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Genetic variability among *Andrographis paniculata* in Chhattisgarh region assessed by RAPD markers

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Random amplified polymorphic DNA (RAPD) markers were used to estimate the genetic variability and dissimilarity among the *Andrographis paniculata* (family- Acanthance), an important medicinal herb. Twenty-four (24) plants were collected from five districts of different places of Chhattisgarh region. Sixteen (16) primers generated a total of 159 polymorphic bands out of 182 total bands (79.95% polymorphism), with an average of 11.37 amplified bands per primers and 23 bands showed monomorphic banding pattern with an average of 1.43 per primers. A dendrogram was constructed based on the unweighted pair group method using arithmetic averages. Cluster analysis of data using UPGMA algorithm placed the 24 accessions of *A. paniculata* into two major clusters I and II which further sub-divided into many subclusters. Genetic dissimilarity coefficients calculated from RAPD data ranged from 0.3635 to 2.0160, with the highest value of 2.0160 between AP10 and AP16 and the lowest value of 0.3635 between accessions AP3 and AP5. The principal component analysis (PCA) clustering pattern corresponded well with the dendrogram. The results indicate that RAPD could be efficiently used for genetic diversity study in wild species of approaching value as it is quick, unswerving and superior to those on pedigree information.

Key words: Acanthance, dendrogram, principle component analysis (PCA), Random amplified polymorphic DNA (RAPD), variability.

INTRODUCTION

Andrographis paniculata (Burm.F) Nees, is an important medicinal herb. The whole plant is used as blood purifier and liver tonic (Chandra and Pandey, 1985; Chandra et al., 1985, 1987). Recently, it has been utilized as a treatment for HIV, hepatitis, diabetes, cancer and kidney disorders (Valdiani et al., 2012). In Chhattisgarh state, the tribal people of Raigarh districts externally applied the plant extract in snakebite (Jain and Singh, 2010), but the tribal of Balaghat district of Madhya Pradesh, used the whole plants for the treatment of chickenginia and malaria (Jain et al., 2011). It is known as a hermaphroditic, self-compatible and a habitual inbreeding plant (Latto et al., 2006) and there is an assumed rate of 28% crosses pollination for it (Sabu, 2002).

A. paniculata (Acanthance) is an annual herb commonly known as Kalmegh or Bhuineem (Sharma et al. 2009; Mishra et al. 2007). It is distributed in Southeastern Asia, including India, Sri Lanka, Pakistan, Indonesia (Mishra et al., 2007) but it is currently cultivated in Southwestern Nigeria (Fasola et al., 2010).

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In India, it is also cultivated as a Kharif season crop. Propagation is generally done through seeds; however, it can also be propagated by inducing rooting in cuttings (Zhou, 1987). The main constituents of *A. paniculata* are diterepene lactones, andrographolide, neoandrographolide, andrographiside and flavones viz., oroxylin, wogonin, andrographidines A, B, C, D, E, F (Martindale et al., 1972). Among them, andrographolide is widely used as a hepatoprotective agent. It also shows choleretic, antidiarrhoeal, immunostimulant, and anti-inflammatory activities (Kokate et al., 1999; Anonymous, 1999; Singh et al., 2003).

A. paniculata has been existing in the list of highly traded Indian medicinal plants (Sajwan, 2008) and it also has been positioned as the 17th crop among the 32 prioritized medicinal plants of India with a demand of 2197.3 tons in the year 2005-2006 and annual growth of 3.1% (Sharma et al., 2009; Anonymous, 2007; Kala et al., 2006). Priority of *A. paniculata* is regarded by herbal industries of developing countries such as Malaysia (Moideen, 2008), Thailand (Chuthaputti and Chawapradit, 2008) and Nigeria (Fasola et al., 2010). In Chhattisgarh State, Kalmegh was cultivated in various places such as Bilaspur, Rajnandgaon, Kabirdham, Bijapur, Kanker, Sukma, Khairagarh, Udanti and East Raipur. Production of Kalmegh in CG State as per market survey report 2006 by CGMFPLTD Raipur was 13950 (Qtnl) (CSMPB Raipur, 2008). Random amplified polymorphic DNA (RAPD) analysis has been applied in herbal medicine to discriminate between species in various genera (Shcher and Carles, 2008). RAPD markers were used to assess genetic variability among the various accessions of *A. paniculata.*

