Genetic diversity and relationship of *Hedychium* from Northeast India as dissected using PCA analysis and hierarchical clustering

CrossMark

Supriyo Basak [a], Aadi Moolam Ramesh [a], Vigya Kesari [a], Ajay Parida [c], Sudip Mitra [b], Latha Rangan [a,*]

[a] *Department of Biotechnology, Indian Institute of Technology, Guwahati, Assam 781 039, India*
[b] *Department of Environmental Science, Tezpur University, Assam 784 028, India*
[c] *M S Swaminathan Research Foundation, 3rd Cross Street, Taramani Institutional area, Chennai 600 113, India*

ARTICLE INFO

ABSTRACT

Molecular genetic fingerprints of eleven *Hedychium* species from Northeast India were developed using PCR based markers. Fifteen inter-simple sequence repeats (ISSRs) and five amplified fragment length polymorphism (AFLP) primers produced 547 polymorphic fragments. Positive correlation ($r = 0.46$) was observed between the mean genetic similarity and genetic diversity parameters at the inter-species level. AFLP and ISSR markers were able to group the species according to its altitude and intensity of flower aroma. Cophenetic correlation coefficients between the dendrogram and the original similarity matrix were significant for ISSR ($r = 0.89$) compared to AFLP ($r = 0.83$) markers. This genetic characterization of *Hedychium* from Northeast India contributes to the knowledge of genetic structure of the species and can be used to define strategies for their conservation and management.

Introduction

Hedychium J. Köenig (*Zingiberaceae*) is an economically important genus, consisting of 65 species worldwide of which Northeast (NE) India has the highest species concentration (24 out of 65) (Wood et al., 2000). *Hedychium* species are widely cultivated for their perfume, as a useful raw material for manufacturing paper, medicinal and for its horticultural significance. The medicinal efficacies of the essential oil extracted from leaves, flowers and rhizomes of these plants including cercaricidal properties (Warren and Peters, 1968), molluscicidal activity (Saleh et al., 1982), antimicrobial activities (Gopanraj et al., 2005), and anti-inflammatory and analgesic effects (Shrotriya et al., 2007) are well established.

In spite of huge ornamental and medicinal significance, limited knowledge is available for many members of *Hedychium*. In addition, high intra and interpopulation variation has led to a debate concerning species concepts and boundaries. Also, the genus is facing the loss of genetic diversity, due to uncontrolled uprooting of the rhizome and the whole plant in huge quantities for preparation of traditional herbal medicine. Species level taxonomy of *Zingiberaceae* propagating vegetatively is unresolved because morphological differences are inconclusive due to the short lived flowers and owning to the fact that plants flower in monsoon season. So there is an urgent need for sustainable management to conserve the germplasm; however sustainable management has so far not succeeded and further aggravating the extinction. For any future analysis using *Hedychium*, it may be practical to have information regarding the genetic relatedness of all species. The data on genetic similarity is a mandatory requirement to study the evolutionary history of a species, as well as for other studies, such as intraspecific variations, genetic resources conservation, etc. In the traditional taxonomy of *Hedychium*, the taxonomic characters are in bract arrangement, number of flowers per bract and petal colors. Such characters simply offered each species a relative, but arbitrary location in the genus, without any quantification of similarity. The quantification of similarity, however, can be easily realized by using molecular techniques.

Molecular markers are used as the taxonomic aid because of advantages of high polymorphism, non-pleiotropy and clear identifying alleles, etc. The major challenge associated with any molecular method is to determine the appropriate taxonomic level at which it is most informative and to correlate it with morphologically definable taxonomic groupings. Inter-simple sequence repeat (ISSR) markers are useful in detecting genetic polymorphisms between and within the species by generating a large number of markers that target multiple microsatellite loci distributed across the genome (Das et al., 2011). Among many researchers, amplified fragment length polymorphism (AFLP) is the marker technology of choice since it combines the reliability of classical restriction-based fingerprinting with the speed and convenience of polymerase chain reaction (PCR)-based marker techniques (Das et al., 2011; Lu et al., 2002; Anderson et al., 1993). The AFLP technique rapidly generates hundreds of highly replicable DNA markers, thus allowing high resolution genotyping. These DNA fingerprinting techniques have been used successfully for confirmation of relationship of *Boesenbergia* (Vanijajivaa et al., 2005); phylogenetic analysis in *Amomum* (Kaewsri et al., 2007) and genetic relationship in *Curcuma* (Das et al., 2011). There is only one report on the phylogenetic analysis and genetic mapping of Chinese *Hedychium* using sequence related amplified polymorphism (SRAP: Gao et al., 2008). Therefore, the present study was undertaken to investigate the intra- as well as inter-species genetic relationship using principal component analysis (PCA) and hierarchical clustering (HCA) among the species of the *Hedychium* occurring in NE India by PCR based molecular markers.

