



RESEARCH ARTICLE

Genetic diversity and population structure of *Hemidesmus indicus* (L.) R.Br. populations of Northeast India revealed by inter-simple sequence repeat (ISSR) markers

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Abstract

Hemidesmus indicus (L.) R.Br. is a highly valued medicinal herb in both ancient and contemporary Indian medicinal systems. However, the species has recently become vulnerable due to indiscriminate collection and the consequent rapid decline of its natural populations. Therefore, assessing its genetic diversity is essential to support future conservation and restoration initiatives. In the present study, genetic diversity was evaluated across five natural populations of *H. indicus* distributed across the north-eastern states of India using 12 inter-simple sequence repeat (ISSR) primers. A total of 54 DNA fragments were generated, of which 27 (50 %) were polymorphic. The polymorphic information content (PIC) ranged from 0.24–0.55, with a mean value of 0.43. The number of observed alleles (N_a) varied from 1.16–1.25, the effective number of alleles (N_e) from 1.12–1.20 and Shannon's information index (I) from 0.10–0.16. Nei's genetic differentiation index ($G_{ST} = 0.437$) indicated moderate genetic differentiation among populations, while analysis of molecular variance (AMOVA) revealed that 73 % of the total genetic variation was distributed within populations. Cluster analysis based on Jaccard's similarity coefficient separated the five populations into two major groups, which was further supported by the results of principal component analysis (PCA). The present findings provide baseline information on the genetic structure of *H. indicus* in Northeast India and demonstrate the effectiveness of ISSR markers in assessing genetic variation. These results are valuable for formulating conservation strategies, germplasm management and future improvement and domestication programmes of this important medicinal species.

Keywords: conservation genetics; genetic diversity; *Hemidesmus indicus*; inter-simple sequence repeat; Northeast India; population structure

Introduction

Hemidesmus indicus (L.) R.Br. also referred as Indian sarsaparilla or false sarsaparilla, is a species within the Apocynaceae family, colloquially also known as 'Anantamul' ('Anant' = eternal + 'Mul' = root) is primarily garnered for its fragrant resilient roots (1). The generic name *Hemidesmus* comes from the Latin word "Hemidesmos" meaning half bond, referring to its sub connate filaments that are joined at the base by pods and stamens. It has two varieties namely black variety, also called as Krishna Sariva and white variety which is called as Sariva (2). It is a twinning shrub, semi-erect or prostrate that grows under mesophytic to semi-dry conditions. The plant possesses laticiferous branches with purplish bark and woody roots and found all across India (3). Initially the plant was classified under the Asclepiadaceae family and later transferred to the Periplocaceae family based on pollination traits and finally added to the Apocynaceae family based on the phylogenetic classification (4).

Traditionally *H. indicus* has been widely employed to treat a range of human ailments that comprises rheumatism, sore mouth,

skin disorders, headache, urinary tract infections and certain sexually transmitted infections such as syphilis and gonorrhoea (5). In addition, *H. indicus* have been reportedly been used in the manufacturing of cosmetics and related herbal goods (6). The root is particularly valued and significantly used in conventional medical practice (Ayurveda and Unani) as antipyretic, anti-diarrheal and as a blood purifier. It is also suggested for the treatment of skin diseases, fever, bronchitis, asthma, kidney and urinary disorders and rheumatism (7). Considering its significant medicinal and industrial relevance, continuous exploitation coupled with habitat disturbance may pose a threat to the genetic resources of this species. In this regard, the assessment of genetic diversity is essential for elucidating patterns of genetic variation within and among populations, identifying genetically distinct or vulnerable populations and providing a scientific basis for the development of effective conservation, management and future breeding strategies. Formulating an effective breeding and conservation studies demands a crucial insight of the genomic makeup of plant genetic resources (8).