Genetic tools that use hybridization, polymerase chain reaction (PCR), and sequencing techniques provide more objective and reliable methods for authenticating herbal medicines (Zhang et al., 2007; Shcher and Carles, 2008). Recently, RAPD analysis has become one of the most effective methods for estimating genetic diversity in plant populations or cultivars because it can reveal high levels of polymorphism. However, it is less reproducible than other methods (Hosokawa et al., 2000; Agrawal et al., 2007; Shcher and Carles, 2008). RAPD also has many advantages, such as its high speed, low cost, and requirement of minute amounts of plant material (Williams et al., 1990; Penner et al., 1993). RAPD markers have been already successfully used on other medicinal and aromatic crops (Yang et al., 2012; Leelambika and Sathyanarayana, 2011; Kasaian et al., 2011; Khan et al., 2010; Verma et al., 2009; Bharmauria et al., 2009; Padmalatha and Prasad, 2007). The RAPD based molecular markers have been found to be useful in *A. paniculata* (Padmesh et al., 1998; Maison et al., 2005; Latto et al., 2006; Kumar and Shekhawat, 2009). Inspite of immense therapeutic value, lack of genetic variability information in *A. paniculata* occurs in Chhattisgarh State. The objective of this study was to use RAPDs to assess genetic variability and dissimilarity among *A. paniculata* of 24 accessions collected from five districts of different places in Chhattisgarh region and to estimate genetic relationships among the accessions which can be utilized for breeding program and phytochemical study.

MATERIALS AND METHODS

Plant materials

A. paniculata plants are found in pine, evergreen and deciduous forest areas, and along roads and in villages. However, it is possible to cultivate them during rainy phase of summer season. A total of 24 accessions, of *A. paniculata* collected from natural sources of five districts of different places in Chhattisgarh region were used for this study. All the accessions collected from different places were effectively maintained in the medicinal garden of the Pt. Ravishankar Shukla University Raipur (C.G) (Table 1).

DNA extraction

Genomic DNA was extracted from fresh young leaves of 24 accessions of *A. paniculata* using cetyltrimethyl-ammonium bromide (CTAB) method (Doyle and Doyle, 1990) with modifications as described by Khanuja et al. (1999). Three grams (3 g) of fresh young leaves of each 24 samples were ground to a fine powder in a mortar in liquid nitrogen and then transferred to Tarson tubes filled with 15 ml of freshly prepared and preheated 2.5% CTAB extraction buffer. Extraction buffer consisted of 100 mM Tris - HCl (pH 8.0), 25 mm EDTA (pH 8.0), 1.5 M NaCl, 1% PVP and 0.2% β - mercepto-ethanol. The suspensions of samples were incubated at 65°C for 1 h. After cooling at room temperature, an equal volume of chloroform-isoamyl alcohol (24:1) was added and centrifuged at 10 K rpm for 10 min at room temperature. DNA from aqueous layer was precipitated by adding 5 M NaCl (30% of supernatant) and 0.6 volumes of chilled isopropanol. The mixture was centrifuged at 10 K rpm for 10 min. Pellets were washed with 80% alcohol and let the pellet dry. Pellets were dissolved in high salt TE buffer then RNase A was added and incubated at 37°C for 30 min. Again, it was extracted with equal volume of chloroform - isoamylalcohal (24:1). Two volumes of cold ethanol was added in aqueous layer and centrifuged at 10 K rpm for 10 min. Pellets were washed with 80% alcohol and dissolved in TE (Tris- Cl- EDTA) buffer after drying. DNA concentration and purity were determined by nanospectrophotometer (UK) A260/280, ratio ranged from 1.83 to 2.15 and averaged 2.04 for all the samples and electrophoresis in a 0.8% agarose gel with known standards was done. For PCR amplification, the final concentration of each DNA sample was diluted to approximately 15 to 20 ng with millique water.