Materials and methods

Plant material

The materials for the present study consisted of 11 species of the genus *Hedychium*, collected from different locations of NE India (Fig. 1, Table 1). Two individuals of *Hedychium coronarium, Hedychium chrysoleucum, Hedychium stenopetalum, Hedychium spicatum, Hedychium gardnerianum* and *Hedychium flavescens* were collected and only a single individual of the remaining five species were collected. Plantlets were maintained in a greenhouse under semi-shade and high humidity (RH 80%) with a 16 h photoperiod at 28 ± 2 °C for hardening. Intermittent mist was supplied for 30 s at 15 min intervals.
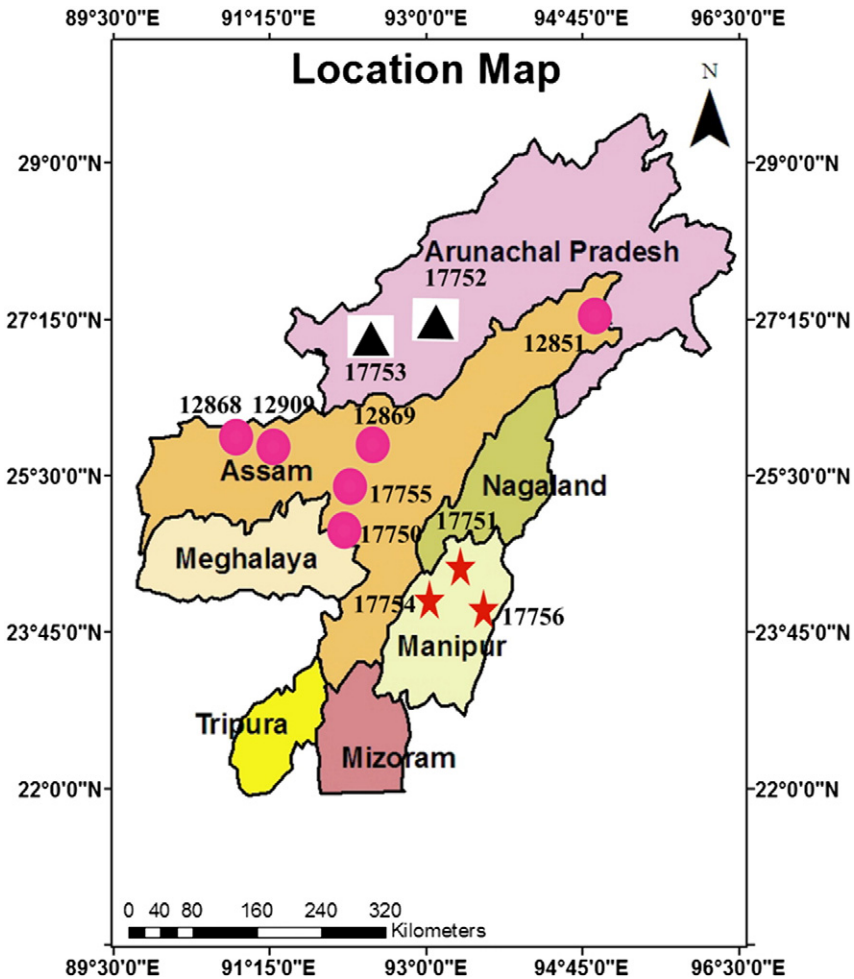
Fig. 1. Geographical distribution of the *Hedychium* species studied. The numbering of the dots (Assam collections) and triangles (Arunachal Pradesh) and stars (Manipur collections) corresponds to the *Hedychium* species according to the collection places. Samples have been shown in the figures by the voucher numbers. *H. coronarium* J. Koenig (17755.1, 17755.2); *H. dekianum* A.S.Rao & Verma (12868); *H. flavum* Roxb. (12909); *H. stenopetalum* Lodd (12869.1, 12869.2); *H. spicatum* Lodd (17750.1, 17750.2); *H. chrysoleucum* Hook (12851.1, 12851.2); *H. gardnerianum* Wall. ex Spreng (17753.1, 17753.2); *H. marginatum* Clarke (17754); *H. flavescens* Lodd (17752.1, 17752.2); *H. aurantiacum* Wall (17751); 11. *H. coccineum* Wall (17756).

