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Assessment of Genetic Variations among *Hedychium coronarium* (L.) Genotypes using RAPD and ISSR Analysis

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Abstract

In the realm of herbal remedies, *Hedychium coronarium* is a significant species of medicinal plant with a variety of applications. In order to rapidly and effectively determine the closeness of genotypes, a molecular markers-based assessment of *Hedychium coronarium* genotypes gathered from various places was carried out utilizing 14 inter simple sequence repeats and 12 random amplified polymorphic DNA markers. Using Jaccard's similarity coefficient of independent and combined markers, a dendrogram was created using sequential agglomerative hierarchical and nested (SAHN) clustering and the unweighted pair group method with arithmetic mean (UPGMA) analysis. In every case, two clusters were discovered. Furthermore, the degree of genetic similarity between germplasms obtained from various locations was also shown by the clustering patterns. The greatest possible realization of this technique's potential would enable the identification and tagging of a significant novel gene in various taxa that has not yet been fully investigated, hence promoting the advancement of this significant medicinal plant species. The results would be very important enough to support current biotechnological approaches to the conservation and characterization of related medicinal plants.

***Key words*:** *Hedychium coronarium*, Genetic variability, Plant genetic resources, Medicinal plant, Clustering, Germplasm

Hedychium coronarium Koen. is a perennial herbaceous aromatic plant native to tropical and subtropical areas, such as India, Southern China, Japan, and Southeast Asia [1]. Due to its eye-catching foliage and vibrant blossoms, it is becoming more and more important in horticulture globally. Because of its beautiful scent, rhizomes and flowers are used in perfumery [2]. The plant's stems are used to make paper because they have a high cellulose content (43–48%). Rhizomes are utilized as food flavourings and spices in South East Asia. In Manipur, a traditional delicacy known as "eronba" is prepared using rhizome. In Malaysia, betel nut and leaf consumption are used to treat stomach pain [3]. *H. coronarium* rhizomes are used to treat rheumatism, fever, flatulence, headaches, and inflammation [1]. Furthermore, rhizomes are utilised to treat conditions connected to the skin and neck [4]. Significant biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and mosquito larvicidal properties, have been reported by pharmacological studies on *H. coronarium* essential oil.

In many forests in Central and North Eastern India, the plant is going extinct. It is listed as endangered in several states of India due to land clearing, overexploitation of the wild, and the indiscriminate uprooting of rhizomes for the preparation of indigenous herbal medicine [5-6]. The current wild resources are under a great deal of strain as a result. Furthermore, as the plant material is replicated vegetatively, it is crucial to comprehend the genetic variety of *H. coronarium* in order to maintain elite genotypes and choose high yielding parental lines.

The best way to estimate genetic variety is to use DNA based molecular markers [7]. Because morphological attributes are susceptible to environmental fluctuation, molecular characterization has been done in order to determine genetic diversity instead of relying solely on morphological analysis and phenotypic character analysis. Among the several molecular markers, multi-locus profiling techniques such as RAPD and ISSR have been employed in the past to characterize genetic diversity [8]. Because RAPD can amplify many loci at once and ISSR can amplify intron regions of the genome and does not require prior sequence information for amplification, the combination of both markers allows for a higher degree of genomic coverage [9].

There are currently very few papers on the molecular characterization of *Hedychium coronarium* with ISSR and RAPD primers. There have been reports on the variations in Hedychium populations based on morphological traits [2], [10] and essential oil composition [1]. However, there are currently no reports on the variability based on phenotypic, genetic, and chemical traits. Furthermore, the selection of exceptional accessions is a necessary requirement to enhance the species' genetic pool, and this can be accomplished through genetic diversity studies. Considering the above facts, the present study

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was carried out to assess the molecular diversity among accessions of *H. coronarium* to understand the relationship among them.

MATERIALS AND METHODS

Plant material

In the present investigation, *Hedychium coronarium* was collected from the different areas of Madhya Pradesh. After collection, the rhizomes of these medicinal plant samples were grown and further analysis was done using leaf samples.