PCR amplication

RAPD assays were carried out in 12.5 µl reaction mixture containing template 2 µl DNA (15-20 ng), 1.25 µl of 10X PCR Buffer (10 mM Tris HCL, 25 mM $MgCl_2$), 1 µl of 10 mM dNTPs, 0.125 µl of 20 mM primer, and 0.75 µl 1.0 of U Taq DNA polymerase, (Bangalore genei) and distilled water up to 6.25 µl. Amplification was performed in a PTC-100 programmable thermal cycler (MJ Research, USA). DNA amplification was obtained as follows: 94°C for 5 min, 32 cycles of 94°C for 30 s, 37°C for 30 s, and 72°C for 1 min, followed by one cycle of 72°C for 7 min and a hold temperature of 4°C at the end. A negative control with all PCR cocktail except the template DNA was included with each set of PCR amplification reactions. The negative control contained sterile

Table 1. Collection of *Andrographis paniculata* accessions (AP1-AP24) used for the study.

State	District	Locality	Source	Accessions number
Chhattisgarh	Korba	Kusmunda	Cultivated	AP1
		Katghora	wild	AP2
		Madanpur	Wild	AP3
		Kartala	Wild	AP4
		Saglawa	Wild	AP5
		Pasan	Wild	AP6
		Nakoya	wild	AP7
	Kawardha	Salewara	Wild	AP8
		Bhoramdeo	Wild	AP9
		Pandari	Wild	AP10
		Neur	Wild	AP11
	Surguja	Ambikapur	Wild	AP12
		Mainpat	Wild	AP13
		Partabpur	Wild	AP14
		Wadrefnagar	Wild	AP15
		Tattapani	Wild	AP16
	Raigarh	Sarangarh	Wild	AP17
		Kharisa	Wild	AP18
		Gharghoda	Wild	AP19
		Leonga	Wild	AP20
		Dharamjaygarh	Wild	AP21
	Bastar	Balenga	Wild	AP22
		Kondagaon	Wild	AP23
		Jagdalpur	Wild	AP24

distilled water instead of template genomic DNA. The amplified PCR products were resolved on 1.8% (w/v) agarose gels, in 1X TBE Buffer at 90 V for 2 h 10 min, visualized with ethidium bromide (0.5 µg/ml) staining and photographed under Gene Snap software of gel Documentation System (Gene Genius, Syngene, U.K.). Lambda DNA double digest ladder (1.5 Kb DNA ladder, Bangalore Genei) was used as molecular marker to know the size of the fragments. For each experiment, the reproducibility of the amplification products was tested thrice using similar reaction conditions at different times.

Screening of specific RAPD primers

Thirty (30) random primers (R-01 - R-30) from R-series, (purchased by Integrated DNA Technologies, Inc., U.S.A) were screened by RAPD for identification of specific markers. The screening of primers resulted in 16 R-series decamer primers which show polymorphisms with all 24 samples. The other 14 Integrated DNA primers could not amplify all the 24 samples.

RAPD data analysis

The amplified bands were scored from photographs into a binary data matrix as 1 (present) or 0 (absent). Based on presence/absence data, genetic similarity was calculated to estimate all pair wise differences in the amplification product for all isolates. The genetic associations between strains were computed based on Jaccard's similarity coefficient (Jaccard, 1908) using 'SAHN' (sequential, agglomerative, hierarchical nested clustering method) sub program of NTSYS-pc 2.20 (q). The program also generated a dendrogram on the basis of above index by unweighted pair group method with arithmetic averages (UPGMA) subprogram of NTSYC-pc 2.20 (q). The data generated from polymorphic fragments were analyzed according to the study of Nei and Li (1979) formula given below:

$$\text{Similarity (F)} = \frac{2\,Mx}{My + Mz}$$

Dissimilarity = 1 - F

Where, Mx is the number of shared fragments between genotypes y and z; My is the number of scored fragments of genotype y; Mz is the number of scored fragments of genotype z. Only clear and unambiguous bands were taken into account and the bands were not scored if they were faint or diffused. The RAPD data were also evaluated by principle component analysis (PCA).