Herbarium vouchers for the seventeen individuals were deposited in the Gauhati University Botanical Herbarium (GUBH, recognized by American Botanical Society).

DNA extraction

Total genomic DNA was extracted from fresh tender leaves using a DNeasy Plant mini kit. For DNA extraction two different leaves from each individual of *Hedychium* were used and subsequent experimental analysis was also carried out in duplicates. The quality and quantity of the extracted DNA were confirmed to be consistent by running the extracted DNA on 0.8% agarose gel, stained with ethidium bromide (0.5 μg/μL). The DNA samples having the $A_{260/280}$ ratio in between 1.6 and 1.8 and $A_{260/230}$ ratio in between 2.0 and 2.2 (Wilfinger et al. 1997) were taken into consideration for subsequent marker analysis.

Table 1
List of *Hedychium* species used in the study with their geographical origin, morphological information and habitat description.

S. no.	Species name	Voucher no	Morphological description	Habitat
1	*Hedychium coronarium* J. Koenig	17755.1 17755.2	Leaves: along or ablong-lanceolate; bracts: large ablong imbricate; flowers: white	Swamps and wet meadows
2	*H. dekianum* A.S.Rao & Verma	12868	Leaves: along or ablong-lanceolate; bracts: large ablong; flowers: white	Lowlands shaded areas
3	*H. flavum* Roxb.	12909	Leaves: along or ablong-lanceolate; bracts: large ablong; flowers: yellow	Humid and shaded areas
4	*H. stenopetalum* Lodd.	12869.1 12869.2	Leaves: along or ablong-lanceolate; Bracts: ablong; flowers:light orange	Humid and shaded areas
5	*H. spicatum* Lodd.	17750.1 17750.2	Leaves: along or ablong-lanceolate; bracts: large ablong; flowers: white	From moderately to highly wet areas
6	*H. chrysoleucum* Hook.	12851.1 12851.2	Leaves: along or ablong-lanceolate; bracts: large ablong; flowers: white tinged with yellow	Humid and shaded areas
7	*H. gardnerianum* Wall. ex Spreng.	17753.1 17753.2	Leaves: ablong, white pulverulent beneath; bracts: large ablong; flowers: bright lemon yellow	Windward hilly slopes
8	*H. marginatum* Clarke	17754	Leaves: along or ablong-lanceolate; bracts: ablong; flowers: yellow flower with reddish-brown inflorescence	Windward moist hilly slope
9	*H. flavescens* Lodd.	17752.1 17752.2	Leaves: along or ablong-lanceolate; bracts: large ablong imbricate; flowers: sulfur yellow	Windward hilly slope
10	*H. aurantiacum* Wall.	17751	Leaves: long ablong; bracts: ablong; flowers: bright orange yellow	Foot hills receiving heavy rainfall
11	*H. coccineum* Wall.	17756	Leaves: lanceolate; bracts: ablong; flowers: small bright crimson	Foot hills and moist grassland

ISSR and AFLP analyses

The experimental procedures for ISSR and AFLP analyses were as described previously (Das et al., 2011). Fifteen primers were used for ISSR analysis and five sets of primers were used for AFLP fingerprinting (Table 3).

Data analysis

For scoring and analysis of data from two molecular marker systems, duplicate samples from each individual were tested. The total number of monomorphic and polymorphic bands which were clear, unambiguous and reproducible was scored for all the tested primers. Data counting/scoring was carried out by using a binary number system for the presence or absence of each fragment in each sample. To avoid taxonomic ambiguities, all bands were taken into consideration, only the presence of a band was taken as an indicative. To compare the efficiency of each primer polymorphic information content (PIC); as a marker discrimination power, was computed using the formula $PIC = 1 - \sum p_i^2$, where p_i is the frequency of ith allele at a given locus and also a marker index (MI) was calculated (Powell et al., 1996). MI defined as the product of the polymorphism percentage and PIC, is used to estimate the overall utility of each marker system and was calculated according to Kesari et al. (2010). Measure of degree of similarity among 11 species was established as a percentage of polymorphic bands, and a matrix of genetic similarity compiled using Dice's coefficient (Dice, 1945). A dendrogram representing the genetic relationship among all *Hedychium* species was generated for each marker system (ISSR and AFLP) by applying unweighted pair-group arithmetic average (UPGMA) method (Sneath and Sokal, 1973) using the SHAN subroutine through the NTSYS-pc (numerical taxonomy system, 2.2 version) (numerical taxonomy system, Applied Biostatistics, NY) (Rohlf, 2005). To know the goodness of fit for 11 species of *Hedychium* to a specific cluster in the UPGMA algorithm, the relationships between the original similarity indices and cophenetic values were evaluated, and the Mantel's test (Mantel, 1967) was executed for it. PCA was also performed with modules STAND, CORR, and EIGEN of NTSYS-pc using the Euclidean distances derived from the standardized values using the NTSYS-pc-2.2 for only polymorphic bands. The filtration of data was carried out by STAND module before PCA analysis (Milligan and Cooper, 1987). Genetic diversity was estimated