Isolation of genomic DNA

Young leaves of *H. coronarium* were used to isolate genomic DNA, with minimal modifications made to the procedure described in [11]. In a cold mortar and pestle, 2 g of fresh, young leaf samples were ground into a fine powder using 2% insoluble PVPP and repeated additions of liquid nitrogen. In order to lessen DNA shearing, thawing was avoided. After that, the powder was put into a 50 ml centrifuge tube and thoroughly mixed with 10 ml of preheated (60°C) 2% CTAB-DNA extraction buffer (10% CTAB; 4M NaCl; 0.5M EDTA, pH 8; 1M Tris-HCl, pH 8; 2% β-mercaptoethanol). For an hour, the mixture was incubated at 65°C in a water bath with gentle shaking intervals. Following incubation, the mixture was allowed to cool to ambient temperature before being gently mixed and emulsified with a 25:24:1 ratio of phenol, chloroform, and isoamyl alcohol. After that, it was centrifuged at ambient temperature for 20 minutes at 10,000 rpm in a cooling centrifuge. Using a micropipette, the top aqueous phase was pipetted out into a second 50 ml centrifuge tube and combined with 2.5 volume of dehydrated ethanol that had been refrigerated beforehand. Following a brief inversion, DNA was spooled out with a bent glass Pasteur pipette, stored in a 1.5 ml microcentrifuge tube, and twice cleaned with 70% ethanol before being dried. The excess T10 E1buffer (Tris-Cl 10 mmol, EDTA 1 mmol, pH 8) was used to dissolve the dried DNA.

Purification of genomic DNA

The crude DNA was purified and the RNA was eliminated since the dissolved DNA was tainted with proteins, RNA, and, in certain cases, polyphenols. RNase treatment was used to extract the RNA. After adding 60 µg of RNase A to 1 ml of crude DNA solution, the mixture was continuously shaken in a water bath at 37 °C for one hour. It was taken out of the water bath after an hour, and the same volume of phenol, chloroform, and isoamyl alcohol (25:24:1) was added. This was carefully combined. After centrifuging the mixture for 20 minutes at 20 °C at 10,000 rpm in a cooling centrifuge, the upper aqueous phase was pipetted out. It was centrifuged at 10,000 rpm for 20 minutes at room temperature after being once more cleaned with chloroform and twice with isoamyl alcohol (24:1). Following centrifugation (as previously mentioned), the top aqueous phase was removed and combined with 1/10th volume of 3M sodium acetate (pH 4.8). After adding 2.5 volumes of cold 100% ethanol, DNA was precipitated and spun to pelletize it. The pellet was meticulously cleaned twice with 70% ethanol before being vacuum-dried. The dried DNA was dissolved in the least quantity of pH 8 T10 E1 buffer.

Qualitative and quantitative analysis of the purified DNA

The UV-vis spectrophotometer was used to measure the quantity and quality of DNA. The amount of DNA in total was determined by measuring the absorbance at a wavelength of 260 nm. The DNA's purity was verified by comparing the absorbance ratios at 260 and 280 nm. DNA quality is high if the ratio is between 1.8 and 2.0. In order to verify the final quality and quantity of DNA, the sample was electrophoresed in a 0.8% agarose gel with diluted uncut lambda DNA as a standard. All of the DNA samples were found to be of extremely high quality. Following measurement, the DNA was diluted to a working concentration of 25 ng/µl using T_{10} E_1 buffer in order to perform RAPD and ISSR analyses.

RAPD analysis

Random decamer operon primers were diluted to a working concentration of 15 ng/µl in double-sterilized T10E1 buffer (pH 8.0) for RAPD analysis. For the RAPD study, a small number of primers chosen based on polymorphism amplification pattern and repeatability were employed. 250 µL of 10X assay buffer (100 mmol Tris-HCl, pH 8.3, 500 mmol KCl, 1.5 mmol $MgCl_2$, and 0.1% gelatin), 200 µM of each dNTP (dATP, dCTP, dGTP, and dTTP), 15 ng of primer, 0.5 unit of Taq DNA polymerase, and 50 ng of template DNA were included in each 25 µl volume amplification reaction mixture.

In PCR, the amplification reaction was conducted. Three PCR stages were involved in the amplification process. The template DNA underwent one cycle of initial denaturation, which lasted five minutes at 94 °C. The second process took 45 cycles, with each cycle consisting of three temperature steps: the template was denaturated for one minute at 92 °C, primer annealing took place for one minute at 37 °C, and primer extension took place for two minutes at 72 °C. The last stage only required one cycle, or seven minutes at 72 °C to complete the polymerization. 4 °C was the soaking temperature. 2.5 µl of 6X loading dye was added to the amplified products after the PCR was finished, and they were kept at -20 °C until needed.

ISSR analysis

Thirty ISSR primers were first evaluated for ISSR analysis, and fourteen ISSR markers in total were chosen for additional research based on the polymorphic banding pattern. Twenty-five ng of template DNA, 2.5 µl of 10X assay buffer (100 mmol Tris-HCl pH 8.3, 500 mmol KCl, 1.5 mmol MgCl2, and 0.1% gelatin), 200µM of each of the dNTPs (dATP, dCTP, dGTP, and dTTP), 40 ng of primer, and 0.5 unit Taq DNA polymerase were included in each 25 µl amplification reaction mixture. A thermal cycler was used to perform the amplification. The initial cycle included of five minutes of 94 °C denaturation of the template DNA, one minute of primer annealing at a particular primer's temperature, and two minutes of primer extension at 72 °C. The denaturation duration was shortened to one minute in the next forty-five cycles, but the primer annealing and primer extension times remained the same as in the initial cycle. The amplified products were resolved in a 2% agarose gel and stained with ethidium bromide during the final cycle, which involved only primer extension at 72 °C for 7 minutes.

Agarose gel electrophoresis

Whereas the ISSR results were resolved in a 2% agarose gel, the PCR products for RAPD were separated on a 1.5% agarose gel. TAE buffer (40 mmol Tris base, 20 mmol sodium acetate, 20 mmol EDTA, glacial acetic acid; pH 7.2) was used to prepare the agarose gel. 125 ml of 1X TAE buffer was added to a 500 ml conical flask along with 1.875 g of agarose. The mixture was then heated to completely melt the agarose and cooled to 50 °C. Once the gel solution had cooled, 6.25 µl of ethidium bromide solution (10 mg/ml) was added, thoroughly mixed, and then the gel solution was poured onto the gel casting tray. The gelling process was allowed to occur for an hour. After that, the gel was placed within a gel tank that held 1X

TAE buffer. The comb was taken out before the samples were loaded. Each well in the submerged gel held 27 µl of the PCR amplified samples that were dyed with tracking dye. After the amplified samples were placed into other wells to determine the size of the amplified DNA fragment, a standard 1 kb DNA ladder was added into the first well. For three hours, the electrophoresis was run at 62 volts. Following electrophoresis, the gel was seen under a UV light source and captured on camera using a gel documenting system to score the bands. By contrasting the amplicons' diameters with the ladder's, the sizes of the two were found. To ensure reproducibility, every step of the procedure was carried out at least twice.

Band scoring and data analysis

For each primer genotype combination used in the RAPD and ISSR analyses, the data was assigned a score of "1" for the existence of the band and "0" for its absence. Every band was thought to represent an underestimate of the genetic similarity. Polymorphism information content (PIC) was calculated as PIC = $1-\Sigma P^2_i$, where, P_i is the band frequency of the i^{th} allele. After measuring the Jaccard's coefficient of similarity [12] a dendrogram based on the similarity coefficients was produced using the unweighted pair group method with arithmetic averages (UPGMA) [13], yielding the SAHN clustering. The statistical programme NTSYS-pc 2.02e was used to carry out the complete analysis [14].

RESULTS AND DISCUSSION

The present investigation was undertaken to analyze RAPD and ISSR markers-based variability among *Hedychium corornarium* genotypes. The results of the investigation are given in the following sections.

RAPD analysis

Twenty-five decamer primers were examined in the beginning. Twelve polymorphic primers were chosen for the current study from a set of twenty-five primers based on their banding pattern clarity and amplification. Every *H. coronarium* sample was utilized in triplicate and yielded unique, repeatable amplicons. The average value of the 77 bands that were amplified was 6.41. 49 of the total amplicons, with an average value of 4.08, were determined to be polymorphic in nature. Primers OPD3 and OPN4 amplified the least amount of bands (5), while primer OPN18 amplified the greatest number of bands (10). With all of the primers, no distinct bands were discovered. The highest percentage (83.3) of polymorphism was demonstrated by primer OPN16. However, the lowest percentage (33.3) of polymorphism was produced by primer OPC2. PIC values for RAPD markers were ranged between 0.189 (OPC2) to 0.489 (OPN16) with an average value of 0.289.

Jaccard's similarity coefficient was used to construct UPGMA based dendrogram. RAPD markers based dendrogram divided *H. coronarium* genotypes into two groups. Major group consisted seven genotypes including HC1, HC2, HC4, HC5, HC6, HC7 and HC8. The major cluster was further divided into two sub groups. The first sub group had HC1, HC2 and HC5 while, the second sub group consisted HC4, HC6, HC7 and HC8. However, the minor group had only one genotype namely HC3. Separate clustering of this genotype indicates higher genetic variability of this genotype from rest of the genotypes. The highest percentage of similarity was found between HC7 and HC8 i.e. 80.9% and both of the genotypes clustered together. Higher similarity indicates higher resemblance between both of the genotypes. Two-dimensional clustering does not follow the clustering pattern of the genotypes according to the dendrogram. In 2D clustering HC6 and HC8 showed higher resemblance. However, the grouping of HC3 was similar to the dendrogram. As this genotype showed higher distance from rest of the genotypes. Three-dimensional scaling also followed similar clustering of genotypes as it is in the two-dimensional clustering. Genotype HC3 showed higher genetic distance from rest of the genotypes in 3D scaling.

Table 1 Data for RAPD primers used for analyzing genotypes of *Hedychium corornarium*

Markers	Sequence 5'-3'	Total bands	Polymorphic bands	Percentage of polymorphism	Polymorphism information content
OPA-03	AGTCAGCCAC	8	6	75.0	0.387
OPA-04	AATCGGGCTG	7	4	57.1	0.248
OPA-09	GGGTAACGCC	6	3	50.0	0.221
OPA-18	AGGTGACCGT	8	5	62.5	0.256
OPC-02	GTGAGGCGTC	6	2	33.3	0.189
OPC-05	GATGACCGCC	8	5	62.5	0.256
OPD-03	GTCGCCGTCA	5	3	60.0	0.264
OPD-07	TTGGCACGGG	8	6	75.0	0.395
OPN-04	GACCGACCCA	5	4	80.0	0.485
OPN-16	AAGCGACCTG	6	5	83.3	0.498
OPN-18	GGTGAGGTCA	10	6	60.0	0.271
Total		77	49	-	3.47
Average		6.41	4.08	-	0.289

Table 2 RAPD markers based Jaccard's similarity coefficient among genotypes

	HC1	HC2	HC3	HC4	HC5	HC6	HC7	HC8
HC1	1.000							
HC2	0.788	1.000						
HC3	0.612	0.543	1.000					
HC4	0.603	0.625	0.597	1.000				
HC5	0.662	0.639	0.636	0.539	1.000			
HC6	0.686	0.639	0.688	0.696	0.735	1.000		
HC7	0.595	0.616	0.588	0.671	0.639	0.761	1.000	
HC8	0.708	0.708	0.614	0.743	0.662	0.783	0.809	1.000

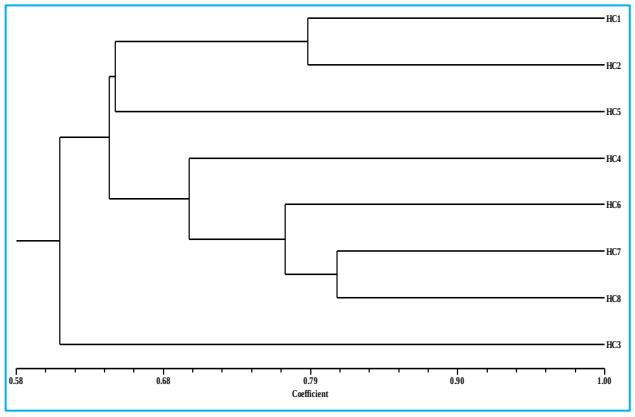

Fig 1 Dendrogram based on RAPD data showing relationship among eight *H. coronarium* genotypes

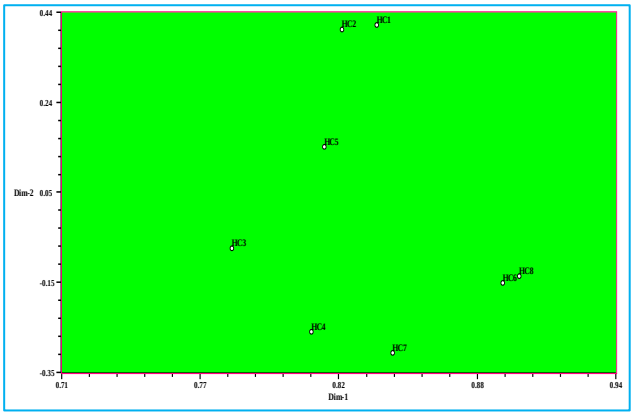

Fig 2 RAPD markers based two-dimensional grouping of eight *H. coronarium* genotypes

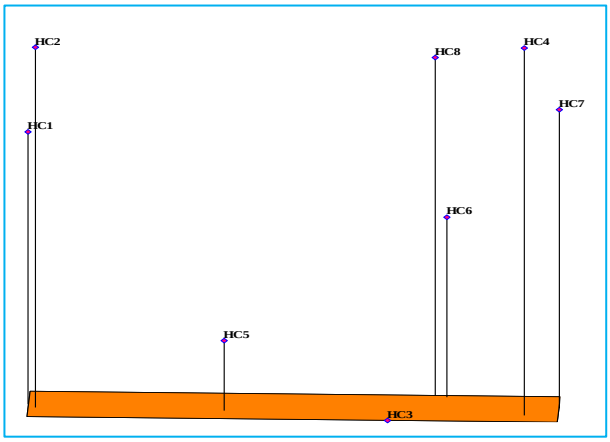

Fig 3 RAPD markers based three-dimensional scaling of eight *H. coronarium* genotypes

ISSR analysis

At initial stage, to select polymorphic markers total 30 ISSR markers were applied. Out of these 30 markers, only 14 were selected on the basis of their polymorphic banding pattern. Total 123 amplicons were produced by ISSR markers. The average numbers of amplicons were 8.79. The highest numbers of amplicons (15) were produced by the marker ISSR825, while lowest numbers (5) of amplicons were produced by ISSR814. Out of total amplicons, 102 amplicons were found to be polymorphic. The average numbers of polymorphic amplicons were 7.29. Lowest percentage (37.5) of polymorphism was demonstrated by primer ISSR812. However, the highest percentage (100) of polymorphism was produced by multiple markers including ISSR811, ISSR834, ISSR835, ISSR853, ISSR860 and ISSR882. The PIC values were ranged from 0.511 (ISSR853) to 0.211 (ISSR812). The average PIC value for ISSR markers was 0.41.

Table 2 Details of ISSR markers used for analysis of molecular diversity among *Hedychium corornarium* genotypes

Marker	Sequence 5'-3'	Total bands	Polymorphic bands	Percentage of polymorphism	Polymorphism information content
ISSR808	AGAGAGAGAGAGAGAGC	8	5	62.5	0.298
ISSR810	GAGAGAGAGAGAGAGAT	7	4	57.1	0.247
ISSR811	GAGAGAGAGAGAGAGAC	9	9	100.0	0.498
ISSR812	GAGAGAGAGAGAGAGAA	8	3	37.5	0.211
ISSR814	CTCTCTCTCTCTCTCTA	5	4	80.0	0.416
ISSR824	TCTCTCTCTCTCTCTCG	7	5	71.4	0.386
ISSR825	ACACACACACACACACT	15	10	66.7	0.312
ISSR827	ACACACACACACACACG	9	7	77.8	0.398
ISSR834	AGAGAGAGAGAGAGAGYT	9	9	100.0	0.523
ISSR835	AGAGAGAGAGAGAGAGYC	8	8	100.0	0.499
ISSR842	GAGAGAGAGAGAGAGAYG	8	8	100.0	0.478
ISSR853	TCTCTCTCTCTCTCTCRT	13	13	100.0	0.525
ISSR860	TGTGTGTGTGTGTGTGRA	10	10	100.0	0.501
ISSR882	VBVATATATATATATAT	7	7	100.0	0.448
Total		123	102	-	5.74
Average		8.79	7.29	-	0.41

Single-letter abbreviations for mixed base positions: R = (A, G); Y = (C, T)

The ISSR markers based dendrogram was generated using NTSyS software. This unweighted pair group method with arithmetic averages (UPGMA) based dendrogram divided *Hedychium coronarium* genotypes into two group. The major group consisted total seven genotypes namely HC1, HC2, HC3, HC5, HC6, HC7 and HC8. However, the minor group had only one genotype namely HC4. The separate clustering of this particular genotype indicates the highest genetic diversity of this genotype from rest of the seven genotypes. The major cluster was divided into two sub clusters. The major sub cluster consisted five genotypes namely HC2, HC5, HC3, HC6 and HC7; however, the minor sub cluster had only two genotypes namely HC1 and HC8. The highest genetic similarity was noticed between HC2 and HC5 i.e. 57.1% and both of the genotypes clustered together. This close clustering indicates higher genetic resemblance between both of the genotypes. The 2D scaling followed similar clustering of the genotypes HC2 and HC5. In the similar way HC4 showed distance from rest of the genotypes. Similar pattern of clustering of genotypes was followed in 3D scaling.

Table 3 ISSR markers base Jaccard's similarity coefficient among genotypes

	HC1	HC2	HC3	HC4	HC5	HC6	HC7	HC8
HC1	1.000							
HC2	0.484	1.000						
HC3	0.516	0.568	1.000					
HC4	0.423	0.421	0.392	1.000				
HC5	0.460	0.571	0.554	0.386	1.000			
HC6	0.412	0.473	0.558	0.456	0.511	1.000		
HC7	0.402	0.448	0.482	0.447	0.440	0.506	1.000	
HC8	0.548	0.396	0.477	0.459	0.389	0.500	0.474	1.000

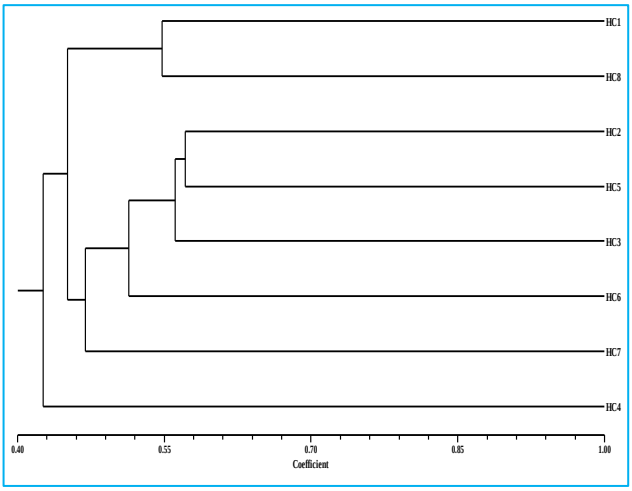

Fig 4 ISSR markers based dendrogram showing relationship among eight *H. coronarium* genotypes

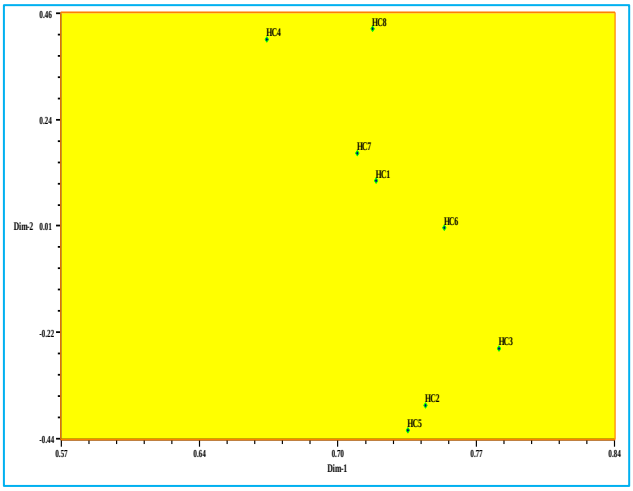

Fig 5 ISSR markers based two-dimensional scaling of eight *H. coronarium* genotypes

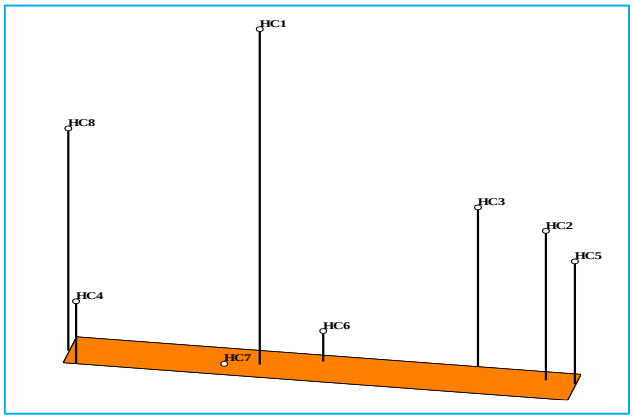

Fig 6 ISSR markers based three-dimensional clustering of eight *H. coronarium* genotypes

RAPD and ISSR combined analysis

Data for RAPD and ISSR markers were combined and analyzed to understand the genetic diversity between and among the *H. coronarium* genotypes. The combined data based dendrogram divided all of the genotypes under study into two groups. Each cluster contained four genotypes. The first cluster had HC1, HC2, HC3 and HC5 while the second cluster contained HC4, HC6, HC7 and HC8 genotypes. In the first cluster, genotypes HC1 and HC2 had the highest similarity i.e. 60.9%. However, in the second cluster the highest similarity (63%) was noticed between HC7 and HC8. In 2D scaling three genotypes namely HC1, HC2, HC3 and HC5 showed close relationship among them. 2D clustering followed similar clustering pattern as it was in the dendrogram. The similar grouping was also noticed in 3D for all of the genotypes of *H. coronarium.*

Table 4 Table Jaccard's similarity coefficient based on combined data of RAPD and ISSR markers

	HC1	HC2	HC3	HC4	HC5	HC6	HC7	HC8
HC1	1.000							
HC2	0.609	1.000						
HC3	0.556	0.557	1.000					
HC4	0.500	0.509	0.476	1.000				
HC5	0.544	0.601	0.589	0.452	1.000			
HC6	0.527	0.546	0.613	0.560	0.606	1.000		
HC7	0.488	0.525	0.529	0.548	0.528	0.622	1.000	
HC8	0.622	0.534	0.538	0.587	0.509	0.629	0.630	1.000

The core task in the field of a species or crop improvement is genetic diversity assessment. For the purpose of identifying species, developing cultivars, obtaining certification, and defending breeders' rights, precise identification and characterization of various germplasm resources are crucial [15]. Since the development of molecular

biology techniques, morphological, cytological, and biochemical characters in studies of phylogenetic and evolutionary relationships, varietal identification, clonal fidelity testing, assessment of genetic diversity, and marker-assisted selection and gene tagging have all been greatly enhanced by DNA-based markers. Deoxyribonucleic acid (DNA) markers are simpler, more effective, and need less time because of their flexibility, universality, and stability—especially in perennials where there are few physical identifiers [16]. Molecular markers for genotype identification and characterization, genetic fingerprinting for gene identification and cloning of key genes, marker-assisted selection, and the comprehension of molecular interrelationships have all received more attention in recent years. Technologies like RAPD (Random Amplified Polymorphic DNA), ISSR, AFLP, and microsatellites that rely on the polymerase chain reaction (PCR) are well-known for their simplicity, affordability, and genetic integrity. With the exception of few genetic fidelity studies of micro-propagated plants and isozyme-based characterization, work on the molecular characterization of Zingiberaceous species is still in its infancy, despite attempts to characterize its morphology [17-19].

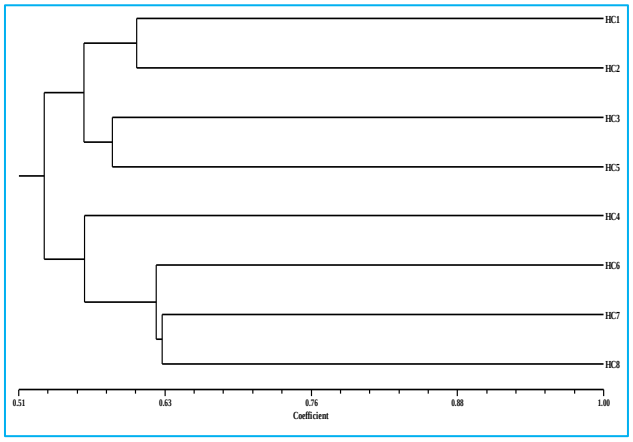

Fig 6 Dendrogram based on the combined data (RAPD and ISSR) showing relationship among *H. coronarium* genotypes

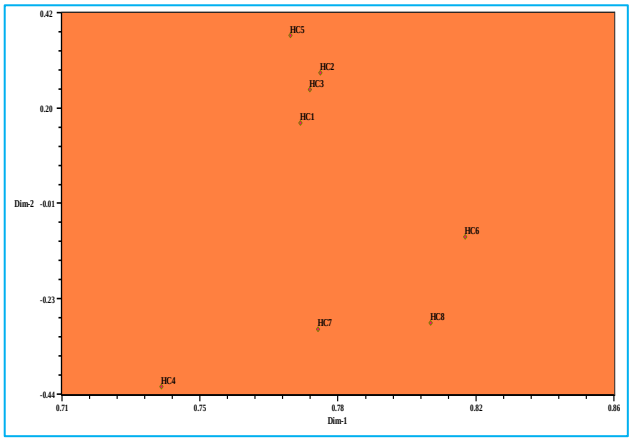

Fig 7 Two-dimensional clustering of the eight *H. coronarium* genotypes based on the combined (RAPD and ISSR) data

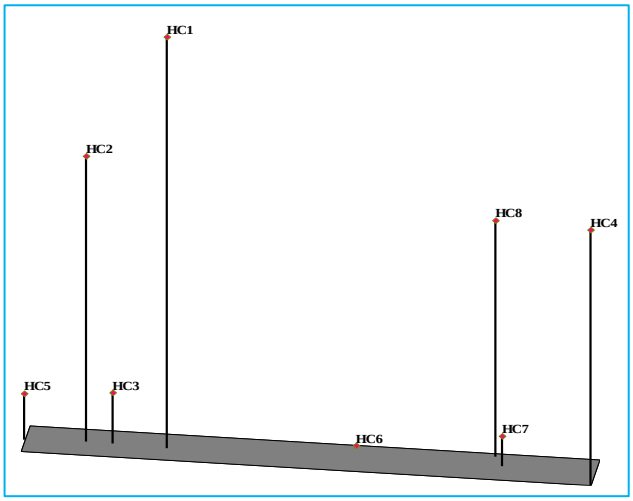

Fig 7 Three-dimensional clustering of the eight *H. coronarium* genotypes based on the combined (RAPD and ISSR) data

Hedychium, sometimes referred to as "butterfly lily" or "ginger lily," is highly valued for its ornamental and therapeutic uses. There is no information on the genetic links of the Hedychium species in Central India, and the genus classification has been controversial. Because physical characteristics aren't always a thorough representation of the genetic structure, relying heavily on them alone for species classification has its own limits. Molecular markers are extremely important since they allow for the detection of polymorphism by analyzing portions of the overall variance in DNA sequence in a genome.

Both marker systems (RAPD and ISSR) produced a greater degree of genetic variability within and between the genotypes of *H. coronarium* that were the subject of our investigation. A prior study employed amplified fragment length polymorphism (AFLP) markers to describe the genetic diversity of *H. coronarium* cultivars and natural populations. Three AFLP primer combinations were used to assess twelve populations in Tunisia. 178 (86%) of the 207 reproducible bands that were found were polymorphic. Similar to the findings of the current study, it was shown that AFLP markers have a high degree of discriminative power and can accurately depict the genetic relationships among Hedysarum plants. High levels of genetic variability within and between populations have been shown in *H. coronarium* by AFLP technology. Molecular markers associated with the plants' geotropism were obtained using AFLP banding patterns. Furthermore, AFLP markers can distinguish between cultivars and wild accessions. Furthermore, population clustering did not correlate with geographic origins [20]. Similar to the present study, Basak *et al.* [21] conducted an experiment on use of AFLP and ISSR markers for identification of genetic variability among eleven *Hedychium* species from Northeast India. Both marker systems were able to differentiate the populations under investigation.

The present investigation also demonstrated the discrimination power of RAPD and ISSR markers systems. As a result, ISSR and RAPD are trustworthy techniques for determining genetic links reflected in coding and non-coding sections of the genome, and they may be applied to help classify and identify Zingiberaceae species by utilizing many species within each genus [22]. We therefore choose to mix the similarity matrix data from other markers given their association, since this generally helps to overcome mistakes or introgression at one locus and to boost explanatory power.

CONCLUSION

Genetic diversity analysis at DNA level is important to understand the variability present among or between individuals. *Hedychium corornarium* is an important medicinal plant species with immense therapeutic values. Two marker systems i.e. RAPD and ISSR were applied to analyze molecular

variability among the collected individuals of *Hedychium corornarium.* Both marker systems were able to demonstrate higher level of genetic variability among the studied genotypes. This medicinally significant plant species is not much exploited to understand molecular level genetic variations. The obtained results in the present investigation may work as fundamental to plan further strategies for conservation and improvement of this medicinal plant species.

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