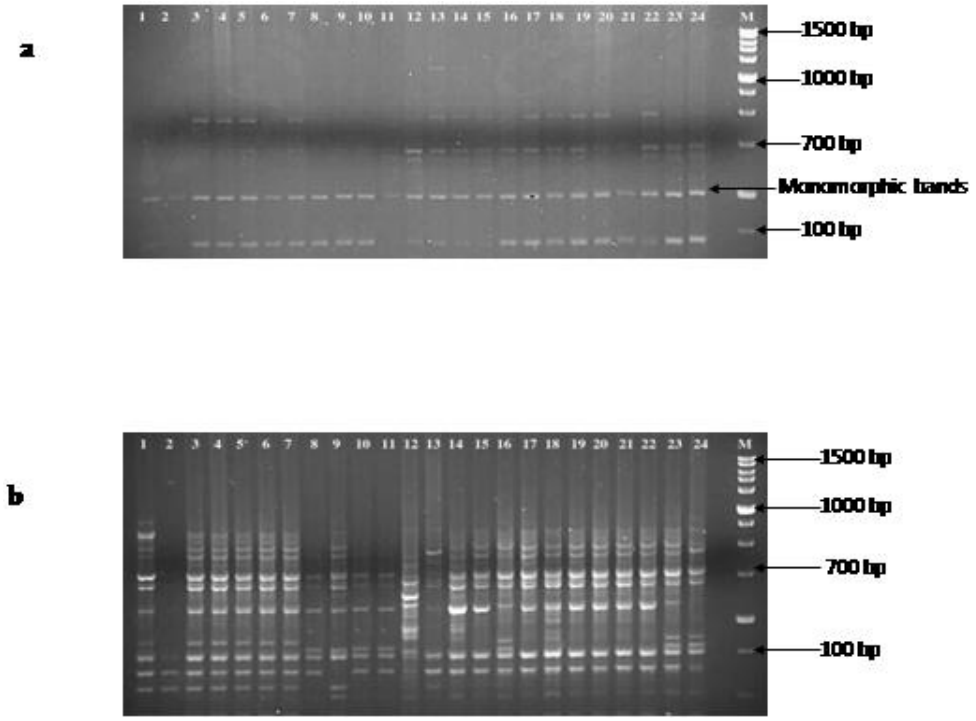

Figure 1. RAPD profiles of *A. paniculata* using R-series 10-decmer primers. a, R-09; b, R-17. The arrowheads indicate the precise size of RAPD markers. M represents 1 Kb molecular size marker, Lane numbers code for each accessions 1 to 7 Korba, 8 to 11 Kawardha, 12 to 16 Surguja, 17 to 21 Raigarh, 22 to 24 Bastar.

RESULTS AND DISCUSSION

RAPD analysis was used to evaluate the degree of polymorphism and genetic variability among 24 accessions of *A. paniculata* was further analyzed according to the resultant RAPD markers. The PCR reactions were repeated three times for each of the 24 accessions and the resultant DNA bands were highly reproducible. Results show that each accession collected from different localities showed genetic variability and dissimilarity in RAPD profiles by using different primers.

Of the 30 random primers screened, 16 R-series (= R) primers produced distinct, highly reproducible amplify-cation profile for all the screened samples (Figure 1a,b). These 16 primers were selected for further ana-lysis of the plant materials. The polymorphism exhibited by RAPD primers among 24 accessions of *A. paniculata* are presented in Table 2. A total 182 marker levels were amplified across the accessions, of which 159 (79. 95) were polymorphic bands.

A wide variation in the number of polymorphic bands ranging from 3 to 21 and monomorphic bands 1 to 3 was observed. The highest 22 bands were observed from primer R-17 and four bands for primers R-07 and R-09 (Figure 1a, b). The average number of polymorphic (9.93), monomorphic (1.43), unique bands (1.68) per primer and the percentage of polymorphism ranged from 50.0 (R-16) to 93.70 (R-13 and R-19) (Table 2). Primer R-17 amplified the highest number of polymorphic bands (21), while the lowest number (3) was observed with primer R-07, R-09 and R-16.

The size of amplified fragments varied with the different primers, ranging from 100 to 1500 bp. The monomorphic bands were observed in all primer whereas most of the primer shows monomorphic bands at positions less than 100 bp. Thus, the present results indicate the variable potentiality of the primers in resolving the variation in accessions studied.

A genetic distance matrix was computed based on Jaccard's similarity coefficient (Jaccard, 1908) using 'SAHN' (sequential, agglomerative, hierarchical nested clustering method) sub program of NTSYS-pc 2.20 (q) software package. The genetic dissimilarity matrix bet-ween accessions that ranged from 0.3635 and 2.0160 indicate that, the genetic distance among the accessions fall in medium range of variability (Table 3).

