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DNA Fingerprinting and Phylogenetic Relationship of the Genus *Chlorophytum* Ker-Gawl, from India using AFLP

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ABSTRACT

Chlorophytum Ker - Gawl, is a medicinally important plant genus employed since ancient time as a key component in Ayurvedic and Unani medicine. Genus represented with more than 217 species out of which 17 species have been reported from India. The main objective of this study is to evaluate molecular phylogeny of *Chlorophytum* species. In this study phylogenetic analysis of *Chlorophytum* species was carried out using AFLP marker. Total 16 selective primer combinations were scored as presence and absence of alleles for all the 17 species, resulting in total 938 allele, out of which 291 allele were found to be polymorphic. The percentage of polymorphism ranged from 18.3% (in the combination E2M3) to 42% (in the combination E1M1).The phylogenetic tree is divided into two clade, each clade contains species with similar morphological characters. The extent of variations within species is discussed.

KEYWORD: *AFLP, Chlorophytum, Fingerprinting, phylogenetic relationship*

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1. INTRODUCTION

Genus *Chlorophytum spp. Were* considered to be one of the primitive Angiosperms that well diversified, comprising 250 species, six subspecies and eight varieties distributed in the old world tropics especially in Africa and India (Chandore*et al.* 2012 and Malpure and Yadav 2009). Seventeen species were reported from India, out of which 15 species occur in the Western Ghats (Adasul 2015). Indian species of *Chlorophytum* were adapted to varied habitats ranging from forest undergrowth and steep slopes amongst grasses to open exposed rocky lateritic plateaus (Lekhak*et al*., 2012; Karthikeyan*et al*., 1989; Chandore*et al.,* 2012; Malpure and Yadav, 2009; Sardesai*et al*., 2006). In India, *Chlorophytumborivilianum,* commonly known as safedmusali, is an important species in Indian folk medicine and has been reported in ancient medicinal text "Ayurveda" (Haque*et al*., 2011). Ayurveda has a long history in India where the plants were used as medicine. *Chlorophytum borivilianum* (Safedmusali) was considered as a "wonder drug" in the Indian system of medicine due to its aphrodisiac and natural sex tonic properties (Mishra, 2012; Singh *et al*. 2012). It is a rich source of over 25 alkaloids, vitamins, proteins, carbohydrates, steroids, saponins, potassium, calcium, magnesium, phenols, resins, mucilage, and polysaccharides and also contains high quantity of simple sugars, mainly sucrose, glucose, fructose, galactose, mannose and xylose. Because of its high therapeutic importance, *Chlorophytum* species tubers contained major constituents of more than 100 Ayurvedic preparations (Thakur *et al*., 2009; Haque *et al*., 2010; Kaushik, 2005; Patil *et al*., 2011; and Lakshmi *et al*., 2009;)

A survey of market samples revealed that other *Chlorophytum* species were often marketed as "Safedmusali" (*C. borivilianum.).* Medicinal properties of certain species were well known but many species were still not explored for their medicinal usage. Taxonomy of quite a few species in this genus was considered to be problematic. (Kale and Thakare, 2013; and Lekhak*et al*., 2012) *Chlorophytum* species showed the continuous variability of morphological characters, especially of aerial parts such as leaves and tubers. A solution to these problems was to evaluate phylogenetic relationships between *C. borivilianum* and sixteen other species reported from India (Lekhak*et al*., 2012). Molecular markers, which directly display differences on the DNA level and which are independent of the phenotype, represent a significant resource for creating genetic and physical genome maps, distinguishing individuals, investigating genetic relatedness and studying genome organization and marker for DNA fingerprinting (Percifield*et al*., 2007; Shasany, *et al*., 2005; Bayder*et al*., 2004; Zerega*et al*., 2002). Molecular markers have been applied in a number of research projects to investigate the polymorphism in *Chlorophytum,* but the reported studieswas restricted to *C. borivillianum*and its cultivated varieties and

accessions by using Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment length polymorphism (AFLP) (Samantaray and Maiti, 2010; Tripathi*et al*., 2012). Here, for the first time in India, we present a study on the phylogenetic relationship among all *Chlorophytum* species reported in India by using AFLP based DNA fingerprinting.

Table 1- List of *Chlorophytum* species reported from India with their voucher numbers, abbreviations, altitude and longitude.

