based on dendrogram and PCO analysis was similar in pattern.

ISSR analysis: Of the 25 ISSR primers screened, 24 primers produced amplification products, of which 19 primers revealed polymorphic loci across the *Aloe vera* accessions tested. A total of 105 bands were amplified of which 85 amplicons were observed to be polymorphic resulting in polymorphism of 80.9%. The average number of polymorphic amplicons per primer was 4.47%. The number of bands amplified per primer varied between 2 (UBC primer No. 860) and 14 (UBC primer No. 867). The size of the amplicons varied between 100-2800 bp and

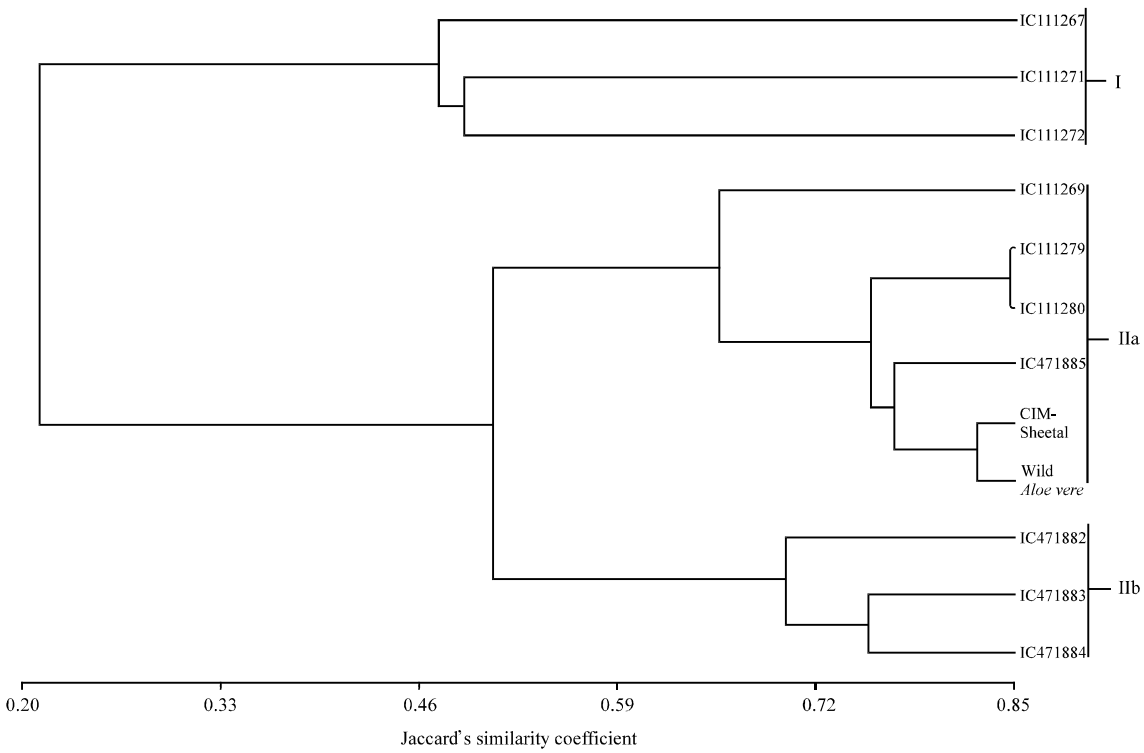

Fig. 2: Dendogram (UPGMA) representing genetic relationships among 12 accessions of *Aloe vera* based on Jaccard's similarity coefficients obtained using the pooled allelic profile of RAPD+ISSR primers

several accession specific bands have been identified as in Fig. 1b. The extent of polymorphism varied with ten alleles between UBC 867 (0.98%) to UBC 861 (0.82%) with two alleles per primer. Similarity matrix values based on ISSR markers ranged from 0.171 between IC111280 and IC111267 to 0.745 between accessions IC471883 and IC471882. Dendrogram analysis separated the accessions into two clusters at 29% similarity. Clustering of accessions based on dendrogram and PCO analysis was similar for most accessions except for the accessions IC111269 which is separately sub-grouped in cluster II.

Combined RAPD and ISSR analysis: The genetic similarity matrix data generated using RAPD and ISSR systems were compared. Mantel test for congruence of RAPD and ISSR data matrices indicated a goodness of fit (r = 0.87158) indicating good correlation between the two molecular marker systems. Although the two marker systems sampled different segments of the genome, the clustering pattern of the genotypes was almost similar with both the marker systems and most of the accessions were placed in their respective clusters with minor changes.