for intra and inter-species variation using POPGENE, version 1.32 (Yeh et al., 1997). The levels of genetic variability within species were estimated using five variables: the observed number of alleles per locus (n_a), the effective number of alleles per locus (n_e), proportion of polymorphic loci (P) per species, Shannon information index (I), Nei's gene diversity (h) (McDermott and McDonald, 1993). HCA was further created for *Hedychium,* where each profile is a string with 11 entries (number of species analyzed). HCA was performed with Cluster (v. 3.0) and visualized with TreeView (Eisen et al., 1998). Pair wise linkage was used as the routine for hierarchical clustering (de Hoon et al., 2004). Species in the study were clustered according to the similarity of their phylogenetic profiles and if there was a similarity of one species to that of the other species, the value 1 was assigned at that position (red), if not, 0 was assigned (black).

Results

ISSR and AFLP polymorphisms

The intra-species genetic diversity was studied among 12 individuals of six species of *Hedychium*. Out of the 20 ISSR primers, 5 gave successful amplification with a total of 28 bands, of which 25 were polymorphic with polymorphism of 89.28%. The highest number of polymorphic fragments was observed for 826 (7) and the lowest number of polymorphic fragments was observed for 17898A (3), with an average of 5.0 per primer. The PIC varied from 0.31 (807) to 0.41 (17898A) with an average PIC of 0.37 (Table 2).

The inter-species genetic diversity was studied for the 11 *Hedychium* species of NE India. Of the 20 ISSR primers, 15 gave the successful amplification with a total of 141 bands, of which 131 were polymorphic with polymorphism of 91%. The highest number of polymorphic fragments obtained was for HB 13 (13) and the lowest for 17898B (4) with an average of 9.00 per primer. The polymorphic information content (PIC) varied from 0.29 (811) to 0.45 (HB13) with an average PIC of 0.37. Of the 8 AFLP primer combinations five gave the successful amplification with a total of 416 polymorphic fragments with 100% polymorphism. The PIC varied from 0.27 (*Mse*I-CAC/*Eco*RI-ACT) to 0.32 (*Mse*I-CAG/*Eco*RI-ACT) with an average value of 0.29. The MI varied from 27 (*Mse*I-CAC/*Eco*RI-ACT primer combination) to 32 (*Mse*I-CAG/*Eco*RI-ACT primer combination: Table 3).

Gene diversity

Intra-species diversity among the 12 individuals of 6 species was found to be low as revealed by ISSR markers. The average value of n_a, n_e, h, I and % P varied from 1.01 ± 0.14 to 1.33 ± 0.48, 1.01 ± 0.09 to 1.24 ± 0.34, 0.01 ± 0.00 to 0.14 ± 0.01, 0.01 ± 0.00 to 0.20 ± 0.02, and 1.85 to 33.33 respectively. But the inter-species gene diversity was found to be high among the 11 species of *Hedychium* as revealed by ISSR and AFLP markers. The average value of n_a and n_e, varied from 1.99 to 2.00, and 1.51 to 1.53, respectively. The average values of h and I were found to be 0.31 and 0.48, respectively (Table 4).

Table 2

Degree of polymorphism and polymorphic information content for intra-species genetic relationship in *Hedychium*.

Primer code no	TNB	NPB	% P	PIC	MI
807	6	5	83.33	0.31	25.46
826	7	7	100.00	0.37	36.80
17898A	5	3	60.00	0.41	25.0
17899A	6	6	100.00	0.40	40.04
HB12	4	4	100.00	0.38	37.84
Total	28	25			
Mean	5.6	5.0	89.28	0.374	33.03

Table 3
Degree of polymorphism and polymorphic information content for inter-species genetic relationship in *Hedychium*.