Sr. No	Species Name	Abbreviation	Vouchar No.	Altitute	Longitute
1	*C. heynei,*Rottel ex Baker	CHY	KUKCHCH2	14^{0}50'07.59	74^{0}22'52.73
2	C. attanetum, *Baker*	CAT	KUKCHAT6	18^{0}16'55.81"N	73^{0}50'59.91"E
3	*C. aurndianaceum Baker*	CAR	KUKCHAR1	21^{0}23'38.03"N	77^{0}14'02.94''E
4	*C. belgaumense, Chandore, Malpure, Adsul&Yadav*	CBS	KUKCHBS1	15^{0}40'15.35" N	74^{0}30'03.08" E
5	*C. bharuchii,* Ansari, Sundaragh&Hemandri	CBH	KUKCHBH2	19^{0}55'13.57"N	75^{0}18'29.56''E
6	*C. borivillianum,* Sant.fern	CBR	KUKCHBO3	21^{0}23'33.56"N	77^{0}15'50.04''E
7	*C. breviscapum,* Dalzell	CBRE	KUKCHBR4	17^{0}48'05.55"N	73^{0}10'23.12''E
8	*C. comosum,* (Thumb) Jacq.	CCM	KUKCHCM1	20^{0}55" 25.57N	77^{0}47''40.43'E
9	*C. glaucum,* Dalzell	CGL	KUKGLALU6	16^{0}06'38.48"N	74^{0}32'00.96''E
10	*C. glaucoides,* Blatt	CGLO	KUKCHGLA5	17^{0}41'23.78'N	73^{0}57'35.94'E
11	*C. gothanese,* Malpure& SR Yadav	CGO	KUKCHGO1	15^{0}05'38.00	73^{0}45'42.62
12	*C. kolhapurense,* Sardesai, SP Gaikwad&SRYadav	CKO	KUKCHKO12	16^{0}28'43.35"N	74^{0}118'38.96''E
13	*C. laxsum,* R.Br.	CLA	KUKCHLX1	21^{0}23'25.79"N	77^{0}26'34.62''E
14	*C. malbaricum,* Baker	CMA	KUKCHMA4	13^{0}30'18.23	75^{0}44'10.07'
15	*C. nepalense,* (Lindl) Baker	CNP	SUMONA49		
16	*C. nimmonii,* Dalzell	CNI	KUKCHNIM8	18^{0}23'09.19"N	73^{0}00'55.81''E
17	*C. tuberosum,* (Roxb.) Baker	CTU	KUKCHTU2	21^{0}21'59.44"N	77^{0}18'51.96''E

Table 1 The List of Chlorophytum species reported from India; voucher number; Abbreviation

2. Materials and Methods

2.1. Collection of *Chlorophytum* species and DNA extraction

Species of *Chlorophytum* were collected from different parts of India during 2009 to 2012. Collected specimens were identified and confirmed by matching with the herbarium specimens at Botanical Survey of India, Pune, Maharashtra, and by extensive literature survey. The specimens were deposited at the herbarium of Botanical Survey of India, Pune, Maharashtra. The Global Positioning System (GPS) recorded spatial data of the different species found in India. Finally, the distributions of different species were displayed in the various parts of India (Table 1).

DNA was extracted from young fresh or silica gel-dried leaves using a modified CTAB procedure of Doyle, and Doyle (1987).

2.2. AFLP fingerprinting

Template preparation and adaptor ligation

The AFLP procedure was performed according to the protocol of Vos *et. al*., (1995) with some modification to improve results. 200 ng DNA of each species was used for initial digestion by 1U *MseI* (Thermo scientific) and 1U *EcoRI* (Thermo scientific) 10 X fast digested green buffer. The final volume of the reaction mixture was adjusted to 40 µl and incubated at 37^o C for 1 hr. The enzyme activity was inactivated by heating at 80^0 C for 20 min. *MseI* and *EcoRI* adaptors were ligated to the digested DNA to generate template *MseI* adaptor 7.5 pmol, *EcoRI* adaptor 7.5 pmol, T_4 ligase enzyme 1U, (10X) T_4 ligase buffer 1X, and the final reaction volume was adjusted to10 µl. To this ligated mixture, digested DNA was added and volume was adjusted to 50 µl. The reaction mixture was incubated at 20^0 C for 2 hr. The ligation products was diluted 10 fold and 5 µl was added to preamplification reaction containing Deam *Taq* Green DNA Polymerase 1U (Thermo scientific), Deam*Taq* Green buffer 1X (Thermo scientific), $MgCl_2$ (Thermo scientific) 2.5mM, dNTP (Thermo scientific), 0.3 mM Pre amplification primer (E+A) 10 pmol, Pre amplified primer (M+C) 10 pmol, final volume of reaction was 50 µl. Preamplification was carried out for 20 cycles at 94 ^{0}C for 30s, at 56^{0}C for 1min., at 72^{0}C for 2 min. and amplified product diluted 50 fold and stored at -20^{0}C.

