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Full Length Research Paper

Assessment of genetic diversity among collected genotypes of *Chlorophytum borivilianum* using random amplified polymorphic DNA (RAPD) markers

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Chlorophytum borivilianum **(Family: Liliaceae), an important threatened medicinal herb is designated as 'Rare' in Red Data Book of Indian plants. Morphological as well as biochemical markers used in the authentication of herbal drugs have many limitations due to the impact of environmental conditions. Molecular markers therefore, are an important tool in quality assurance and preservation of germplasm of medicinal plant species in the plant kingdom. In this study, RAPD markers were used to assess genetic diversity in nine genotypes of *C. borivilianum* collected from different geographical regions of Chhattisgarh, Madhya Pradesh and Uttar Pradesh. Out of the 43 bands obtained, 26 were polymorphic, with (67.49) percent polymorphism, thus, revealing a high degree of polymorphism. Percentage of polymorphism detected with each primer was as high as 80.00 (OP L18) and as low as 33.30 (OP L12). The Jaccard's similarity coefficient ranged from 0.609 to 0.810. The maximum similarity value was noticed between V_3 and V_5 genotypes collected from Lamni (C.G.) and Chitrakoot (U.P.), respectively.**

Keywords: Genetic diversity, random amplified polymorphic DNA (RAPD)-PCR, UPGMA, *Chlorophytum borivilianum*.

INTRODUCTION

Chhattisgarh, the herbal state, is bestowed with a wealth of medicinal and aromatic plants, most of which have been traditionally used in Ayurveda, Yunani systems of medicines and by tribal healers for generations. *Chlorophytum borivilianum* belongs to the family Liliaceae, it is a traditional medicinal plant found in the natural forest. Besides its extensive use in Ayurveda and other conventional medicinal systems in Asia, it is also gaining increasing acceptance as a vitalizer and health-giving tonic, a curative for pre-natal and post-natal problems, and as a remedy for diabetes and arthritis. Since the introduction of random amplified polymorphic DNA (RAPD) markers in 1990 (Williams et al., 1990), its use in plant genetic analysis have increased in exponential manner. RAPDs are relatively quick, inexpensive and require no prior information of target genome. Presence or absence of DNA bands in the gel is used as RAPD markers to study close genetic relationship (Sang-Bok and Rasmussen, 2000), inter and intra-specific genetic variations (M'ribu and Hilu, 1994), for the identification of specific genes (Paran et al., 1991) and to study the pattern of gene expression (Valle et al., 2000). In this study, *C. borivilianum* accessions collected from the wild forest regions of Chhattisgarh, Madhya Pradesh and Uttar Pradesh were characterized using PCR based molecular markers (RAPD) in order to find out genetic diversity among the collected accessions.

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MATERIALS AND METHODS

Safed musli is an important ingredient of more than a hundred Ayurvedic, allopathic, homeopathic and Unani medicinal preparations. Musli is a tuberous plant. It is a small perennial herb which is full of radical leaves appearing over ground with the advent of summer rains. These are fascicled, sessile, cylindrical, 1 to 8 in number, brown to black skinned and white after peeling; the tubers

Table 1. Collection of *C. borivilianum.*

Genotype	Collection site
V_1	Chhaparawa 1 (C.G.)
V_2	Chhaparawa 2 (C.G.)
V_3	Lamni (C.G.)
V_4	Kewachi (C.G.)
V_5	Chitrakoot (U.P.)
V_6	Shivtarai (C.G.)
V_7	Achanakmar (C.G.)
V_8	Amarkantak (M.P.)
V_9	Ambikapur (C.G.)

are 3 to 10 cm long at maturity. The flowers are bracteate, pedicellate, usually arranged in alternate clusters, each consisting of 3 flowers. This is an insect pollinated plant. The fruit is a loculicidal capsule, green to yellow, triquetrous to 3-b sulcate, almost equal in length and width. The seeds are onion-like, black in appearance with angular edges. The genus *Chlorophytum* is represented by about 175 valid taxa of rhizomatous herbs. It is distributed predominantly in the tropical parts of the world (Hooker and Jacksn, 1894), in Index Kewensis, it has more than 300 species in the genus. Sheriff and Chennaveeraiah (1972) reported that nearly 13 species are known to occur in India, of which 8 are endemic to the subcontinent. This is supported by the study of Nair (1974). *C. borivilinaum* produces the highest yield and highest saponin content. The experimental materials consisted of nine accessions of *C. borivilianum* L. Planting material of *C. borivilianum* was collected from different forests of Chhattisgarh and its adjoining regions as well as some parts of Madhya Pradesh and Uttar Pradesh. Plants together with the root tubers were kept at the experimental field of the Department of Biotechnology, Indira Gandhi Agricultural University, Raipur. The accessions were raised in randomized block design (RBD) with three replications. Each accession was raised in a single row of 5 m length by adopting a spacing of 45 x 15 cm. FYM at 2.5 kg/bed was applied in the field and no inorganic fertilizer was used.