Primer code no	TNB	NPB	% P	PIC	MI
ISSR primer					
17898B	5	4	80.0	0.39	31.20
826	11	11	100.0	0.35	35.00
HB12	13	13	100.0	0.39	39.00
HB13	8	7	87.5	0.45	39.37
17899A	11	11	100.0	0.34	34.00
816	9	9	100.0	0.38	38.00
817	9	8	88.8	0.40	35.52
811	10	6	60.0	0.29	17.40
825	12	12	100.0	0.33	33.00
807	9	9	100.0	0.45	45.00
824	10	9	90.0	0.33	29.70
HB15	7	6	85.7	0.33	28.28
814	12	12	100.0	0.39	39.00
17899A	6	5	83.3	0.42	34.98
809	9	8	88.8	0.39	34.63
Total	141	131	1364.10	5.24	514.08
Mean	9.71	9.00	90.94	0.37	34.27
AFLP primer					
MseI-CAC/EcoRI-ACT	111	111	100	0.27	27
MseI-CAG/EcoRI-ACT	46	46	100	0.32	32
MseI-CAT/EcoRI-ACT	110	110	100	0.30	30
MseI-CTC/EcoRI-ACT	51	51	100	0.29	29
MseI-CTG/EcoRI-ACT	98	98	100	0.28	28
Total	416	416	500	1.46	146
Mean	83.2	83.2	100	0.292	29.2

TNB total number of bands, NPB number of polymorphic bands, % P percentage of polymorphism, PIC polymorphic information content, MI marker index.

Genetic similarity analysis

Cluster analysis and PCA are valuable for determining relationships among individuals of the same and different species (Crawford, 1990; Lee, 1945). The PCA plot and UPGMA dendrogram of the intra-species genetic diversity study by ISSR markers showed two clusters for 12 individuals of *Hedychium*. Cluster I consisted of the individuals from *H. coronarium*, *H. chrysoleucum*, *H. flavescens*, *H. stenopetalum* and *H. spicatum*. Cluster II consisted of the individuals from *H. gardnerianum*. Dendrogram showed the highest genetic similarity between the individuals of *H. chrysoleucum* and the lowest genetic similarity between the individuals of *H. coronarium*. Mantel test of cophenetic correlation matrix and the similarity matrix

Table 4
Genetic diversity parameters for *Hedychium*.

	Population	Sample size (n)	Observed no of alleles (n_a)	Effective no of alleles (n_e)	Nei's gene diversity (h)	Shannon's information index (I)	% P
Intra-species	ISSR						
	H. coronarium	2	1.33 ± 0.48	1.24 ± 0.34	0.14 ± 0.01	0.20 ± 0.02	33.33
	H. chrysoleucum	2	1.01 ± 0.14	1.01 ± 0.09	0.01 ± 0.00	0.01 ± 0.00	1.85
	H. stenopetalum	2	1.05 ± 0.23	1.03 ± 0.16	0.02 ± 0.01	0.03 ± 0.01	5.56
	H. spicatum	2	1.05 ± 0.23	1.03 ± 0.16	0.02 ± 0.01	0.03 ± 0.01	5.56
	H. gardnerianum	2	1.05 ± 0.23	1.03 ± 0.16	0.02 ± 0.01	0.03 ± 0.01	5.56
	H. flavescens	2	1.05 ± 0.23	1.03 ± 0.16	0.02 ± 0.01	0.03 ± 0.01	5.56
	Total gene diversity	12	1.92 ± 0.26	1.60 ± 0.30	0.19 ± 0.14	0.09 ± 0.19	40.00
Inter-species	Total gene diversity (ISSR)	11	1.99 ± 0.08	1.53 ± 0.32	0.32 ± 0.15	0.48 ± 0.18	90.94
	Total gene diversity (AFLP)	11	2.00 ± 0.08	1.51 ± 0.32	0.31 ± 0.15	0.48 ± 0.18	100.00

revealed a very good degree of confidence (r = 0.96) in the association obtained for the 12 individuals of *Hedychium* (Supplementary Fig. 1)

For inter-species genetic relationship among 11 species of *Hedychium*, PCA of 15 ISSRs and 5 AFLP primer combinations displayed differential grouping patterns. The highest cumulative contribution of the first three principal components to total variation was found to be by ISSR markers (57.24%) compared to AFLP (42.94%) marker. The highest percent contribution of Eigenvalues of ISSR marker system is implicating better information of relatedness for the species studied. PCA analysis of ISSR and AFLP data-set for inter-species genetic relationship showed two discrete clusters (Fig. 2).