Selective amplification was carried out with *EcoRI* and *MseI* primers (Table 2) each carrying extra three and two selective nucleotides respectively. The PCR reaction contained Deam *Taq* Green DNA Polymerase 1U (Thermo scientific), 10 X Deam*Taq* Green buffer 1X (Thermo scientific), $MgCl_2$ (Thermo scientific) 2.5mM, dNTP (Thermo scientific), 0.3 mM, selective *MseI* and *EcoRI* 10pmol respectively. Final volume of reaction was adjusted to 20 µl. The PCR selective amplification temperature profile were as follows: one cycle at 94^{0}C for 30 s, at 65^{0}C for 30 s, and at 72^{0}C for 60 s, followed by 12 cycles of touchdown PCR in which the annealing temperature decreased by 0.6^{0}C every cycle until a 'touchdown' annealing temperature of 56^{0}C was reached. Once reached, another 23 cycles were conducted as 94^{0}C for 30 s, 56^{0}C for 30 s, and 72^{0}C for 2 min followed by final extensions at 60^{0}C for 30 min. Reaction product was mixed with equal volume of 98% form amide. Denatured by incubating at 90^{0}C for 3 min and quickly cooled on ice. The products were analysed on 4 % (w/v) denaturing polyacrylamide gels. The gel was run at constant power (500 V) until the xylene cyanol has reached two-thirds down the length of the gel. The gels were silver stained and scanned with scanner for analysis and documentation.

Table 2: Oligonucleotide sequences used in AFLP Analysis

Primer	Code	Sequences
*EcorI*AAG	E1	GACTGCGTACCAATTCAAG
*EcorI*ACG	E2	GACTGCGTACCAATTCACG
*EcorI*AGC	E3	GACTGCGTACCAATTCAGC
*EcorI*AAC	E4	GACTGCGTACCAATTCAAC
*MseI*CAG	M1	GATGAGTCCTGAGTAACAG
*MseI*CTC	M2	GATGAGTCCTGAGTAACTC
*MseI*CTA	M3	GATGAGTCCTGAGTAACTA
*MseI*CTT	M4	GATGAGTCCTGAGTAACTT

2.3. Phylogenetic analysis

AFLP analysis

To analyze the AFLP markers data, the amplified fragments were scored qualitatively as a dominant allele at a unique locus. Polymorphic amplified fragments were scored manually as '1' for the presence and '0' for the absence of an allele at a particular locus across all the 17 species for each primer combination. Only clearly distinct alleles were scored for data analysis. Binary data obtained for the AFLP primer combinations was used for assessing the discriminatory power of AFLP primer combinations. PIC was calculated using the formula PIC=1—ΣP^2ij where, *Pij* is the frequency of the *jth* allele for *ith* locus summed across all alleles for the locus. The polymorphic information content (PIC) was calculated for each primer combination (Table 3). The marker index was calculated for each AFLP primer combination as MI = PIC x $n\beta$ where PIC is the mean PIC value, *n*the number of alleles, and β is the proportion of polymorphic alleles (Table 3) (Powell *et. al.,* 1996; Mateescu *et. al*., 2005; and Poroceddu *et. al*., 2002). Dendrogram was generated by using NTSYS-pc (Rohlf, 2000).

