Chapter 4

Evaluation of Genetic Diversity in *Chlorophytum borivilianum* (Santp. and Fernan.) Using Molecular Markers: An Endangered Medicinal Plant

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Additional information is available at the end of the chapter

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Abstract

Chlorophytum borivilianum is a traditional medicinal plant distributed throughout the tropics and subtropics. In the present investigation, RAPD and ISSR analyses were used to assess the genetic diversity among 21 accessions collected from different geographical regions of India using 20 RAPD primers and 6 ISSR primers. RAPD and ISSR primers revealed 92.26% and 82.76% polymorphism, respectively. Similarity in coefficient values ranged from 0.321 to 0.707 for RAPD and 0.363 to 0.846 for ISSR markers. The dendrogram developed by RAPD and ISSR marker-based analysis grouped the 21 accessions into different clusters. Mantel test employed for detection of goodness of fit established the cophenetic correlation value for both the primer systems and it was observed to be significant. Clustering of accessions within groups was also similar based on RAPD- and ISSR-derived dendrograms. In our study, both marker systems were similar except for the percentage polymorphism which was found to be greater using RAPD, thus indicating the greater effectiveness of RAPD primers for estimating genetic variation of *C. borivilianum*.

Keywords: *Chlorophytum borivilianum*, ISSR, RAPD, Diversity, medicinal plant

1. Introduction

All the plants considerably possess some medicinal or perfumery or mixed properties. In the course of time, the human beings were able to distinguish between the harmful and useful plants. The world Health Organization (WHO) has listed over 21,000 plant species that have been reported for medicinal uses around the world. Among these, over 100 botanicals are reported to have consistent large demand and are traded in major drug markets in the world.

In the developing countries, about 80% of the people depend upon the traditional system of the medicine, as it shows no or less side effects [1]. India is considered as a veritable emporium of medicinal and aromatic plants. In India, about 2500 plant species belonging to more than 1000 genera are used by traditional healers and about 500 plant species are utilized by 159 different pharmaceutical companies [2].

Many of the medicinal plant species are facing threats of extinction due to over and improper exploitation, habitat loss, degradation of land, urbanization, etc. On the other hand, the increasing global demand for the medicinal plants necessitates an accelerated cultivation and conservation of them. However, before the widespread domestication of such plant species is implemented, it would be important to determine their genetic diversity so that the useful genotypes could be effectively used as cultivars by farmers or breeders and it would, in turn, facilitate the efficient conservation, management and utilization of the species. For the purpose of conservation and to carry out successful breeding programmes, proper identification of the plant is of prime importance, for which an accurate, reliable and more authentic system of classification is required. Conventionally, identification and classification of plant groups are solely based on similarities and dissimilarities in morphological features, particularly, the floral character which are considered to be consistent. As already established, expression of morphological characters is the outcome of interaction between the environment and the genotype and is highly influenced by climatic and edaphic factors. Certain biochemical markers such as isozymes and storage proteins are used for identification of cultivars as well as characterization of somaclonal variation. However, the number of genetic loci generated with chemical/ biochemical (isozymes) were quite lower than detected with DNA markers [3]. Molecular techniques are very much useful not only to identify the genotypes for authentication but also to assess and exploit the genetic variability [4]. DNA fingerprinting of all the genetic resources of the medicinal plants is necessary to generate a molecular database as well as to utilize the information in a systematic manner.

During last 20 years, the advent of the PCR and the DNA sequencing techniques has allowed a very significant development of this approach, which leads to great change in the traditional vision of the classification of the organisms. The DNA marker systems are considered to be the best tools for determining the genetic diversity, as they are unlimited in number, show high polymorphism and are independent of environmental interaction, i.e. they are highly heritable. The application of the DNA marker systems in agricultural research has progressed rapidly over the past few years, especially, in the area of cultivar identification and characterization [5] as well as determination of population diversity in many plant species [6]. Among these, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers provide a larger number of potential markers that are useful for the analysis of genetic diversity, often using fast, simple and reliable protocols that minimize the amount and quality of DNA required. Genetic diversity in a population is considered to represent its evolutionary potential. Genetic variation has implications for conservation at the species level. Evidently, molecular markers could be used to derive genetic relationship with an increased level of accuracy and also can provide valuable data on diversity through their ability to detect variation at the DNA level.

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Chlorophytum borivilianum Santapau and Fernandes belong to the family Liliaceae and are popularly known as safed musli. It is an important medicinal plant. Its peeled and dried fasciculated roots are considered to be a wonder drug in traditional Indian systems of medicine due to its aphrodisiac and natural tonic properties [7]. About 100 Ayurvedic preparations are available in the Indian market using *C. borivilianum* as a major ingredient [8]. Its roots are widely used for various therapeutic applications in the Ayurvedic and Unani [9] systems of medicine. Though many tribal communities of India use the fresh leaves of safed musli as Pot herb [10], however, the roots are the useful part of the plant for medicinal purposes. Dried roots of Chlorophytum contain 42% carbohydrate, 8–9% protein, 3–4% fibre and 2–17% saponin [11]. It is known to cure many physical illness and weaknesses. In recent years, its effectiveness in increasing male potency has become very popular and is now considered as an alternative to 'Viagra'. Excessive collections from its natural stands and destructive harvesting techniques coupled with poor seed germination and low vegetative multiplication ratio have made this species endangered and simultaneously provided the justification for its conservation. The cost of genetic conservation should be reduced ensuring representation of maximum genetic variation for which a set of accessions should be selected to represent the genetic diversity of a base collection with minimum redundancy [12]. Thus, the present study aimed at characterizing the genetic diversity in 21 accessions of *C. borivilianum* collected from different regions of India using RAPD and ISSR.