Molecular markers are among the most widely used tools for studying genetic variation (9). Polymerase chain reaction (PCR) technique based on inter-simple sequence repeats (ISSR), is one of the molecular marker techniques that has been extensively employed in studies of population genetics of many plant species, covering a number of medicinal herbs and different crops. The ISSR markers were selected for the current study owing to their several advantages, particularly for non-model medicinal species such as *H. indicus*, where genomic information remains limited. In contrast to simple sequence repeat (SSR) markers, ISSRs do not require prior sequence data for primer development, making them relatively simple, cost-effective and less labour-intensive. Compared with high-resolution systems such as single nucleotide polymorphism (SNP markers), ISSRs provide a rapid and economical tool for preliminary assessment of population genetic diversity, especially when a large number of accessions are involved (10–13). In addition, ISSR stands out for its high reliability, attributed to its high annealing temperatures of its primers and the generation of longer sequence DNA fragments, thereby facilitating precise molecular investigations (14). Applications of ISSR have produced useful data, in studies involving interrelated as well as distantly related species in evolutionary biology, genome mapping, genetic diversity assessment and phylogenetic investigations (15). Previous studies have suggested that ISSR may be able to generate more consistent and repeatable bands due to its high annealing temperature, longer primer sequence and do not necessitate prior knowledge of target sequences contrary to SSR markers (16). In this work, the genetic diversity of *H. indicus* populations from Northeast India was investigated using ISSR markers. To the best of our knowledge, this is the first report on genetic diversity of *H. indicus* populations from this region, with an aim to offer insights, crucial for enhancing conservation management strategies.

Materials and Methods

Collection of plant samples

For the present study, samples of *Hemidesmus indicus* plants were collected from different parts of north-eastern states of India (Fig. 1 and 2). The collected accessions were maintained under greenhouse conditions mainly for *ex situ* conservation and propagation. Young leaf samples used for DNA isolation were obtained from these preserved accessions, where greenhouse maintenance served only to sustain healthy and uniform plant material for analysis, without affecting the inherent genetic composition of the samples. Details of the collected samples are listed in Table 1.



Fig. 1. *Hemidesmus indicus*.