For proper growth and development of tubers and proper expression of planting material, raised beds were prepared. Tubers were treated with 0.2% bavistin solution before planting to reduce the rotting of fleshy roots. Irrigation was provided after an interval of 10 to 15 days. The complete list of the accessions observed is presented in Table 1.

DNA extraction and RAPD analysis

Leaves of 6 week old plants were collected for DNA isolation. Total DNA was extracted from fresh leaf tissues as described by Dellaporta et al. (1983). The collected pellet was washed with 70% ethyl alcohol and air-dried. DNA quantification was done on 1% agarose gel with Lambda DNA or corresponding amounts of genomic DNA samples were placed in a quartz spectrophotometric cuvette. Absorbance (optical density, OD) readings at 260/280 nm were recorded using a Shimadzu UV-spectrophotometer. The conditions for PCR amplification on PTC 100 (Programmable thermocycler) of MJ Research Pvt. Ltd., USA were optimized for template DNA, *Taq* polymerase and $MgCl_2$ concentration. PCR amplifications were performed with 30 random primers. Amplifications were carried out in 20 µl reaction volume containing 20 ng of genomic DNA, 10X PCR buffer containing $MgCl_2$, dNTPs (2.5 mM) (Bangalore Genei Ltd., India), Primers (Operon Technologies Inc., USA), *Taq* polymeras e (3 units/µL) (Bangalore Genei Ltd., India). Amplification conditions included Profile 1: 94°C for 2 min Initial denaturation, profile 2: 94°C for 1 min denaturing, profile 3: 37°C for 1 min annealing, profile 4: 72°C for 2 min extension, profile 5: 72°C for 5 min final extension and profile 6: 4°C to hold the samples for infinity. Profiles 2, 3 and 4 were programmed to run for 31 cycles. The PCR products were separated on 1.5% agarose gel run in 1 X TBE and stained with ethidium bromide. The gels were visualized with a UV trans-illuminator. Gel photographs were scanned through Gel Doc system (Gel Doc, 2000, BioRad, USA) and the amplification product sizes were evaluated using the software Quantity (Bio Rad). All PCR reactions were run in triplicate and only reproducible and clear bands were scored.

Data scoring and statistical analysis

Each reproducible band was visually scored '1' for presence and '0' for absence. The data were analyzed using NTSYSpc ver. 2.1 (Rohlf, 2002). Similarity matrices, generated according to the coefficient of Jaccard (Sneath and Sokal, 1973) were used to perform cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) (Sokal and Michener, 1958). Dendrogram was constructed with the TREE programme of NTSYSpc.

RESULTS AND DISCUSSION

Out of the 30 RAPD primers screened, 10 primers produced clear polymorphic bands in all the accessions on preliminary screening and were selected for further analysis. A total of 43 bands were detected with the 10 primers (OP L1, OP M12, OP L 18, OP L6, OP L11, OP L12, OP K8, OP D15, OP K4 and OP B18; Figure 1). The number of bands per primer ranged from 3 (OP L12 and OP K8) to 6 (OP M12) with an average of 4.3 scorable bands per primer. Out of the 43 bands, 26 were polymorphic (67.49%) revealing a high degree of polymorphism (Table 2).

Genetic similarity matrix was calculated on the basis of Jaccards algorithm for RAPD data. The pair wise similarity values ranged between 0.609 and 0.810 (Table 3). The maximum similarity value was noticed between V_3 and V_5 accessions. The wide range of similarity indicated the presence of high genetic diversity in *Chlorophytum* collection. The clustering of RAPD based genetic similarity values using the UPGMA method are presented in a dendrogram (Figure 2). In the dendrogram, similarity coefficients between all possible pairs of genotypes ranged between 0.50 and 1.00.

A persual of dendrogram indicates that there were two clusters, that is, cluster I and II. Cluster I consists of two genotypes V_1 and V_2, whereas cluster II consists of 7 genotypes V_3, V_5, V_4, V_8, V_8, V_6 and V_7. Cluster II is again subdivided into two- group IIA and IIB. Group II A consists of V_3, V_5, V_4, V_8 and V_9, whereas, group IIB consists of V_6 and V_7. RAPD analysis on *C. borivilianum* indicated the maximum similarity between V_3 and V_5 which was shown by the coefficient of 0.81%. Regenerated plants derived from somatic embryos of leaf explants origin showed a very little variation (0.62%) in

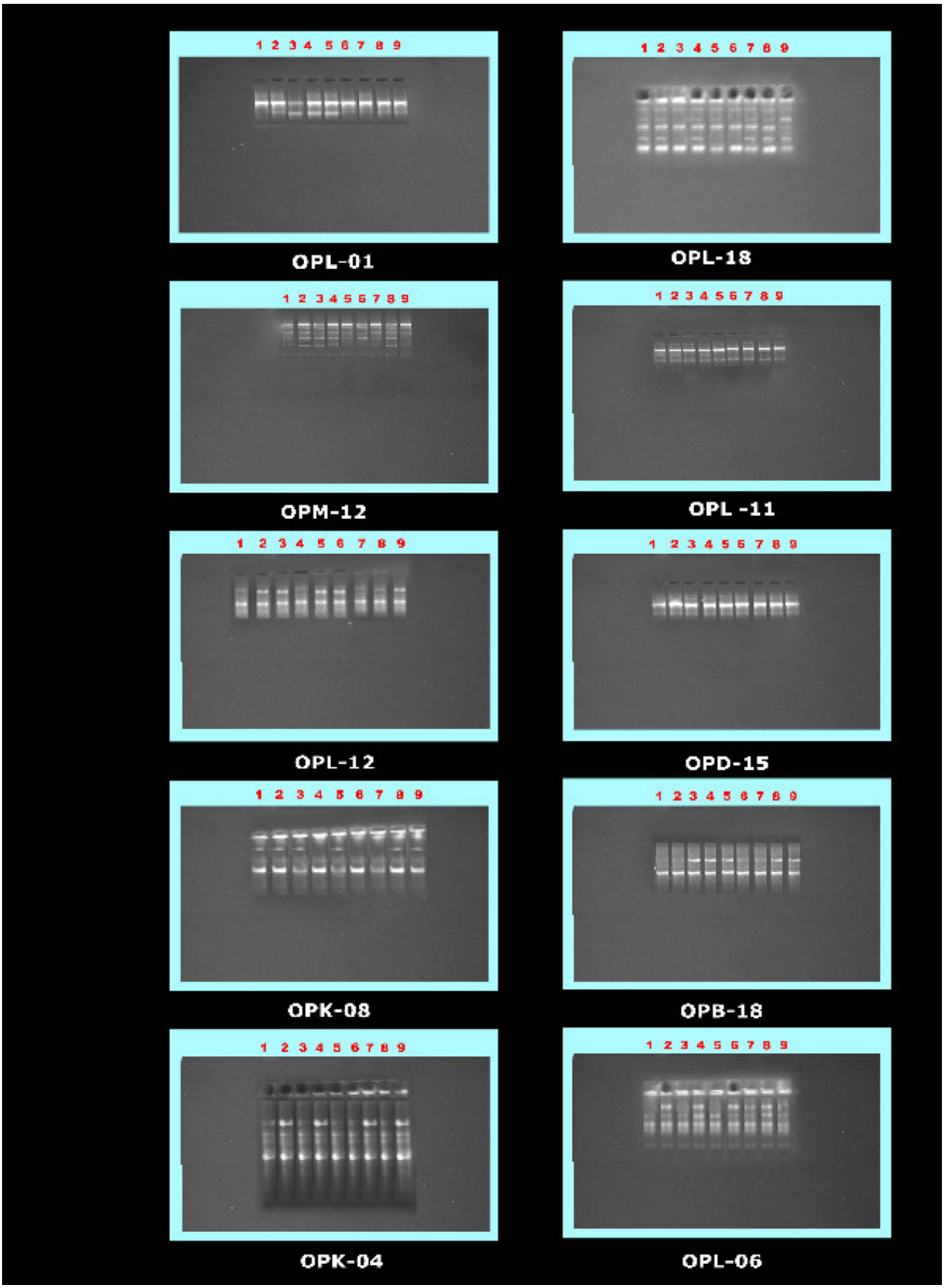

Figure 1. RAPD profiles of *C. borivilianum* using different primer.