2. Materials and methods

2.1. Plant materials

A total of 21 accessions of *C. borivilianum* were collected from different parts of India (Gujarat, Rajasthan, Madhya Pradesh and Maharashtra) and maintained under uniform growth conditions at DMAPR, Anand, Gujarat (**Figure 1**). The name and place of collection of the material used in the study are given in **Table 1**. *C. borivilianum* is reported in Bastar Forests (Chhattisgarh), Dangs forest (Gujarat), Mount Abu, Mahi and Aravalli hills (Rajasthan) of India. It is also reported to occur in some parts of Pakistan. It is now widely cultivated in different parts of India like Andhra Pradesh, Rajasthan, Gujarat and Maharashtra on commercial basis. The CAMP workshop at IIFM, June 1999 reported the natural habitat of this plant as endangered.

2.2. Molecular characterization

2.2.1. Isolation, purification and quantification of genomic DNA

Since isolation of DNA from *C. borivilianum* was encountered with lots of problems, because of the high polysaccharides and saponin contents, a method was standardized for isolation of genomic DNA of this species. Total genomic DNA was extracted from young leaves derived from field-grown plants following the CTAB method [13] with major modifications. At the time of homogenization of the leaves, 20 mg PVP and 6.5 mM dithiothreitol (DTT) were added. The extraction buffer consisted of 4% (w/v) CTAB (cetyl trimethyl ammonium

Figure 1. An overview of 21 accessions of *C. borivilianum* collected from different regions of India and grown in the field.

bromide), 3 M NaCl, 20 mM EDTA (pH, 8.0), 100 mM Tris-Cl (pH, 8.0), 50 mM ascorbic acid, 40 mM diethyl dithiocarbamic acid and 2% (v/v) ß-mercaptoethanol. The quality and quantity of the DNA were checked by 0.8% agarose gel followed by spectrophotometric measurement.

2.2.2. RAPD and ISSR analysis

RAPD analysis was performed using randomly and arbitrarily 10-base primers (Operon Technologies Inc., Alameda, California). A preliminary screening was carried out using 100 RAPD primers following the protocol of Williams et al. [14] with minor modifications. ISSR analysis was carried out using ISSR primers (Banglore Genei, India) based on the protocol of Zietkiewicz et al. [15] with some modifications. The amplified products were separated in 1.5% agarose gel for both the markers used. After electrophoresis, the gel was visualized under the UV light and photographed in a gel documentation system (Syngene, United Kingdom). The sizes of the amplicons were determined by comparing them with that of the ladder.

2.2.3. Scoring of the data for RAPD and ISSR

The data were scored as 1 for the presence and 0 for the absence of the band for each primer-accession combination for RAPD and ISSR analysis.

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Sr. no.	Germplasm (name)	Place of collection
1	Ch1	Madhya Pradesh
2	Ch2	Madhya Pradesh
3	Ch3	Madhya Pradesh
4	Ch4	Madhya Pradesh
5	Ch5	Madhya Pradesh
6	Ch6	Madhya Pradesh
7	Ch7	Madhya Pradesh
8	Ch8	Madhya Pradesh
9	Ch9	Madhya Pradesh
10	Ch10	Rajasthan
11	Ch11	Rajasthan
12	Ch12	Rajasthan
13	Ch13	Rajasthan
14	Ch14	Rajasthan
15	Ch15	Maharashtra
16	Ch16	Maharashtra
17	Ch17	Maharashtra
18	Ch18	Gujarat
19	Ch19	Gujarat
20	Ch20	Gujarat
21	Ch21	Gujarat

Table 1. Detailed information of 21 accessions of *C. borivilianum*.

2.2.4. Data analysis

2.2.4.1. Resolving power (Rp)

Resolving power of the primer/primer combination was calculated as per Prevost and Wilkinson [16] as: $Rp = \Sigma IB$ [IB (band informativeness) = 1- [2 × (0.5 – P)], P is the proportion of the 21 accessions containing the loci.

2.2.4.2. PIC and primer index

The primer index (PI) was calculated from the polymorphic index (PIC). The polymorphic index was calculated as PIC = ΣP_{2i}, P_i is the band frequency of the ith allele [17] Here, the PIC was considered to be 1-p2-q2, where p is the band frequency and q is no band frequency [18]. The PIC value was then used to calculate the RAPD and ISSR primer index. PI is the sum of the PIC of all the markers amplified by the same primer.

2.2.4.3. Jaccard's similarity

Jaccard's coefficient of similarity [19] was measured and a dendrogram based on similarity coefficients generated by the unweighted pair group method using arithmetic averages (UPGMA) [20], and the sequential agglomerative hierarchial and nested (SHAN) clustering was obtained. The entire analysis was performed using the statistical package NTSYS-pc 2.02e [21].

2.2.4.4. Cophenetic correlation

The correlation among different dendrograms generated from the data obtained from RAPD and ISSR was calculated separately and the cophenetic correlation for both the markers was also calculated from the total data.

2.2.4.5. Principal co-ordinate analysis

To visualize the genetic variation among 21 accessions of *C. borivilianum* in detail, PCA was performed with 20 RAPD markers and 06 ISSR markers. This technique helps in converting a set of variables into a few dimensions using which the genotypes under study can be depicted in a two- or three-dimensional space [22] so that the variations of several individuals will be condensed into a set of limited axes. Such a graphical analysis helps in identifying the individuals that tend to cluster together. Principal co-ordinate analysis was also performed using the NTSYS-pc 2.02C software.

To study the efficiency of each marker technique, the Mantel 'Z' test was performed for the comparison of each marker system with the combined data and between the marker systems [23].

3. Results

The present work pertains to the study of genetic diversity among 21 accessions of *C. borivilianum* collected from different regions of India (Gujarat, Madhya Pradesh, Maharashtra and Rajasthan) using molecular markers.