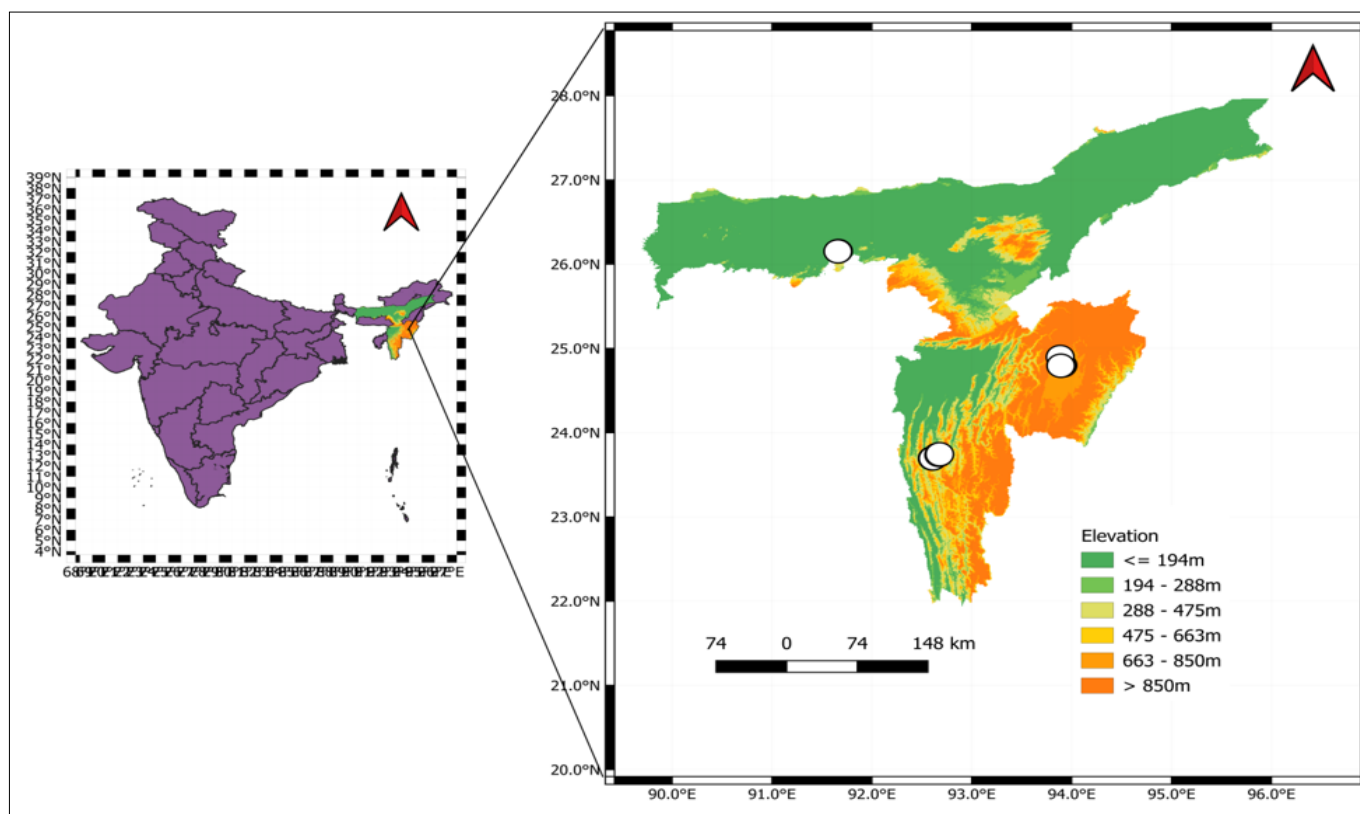


Fig. 2. Map of *Hemidesmus indicus* accession collection sites in Northeast India.

Source: QGIS version. 4.0.0.

Table 1. Details of samples collected from northeastern region used in the study

Sl. No	Sample ID	Location	State	Latitude	Longitude	Elevation (m)
1	TRS-M1	Mizoram University	Mizoram	23°44'13.4" N	92°39'33.6" E	762
2	TRS-M2	Mizoram University	Mizoram	23°44'11.3" N	92°39'53.3" E	818
3	TRS-M3	Mizoram University	Mizoram	23°44'02.3" N	92°39'34.9" E	807
4	TRS-M4	Mizoram University	Mizoram	23°44'02.0" N	92°39'31.7" E	798
5	TRS-M5	Mizoram University	Mizoram	23°44'16.9" N	92°40'02.7" E	868
6	TRS-R1	Reiek	Mizoram	23°41'43.5" N	92°36'20.2" E	1208
7	TRS-R2	Reiek	Mizoram	23°41'44.0" N	92°36'19.2" E	1203
8	TRS-R3	Reiek	Mizoram	23°41'43.1" N	92°36'18.8" E	1203
9	TRS-R4	Reiek	Mizoram	23°41'39.3" N	92°36'23.8" E	1236
10	TRS-R5	Reiek	Mizoram	23°41'42.5" N	92°36'38.4" E	1133
11	TRS-MZ1	Tanhrlil	Mizoram	23°44'14.3" N	92°40'13.6" E	926
12	TRS-MZ2	Tanhrlil	Mizoram	23°44'15.7" N	92°40'20.4" E	961
13	TRS-MZ3	Tanhrlil	Mizoram	23°44'37.6" N	92°40'44.4" E	952
14	TRS-IM1	Imphal west	Manipur	24°47'58.8" N	93°54'44.4" E	803
15	TRS-IM2	Imphal west	Manipur	24°47'56.9" N	93°54'31.9" E	801
16	TRS-IM3	Imphal west	Manipur	24°53'54.9" N	93°53'09.1" E	847
17	TRS-IM4	Imphal west	Manipur	24°47'43.2" N	93°53'30.3" E	830
18	TRS-G1	Guwahati	Assam	26°09'20.2" N	91°39'53.4" E	75
19	TRS-G2	Guwahati	Assam	26°09'10.4" N	91°39'38.0" E	82
20	TRS-G3	Guwahati	Assam	26°09'09.0" N	91°39'37.4" E	85