The genetic relatedness of the accessions was determined using Jaccard's similarity coefficients. Cluster analysis (UPGMA) performed from combining polymorphic data of both markers (RAPD and ISSR) generated a dendrogram that separated the accessions into two major clusters at 48% of variation (Fig. 2). The first cluster (I) comprised IC111267, IC111271 and IC111272. The second cluster (II) further divided into 2 sub-clusters. Among them the first sub-cluster (IIa) comprised of IC111269, IC111279, IC111280, IC 471885, wild *Aloe vera* and *Aloe CIM-Sheetal* (CAL14) and the second sub-cluster (IIb) comprised of IC471882, IC471883 and IC471884. The accession IC111280 and IC111279 appeared to be closer to each other with a similarity coefficient of 0.847 (Table 2), while the divergent accessions were IC111279 and IC111272 (similarity coefficient 0.143). Principal Coordinate Analysis (PCO) based on genetic similarity showed the relationship among accessions in two dimensional spaces. The PCO analysis based on pooled data of RAPD and ISSR primers grouped the accessions into three groups as given in Fig. 3 which is similar to the UPGMA clustering pattern.

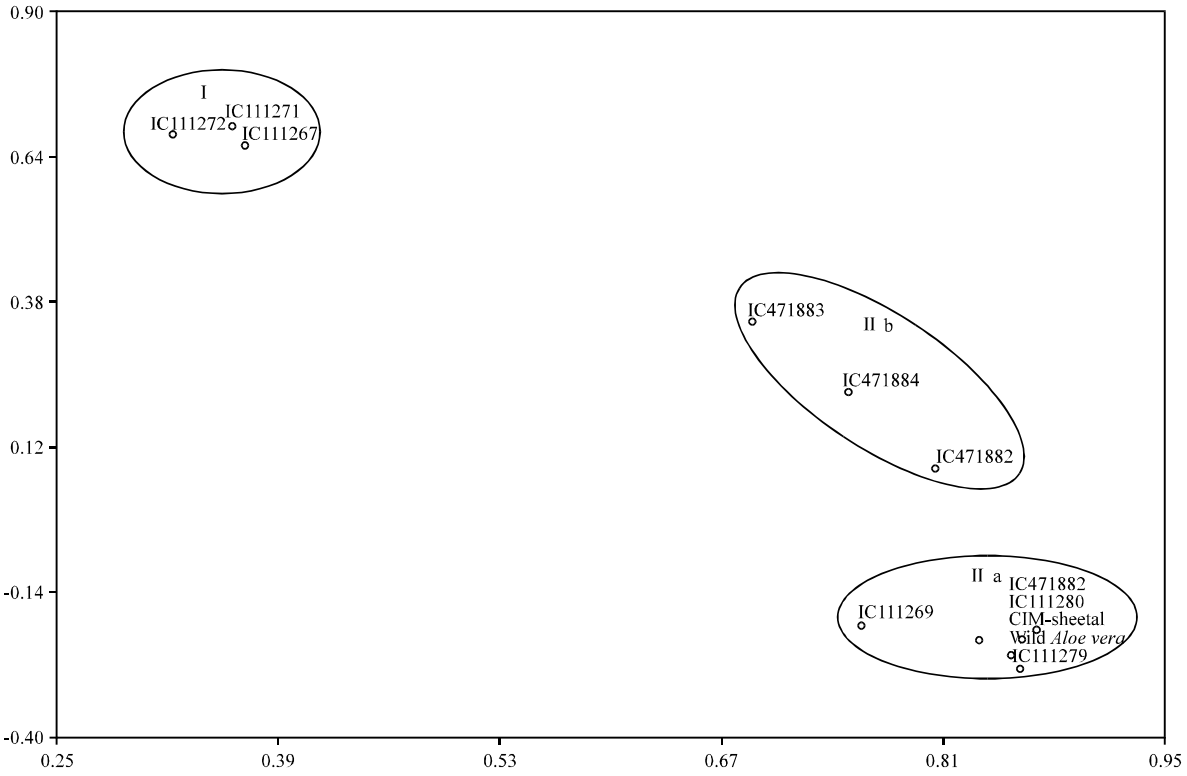

Fig. 3: Two-dimensional scaling of *Aloe vera* accessions by principal component analysis using pooled data of RAPD and ISSR primers

DISCUSSION

An understanding of the extent of genetic diversity is important for both plant breeding and germplasm collection. In *Aloe vera* traditional methods like horticultural traits are relatively less reliable and inefficient for precise discrimination of closely related genotypes (Nejatzadeh-Barandozi *et al*., 2012). Hence, selection based on genetic information using neutral molecular markers is essential as it is more reliable and consistent. Among different marker systems available, RAPD and ISSR markers became popular in diversity studies because of simplicity, rapid, inexpensive and applicable to any genome without any prior information regarding the genome of the plant.