Table 2. List of primers and number of bands generated through amplification.

S/N	Primer code	Total number of band	Number of polymorphic band	Percentage of polymorphism
1	OPL-01	4	3	75.00
2	OPL-18	5	4	80.00
3	OPM-12	6	4	66.66
4	OPL-11	4	2	50.00
5	OPL-12	3	1	33.33
6	OPD-15	5	3	60.00
7	OPK-08	3	2	66.60
8	OPB-18	5	3	60.00
9	OPK-04	4	2	50.00
10	OPL-06	4	3	75.00
Total number of bands		43	26	67. 49
Mean number of bands per primer		4.3	2.6	

Table 3. Genetic similarity matrix for *C. borivilianum* genotypes generated using Jaccard´s similarity coefficient.

Genotype	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
V_1	1								
V_2	0.78	1							
V_3	0.6	0.65	1						
V_4	0.7	0.75	0.65	1					
V_5	0.72	0.72	0.81	0.69	1				
V_6	0.69	0.65	0.72	0.74	0.71	1			
V_7	0.78	0.65	0.64	0.74	0.67	0.72	1		
V_8	0.72	0.68	0.76	0.77	0.79	0.76	0.71	1	
V_9	0.73	0.69	0.72	0.78	0.8	0.64	0.68	0.8	1

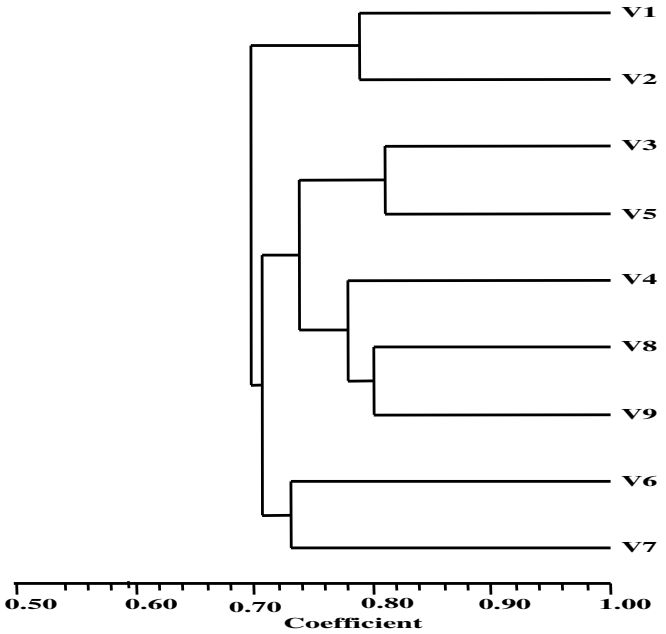

Figure 2. Dendrogram developed from UPGMA cluster analysis showing genomic relationship among the genotypes of *C. borivilianum*

RAPD fingerprinting (Arora et al., 2006). The genetic fidelity test of micro-cloned, bio-hardened progeny of *C. borivilianum* based on a RAPD analysis using 40 random decamer DNA primers indicated a strong uniformity in relation to the parent genotype (Mathur et al., 2008).

RAPD analysis detects large differences even in closely related taxa and, at least if detection conditions are adjusted very carefully, can find reliable phylogenetic signal even above the species level. The high level of polymorphism observed agreed with the results of previous studies carried out on *C. borivilianum* and also in other plant species like *Allium* (Al-Zahim et al., 1997; Bradley et al., 1996; Maas and Klaas, 1995), *Gloriosa superba* (Ghosh et al., 2009) and *Populus deltoides* Marsh (Rani et al., 1995), thus confirming the high diversity within the germplasm. Number of bands obtained per primer indicated the efficiency of the primers to characterize the germplasm.

The polymorphic pattern observed in the different *C. borivilianum* genotype and the extent of information generated from RAPD analysis varied with the primers. The observation of molecular data reveals significant levels of diversity among genotypes. The greatest advantage of the RAPD approach is its technical

simplicity and the independence of any prior DNA sequence information. The polymorphism indicated the effectiveness of RAPD markers in detecting polymorphism at the intra specific level.

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