DNA extraction and PCR amplification

Young leaf tissues of *H. indicus* were subjected to DNA isolation using the optimised cetyltrimethylammonium bromide (CTAB) method (17). The quality of the extracted DNA was verified by electrophoresis on 1 % agarose gel. DNA quantity was ascertained using the Multiskan GO microplate spectrophotometer (thermo scientific) and modified to formulate a stock of 50 ng with TE buffer for further analysis.

A total of 27 University of British Columbia (UBC) ISSR primers were employed in the study. The PCR amplification was performed using a thermocycler (Bio Rad, C1000™) with a 50 µL reaction mixture comprising 10x buffer, 10–50 mg of genomic DNA, 0.2 mM deoxyribonucleotide triphosphate (dNTP (Himedia, India)), 1.5 mM magnesium chloride (MgCl₂), 0.5 µM of the primers and 1 U taq polymerase (Sigma-Aldrich Pvt. Ltd, Bangalore). The reaction protocol began with a preliminary denaturation step of 5 min at 94 °C, followed by 35 cycles consisting of 45 sec at 94 °C, 45 sec at 40–55 °C and 1 min at 72 °C, concluding with an extended synthesis stage of 7 min at 72 °C. Details of the primer sequences and their respective annealing temperatures are provided in Supplementary Table 1. The generated amplicons were subsequently loaded onto a 2 % agarose gel and visualised on a gel documentation system (BIO-PRINT, Vilber). The estimation of the observed band length was achieved with reference to a 1 kb ladder (Invitrogen, USA).

Data analysis

Analysis of the binary data matrix was done with BioVision software (Vilber) and only distinct, well-defined bands were scored. These markers were then subsequently encoded as 1, indicating presence and 0 indicating absence. Polymorphism information content (PIC) values were estimated from the binary matrix using the formula:

$$PIC_i = 2fi(1 - fi)$$

Where,

PIC_i is polymorphic information content of marker *i*,

f_i is the frequency of present fragments and

(1 – *f_i*) is the frequency of absent fragments (18).

Additionally, marker index (MI) was determined as the product of PIC and effective multiple ratios (EMR), where EMR is expressed $\beta \times n$, where β represents the proportion of polymorphic loci and *n* denotes the number of polymorphic loci.

Five parameters were calculated to estimate genetic diversity, including number of alleles (Na), effective alleles (Ne), Nei's genetic diversity (H) and Shannon's information index (I) by using GenAlex v6.5 (19). POPGENE version 1.32 was used to estimate the gene flow (Nm) and coefficient of gene differentiation (Gst) (20). Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were also estimated using GenAlex v6.5. A dendrogram was generated to illustrate the genetic similarity derived using Jaccard's coefficient from the binary data and clustered by the unweighted pair group method with arithmetic mean (UPGMA) using NTSYS-pc version 2.02 software (21).

Genetic structure of the populations was analysed in STRUCTURE (version 2.3.4) under the admixture model. Analyses were performed with 10000 burn-in steps and 100000 Markov chain Monte Carlo (MCMC) iterations across 10 replicate runs, with K values ranging from 2–7. The delta K (K) method for estimating the optimal population, utilising STRUCTURE SELECTOR was used to determine the optimal value of K (22).