In the present study, a set of 12 elite *Aloe vera* accessions (Table 1) were analyzed using 64 RAPD and 25 ISSR markers to describe the genetic structure among the accessions. The RAPD primers revealed 71.8% polymorphism with 4.34 polymorphic bands/primer, while ISSR primers revealed 80.9% polymorphism with 4.47 polymorphic bands/primer indicating wide genetic variation among the accessions. ISSR primers detect more polymorphism than RAPD primers because of variability in microsatellite loci due to DNA slippage (Williams *et al*., 1990). The RAPD markers cover the entire genome in coding and non coding regions including repeated or single-copy sequences, while ISSR markers disclose polymorphism from sequences between two microsatellite primer sites (Williams *et al*., 1990; Zietkiewicz *et al*., 1994). The ISSR method has been reported to be more reproducible (Goulao and Oliveira, 2001) and produces more complex marker patterns than the RAPD approach (Chowdhury *et al*., 2002; Parsons *et al*., 1997) reported that is an advantageous when differentiating closely related cultivars. Both the marker techniques provides a useful approach for evaluating genetic differentiation, significantly in those species that are poorly known genetically and are propagated vegetatively like monocot genus in *Musa* (Bhat and Jarret, 1995) and *Lilium* (Haruki *et al*., 1998).

The PCO analysis and dendrogram constructed based on RAPD+ISSR polymorphism showed similar clustering pattern and disclosed predominantly two major clusters (I and II). The similarity values ranging from 14 to 85% indicating that there is a remarkable genetic

variation among *Aloe vera* accessions used in the present study. The highest similarity (85%) was recorded between IC111280 and IC111279 accessions followed by 82% similarity between wild *Aloe vera* and *Aloe CIM-Sheetal* accessions which were collected from CIMAP Research Center, Hyderabad, India. Recently, AFLP based characterization of *Aloe vera* accessions from different location of Madhya Pradesh, India has reported modest level of genetic variability (Tripathi *et al*., 2011). This low level of variability could be because of small sample size collected from the limited geographical regions. Similarly, Darokar *et al*. (2003) has also reported the morphological similarity 78.8-99% in *Aloe vera* accessions revealed by RAPD and AFLP analysis. Nayanakantha *et al*. (2010) have reported a good amount of genetic variability among *Aloe vera* accessions based on RAPD analysis.

CONCLUSION

Apparently, the present study constitutes the presence of wide genetic variability among the *Aloe vera* accessions obtained from NBPGR, New Delhi, India. This variability can be used for genetic improvement through breeding programs and the accession specific bands were identified in this study will provide tags for future genetic improvement as well as in authenticating the genotypes. Both the markers techniques (RAPD and ISSR) have been shown to be useful in detecting small genetic variations within and among *Aloe vera* populations.

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REFERENCES

Bhaludra, C.S.S., R.R. Bethapudi, A.C. Murugulla, C. Pullagummi and T. Latha *et al*., 2013. Cultivation, phytochemical studies, biological activities and medicinal uses of *Aloe ferox*, Grandfather of Aloes an important amazing medicinal plant. Int. J. Pharmacol., 9: 405-415.

Bhat, K.V. and R.L. Jarret, 1995. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. Genet. Resour. Crop Evol., 42: 107-118.

Boudreau, M.D. and F.A. Beland, 2006. An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Mill.), *Aloe vera*. J. Environ. Sci. Health Part C: Environ. Carcinog. Ecotoxicol. Rev., 24: 103-154.

Chowdhury, M.A., B. Vanderberg and T. Warkentin, 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica, 127: 317-325.

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* spp. using RAPD and AFLP analysis. J. Med. Arom. Plant Sci., 25: 354-361.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Goulao, L. and C.M. Oliveira, 2001. Molecular characterisation of cultivars of apple (*Malus*× *domestica* Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica, 122: 81-89.

Haruki, K., T. Hosoki and Y. Nako, 1998. RAPD analysis of *Lilium japonicum* Thunb. native to Shimane and other prefectures (Japan). J. Japanese Soc. Hort. Sci. (Japan), 67: 785-791.

Klein, A.D. and N.S. Penneys, 1988. *Aloe vera*. J. Am. Acad. Dermatol., 18: 714-720.

Manvitha, K. and B. Bidya, 2014. *Aloe vera*: A wonder plant its history, cultivation and medicinal uses. J. Pharmacogn. Phytochem., 2: 85-88.

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.

Nejatzadeh-Barandozi, F., M.R. Naghavi, M.E. Hassani, Y. Mostofi, A. Mousavi and S. Tahmasebi Enferadi, 2012. Diversity of Iranian Aloe (*Aloe vera* L.) genotypes based on aloenin contents and some morphological traits. The J. Hortic. Sci. Biotechnol., 87: 673-677.

Parsons, J.B., H.T. Newbury, M.T. Jackson and B.V. Ford-Lloyd, 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breed., 3: 115-125.

Rohlf, F.J., 1997. NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System. Version 2.02i, Exerter Software, Setauket, New York.

Shioda, H., H. Satoh, F. Nagai, T. Okubo and T. Seto *et al*., 2003. Identification of *Aloe* species by random amplified polymorphic DNA (RAPD) analysis. Shokuhin Eiseigaku Zasshi, 44: 203-207.

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.

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.

Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by Simple-Sequence Repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.