The highest genetic dissimilarity revealed by RAPD analysis (2.0160) detected between accessions AP10 and AP16 while the lowest dissimilarity (0.3635) between accessions AP3 and AP5. The dendrogram of *A. paniculata* was constructed based on RAPD data (Figure 2). All accessions initially fall under two major clusters which levels. Cluster I contains six accessions (AP1, AP2, AP8, AP10, AP11 and AP9) collected from Kusmunda,

Table 2. Polymorphism exhibited by RAPD primers.

Number	Primer	Sequence of primer	Amplified band	Number of monomorphic bands	Number of polymorphic bands	Unique bands	% of polymorphic bands
1	R-02	5'-GGCGCGTTAG-3'	10	1	9	1	90.0
2	R-03	5'-GGAACCCACA-3'	8	2	6	1	62.5
3	R-07	5'-ACCACCCGCT-3'	4	1	3	0	75.0
4	R-08	5'-AGTCGGCCCA-3'	13	3	10	1	84.6
5	R-09	5'-GGTCCTACCA-3'	4	1	3	0	75.0
6	R-11	5'-AACCGCGGCA-3'	9	1	8	3	77.7
7	R-13	5'-GGAGCGTACT-3'	16	1	15	3	93.7
8	R-14	5'-ACTGCCCGAC-3'	17	3	14	1	76.4
9	R-15	5'-GGTTACTGCC-3'	15	1	14	2	93.3
10	R-16	5'-GGGATGACCA-3'	6	3	3	1	50.0
11	R-17	5'-AAGCCCCCCA-3'	22	1	21	1	81.8
12	R-18	5'-ACGGCACGCA-3'	8	1	7	2	87.5
13	R-19	5'-GTGGCCGATG-3'	16	1	15	3	93.7
14	R-20	5'-AAGTGCACGG-3'	14	1	13	6	85.7
15	R-22	5'-GGTTGGAGAC-3'	9	1	8	0	88.8
16	R-28	5'-GTGCGCAATG-3'	11	1	10	2	63.6
Total			182	23	159	27	79.95
Average/primers			11.37	1.43	9.93	1.68	

Katghora, Salewara, Pandari, Neur and Bhoramdeo, respectively. Cluster I showed a high level of genetic variation among the accessions and was further sub-divided into two sub-clusters. Sub-Cluster Ia contains five accessions (AP1, AP2, AP8, AP10 and AP11) belonging to different areas such as Kusmunda, Katghora, Salewara, Pandari, Neur while Sub-Cluster Ib contained single genotype (AP9) belonging to Bhoramdeo. Cluster II was further divided in two subclusters (SC IIa and SC IIb). Sub Cluster (SC IIa) had a single accession 'AP24' collected from Jagdalpur, placing a separate position in dendrogram thus demonstrating the distinctiveness of the genetic background of these accession from all the other accessions.

The Subcluster (SC IIb) is further divided into two subcluster; II ba and II bb. Sub-Cluster SCII (ba) contained AP12 accessions and the subcluster SCII (bb) was further sub-divided into two subclusters; SCII (bbi) and (bbii). Sub-Cluster SCII (bbi) contained AP15 and sub-clusters SCII (bbii) is further divided two sub-clusters; SCII (bbii a) (bbii b) showing 90% dissimilarity level with all accessions. SCII (bbii a) comprised of two accessions (AP16 and AP23) and both of them belonged to a different area. SCII (bbii b) is further divided into two subclusters; SCII (bbii ba) and (IIbbii bb). SCII (bbii ba) has AP14 showing 97% dissimilarity level with all accessions and the subcluster SCII (bbii bb) is again divided into two subclusters; SCII (bbii bba) and SCII (bbii bbb). SCII (bbii bba) contained AP13 showing 89% dissimilarity levels. SCII (bbii bbb) was again divided two sub-clusters; SCII (bbii bbb x) and (IIbbii bbb y) showing 87% dissimilarity levels with all accessions. SCII (bbii Bbbx) contained six accessions (AP17, AP18, AP19, AP20, AP21 and AP22), five belongs to Sarangarh, Kharisa, Gharghoda, Leonga, were grouped into several subclusters. Dendrogram clusters can be divided into Clusters I (CI) and Clus-ters II (CII) at 1.36 dissimilarity Dharamjaygarh and one single accession belongs to Balenga. SCII (bbii bbb y) contained other accessions (AP4, AP7, AP3, AP5,

Table 3. Genetic dissimilarity matrix of 24 accessions of *Andrographis paniculata* obtained from RAPD markers.