Results

Among the 27 ISSR primers utilised to amplify DNA fragments across 20 accessions of *H. indicus*, 12 primers produced visible, reproducible, high quality bands and were therefore deemed as suitable (Fig. 3). The 12 ISSR primers resulted in the amplification of 54 distinct bands across the accessions examined. The lowest number of bands was recorded for primer UBC867 while UBC896 showed the highest number of bands (Table 2). Binary data of the PCR products generated with the UBC-ISSR primers by the different samples are provided in Supplementary Table 2. Gel images of the PCR amplifications obtained are also provided in Supplementary Fig. 1. The percentage of polymorphic bands (PPB) ranged from 25–75 % with an average of 51.34 %. The PIC score of the studied primers extended from 0.24 (UBC876)–0.55 (UBC897) recording an average of 0.43 (Table 2). The MI of the primers used in the study ranged from 0.06 (UBC866)–1.23 (UBC897) with an average of 0.57.

Among the *H. indicus* samples studied, Na varied from 1.25 (UBC866)–1.75 (UBC873) with a mean of 1.513, Ne varied from 1.056 (UBC876)–1.53 (UBC873) with a mean value of 1.286 (Table 3). Shannon information index (I) recorded between 0.09–0.446 with a mean of 0.107, H varied between 0.047–0.305 with an overall mean of 0.076. The highest degree of genetic diversity (H = 0.305, I = 0.446)

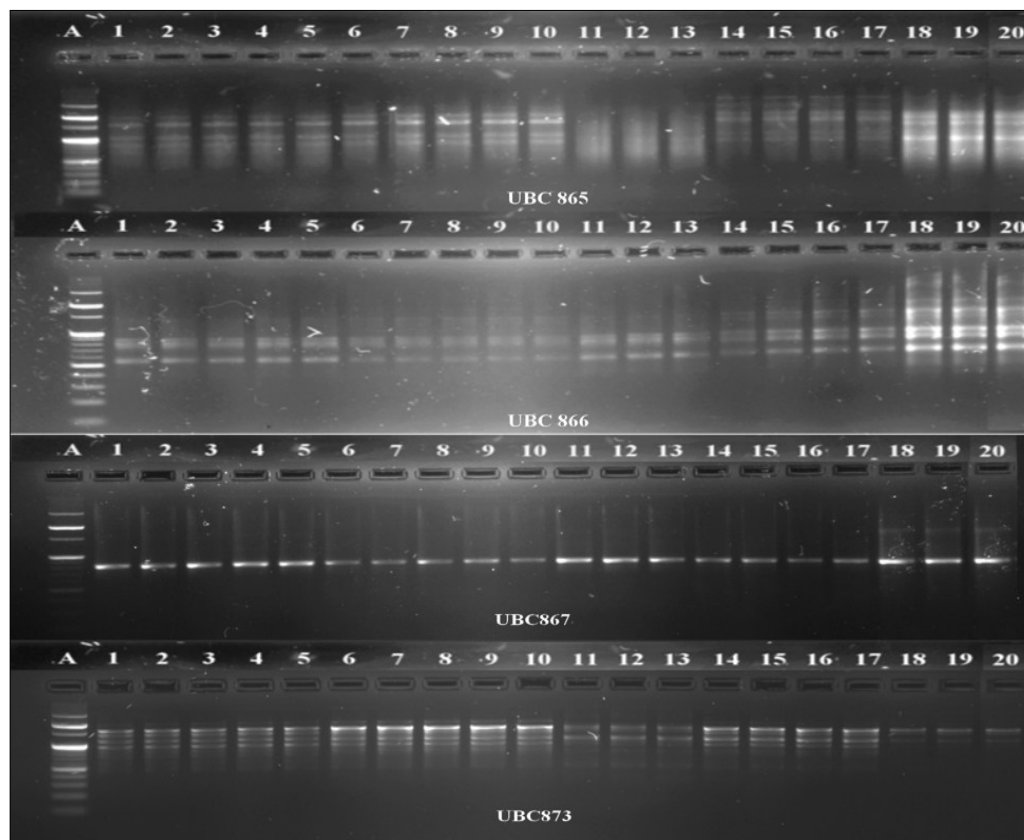


Fig. 3. PCR amplification profiles for (UBC865, UBC866, UBC867 and UBC873) of 20 *Hemidesmus indicus* accessions.