3. Results and Discussion

3.1. Allele Scoring.

Eight different primers (Table 2) were used in sixteen combinations to generate AFLP fingerprintings. A total of 938 AFLP alleles were identified in the study, of which 291 were polymorphic with clear and reliable reading. The number of polymorphic alleles ranged from 11 to 26 per gel with an average of 18.19 ± 3.98 per primer combination. The percentage of polymorphism ranged from 18.3% (in the combination E2M3) to 42% (in the combination E1M1) (Table 3). Out of 291 polymorphic alleles, 55 alleles were found to be unique for species for 16 primers combination; the primer combination E1M1 amplified maximum number of specific alleles (7) while E3M4 generated only one specific allele. All the 17 species of *Chlorophytum* possessed specific alleles, *C. kolhapurense,* exhibited highest specific alleles (19). *Chlorophytumbharuchea* had 18 specific alleles and *C. borivilianum*exhibited 15 specific alleles. *Chlorophytumbelgaumense*, *C. breviscapum* and *C. glaucoides* had 13 specific alleles each, *C. arundinaceum* and *C. glaucum* had 12 alleles each and *C. tuberosum*, *C. nepalense* and *C. Malbaricum* and *C. heynei* exhibited 10 and 9 specific alleles respectively. *Chlorophytumnimmonii*had 8 alleles while *C. attenuatum* had 7 specific alleles. *Chlorophytumcomosum* had 6 alleles, while *C. gothanese* and *C. laxum* exhibit 5 alleles. (Table 4). The marker index per primer combination varied from 9.23 to 23.66 with an average of 16.20 (Table 3). PIC values ranged from 0.83 to 0.92, and average value was at 0.88 ± 0.05.

Table 3: Characterization of the degree of polymorphism and quality of AFLP data generated with 16 primer combination.

Sr. No	PC.	TNB	NPB	PPB	PIC	MI
1	E1M1	50	21	42	0.92 ± 0.03	19.32
2	E1M2	55	14	25.45	0.92 ± 0.03	12.87
3	E1M3	65	17	26.15	0.88 ± 0.05	14.95
4	E1M4	58	21	36.20	0.93 ± 0.03	19.53
5	E2M1	60	17	28.33	0.90 ± 0.04	15.30
6	E2M2	56	17	30.35	0.88 ± 0.05	14.96
7	E2M3	60	11	18.33	0.83 ± 0.08	9.13
8	E2M4	68	18	20.47	0.90 ± 0.05	16.20
9	E3M1	55	15	27.27	0.88 ± 0.06	13.20
10	E3M2	70	20	28.57	0.91 ± 0.04	18.21
11	E3M3	80	25	31.25	0.89 ± 0.05	22.25
12	E3M4	56	15	26.47	0.83 ± 0.08	12.45
13	E4M1	71	26	36.61	0.91 ± 0.04	23.66
14	E4M2	49	15	30.61	0.84 ± 0.07	12.60
15	E4M3	63	21	33.33	0.89 ± 0.05	18.69
16	E4M4	67	18	26.86	0.89 ± 0.05	16.02
	Mean	61.43	18.19	29.27	0.88 (± 0.05)	16.20
	Total	938	291			

PC –Primer Combination
TNB- Total Number of Polymorphic allele
NPB- Number of polymorphic allele
PPB- Percentage of polymorphic allele
PIC- Polymorphic Information Contain
MI- Marker Index

