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Analysis of genetic variability in endemic medicinal plants of genus *Chlorophytum* from the Indian subcontinent using amplified fragment length polymorphism marker

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ABSTRACT

The genus *Chlorophytum* consists of medicinally important species like *Chlorophytum borivilianum*, *C. tuberosum* and *C. attenuatum*. Uncontrolled harvest of this plant from wild habitat due to its high commercial value made the species of this genus be listed in the *Red Data Book of Indian plants* as an endangered species. In India, approximately nineteen species of *Chlorophytum* are found; out of these, only *C. borivilianum* is cultivated commercially. The objective of this study was to measure genetic diversity, population structure and phylogenetic relationship among the species using Amplified Fragment Length Polymorphisms (AFLP). Fifteen pairs of primer (out of 64 primer pairs screened) were used to analyse the genetic diversity in eighteen species of genus *Chlorophytum*. Cluster analysis, estimation of the gene flow among the species and of the phylogeographic distribution of this genus were carried out using an AFLP data matrix. A high level of genetic diversity was observed on the basis of the percentage of polymorphic bands (99.91%), Shannon's information index (0.3592) and Nei's gene diversity (0.2085) at species level. Cluster analysis of UPGMA dendrogram, principal component analysis and Bayesian method analysis resolved these species in three different clusters, which was supported by morphological information. The Mantel test ($r = 0.4432$) revealed a significant positive correlation between genetic and geographic distances. The collected data have an important implication in the identification, authentication, and conservation of the species of the genus *Chlorophytum*.

1. Introduction

The genus *Chlorophytum* belongs to the family *Asparagaceae* and comprises about 215 species, six sub-species and eight varieties. It is considered to be a native of the old world and is mainly distributed in the tropical and subtropical regions of Africa, Madagascar, and India [1,2]. The species of *Chlorophytum* are well known for their medicinal use in Ayurvedic and Unani systems. The root of these species contains a variety of secondary metabolites like alkaloids, saponins and flavonoids, which possess various pharmacological uses [3–5]. The root powder of *C. borivilianum*, *C. attenuatum*, and *C. tuberosum*

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is the commercial name in India of 'Safed musli'. This drug is known to be very effective for enhancing general body immunity, vigour and strength [6]. Because of this commercial importance, the plants are generally harvested from their wild habitats. These plants have been listed as an endangered species in the *Red Data Book of Indian plants* by the Botanical Survey of India because of their habitat destruction and uncontrolled harvesting [7].

It is a general ecological concept that for long-term survival of species, genetic diversity plays a crucial role by assisting plants with adapting to environmental changes. Identification and understanding of genetic diversity in plant species is an important step for conservation. It is very difficult to develop and implement the long-term conservation plan of the *Chlorophytum* species because of the small population size and habitat heterogeneity. This might result in limited gene flow leading to higher genetic differentiation among plant populations. *Chlorophytum* species are very difficult to identify based on morphological characteristics. The genetic diversity of this medicinally important species is largely unknown, but essential to designing effective breeding and conservation programs to sustain natural population [8]. A very limited information on identification and phylogenetic analysis based on molecular markers is available [9]. Also, it is highly desired to authenticate the genus *Chlorophytum* using genome-based approaches before its use as a medicinal plant.

Various molecular markers like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) were used to study the genetic diversity at the molecular level in various wild plant species [10]. Among these markers, AFLP provides dominant, multilocus and genome wide DNA profile. AFLP is a cost effective and highly reproducible tool; it does not require prior sequence information, making it suitable for molecular characterization and DNA fingerprinting [11,12]. The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. It provides high genetic polymorphism, valuable site information, and reveals genetic variations between individuals.

The objective of this study was to evaluate the genetic diversity among the genus *Chlorophytum* accessions collected from the Indian subcontinent using AFLP markers. It seems that taxa remain emblematic to higher altitude geographic locations having higher precipitation rates, mostly in Western Ghats and in the peninsular Deccan plateau of India. The species grows in small patches with limited population. The study aims at contributing to the identification and discrimination of 18 *Chlorophytum* species. It reveals the level of genetic diversity and genetic structure within species.