3.1. RAPD analysis

A total of 100 random decamer oligonucleotide primers from 20 numbers from each series (OPA, OPC, OPD, OPP and OPT) were screened using an accession (Ch12; Raj 4) of *C. borivilianum* for primer optimization out of them 20 primers showed the distinct and reproducible amplicons. Twenty primers produced 168 loci out of which 155 were polymorphic and 13 were monomorphic in nature. Among the polymorphic loci, five loci were found unique in nature (**Figure 2**). The amplicons were observed in the range of 250 to >3000 base pair. The resolving power and primer index of the primers varied from 12.762 to 3.810 and 0.499 to 0.245, respectively. Best resolving power was observed in the primer OPA16 and the minimum (3.810) with primer OPT18. The maximum RAPD primer index (RPI) (0.499) was observed with the

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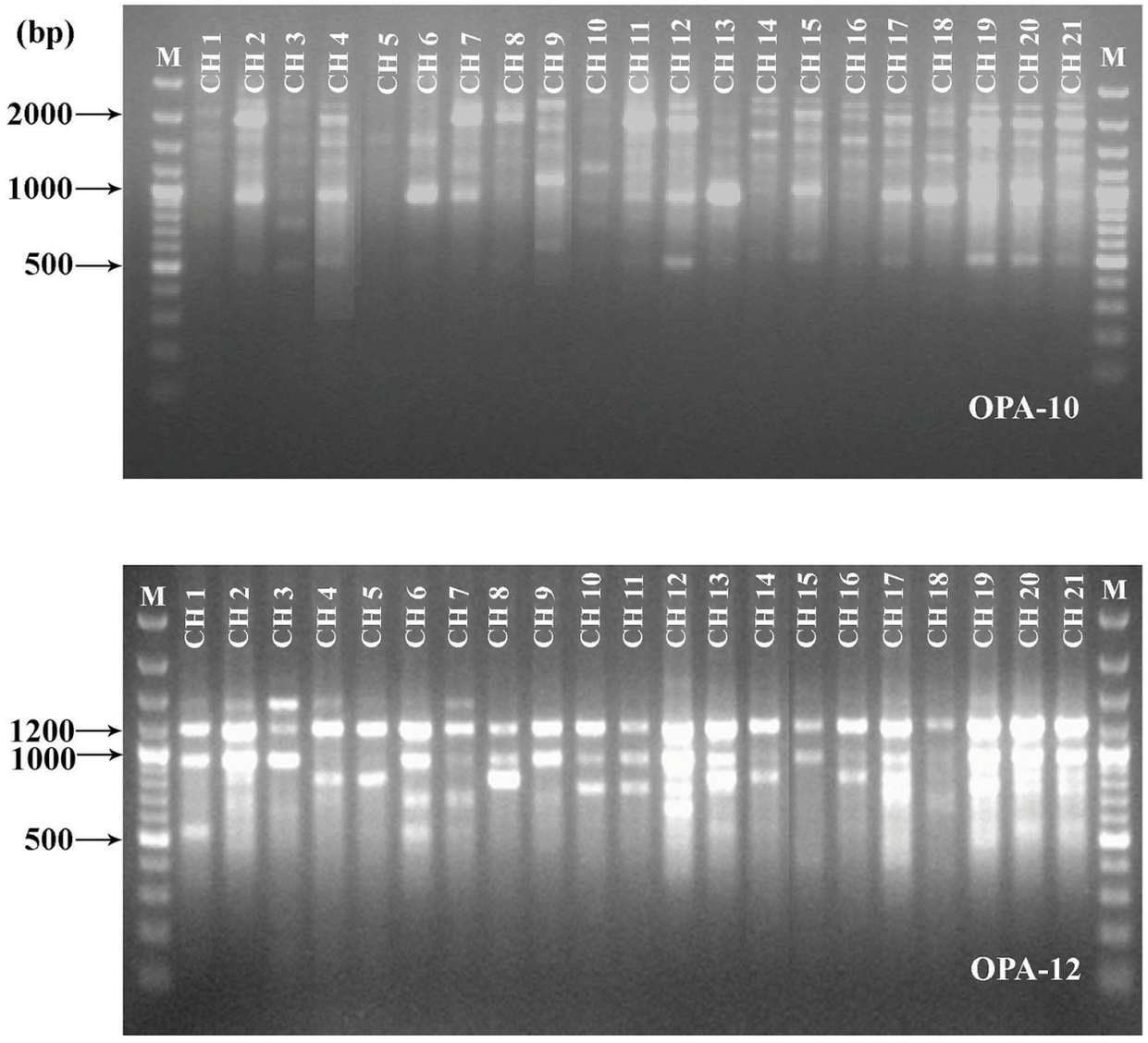

Figure 2. RAPD banding pattern in 21 accessions of *C. borivilianum* with OPA10 and OPA12 primer [M: 100 bp DNA ladder, Lane 2-22: Ch1–Ch21].

primer OPA07, OPA08, OPA10, OPC20 and OPP12 and the minimum (0.245) with primer OPT16.

The Jaccard's coefficient showed that MH2 and MH3 were most closely related with a similarity value of 0.707 followed by MH3 and Akola with similarity value of 0.697. The matrix value ranged from 0.321 to 0.707. It was observed that MP5 and Guj2 were most remotely placed with the similarity coefficient of 0.321. The average similarity coefficient was 0.639 between any two accessions taken into account.

The dendrogram constructed by the UPGMA method of pooled RAPD data led to the segregation of the 21 accessions into two distinct groups. All accessions were distributed between 39 units. Surprisingly, the first contained only one accession, Ch8 collected from Madhya Pradesh and second contained remaining 20 accessions collected from different diversified

regions (Madhya Pradesh, Rajasthan, Maharashtra and Gujarat) though they belong to same species. The second major cluster divided into two sub-major clusters out of which one sub-major cluster IIA contained four accessions (Ch1, Ch4, Ch5 and Ch7) collected from Madhya Pradesh with ~47% similarity and the another one (Group IIB) clutched Ch2 and Ch3 collected from Madhya Pradesh depicting 47% genetic variability. The third sub-major cluster (Group IIC) comprised four accessions Ch6 (Madhya Pradesh collection), Ch15, Ch16 and Ch17 collected from Maharashtra showing genetic similarity at the level of 68%. Another group, IID, consisted of two accessions (Ch12 and Ch13) collected from Rajasthan and other two Ch19 and Ch21 of Gujarat collections. Another group (IIE) holds two accessions (Ch18 and Ch20) collected from Gujarat showed 51% (approx.) genetic diversity. Accession no. Ch10 and Ch11 of Rajasthan collections fall in group IIF depicting 52% genetic similarity whereas cluster (IIG) containing two accessions (Ch9 and Ch14) showed genetic variability of about 51% collected from Madhya Pradesh and Rajasthan, respectively.