The DICE genetic similarity values derived from the ISSR data ranged from 0.58 (*H. flavescens* and *Hedychium dekianum*) to 0.79 (*Hedychium coccineum* and *H. edychium aurantiacum*). Similarly AFLP
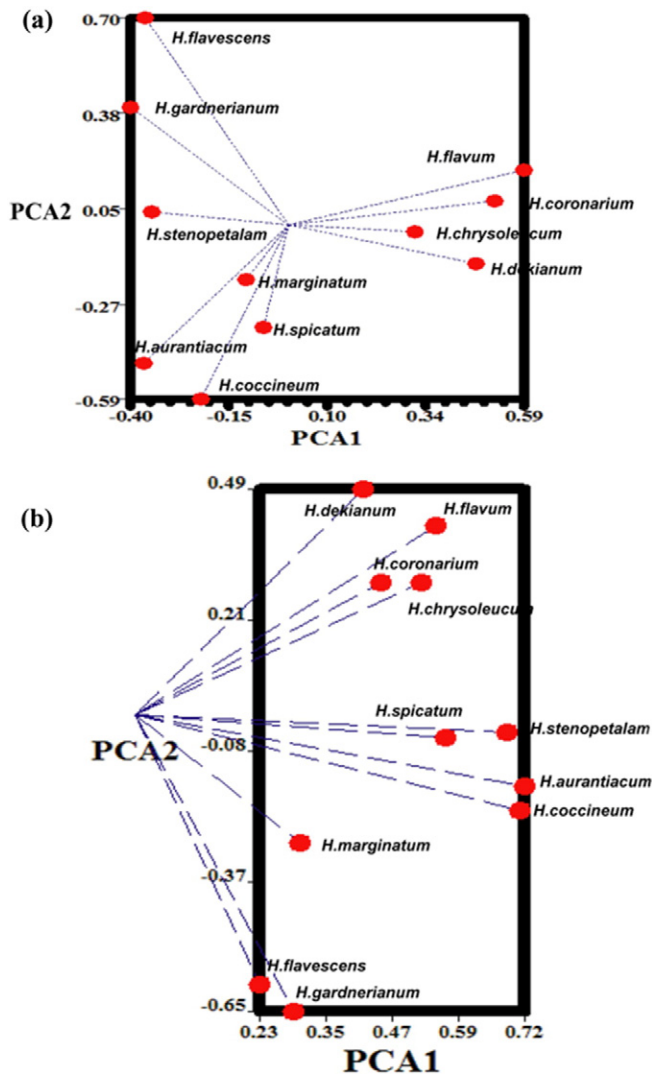

Fig. 2. PCA plots of the 11 *Hedychium* species of Northeast India based on molecular markers. (a). ISSR (b). AFLP. The first three components explained 57.24% and 42.94%, of the variation. PCA analysis of ISSR and AFLP dataset segregates species collected from different altitudes and flower aroma into two groups represented by dotted lines.

showed the lowest genetic similarity coefficient between *H. chrysoleucum* and *H. stenopetalum* (0.63) while the highest value was calculated between *H. coccineum* and *H. aurantiacum* (0.79). This result showed that the inter-species genetic similarity of the genus *Hedychium*is almost the same using two different PCR based molecular markers. Dendrogram constructed based on AFLP similarity matrix, showed two distinct groups for the 11 species of *Hedychium* at the level of similarity of 0.60, placing *H. flavescens* and *Hedychium marginatum* in cluster II and the rest of the species in the cluster I. *H. flavescens* and *H. marginatum* had 63.2% similarity between them. Cluster I formed two subgroups. Subgroup I consisted of *Hedychium flavum*, *H. coronarium*, *H. dekianum* and *H. chrysoleucum*. Subgroup II of cluster I consisted of *H. spicatum*, *H. stenopetalum*, *H. coccineum*, *H. aurantiacum* and *H. gardnerianum*. The level of similarity between *H. coccineum* and *H. aurantiacum* was found to be 0.790, which is of similar magnitude to that of the ISSR (0.787) dendrogram. The species clustering of ISSR dendrogram was similar to AFLP dendrogram in the subgroup I of cluster I (Supplementary Fig. 2a & b). The high amount of red area in ISSR and AFLP HCA compared to the other dendrograms signifies better informativeness (Supplementary Fig. 2c & d).