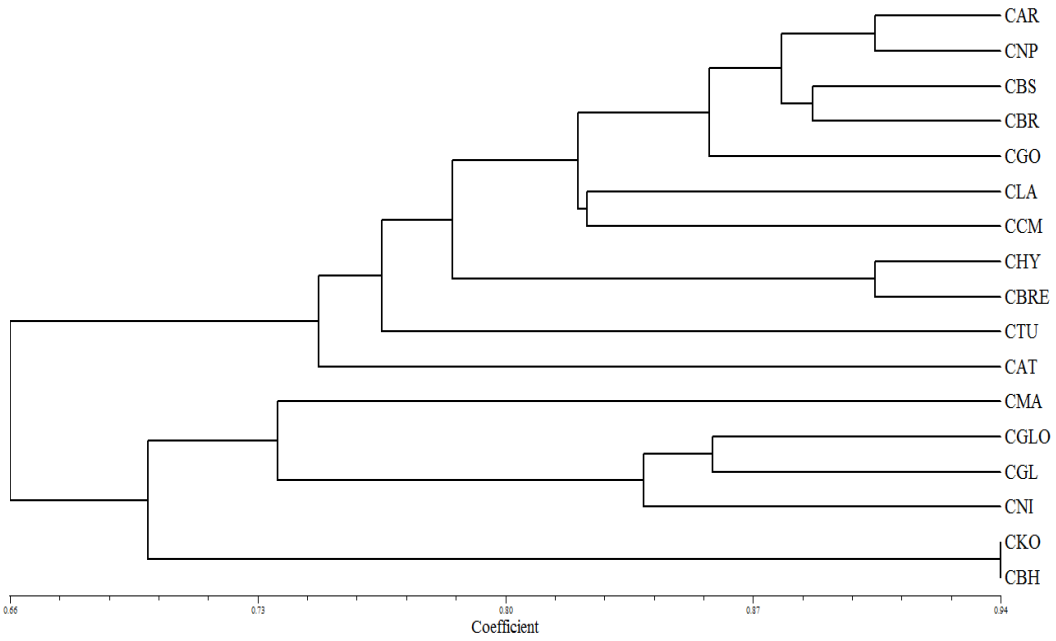

Fig.1 Dendrograms generated using unweighted pair group method with arithmetic average (UPGMA) analysis, showing relationships between 17 *Chlorophytum* species using AFLP.

3.2. Phylogenetic relationships

For the AFLP analysis, each polymorphic fragment was scored as a locus with two allelic classes. Differences in genetic diversity among the species of *Chlorophytum*, attributed to adaptation to varied habitats ranging from forest undergrowth and steep slopes amongst grasses to open exposed rocky lateritic plateaus were evaluated by analysis of variance. PIC values were then calculated for each locus in every species of *Chlorophytum*. The maximum PIC value of an AFLP locus is 0.92. Since the data for different species, the numbers of polymorphic alleles were expected to be higher as compared to the accessions and varieties. Genetic relationships among cultivars were calculated with the Dice's coefficient (Dice, 1945) using NTSYS-pc version 2. 0 software (Rohlf, 2000). The resulting similarity matrix was first subjected to cluster analysis by the unweighted pair-group method with the arithmetic averages (UPGMA) using NTSYS-pc (Rohlf, 2000). The high level of polymorphism was reported in the study. The relationship observed using molecular markers may provide information on the history and biology of species, but it does not reflect any correlation with medicinal properties. However, we found that, there was no relationship between morphological divergence and geographical origin of species, as same cluster contain species found in different geographical condition. To evaluate the phylogenetic relationships among Indian species of *Chlorophytum*, a dendrogram based on 291 AFLPs loci was constructed by using UPGMA (Fig. 1). The Dice's coefficient of similarity among the cultivars based on these 291 polymorphic AFLP loci showed a normal distribution, with an average of 0.74 ± 0.07. Similarity coefficients ranged from 0.52 (*C. attenuatum Baker*and *C. glaucum* Dalzell) to 0.93 (*C. Kolhapurense* Sardesai, Gaikwad and Yadavand *C. Bharuchea*Ansari, Sundaragh. and Hemadri) (Fig-2). The dendrogram was dividedinto two clades: clade I comprise *Chlorophytum* species with unbranchin florescence while lade II were represented by *Chlorophytum* species with branched inflorescence. In addition to this, we proposed some other characters such as the presence or absence of papillae on the filaments and length of filaments with respect to anthers which were of diagnostic value and strongly supported the AFLP data. In clade I *C. arudinaceum*and *C. nepalense* (0.9) had papillose filament and filament shorter than anther, both species exhibits 12 and 10 specific alleles, out of which 3 alleles were species specific (Table 4), and showed similar morphological characters. *C. borivilianum and C. belgaumense* (0.88) showed simple and long filament as compared to anthers, exhibited 15 and 13 specific alleles of which 4 were specific species and they were in same grouped. (Table 4). *C. laxsum and C. comosum* (0.9)had simple filament and anther were shorter as compared to filament, both species were grouped in same sub clade. *C. breviscapum and C. heynei* were group in same sub clade, had papillose filaments and anthers shorter than filaments while both species had 8 species specific allele. *C. gottanese*were showed the individual evolution from *C. borivilianum* clade and simple filaments and anthers was longer than filaments. The *C. attenuatum,* and *C. tuberosum* had simple filament but in *C. tuberosum* anther were shorter than filament while *C. attenuatum* had equal length of anther and filament, all these species do not share any common alleles (Fig1). In Clade II *C. glaucum,* and *C. Glaucoides* (0.85)had papillose filament and anthers greater than filament, *C. nimmonii* showed individual evolution from *C. glaucum* clade and had papillose filament and filament shorter than anther, which exhibits 12 and 13 specific alleles out of which 7 were found to species specific andshowed common evolution with*C. barucheaand C. Kolhapurense* (0.93) had papillose and simple filaments respectively but anther were shorter than filaments, which exhibits 18 and 19 specific alleles out of which 6 were unique to species and grouped in same clade and *C. malbaricum*not share any group, thus were genetically diverse fromclade II (Table 4) (Fig 1). Fischer (1935) used certain floral characters such as the number of nerves in the perianth segments to distinguish certain South Indian species. According to Naik (1977) these features along with relative flower size, including lengths of perianth segments, filaments and anthers appear to be fairly constant and can be used in classifying Indian species. Traditionally, *Chlorophytum* species were identified by using morphological characters but problem was encounter when we had working on closely related species which showed common character at that time traditional way had limitation resulted in wrong identification. Recent advances in DNA based identification technique allows identification of species on the basis of molecular marker, if we considered each alleles as morphological characters then we can standardized the molecular key (identical with in taxonomical key). In taxonomy common character represents the relativeness of species