The cophenetic correlation showed maximum (0.7065) correlation between Ch15 (MH2) and Ch16 (MH3); both collected from Maharashtra. The average cophenetic correlation between two accessions was found to be 0.584.

3.2. ISSR analysis

A total of 10 ISSR primers were used out of which 06 primers resulted in amplification of 29 loci. Out of 29 loci, 24 loci were polymorphic and 05 loci were monomorphic in nature. Among the polymorphic loci, only one (01) locus was found to be unique in nature. The amplicons were observed in the range of 180 to >3000 base pair (**Figure 3**). The resolving power of the primers varied from 1.905 to 8.667 while the primer index varied from 0.091 to 0.499. Best resolving power (8.667) was observed in the primer (CA)8AT and the minimum (1.905) with primer (CA)6GG. The maximum ISSR primer index (0.499) was observed with the primer (CT)9G and the minimum (0.091) with primer (CA)6GG.

The Jaccard's coefficient showed that Ch13 (Raj 5 was most closely related) to Ch 14 (Raj 11) with a similarity value of 0.846 followed by Ch7 (MP7) and Ch10 (Raj1) with a similarity value of 0.833. The matrix value ranged from 0.363 to 0.846. The Rajasthan collected accession (Ch11) and Ch18 (Dangs) of Gujarat collections were most remotely placed with the similarity coefficient of 0.363. Between any two accessions, the average similarity coefficient was observed as 0.627.

Six (06) ISSR primers were used to construct a dendrogram by using the UPGMA method which led to the segregation of the accessions into two distinct groups. All accessions were distributed between 36 units. The resultant dendrogram formed a cluster with the 18 accessions in one group and the other 3 accessions into a separate group. Again 18 accessions discriminated into several groups. Group I consisted of only three accessions, Ch9, Ch11 and Ch19 collected from Madhya Pradesh, Rajasthan and Gujarat, respectively that showed about 35% genetic variability. Group II comprises five groups such as IIA, IIB, IIC, IID and IIE. Group IIC is the largest, which comprises six accessions, Ch3, Ch6 and Ch7 collected from Madhya Pradesh, Ch 10 and Ch12 from Rajasthan and Ch18 collected from Gujarat showed 61% genetic similarity.

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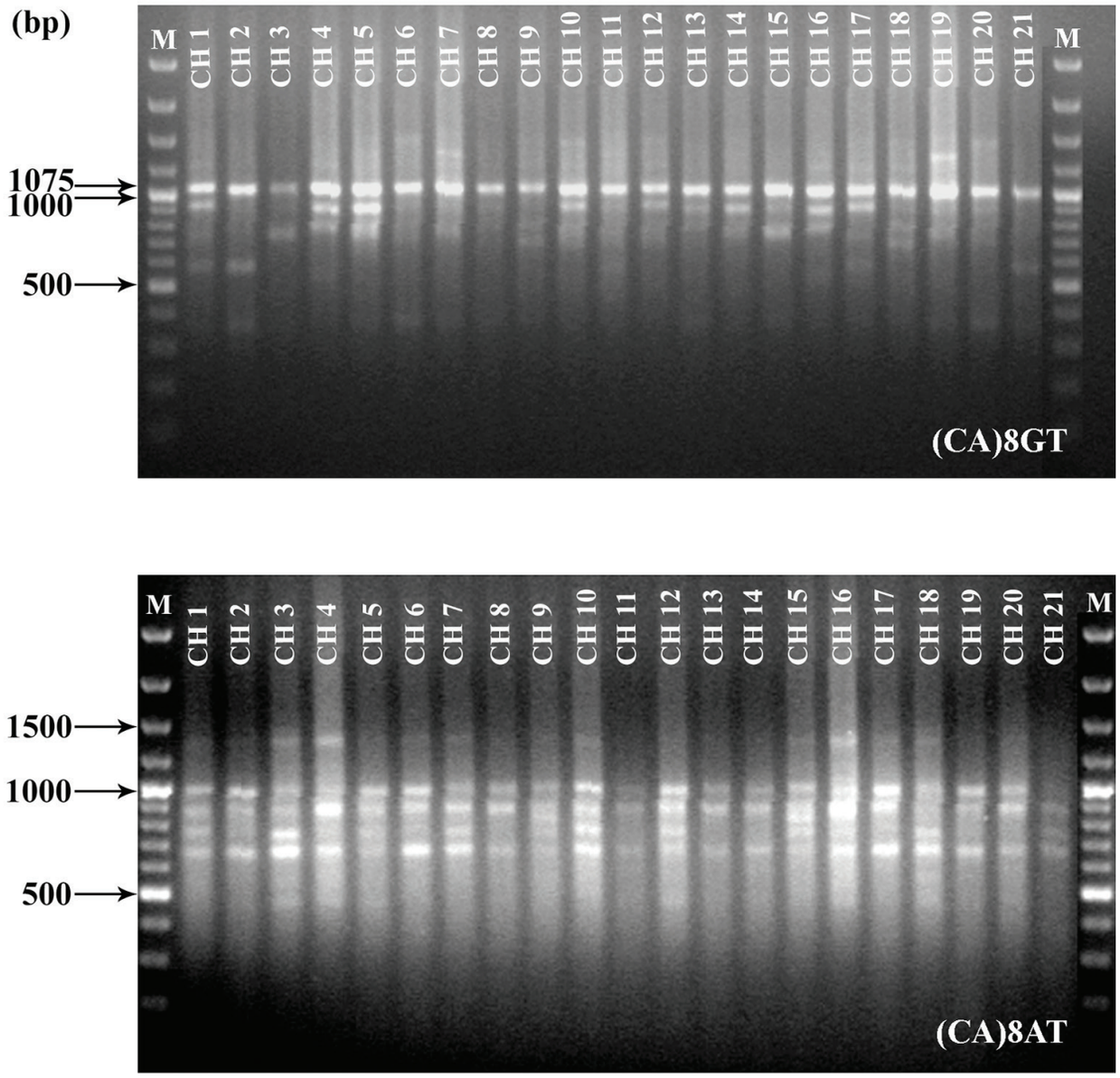

Figure 3. ISSR banding pattern in 21 accessions of *C. borivilianum* (CA)8GT and (CA)8AT primer [M: 100 bp DNA ladder, Lane 2-22: Ch1–Ch21].