The separation approach as revealed by the Mantel test comparing the results of ISSR and AFLP indicated a significant correlation among the 11 different *Hedychium* species. Mantel test yielded results ranging from good fit to very good fit of cophenetic values ($0.83 < r < 0.86$) (Table 5).

Discussion

Hedychium commonly known as 'ginger lily' or 'butterfly lily'is well known for its medicinal and ornamental importance. Classification of genus has been contentious and no report on genetic relationships of *Hedychium* species of NE India exists. Relying much on the morphological characters alone in species delineation has its own limitations since they are not always complete representative of the genetic structure. Molecular markers assume great significance, as these methods detect polymorphism by assaying subsets of the total amount of DNA sequence variation in a genome.

The intra-species variation in terms of percentage of polymorphism, observed number of alleles, effective number of alleles, Nei's gene diversity and Shannon's information index was found to be highest in *H. coronarium* and to be lowest in *H. chrysoleucum*. This might be the result of cross pollination in *H. coronarium* and vegetative propagation in other individuals of the species.

Marker studies separated *Hedychium* into 2 main clusters based on different altitudes and intensities of flower aroma. In the ISSR dendrogram, species of the moist lowlands with strong fragrant flower (*H. coronarium, H. dekianum, H. flavum, H. chrysoleucum, H. stenopetalum, H. spicatum, H. marginatum, H. coccineum, H. aurantiacum*) were combined in cluster I. The strong aroma of the flowers may be the evolutionary derived trait among the species. A small cluster (cluster II) consisted of species of *H. flavescens* and *H. gardnerianum* that possessed geographic similarity of high altitude and has yellow and mildly fragrant flowers. AFLP marker analysis too closely follows the altitude based clustering of ISSR marker analysis. However, our study of PCR based molecular markers deviates from the DNA analysis by other researchers who split *Hedychium* into three groups in terms of the species distribution according to altitude; each group correlated to a separate climatic zone (Wood et al., 2000). The discrepancy of our study from the earlier reported study by SPAR based marker was the positioning of *H. spicatum* which was found to cluster with *H. stenopetalum* (Gao et al., 2008).

The PCA and cluster analyses of a binary matrix (ISSR and AFLP) showed a unique genetic structure in *Hedychium*. These results suggest that the manner of polymorphism differs because of marker specificity. Modest correlation between marker systems was also reported in soybean (Powell et al., 1996), maize

Table 5
Comparison of correlation coefficient between similarity matrices and co-phenetic matrices derived from different markers.

		Similarity matrix values	
		ISSR	AFLP
Cophenetic correlation	ISSR	0.86	0.06
	AFLP	− 0.02	0.83

Note: Above diagonal values represent correlation coefficients between similarity matrices and below diagonal values represent correlation coefficients between co-phenetic matrices. Values on the diagonal represent co-phenetic correlation for markers.

(Pejic et al., 1998), and safflower (Sehgal and Raina, 2005). In addition, the relation is assumed to depend on the genome coverage and sequence type recognized by each marker system (Pejic et al., 1998; Powell et al., 1996; Sehgal and Raina, 2005). Therefore, ISSR and AFLP are reliable methods for calculating genetic relationships reflecting coding and non-coding regions of the genome and they could well be used in aiding identification as well as classification of the *Zingiberaceae* using more species in each genus. Given the correlation of similarity matrix data from different markers, we therefore prefer to combine them in analyses, because this approach generally helps to overcome errors or introgression at one locus, and to increase explanatory power (Edwards and Beerli, 2000; Nixon and Carpenter, 2005).

This is the first report on genetic relationships for *Hedychium* species of NE India producing trees and PCA plot with high topological support for most nodes using ISSR and AFLP markers. The UPGMA, hierarchical clustering and PCA analysis of ISSR and AFLP datasets represent our best hypothesis of genetic relationship among *Hedychium*. We recommend the use of ISSR and AFLP topology as the most complete framework for future studies of phylogenetic comparative analyses, tests of bio-geographic hypotheses and models of trait evolution. This study confirmed that two marker utilizations (ISSR and AFLP) can be crucial for estimation of the relatedness of *Hedychium* at the inter-species level.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2014.05.002.

Acknowledgments

References

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., Sorrells, M.E., 1993. Optimizing parental selection for genetic linkage maps. Genome 36, 181–186.

Crawford, D.J., 1990. Plant Molecular Systematics: Macromolecular Approaches. Wiley, New York.