similarly common alleles indicate that species share common origin and unique alleles act as a key for identification. A multiverient analysis of polymorphic data was performed, for this purpose, a principal coordinate analysis (PCO) implemented in the program PAST (Hammer *et al.,* 2001), using "Gower" general similarity coefficient was performed. All the 17 *Chlorophytum* species divided into four groups due to central axis and each group contain species with similar morphology and showing similarity with UPGMA cluster analysis (Fig 3).

	CAR	CMA	CNP	CGO	CTU	CGLO	CGL	CNI	CBS	CLA	CAT	CCM	CKO	CBH	CBR	CHY	CBRE
CAR	1																
CMA	0.75	1															
CNP	0.9	0.73	1														
CGO	0.88	0.74	0.84	1													
CTU	0.83	0.7	0.77	0.84	1												
CGLO	0.63	0.73	0.65	0.67	0.62	1											
CGL	0.64	0.74	0.66	0.65	0.61	0.85	1										
CNI	0.64	0.73	0.67	0.62	0.56	0.82	0.85	1									
CBS	0.89	0.7	0.85	0.85	0.76	0.63	0.65	0.64	1								
CLA	0.83	0.68	0.79	0.79	0.73	0.55	0.58	0.57	0.86	1							
CAT	0.82	0.63	0.76	0.73	0.74	0.53	0.52	0.53	0.79	0.76	1						
CCM	0.84	0.71	0.81	0.79	0.74	0.57	0.59	0.58	0.83	0.82	0.73	1					
CKO	0.69	0.73	0.7	0.69	0.62	0.68	0.69	0.67	0.71	0.65	0.61	0.67	1				
CBH	0.71	0.73	0.72	0.7	0.64	0.7	0.69	0.69	0.73	0.66	0.62	0.66	0.93	1			
CBR	0.88	0.69	0.87	0.84	0.74	0.65	0.66	0.65	0.88	0.8	0.75	0.82	0.78	0.8	1		
CHY	0.79	0.71	0.79	0.79	0.69	0.65	0.64	0.63	0.78	0.71	0.67	0.7	0.76	0.79	0.86	1	
CBRE	0.82	0.78	0.78	0.82	0.77	0.65	0.65	0.65	0.78	0.76	0.69	0.77	0.7	0.71	0.79	0.9	1

Fig.2 f 17 species of *Chlorophytum* reported in India. Diversity estimates were based on 291 AFLP markers