The second largest Group IIB holds five accessions from which Ch2 and Ch8 collected from Madhya Pradesh, Ch13 and Ch14 from Rajasthan and Ch20 collected from Gujarat showed about genetic variation of about 40%. IID formed a group with three accessions, Ch 15, Ch16 and Ch17 of Maharashtra collections showing about 70% genetic similarity among them. The smallest Group IIE consisted accessions Ch4 and Ch5 of Madhya Pradesh showed 30% genetic variability between them.

The cophenetic correlation showed the maximum cophenetic correlation value (0.846) between Ch13 (Raj5) and Ch16 (Raj11); both are collected from Rajasthan. The average cophenetic correlation between two accessions was found to be 0.605.

3.3. Combined RAPD and ISSR data analysis

Both RAPD and ISSR produced a total of 197 loci of which 179 loci were polymorphic and 18 loci were monomorphic. A dendrogram was constructed by pooled RAPD and ISSR data using Jaccard's similarity coefficient and SHAN clustering.

The Jaccard's similarity coefficient showed a wide range of correlation among all the accessions. The average similarity coefficient between any two accessions was approximately 0.565. Maximum similarity (0.709) was observed between Ch15 and Ch16. However, Ch 8 and Ch17 were found distantly related with a similarity coefficient value of 0.423.

3.4. RAPD- and ISSR-derived dendrogram analysis from Jaccard's coefficient

The dendrogram constructed from combined RAPD and ISSR data segregates a single accession (Ch 8) of Madhya Pradesh Collection from the rest of the accessions sharing a node at 48% level of genetic variation (**Figure 4**). The subsequent sub-cluster contained five groups (IIA, IIB, IIC, IID and IIE). The accessions (Ch1, Ch4 and Ch5) formed a group (IIA) which were collected from Madhya Pradesh. Then group IIB consisted of Ch2 and Ch3 collected from Madhya Pradesh showed 47% genetic variability. The IID holds five accessions out of which Ch12 and Ch13 of Rajasthan collections and Ch19, Ch20 and Ch21 of Gujarat collections showed 52% genetic similarity. Thereafter Group IIC consisted of four accessions among which Ch6 collected from Madhya Pradesh and three accessions such as Ch15,

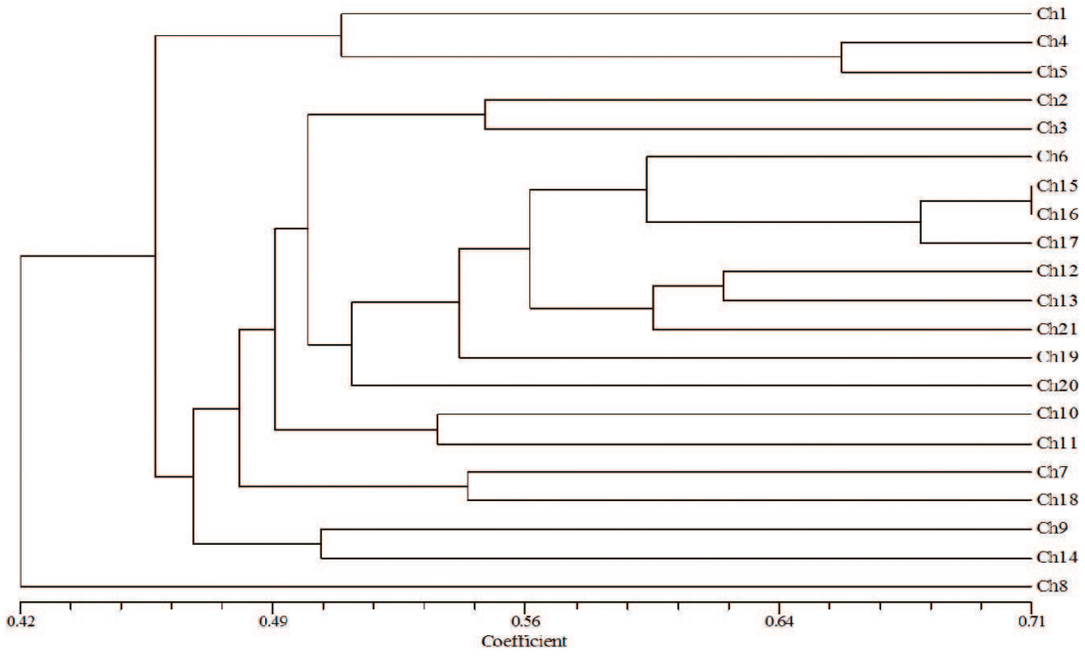

Figure 4. Clustering pattern of 21 accessions of *C. borivilianum* based on combined RAPD and ISSR markers.