	AP1	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**	**AP7**	**AP8**	**AP9**	**AP10**	**AP11**	**AP12**	**AP13**	**AP14**	**AP15**	**AP16**	**AP17**	**AP18**	**AP19**	**AP20**	**AP21**	**AP22**	**AP23**	**AP24**
AP1	0																							
AP2	1.01	0																						
AP3	1.62	1.84	0																					
AP4	1.61	1.79	0.48	0																				
AP5	1.58	1.82	**0.36**	0.54	0																			
AP6	1.61	1.84	0.42	0.61	0.41	0																		
AP7	1.54	1.79	0.53	0.69	0.48	0.63	0																	
AP8	1.39	1.19	1.93	1.87	1.91	1.92	1.87	0																
AP9	1.32	1.38	1.62	1.61	1.62	1.64	1.59	1.73	0															
AP10	1.37	1.16	1.95	1.89	1.92	1.94	1.89	0.35	1.69	0														
AP11	1.33	1.21	1.67	1.61	1.68	1.67	1.65	1.59	1.25	1.51	0													
AP12	1.75	1.88	1.51	1.37	1.31	1.31	1.31	1.93	1.71	1.91	1.71	0												
AP13	1.49	1.68	1.13	1.13	1.07	1.07	1.02	1.76	1.52	1.77	1.57	1.18	0											
AP14	1.61	1.77	1.29	1.31	1.25	1.31	1.19	1.89	1.59	1.88	1.57	1.32	0.99	0										
AP15	1.47	1.51	1.31	1.23	1.27	1.32	1.28	1.65	1.48	1.61	1.38	1.37	1.11	1.23	0									
AP16	1.72	1.92	1.07	1.15	1.11	1.13	1.11	2.01	1.71	**2.01**	1.71	1.42	1.32	1.32	1.43	0								
AP17	1.61	1.84	0.89	0.93	0.88	0.88	0.93	1.91	1.65	1.92	1.63	1.29	0.97	1.11	1.22	1.14	0							
AP18	1.65	1.87	0.88	0.93	0.87	0.89	0.92	1.92	1.66	1.93	1.67	1.18	0.97	1.05	1.19	1.14	0.56	0						
AP19	1.64	1.85	0.95	1.02	0.97	0.99	0.89	1.91	1.63	1.91	1.62	1.21	0.89	1.11	1.22	1.09	0.67	0.63	0					
AP20	1.62	1.84	0.96	1.01	0.98	1.03	0.87	1.89	1.62	1.91	1.63	1.28	0.95	1.02	1.26	1.14	0.68	0.67	0.59	0				
AP21	1.66	1.87	0.98	1.02	0.97	0.97	0.99	1.91	1.68	1.93	1.65	1.34	1.03	1.12	1.27	1.17	0.51	0.66	0.72	0.64	0			
AP22	1.71	1.91	1.01	1.04	1.02	1.04	1.04	1.95	1.69	1.95	1.71	1.33	1.02	1.18	1.24	1.21	0.77	0.71	0.81	0.83	0.84	0		
AP23	1.78	1.97	1.14	1.21	1.12	1.19	1.12	1.98	1.81	2.00	1.86	1.46	1.41	1.49	1.46	0.91	1.24	1.21	1.19	1.22	1.22	1.26	0	
AP24	1.41	1.52	1.48	1.51	1.49	1.47	1.48	1.56	1.62	1.57	1.47	1.57	1.36	1.43	1.41	1.47	1.43	1.44	1.46	1.51	1.44	1.49	1.58	0

and AP6); all of them belonged to the same areas showing 48% dissimilarity level with all accessions. The cluster analysis with all the accessions tested showed a genetic variability between different accessions. According to the dendrogram, the accessions considered for this study did not cluster according to the geographical position of their sites. It might be possible that at the time of cross-pollination, these varieties get intermixed with other accession.

Genetic variability among *A. paniculata* was also visualized in detail; PCA was made for 182 bands produced by 16 decamer primers. It is evident from the data that *A. paniculata* were more dispersed on the PCA plot which reflects a wider genetic range. It shows clear- cut partition of 24 accessions into three groups; each group being distinct and showing dissimilarity from other group (Figure 3).

Group a contained AP24, AP13, AP12, AP15, AP14, AP16 and AP23; while group b contained AP8, AP10, AP11 and AP9; AP17, AP21, AP18, AP19, AP20, AP22, AP4, AP7, AP3, AP5, AP6 and AP1, AP2 were the part of group c.

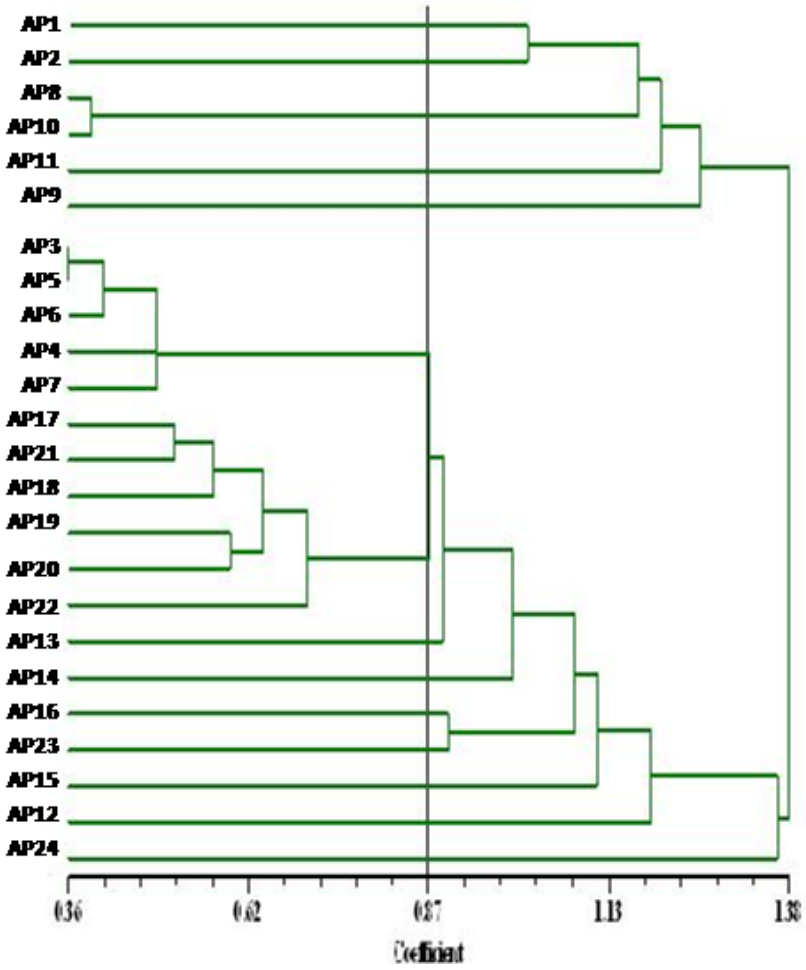

Figure 2. UPGMA dendrogram showing relationship (variation) among various *A. paniculata* accessions using 16 RAPD markers.

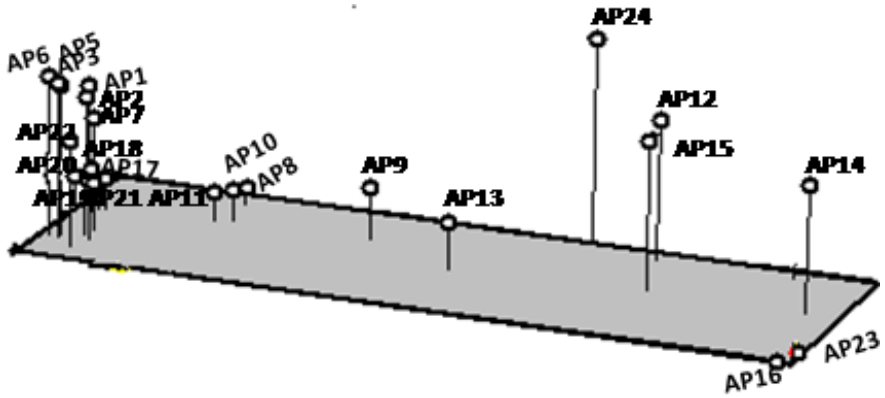

Figure 3. PCA of *A. paniculata* accessions generated by RAPD markers.

It might be possible that at the time of cross-pollination, these varieties get intermixed with other accession. In general, the data obtained from PCA are in concurrence with the dendrogram generated by UPGMA method which is a further evidence of the genetic variability delineated by cluster analysis.