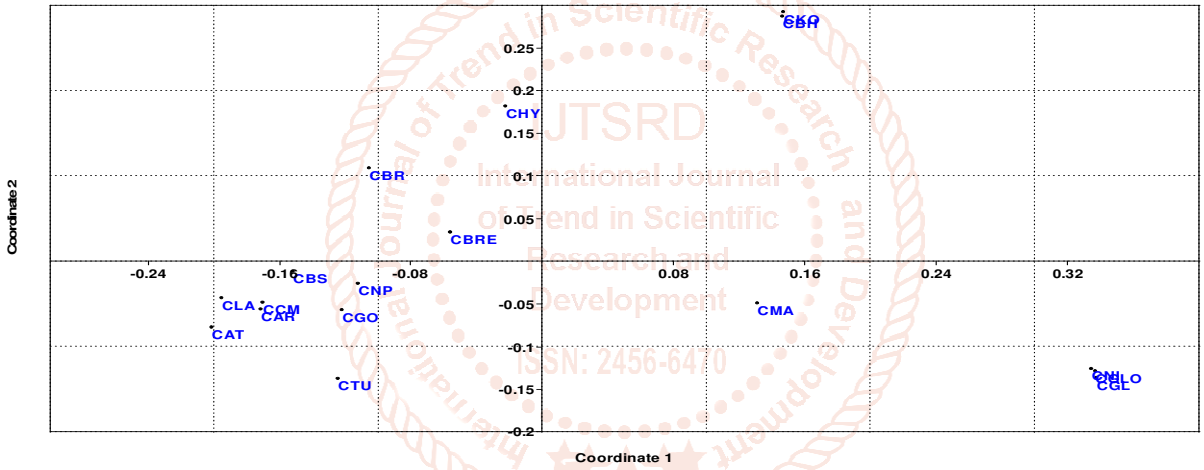

Fig.3 Two dimentionalprincipal coordinate analysis (PCO) of 17 species of *Chlorophytum* reported in India. Diversity estimates were based on 291 AFLP markers

Table 4 AFLP primer combinations amplified species specific allele (base pairs) in 17*Chlorophytum* species. Size of alleles were calculated by using Alpha Imager 2200 gel documentation software

	CBR	CBS	CMA	CTU	CNP	CAR	CNI	CBRE	CGL	CAT	C. HEYNEI	CCM	CBH	CGO	CLA	CGLO	CKO
E1 M1	186, 275	186, 245,275	245	245, 280	186, 245, 275	186, 245, 275		55, 126	275, 310		55, 126	55, 126, 310	55, 126, 186,275	310,	55, 126, 310	310	55, 126,186, 275,310
E1 M2	285			285	240	240, 285			240, 285							285	
E1 M3	89	89			89	89							170			270	170
E1 M4				248		218	181	218, 181		248			218			181	218
E2 M1	132	132	228, 110	180	110	110	165	228, 110	165	180	110	132	228, 180,110				228
E2 M2					219	219		105			219, 105	219, 121	219		121		219
E2 M3	65	65	190, 123					190, 65	65	190	65				190	65	

E2 M4	214, 150, 110	214, 190, 150, 110	190	110			150, 55	190	150	110	190		214	150		150	55
E3 M1	122, 89	89	50			50			50							50	
E3 M2			110		178	178	216, 178		216, 169				210, 178			216, 178	216, 210, 178
E3 M3	316	316					316	300								316, 300	316
E3 M4																	75
E4 M1	280, 130, 100	130		130				130		130			280, 130, 94	280			130, 94
E4 M2			285	110						110				169			
E4 M3				59, 44	208	208	44	59	208, 44	44	59		44			44	59, 44
E4 M4					138						138		239	138		239	

Conclusion

Present study confirms the robustness and the suitability of the AFLP approach for diversity analysis and for the assessment of genetic relationship among 17 *Chlorophytum* species reported in India. Knowledge of the levels and patterns of genetic diversity were important for designing conservation strategies for threatened and endangered plant species. There was concerned that the erosion of the genetic variability might result in reduction of the plasticity of species to respond to changes in climate, pathogen populations, agricultural practices, or quality requirements. As per molecular works of *Chlorophytum species* only few relevant work of *Chlorophytum*species has been identification of *Chlorophytumborivilianum, C. arundinaceum, C. laxum, C. capense*and *C. Comosum* using *rbcl* genesequences, RAPD and *rpl16* species sequences *(*Kaotch *et. al.,* 2010). Molecular phylogenetic relationship of medicinally important *Chlorophytum borivilianum* using AFLP marker (Tripathi *et. al.,* 2012) and Genetic diversity of micropropogated plants *Chlorophytum borivilianum* of RAPD analysis (Samantaray and Maiti, 2010)which itself depicts the need of more molecular works as far as this plant was concerned. So it was quite evident that molecular documentation of this particular genus has not been thoroughly explored. The results obtained in the present study clearly demonstrated that AFLP markers represent a good diagnostic means to differentiate 17 species of the *Chlorophytum*. In addition AFLP marker were preferable, since they provide good information about polymorphism information contained and marker index on species level identification of *Chlorophytum,* simultaneously it provide genomewise wild differences among species and will be helpful for identification of unknown *Chlorophytum* species.

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