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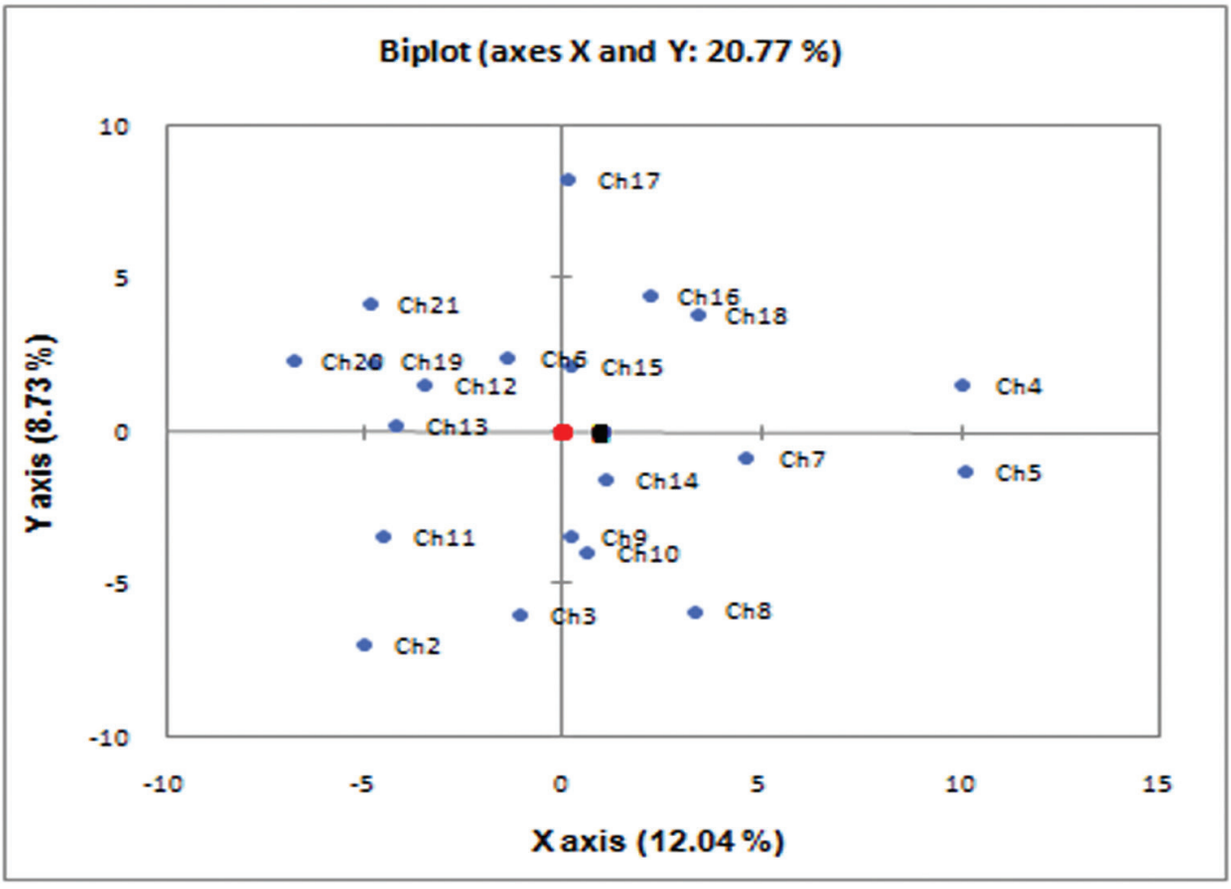

Figure 5. Principal co-ordinate analysis (PCA) in 21 accessions of *C. borivilianum* based on RAPD and ISSR markers.

Ch16 and Ch17 collected from Maharashtra shared 68% genetic similarity among them. About 47% variability was observed between Ch10 and Ch11 of Rajasthan collections which fall in Group IIE. In this study, it was observed that genetic relation/ variability among the accessions were found more or less similar to RAPD analysis. The maximum cophenetic correlation value (0.707) existed between Ch15 (MH2) and Ch16 (MH3); both were collected from Maharashtra. The average cophenetic correlation between two accessions was found to be 0.590.

As can be seen in **Figure 5**, the accessions of *C. borivilianum* were more dispersed on the PCA, which is a reflection of broad genetic base of this species. In general, the result of PCA is in agreement with the dendrogram and is a further confirmation of the genetic relationships delineated by cluster analysis.

Matrix comparison showed the *r* value for RAPD, ISSR and combined RAPD and ISSR to be 0.720, 0.696 and 0.707, respectively, which indicates significant fitness among all the markers studied.