2. Materials and methods

2.1. Population sampling

Plant material from 18 different species of genus *Chlorophytum* was collected from different locations in the Indian subcontinent (Fig. 1). A total of 87 samples, 3–5 individuals per species, were collected from different type localities [6,13]. The plant specimens were maintained in a germplasm field at the botanical garden of the Department of Botany, Shivaji University, Kolhapur. The identification of all species was confirmed by referring to different prologues, floras and comparing with a typed specimen. The representative voucher number and GPS location of specimens is listed in Table 1.

2.2. DNA extraction

Total genomic DNA was extracted using the modified CTAB method [14], but the high level of secondary

Table 1
Sampling details of the populations of genus *Chlorophytum* in the present study.

Species name	Accession number[a]	Sample size	Sampling locality	Longitude (E)	Latitude (N)	Voucher number (collection year)
Chlorophytum kolhapurense	C1 (A-E)	5	Sutagatti ghat, Karnataka.	16.042208	74.489306	SUK 106 (2012)
Chlorophytum arundinaceum	C2 (A-E)	5	Melghats Amravati.	21.399728	77.298472	SUK 109 (2012)
Chlorophytum laxum	C3 (A-E)	5	Shivaji university, Kolhapur.	16.673583	74.254194	SUK 105 (2012)
Chlorophytum glaucoides	C4 (A-E)	5	Tillari, Kolhapur.	17.939908	73.632758	SUK 111 (2012)
Chlorophytum bharuchae	C5 (A-E)	5	Adi Chikkodi, Karnataka.	16.498567	74.351700	ANC 700 (2009)
Chlorophytum belgaumense	C6 (A-E)	5	Khanapur, Belgum.	15.680281	74.502631	Chandore 1113 (2010)
Chlorophytum borivilianum	C7 (A-E)	5	Kasedi, Poladpur.	17.902167	73.437289	SUK 100 (2011)
Chlorophytum amaniense	C8 (A-E)	5	Ornamental plant	–	–	SUK 749 (2009)
Chlorophytum heynei	C9 (A-C)	3	Anamalai Hills, Tamil Nadu.	–	–	SUK 713 (2011)
Chlorophytum breviscapum	C10 (A-E)	5	Marleshwar, Ratnagiri.	17.056186	73.745858	SUK 108 (2012)
Chlorophytum indicum	C11 (A-E)	5	Sultanpeth, Nandi hills, Karnataka.	13.385517	77.667647	SUK 102 (2011)
Chlorophytum gothanense (Gothane Plateau)	C12 (A-E)	5	Gothane Plateau, Sangali.	17.075706	73.764153	SUK 107 (2012)
Chlorophytum malabaricum	C13 (A-E)	5	Nandi Hills, Karnataka.	13.429864	75.756486	SUK 99 (2011)
Chlorophytum glaucum	C14 (A-E)	5	Tillari, Kolhapur.	15.778489	74.171792	SUK 110 (2012)
Chlorophytum tuberosum	C15 (A-E)	5	Ratnagiri, Maharashtra.	17.005517	73.327647	SUK 101 (2011)
Chlorophytum gothanense (Kondushi Plateau)	C16 (A-E)	5	Kondushi, Gargoti.	16.211431	73.990014	SUK 103 (2011)
Chlorophytum sharmae	C17 (A-E)	5	Munnar, Kerala.	10.083411	77.066697	Adsul 2553 (2013)
Chlorophytum nepalense	C18 (A-D)	4	Tengnoupal, Chandel, Manipur.	24.386664	94.143631	SUK 712 (2013)

[a] Same accession numbers have been presented in dendrogram (Fig. 2) and Principal component analysis (Fig. 4).