In this study, we used RAPD markers to evaluate genetic variability and dissimilarity among 24 accessions of *A.*

paniculata and to select accession for further genetic improvement and conservation of genetic diversity for qualitative and quantitative traits. The selection of RAPD technique was motivated by the fact that no DNA sequence information is known about these species collected from five districts of different places of Chhattisgarh. In the present study, a high level of genetic variability and dissimilarity was found within 24 accessions of *A. paniculata* collected from various sites. The cluster analysis with all the accessions tested showed a genetic variability between different accessions.

According to the dendrogram, the accessions considered for this study did not cluster according to the geographical position of their sites. This result shows a large variation among accessions collected from different regions. It may have occurred due to the distribution of the species over a relatively small area and high ratio of cross-pollination. The plants collected for the studied were surrounded by natural environments, such as forest and mountains; there may be extensive chances of pollen and seed migration from one site to another. Our study shows a wide range of dissimilarity among 24 accessions belonging to different places; ranged from 0.3635 to 2.0160. Our results are also supported by those of Kumara and Anuradha (2011) who reported the genetic variability and highest dissimilarity among patchouli cultivars determined by using RAPD markers. El-kamali et al. (2011) determined the genetic variability of two *Sonchus* species collected from two locations in Khartoum State using RAPD technique.

Ambiel et al. (2010) studied the genetic dissimilarity among germ-plasm of six *Brachiaria* species and showed that dissimilarity indexes ranged from 0.262 to 0.907. El-Kamali et al. (2010) studied the genetic relationship and dissimilarity among *Pulicaria undulate* (UN) and *Pulicaria crispa* (CR) and their three putative hybrids (N1, N2 and HY) through RAPD makers. Highest dissimilarity was found between hybrid HY & N1 (0.50%) while the minimum dissimilarity was between *P. undulata* and hybrid N1 (0.22%). Subramanyam et al. (2010) analyzed *Jatropha curcas* germplasm accessions using RAPD markers and found that it is efficient for detecting genetic diversity and relationship of inter and intra populations. Several other reports are available on RAPD based molecular markers for diversity studies in *A. paniculata.* Sharma et al. (2009) reported genetic diversity among 15 genotypes of *A. paniculata* collected from different locations analysed using RAPD to discriminate molecular variability. Latto et al. (2008) compared diversity among 53 accessions collected from five different ecogeographic regions on the basis of morphometric and RAPD analysis for the purpose of genetic improvement and conservation of its variability.

Padmesh et al. (1999) studied the intraspecific genetic variability in Kalmegh by RAPD analysis. The RAPD-based marker also proved a useful technique for genetic diversity analysis in different plant species. Bharmauria et al. (2010) used eight random primers in the RAPD analysis of six samples of *Urtica dioica.* Out of 134 amplified fragments, 107 bands (20.2%) were polymorphic and 79.8% were monomorphic. Ikbal et al. (2010) used 50 arbitrary primers among them; 44 primers generated polymorphic profiles for RAPD analysis of *J. curcas.* The total number of bands was 328 of which 308 (93.90%) were polymorphic. Zou et al. (2011) studied genetic diversity among 33 accessions *Curcuma* species using 21 RAPD primers. The total number of amplified products was 115, including 106 polymorphic bands and represented a level of polymorphism of 92.17%. The present study of *A. paniculata* genetic variability generated a total of 182 reproducible and scorable amplification products by 16 primers across 24 accessions, of which 159 fragments (79.95%) were polymorphic. The highest number of bands generated by each primer varied from 04 (R-07 and R-09) to 22 (R-17). Our results are in agreement with those reported by Bharmauria et al. (2010) and Sharma et al. (2009). It has been shown by the result that RAPD could be effectively used for genetic diversity analysis in wild species as it is rapid, reliable and superior to those based on pedigree information. The RAPD analysis of different regions of *A. paniculata* revealed significant genetic polymorphism within each region. Perhaps, this is due to cross-pollination in the species. The analysis revealed that the species contains high level of genetic variation that enabled its wide distribution and formation of new varieties and species. This report describes the genetic variability in *A. paniculata* collected from five districts of different places in Chhattisgarh using RAPD markers. The result indicates that RAPD could be efficiently used for genetic diversity study in wild species of approaching value as it is quick, unswerving and superior to those on pedigree information.

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