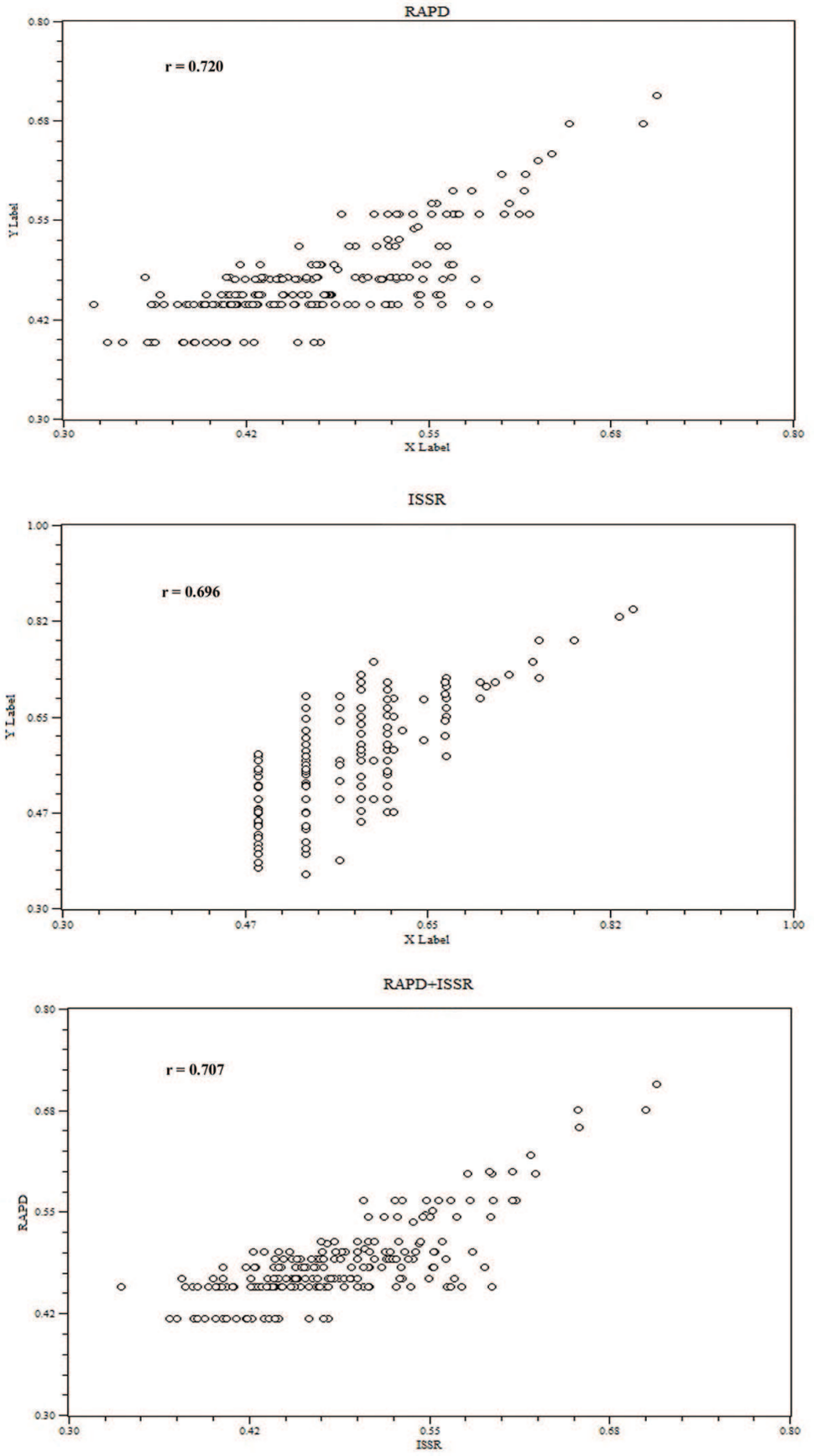

Figure 6. Matrix comparison of combined RAPD and ISSR markers in 21 accessions of *C. borivilianum.*

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The programme takes two symmetric similarity or dissimilarity matrices and plots, one matrix against the other element by element (but with the diagonal values ignored). It also computes the product-moment correlation, *r*, and the Mantel test statistic, *Z*, to measure the degree of relationship between the two matrices (**Figure 6**).

4. Discussion

C. borivilianum is an important traditional and ancient crop of India. Due to its high-therapeutic value, this species is being over-exploited for which it was listed in the endangered category. Therefore, their cultivation area has expanded rapidly during last few decades to cope up with the current internal demand and export. However, it would be important to determine their genetic variation so that the useful genotypes could be effectively used as cultivars by farmers or breeders and it would, in turn, facilitate the efficient conservation, management and utilization of the species. Nevertheless, genetic support to the cultivar development programme still remains limited relative to the scenario in many traditional crops where enormous genetic resource knowledge and saturated linkage maps have become available. Molecular markers can demonstrate genetic similarities and differences between accessions even when a classical morphological description is severely limited. To resolve the nomenclature problem, identification of duplicates and also to develop new cultivars, 21 different accessions of *C. borivilianum* were characterized using RAPD and ISSR markers to assess the variability within and among them. DNA markers are considered to be the most suitable means for estimating genetic diversity because of their abundance polymorphism and the fact that they are independent of the environment [24]. Quite considerable genetic variability does exist among different accessions.

Characterization of genetic resource collection has been greatly facilitated by the availability of a number of molecular marker systems. Different types of molecular markers have been used to assess the genetic diversity in crop species, but no single technique is universally ideal. Therefore, the choice of the technique depends on the objective of the study, sensitivity level of the marker system, financial constraints, skill and facilities available [25].

The molecular markers best studied for detecting genetic diversity should be relatively easy and inexpensive to use and should rapidly evolve enough variable within populations [26]. There are numerous DNA-based molecular marker systems suitable for genetic diversity assessment. Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) marker, in addition to its suitability of genetic diversity study, are highly polymorphic, reproducible, cost effective and require no prior information of sequence [27]. These facts suggest RAPD and ISSR could be unbiased tools to evaluate the changes of diversity in agronomically important crops [28].

RAPD generated a total of 168 bands ranging from 300 to >3000 bp of which 7.73% were monomorphic common to all accessions, 2.97% were unique present in four accessions and 92.26% were polymorphic in many of the accessions. This high degree of polymorphism detected with 20 RAPD primers indicates a high marker index. This is consistent with the

results obtained by Shin et al. [29] in watermelon and Galderisi et al. [30] in fig. Some RAPD primers (OPA16, OPA18) produced more markers probably because genomic DNA sequences possess high frequency of annealing sites [31]. ISSR markers have been used to evaluate the genetic diversity in several medicinal plants [32–35]. The larger number of polymorphic markers (as compared to number of primers used) generated by ISSR-PCR can be attributed to the fact that the centromeric region contains a large amount of repeated sequences [36]. A higher level of polymorphism was detected by both RAPD and ISSR in our study corroborated to other reports [37, 38].

The present results showed that molecular markers, ISSR and RAPD, efficiently identify *C. borivilianum* accessions allowing the characterization of all the different accessions. Moreover, both ISSR and RAPD markers exhibited their efficiency for genotype identification in other medicinal and aromatic plants earlier [39–41].

Though RAPD and ISSR markers demarcated 21 accessions into several groups, clustering of accessions within groups was not similar. A possible explanation for the difference in resolution of RAPD and ISSR is that the two marker techniques targeted different portions of the genome. The inter-simple sequence repeats target regions lying within the micro-satellite, and the amplification loci of RAPD are mainly in the gene expression region [14, 15]. These differences may also be attributed to marker sampling errors and/or the level of polymorphism detected reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars [42, 43]. The putatively similar bands originating for RAPDs in different individuals are not necessarily homologous, although they may share the same size in base pairs. This result sometimes may lead to wrong results when calculating genetic relationships [44].

The dendrogram did not indicate any clear pattern of clustering according to the locations from where germplasm were collected, indicating little and no location specificity among *C. borivilianum* germplasm except in Maharashtra collections. A similar result was observed earlier in 22 accessions of Shisham (*Dalbergia sissoo*) collected from different regions of India using RAPD and ISSR markers [45]. Since different dendrograms were obtained; no conclusive common grouping could be drawn from ISSR and combined RAPD-ISSR analyses. These results suggest that the manner of polymorphism differs because of marker specificity. In addition, the relation is assumed to depend on the genome coverage and sequence type recognized by each marker system [42, 46]. On comparing the diversity from different places of Madhya Pradesh, it was evident that *Chlorophytum* accessions from Madhya Pradesh were more diverse (52%) compared to Maharashtra (31%). Since *C. borivilianum* has been vegetatively propagated for a considerable time, it is reasonable to assume that part of the diversity detected in this study, is of ancient inheritance or accumulation of somatic point mutation in the course of vegetative reproduction events.

Matching the clustering result with their collection sites revealed that the geographical distribution of most accessions was not found to be defined. The majority of accessions in subgroup IIC were from different places of Madhya Pradesh and Rajasthan. Similarly, three of five accessions in the sub-group IID were collected from Maharashtra whereas two were from Madhya Pradesh. Similar conclusions were made by Padmesh et al. [47] in *Andrographis*

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paniculata where one accession collected from Thailand was shown to cluster with genotypes of different parts of Tamil Nadu. All the genotypes in sub-group IIA were from different places of Madhya Pradesh exhibiting 37% diversity. Without exception, two accessions in group IA from Madhya Pradesh showed 79% similarity; it was evident from the dendrogram that most of the accessions assessed, were clustered according to their geographical region.

On the basis of percent of polymorphism (RAPD = 92.26 and ISSR = 82.76), RAPD markers were marginally more informative than ISSR in the assessment of genetic diversity in *C. borivilianum*. This is corroborated with the findings in *Caldesia grandis*, *Dalbergia sissoo* and *Prunus armeniaca* [45, 48, 49]. This may be because of the fact that two marker techniques targeted different portions of the genome. Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral [50, 51]. Some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci [52]. Nevertheless, RAPD have been sometimes been associated with a lack of reproducibility [53]. However, if the PCR conditions are well controlled, a high level of reproducibility is attainable [54]. In this study, a considerable effort has been made to optimize the components of PCR, including the concentrations of $MgCl_2$, dNTP, primer, *Taq* DNA polymerase and the quality and concentration of template DNA.

The Mantel test on the similarity matrices produced by RAPD and ISSR markers showed significant correlation ($r = 0.7638$; $p < 0.001$) between RAPD and ISSR markers in their ability to detect genetic relationships between *Chlorophytum* accessions. The coefficient correlation value, $r = 0.7591$, 0.7263 and 0.7638 for RAPD, ISSR and combined RAPD and ISSR analyses, respectively. This result is corroborated with the earlier report of Dai et al. [55].

In this study, superiority of RAPD markers over ISSR were observed with regards to polymorphism detection, as RAPD detected 92.26% as compared to 82.76% for ISSR markers. This is in contrast to the results as obtained for other several plant species where ISSR was proved to be superior as compared to RAPD [44, 56–58]. More polymorphism, in the case of RAPD than ISSR markers might be due to the fact that out of 10 primers used in the study, only 6 primers amplified 609 numbers of fragments for ISSR. While in the case of RAPD, all the 20 primers which were used in the investigation amplified 3528 number of fragments. The same polymorphism pattern was observed in jatropha, podophyllum and apricot [59–61]. RAPD also provides marker even for cultivars identification [62] determining hybridity among the sexual cross made intentionally to exploit the genetic variability [63, 64] and germplasm evaluation [31].

To conserve the diversity of genetic resources, a large number of accessions are to be conserved for which problems are encountered in documentation, conservation, multiplication and evaluation. Therefore, to minimize the cost of genetic conservation ensuring representation of maximum genetic variation, a set of accessions (core collection) should be selected to represent the genetic diversity of a base collection with minimum redundancy [12]. From the PCA of the 38 accessions, it was evident that some of the accessions were found overlapping each other depicting redundancy which should be eliminated. Since there are different strategies for developing a core collection using morphological or marker data, however molecular markers are more stable and efficient in estimating the genetic relatedness among the individuals [65, 66].

The molecular analyses of RAPD and ISSR markers were extremely useful for studying the genetic relationship/variability between and among *C. borivilianum* accessions. Also, the phylogenetic analysis on the basis of RAPD- and ISSR-derived dendrograms supports the fact that region-specific variation are there, which is because of the multiple generations of the selection carried out after their introduction.

5. Conclusion

The present findings demonstrate the use of RAPD and ISSR markers in estimating genetic diversity and identifying a core collection in *C. borivilianum* for which genotypes representing maximum genetic diversity need to be conserved followed by accessions which complement the previous one. Moreover, sample duplication could be detected in the germplasm collection followed by selection of a core collection to enhance the efficiency of germplasm management for use in crop improvement and conservation. Based on polymorphic features among the accessions of *C. borivilianum* based on RAPD and ISSR study, it may be recommended that any future conservation plans for this species should be specifically designed to include representative accessions with the highest genetic variation for both *in situ* conservation and germplasm collection expeditions. The unique bands that could be identified are likely to provide tags for future genetic improvement as well as in authenticating the genotypes. Further investigation using more sophisticated markers may be helpful for the accuracy and resolution of genetic diversity. ISSR and RAPD markers along with chemical fingerprinting and morphological characters could now be used as coherent tools for the development of core collection of *C. borivilianum.* The RAPD markers along with ISSR markers should complement one another during genetic identification, by coding different regions of *C. borivilianum* genome. Conversion of specific RAPD/ISSR segments into sequenced characterized amplified region (SCAR) markers could enhance the value of these markers for the identification of any variety/ cultivars developed.

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