Das, A., Kesari, V., Satyanarayan, V.M., Parida, A., Rangan, L., 2011. Genetic relationship of *Curcuma* species from Northeast India using PCR-based markers. Mol. Biotechnol. 49, 65–76.

de Hoon, M.J.L., Imoto, S., Nolan, J., Miyano, S., 2004. Open source clustering software. Bioinformatics 20 (9), 1453–1454.

Dice, L.R., 1945. Measures of the Amount of Ecologic Association Between Species. Ecology 26, 297–302.

Edwards, S.V., Beerli, P., 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54, 1839–1854.

Eisen, M.B., Spellman, P.T., Brown, P.O., Botstein, D., 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. U. S. A. 95 (25), 14863–14868.

Gao, L., Liu, N., Huang, B., Hu, X., 2008. Phylogenetic analysis and genetic mapping of Chinese *Hedychium* using SRAP markers. Sci. Hortic. 117, 369–377.

Gopanraj, G., Dan, M., Shiburaj, S., Sethuraman, M.G., George, V., 2005. Chemical composition and antibacterial activity of the rhizome oil of *Hedychium larsenii*. Acta Pharma. 55 (3), 315–320.

Kaewsri, W., Paisooksantivatana, Y., Veesommai, U., Eiadthong, W., Vajrodaya, S., 2007. Phylogenetic analysis of Thai *Amomum* (Alpinioideae: *Zingiberaceae*) using AFLP markers. Kasetsart J. Nat. Sci. 41, 213–226.

Kesari, V., Sathyanarayana, V.M., Parida, A., Rangan, L., 2010. Molecular marker-based characterization in candidate plus trees of *Pongamia pinnata*, a potential biodiesel legume. AoB Plants http://dx.doi.org/10.1093/aobpla/plq017.

Lee, R., 1945. Measures of the amount of ecologic association between species. Ecology 26 (3), 297–302 http://dx.doi.org/10.2307/1932409. JSTOR.

Lu, G., Cao, J.S., Chen, H., 2002. Genetic linkage map of *Brassica campestris* L. using AFLP and RAPD markers. J. Zhejiang Univ. Sci. A 3 (5), 600–605.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27 (2), 209–220.

McDermott, J.M., McDonald, B.A., 1993. Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31, 353–373.

Milligan, G.W., Cooper, M.C., 1987. A Study of Variable Standardization, College of Administrative Science Working Paper Series, 87–63. The Ohio State University, Columbus, OH.

Nixon, K.C., Carpenter, J.M., 2005. On simultaneous analysis. Cladistics 12, 221–241.

Pejic, I., Ajmone-Marsan, P., Morgante, M., Kozumplick, V., Castiglioni, P., Taramino, G., Motto, M., 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet. 97, 1248–1255.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., Rafalski, A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed. 2, 225–238.

Rohlf, F.J., 2005. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.2. Exeter Software, Setauket, NY.

Saleh, M.M., Kamal, R.M., Abdulla, W.A., 1982. A contribution to the molluscicidal activity of *Hedychium gardinarum*. Planta Med. 45 (7), 166.

Sehgal, D., Raina, S.N., 2005. Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints. Euphytica 146, 67–76.

Shrotriya, S., Ali, M.S., Saha, A., Bachar, S.C., Islam, M.S., 2007. Anti-inflammatory and analgesic effects of *Hedychium coronarium* Koen. Pak. J. Pharm. Sci. 20 (1), 47–51.

Sneath, P.H.A., Sokal, R.R., 1973. Numerical Taxonomy. Freeman, San Francisco.

Vanijajivaa, O., Sirirugsab, P., Suvachittanont, W., 2005. Confirmation of relationships among *Boesenbergia* (*Zingiberaceae*) and related genera by RAPD. Biochem. Syst. Ecol. 33, 159–170.

Warren, K.S., Peters, P.A., 1968. Cercariae of *Schistosoma mansoni* and plants: attempt to penetrate *Phaseolus vulgaris* and *Hedychium coronarium* produces a cercaricide. Nature 217 (5129), 647–648.

Wilfinger, W.W., Mackey, K., Chomczynski, P., 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22 (3), 474–476 (478–81).

Wood, T.H., Whitten, W.M., Williams, N.H., 2000. Phylogeny of *Hedychium* and related genera (*Zingiberaceae*) based on ITS sequence data. Edinb. J. Bot. 57 (2), 261–2170.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X., 1997. POPGENE, the User Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta.