The Bioscan 9(1): 07-10, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

DIVERSITY ANALYSIS OF DIFFERENT ACCESSIONS OF *ALOE BARBADENSIS* MILL. (SYN. *ALOE VERA* .L) COLLECTED FROM RAJASTHAN USING RAPD MARKER SYSTEM

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KEYWORDS
RAPD
Diversity analysis
Accessions

Received on :
30.06.2013

Accepted on :
15.10.2013

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ABSTRACT

Under a survey and collection programme of National Agricultural Technology Project (NATP), various accessions of *Aloe vera* were collected from Rajasthan and Gujarat. In this study 10 Accessions from Rajasthan were characterized through RAPD. RAPD revealed 32.08 per cent polymorphic bands detecting 11.9% average diversity among the accessions studied. The diversity ranged from 4.3% to 20.4%. The primers like OPG-15 having high PIC value (0.346) are considered important for diversity studies, whereas, OPG-14 have highest Discrimination index (0.911) and may be used for identification of different accessions. The clustering analysis resulted in the formation of one group, only consisting of eight accessions while Nagour collections remained out of cluster. The diversity pattern did not show any correlation with the site of collection indicating that original introduction consisted of small sample size its spread was random.

INTRODUCTION

Aloe is a xerophytic, succulent and perennial plant with multiple tuberous roots and many fibrous supporting roots penetrating into the soil (Lal *et al.,* 2002). It is not a cactus, but a member of the Lily family, known as *Aloe barbadensis*. There are over 250 species of *Aloe* grown around the world. However, only two species are grown today commercially, viz., *Aloe barbadensis* Mill. (*Aloe vera*) and *Aloe aborescense*. Since the earliest days of recorded history, man has made use of *Aloe* plants. The Chinese were among the earliest people who used the plant for its medicinal qualities. The *Aloe* as the "cure all diseases plant" finds use in traditional medicine in many countries (Crosswhite & Crosswhite , 1984). The commercial use of the *Aloe* plant was in the production of latex called Aloin. Aloin, the main purgative principle is a mixture of anthracene derivatives and chemically, it is 10-glucopyranosyl derivatives of *Aloe* emodin anthrone [10-(1-deoxyglycosyl)]. A number of paramedical publications extol its ability to promote healing of burns and their cutaneous injuries and of ulcers of mucous membranes. It acts as a powerful anti-inflammatory agent, it improves intestinal motility, stimulates the production and intensity of action of immune cells and has the ability to destroy parasites, bacteria, viruses and fungus. The flowers are antihelminthic and useful in biliousness. The leaf gel is good for piles. It posses wound healing, skin protection, anti-allergic, anti-bacterial, antidysentric, antifungal, anti-fertility activities. Whereas, aloin posses cathartic, antibacterial, antiviral, anti-cancer, hepatoprotective and ultraviolet radiation protection activities. Lal *et al.,* (2002).

Among the various DNA marker-assisted techniques available, the randomly amplified polymorphic DNA (RAPD) technique (Williams *et al.*, 1990) has been most popular because of speed, low cost and the use of only minute amounts of plant material for analysis. It is less restrictive than the restriction fragment length polymorphism (RFLP) technique (no hybridization and no use of radioisotope) and amplified fragment length polymorphism (AFLP).

Aloe a native of Africa is an introduced plant in India. Morphologically it shows polymorphism but confirmation is done through RAPD analysis. Thus in the present investigation it is proposed to undertake characterization and RAPD profiling of various accessions.

MATERIALS AND METHODS

Plant material

Present investigation was conducted on *A. vera*. Accessions were obtained from nursery of *A. vera* collected and being maintained under NATP on plant Diversity. From accession No.1 to 15 those were bitter type viz., Anupgarh (1), Ganganager (3), Rajsamand (4), Pushkar (7), Udaipur (8), Suratgarh (11), Bikaner (12), Sambhar (15), Nagour (Ac. No.14, Ac. No.9), were initially taken for the study. Each type was planted in 2 x 3 meter plots, having 3 rows of plants. Each row

having 6 plants (plant to plant 45 cm). The rows spaced at 60 cm. the plants were 2-3 year old.

Molecular analysis of various biotypes

DNA from different accessions was extracted from buds using C-TAB method as described by Doyle and Doyle 1990. Random amplification of polymorphic DNA was done by using 15 primers of OPG series (OPG-1 to 15) obtained from "operon technologies" and PCR reaction were performed in final volume of 25ml containing 1X Assay Buffer, 0.5 units of Taq. DNA polymerase, 200 mM each of dNTPs, 10 pmols/reaction of random primer's and 50 ng of tempelate DNA. The 'Biometra Thermocycler' was programmed for first denaturation step at 94°C for 3 min, 44 cycles, each consisting of: 1 min at 94°C, 1 min at 37°C, 2 min. at 72°C, and last cycle consist of 1 min at 94°C, 1 min at 37°C, 10 min. at 72°C and a final extension at 72°C for 10 min and then held at 4°C prior to analysis. PCR products were evaluated by electrophoresis, and every scorable band was scored as present (1) or absent (0) and bi-variate 1 - 0 data matrix generated.

Statistical analysis for similarity coefficient

The similarity coefficient matrix generated from primers data was subjected to algorithm UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and clusters were generated using NTSYS 2.02pc program (Rohlf, 1998). To compare the efficiency of the markers in varietal identification, a single numerical index of discrimination (D) was calculated based Simpson's index of diversity (Simpons, 1949) and described by Hunter and Gaston (1988). The relative information content of molecular markers was compared by calculation of Polymorphism Information Content (PIC; Anderson *et al.,* 1993).

RESULTS AND DISCUSSION

Although *A. vera* a native of Africa is an introduced in India and has become naturalized in recent past (Panwar *et al.,* 2013), it has become necessary to characterize various biotypes to elucidate significant difference among them to see the prospects of cultivation of accessions useful for medicinal, nutritional and/or cosmetics purposes. Keeping this in mind the differences were analyzed between various accessions of *A. vera* collected from different parts of Rajasthan by using molecular methods.

PCR based markers have been widely used to characterize a wide range of plant species including medicinal plants (Suchar and Carles, 2008) such as, AFLP (Tripathi *et al.*,2011), RAPD (Nayanakantha *et al* 2010; Panwar *et al.,* 2013) to study *Aloe* spp. In present study we observed that flower buds of *Aloe vera* yielded sufficient quantity of quality DNA through Doyle and Doyle (1990) method. On the basis of our previous experience in laboratory, we can conclude that it was easier to isolate DNA from flower buds of *Aloe vera* than using fleshy leaves of it.

During markers amplification, out of 15 decamer primers used for screening, 10 produced amplicons. The amplicons generated ranged in numbers from 3 (OPG-4, OPG-6, OPG-15) to 11 (OPG - 8) depending on the primer used, previous studies suggest that the number of amplicons are primer dependent (Congiu *et al.,* 2000). Advantage with multilocus primer is that with single primer or primer combination a large number of bands are generated and this helps in better genetic diversity but at the same time when more number of bands are generated sometimes it becomes difficult to score all bands correctly and may lead to poor interpretation therefore, smeared and non consistent bands should be avoided during scoring, (Panwar *et al.,* (2013) which has been done in present study and only clear and consistent band has been considered for scoring and for further analysis. Out of 10 amplifying primers, 6 produced polymorphic bands (Table-1). The number of polymorphic bands ranged from 0 to 6 with range of polymorphisms being zero percent (OPG- 2, OPG- 9, OPG-10, OPG- 12) to 100 percent (OPG – 15) (Table 1). The total number of bands generated by ten amplifying primers was 53 with an average amplification of 5.3 bands per primer. Similar results were observed by Panwar *et al.,* (2013). The average polymorphism generated by these bands was 32.08 % whereas, Nayanakantha *et al*. (2010) observed 96.41% which was much higher than the present study but was almost similar (48.5%) as observed by Tripathi *et al*. (2011). Therefore, low polymorphism percentage reported in present study demonstrates that low genetic diversity that exists among *Aloe* accessions collected from different location of Rajasthan.

The primers differed for generating the polymorphism, therefore the relative information content of molecular markers can be compared by calculation of Polymorphism Information Content (PIC; Anderson *et al.,* 1993). Panwar *et al.,* (2013) calculated PIC value for 10 primers during study varied from 0.13 to 0.44. Likewise, in present study the average PIC values of different primers ranged from 0.0 (OPG -2, 9, 10, 12) to 0.346 (OPG-15) (Table 1). The primers like OPG-15 having high PIC value are considered important for diversity studies. The primers having high PIC value usually had high Discriminatory power. The ability of a primer to distinguish between unrelated strains can be determined by the number of types (pattern types) defined by the primer and the relative frequencies of their types. We have calculated a single numerical index of discrimination (D) based on the probability that two unrelated genotypes amplified from the test population would be placed into different typing groups. The value of D is one when all the patterns are unique and 0 when patterns generated for all the varieties contain monomorphic bands. The value of D ranged from 0.0 to 0.911 for different primers used for analysis based on RAPD (Table 1). The primers (OPG-14) with high discriminatory power (0.911) may be used for identification of different accessions.

The banding pattern generated and polymorphism reflected in these patterns was used to calculate the diversity among accessions and cluster analysis taken for present study. The Jaccard's similarity coefficient values ranged from 0.796 (Accession No. 3 and Accession No.9) to 0.957 (Ac. No. 1 and 11, Ac. No.11 and 15, Ac. No. 12 and 15) with an average of 0.881 (Table - 2). In previous studies, Nayanakantha *et al.,* (2010) observed similarity value from 0.068 to 0.610 whereas, Panwar *et al*. (2013) observed value ranged from 0.31 to 1.00. The dendrogram based on RAPD analysis has generated only one group. However, two accessions (Ac. No. 9 & 14)

Table 1: List of arbitrary primers showing total and polymorphic amplicons generated along with discriminatory power and average PIC (per primer) value of each pattern for 10 genotypes of *Aloe vera*

Primers	Sequences(5′→3′)	Total no. of bands (a)	Total no. of polymorphic bands(b)	Polymorphism (b/a × 100)	Discrimination index (D)	Average PIC
OPG-2	GGCACTGAGG	4	0	0	0	0
OPG-4	AGCGTGTCTG	3	1	33.3	0.200	0.060
OPG-6	GTGCCTAACC	3	2	66.7	0.822	0.326
OPG-8	TCACGTCCAC	11	3	27.37	0.778	0.105
OPG-9	CTGACGTCAC	5	0	0	0	0
OPG-10	AGGGCCGTCT	6	0	0	0	0
OPG-12	CAGCTCACGA	5	0	0	0	0
OPG-13	CTCTCCGCCA	5	2	40	0.200	0.072
OPG-14	GGATGAGACC	8	6	75	0.911	0.303
OPG-15	ACTGGGACTC	3	3	100	0.511	0.346
	Total		53	17	-	
	Average	5.3	1.7	32.08		

Table 2: Jaccard's average similarity coefficient among *Aloe vera* genotypes of different areas based on RAPD profiling

	1	3	4	7	8	9	11	12	14	15
1	1.000									
3	0.875	1.000								
4	0.840	0.918	1.000							
7	0.894	0.896	0.898	1.000						
8	0.935	0.857	0.898	0.915	1.000					
9	0.830	0.796	0.800	0.813	0.813	1.000				
11	0.957	0.878	0.880	0.936	0.936	0.872	1.000			
12	0.915	0.878	0.918	0.857	0.896	0.833	0.917	1.000		
14	0.857	0.860	0.863	0.840	0.840	0.816	0.860	0.898	1.000	
15	0.956	0.875	0.878	0.894	0.935	0.830	0.957	0.957	0.896	1.000

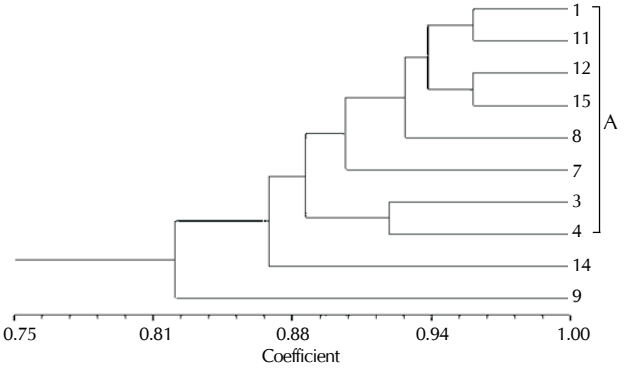

Figure 1: Dendrogram showing relationship among ten *Aloe* vera accessions generated by UPGMA analysis based on RAPD

belonging to Nagour did not cluster with the group. The group consisted of 8 genotypes viz. Anupgarh (1), Suratgarh(11), Bikaner (12), Sambhar (15), Udaipur (8), Pushkar (7), Ganganager (3) and Rajsamand (4). The sub group within the main group consisted of four genotypes viz. Anupgarh (1), Suratgarh (11), Bikaner (12), Sambhar (15) had higher within group similarity of 94.3%, while it was 90.5% for the main group. The two Nagour (Ac. No.14, Ac. No.9) accessions joined this group at the similarity level of 86.4% and 82.2% respectively. However, average diversity estimated was very low 11.09%, with a range from 4.3% to 20.4% diversity.

Results suggested that, related material probably got spread in various areas of Rajasthan. For example biotypes from Anupgarh, Suratgarh, Bikaner, which are closely located, resembled more with Sambhar samples than with Sri Ganganager one. Similarly, the main group consisting of 8 accessions included samples from diverse regions of Rajasthan. Moreover, the two samples collected from Nagour varied from each other and with other accessions collected from different regions. We may thus conclude that may be because of its vegetative propagation, it accumulated less variation. Moreover, the limited variation detected does not show any correlation with the distance of its collection. This may be suggestive of introduction of limited plant material to India especially in Rajasthan from its native place and also accompanied by human interventions such as selection and breeding that might be one of the major contributing factors in the evolutionary status of this economically important species. Similar observations have been made in Coconut (Perera *et al,* 1998), and *Ziziphus* spp. (Singh *et al.,* 2006) where, the level of genetic diversity was shown to correlate with the breeding nature of the plants.

ACKNOWLEDGEMENTS

We are very grateful to our M.Sc. guide Dr. S. Bhargava Associate Professor and Head, Department of Biodiversity, Bikaner, for their guidance and help in writing this manuscript. Plant Biotechnology Center, SKRAU, Bikaner, Rajasthan is highly acknowledged for supply of *Aloe vera* accessions.

REFERENCES

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

Congiu, L., Chicca, M., Cella, R. and Bernacchaia, G. 2000. The use of random amplified polymorphic DNA (RAPD) marker to identify strawberry varieties and forensic application. *Molecular Ecology*. **9:** 229-232.

Crosswhite, F. S. and Crosswhite, C. D. 1984. *Aloe vera*, plant symbolism and the threshing floor: light, life and good in our heritage. *Des Plants*. **6:** 43-44.

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

Hunter, P. R. and Gaston, M. A. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology*. **26:** 2465-2466.

Lal, R. K., Sharma, J. R., Patra, D. D., Farooqui, A. H. A., Kalra, A., Baba, J. R., Singh, A. K. and Khanuja, S. P. S. 2002. *Aloe vera* and its cultivation in India. Farm bulletin: Central institute of Medicinal and Aromatic Plants, Lucknow, India.

Nayanakantha, N. M. C., Singh, B. R. and Gupta, A. K. 2010. Assessment of genetic diversity in aloe germplasm accessions from india using rapd and morphological markers. *Ceylon journal of Science*. **39(1):** 1-9.

Panwar, B. S., Singh, R., Dwivedi, V. K., Kumar, A., Kumari, P. 2013. Genetic diversity among Indian Aloe accessions based on RAPD analysis. *International Journal of Medicinal and Aromatic Plants*. **3(3):** 326-333.

Perera, L., Russel, J. R., Provan, J., McNicol, J. M. and Powel, W. 1998. Evaluating genetic relationship between indigenous coconut (*Cocus nucifera* L.) accessions from Sri Lanka by mean of AFLP profiling. *Theoretical and Applied Genetics*. **96:** 545-550.

Rohlf, F J. 1998. NTSYS-pc ver. 2.02. Numerical taxonomy and multivariate analysis system. Exeter Publishing, Setauket.

Singh, A. K., Sharma, R., Singh N. K., Bansal, K. C., Koundal K. R. and Mohapatra, T. 2006. Genetic diversity in ber (*Ziziphus* spp.) revealed by AFLP markers. *Journal of Horticulture Science and Biotechnology*. **81(2):** 205-210.

Simpson, E. H. 1949. Measurement of species diversity. *Nature*. **163:** 688.

Sucher N. J. and Carles M. C. 2008. Genome-based approaches to authentication of medicinal plants. *Planta Medical*. **74:** 603-623.

Tripathi, N., Saini, N., Tiwari, S. 2011. Assessment of genetic diversity among *Aloe vera* accessions using amplified fragment length polymorphism. *International Journal of Medicinal and Aromatic Plants*. **1(2):** 115-121.

Williams, G. K., Kubelik, A. R., Livak, K. L., Rafalshi, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. **18:** 6531-6535.