A: 100 kb ladder; 1: TRS-M1; 2: TRS-M2; 3: TRS-M3; 4: TRS-M4; 5: TRS-MR5; 6: TRS-R1; 7: TRS-R2; 8: TRS-R3; 9: TRS-R4; 10: TRS-R5; 11: TRS-MZ1; 12: TRS-MZ2; 13: TRS-MZ3; 14: TRS-IM1; 15: TRS-IM2; 16: TRS-IM3; 17: TRS-IM4; 18: TRS-G1; 19: TRS-G2; 20: TRS-G3.

Table 2. Inter simple sequence repeat (ISSR) primer used and their respective markers performance indexes

Primer	Primer sequence	Annealing temperature (°C)	TNB	NMB	NPB	PPB	EMR	PIC	MI
UBC864	ATGATGATGATGATGATG	49	4	2	2	50	1	0.36	0.36
UBC865	CCGCCGCCGCCGCCGCCG	48	5	3	2	40	0.8	0.46	0.36
UBC866	CTCCTCCTCCTCCTCCTC	48	4	3	1	25	0.25	0.26	0.06
UBC867	GGCGGCGCGCGCGCGGC	49	2	1	1	50	0.5	0.35	0.17
UBC868	GAAGAAGAAGAAGAAGAA	50	4	2	2	50	1	0.53	0.53
UBC873	GACAGACAGACAGACA	50	4	1	3	75	2.25	0.36	0.82
UBC876	GATAGATAGACAGACA	50	6	4	2	33.33	0.66	0.24	0.16
UBC881	GGGTGGGGTGGGGTG	53	4	1	3	75	2.25	0.52	1.18
UBC885	BHBGAGAGAGAGAGAGA	49	5	3	2	40	0.8	0.53	0.42
UBC895	AGAGTTGGTAGCTCTTGATC	55	5	2	3	60	1.8	0.49	0.89
UBC896	AGGTCGCGGCCGCNNNNNNATG	55	7	4	3	42.86	1.28	0.49	0.63
UBC897	CCGACTCGAGNNNNNNATGTGG	55	4	1	3	75	2.25	0.55	1.23
Average			54	27	27	51.34	1.236	0.43	0.57

TNB: total number of bands; NMB: number of monomorphic bands; NPB: number of polymorphic bands; PIC: polymorphism information content; MI: marker index.

Table 3. Genetic diversity within *Hemidesmus indicus* accessions as revealed by inter simple sequence repeat (ISSR) markers

Primer	Na	Ne	H	I
UBC864	1.5	1.253	0.164	0.253
UBC865	1.4	1.170	0.114	0.182
UBC866	1.25	1.169	0.100	0.148
UBC867	1.5	1.417	0.227	0.323
UBC868	1.5	1.221	0.153	0.242
UBC873	1.75	1.530	0.305	0.446
UBC876	1.333	1.056	0.047	0.090
UBC881	1.75	1.284	0.194	0.314
UBC885	1.4	1.375	0.193	0.270
UBC895	1.6	1.342	0.196	0.295
UBC896	1.428	1.148	0.094	0.154
UBC897	1.75	1.464	0.278	0.415
Average	1.513	1.286	0.172	0.261
Std deviation	0.168	0.142	0.076	0.107

Na: observed number of alleles; Ne: effective number of alleles; H: Nei's genetic diversity; I: Shannon's information index.

was recorded in primer UBC873 followed by UBC897 ($H = 0.278$, $I = 0.415$) (Table 3). A significant degree of genetic differentiation was detected in the studied samples of *H. indicus* (Table 4). The assessment of genetic variation using ISSR primers showed H_t ranging from 0.0443 (UBC876)–0.2791 (UBC873) with an average of 0.1714. The total gene diversity within accessions (H_s) varied from 0.0382 (UBC866)–0.1775 (UBC873) with a mean of 0.0922. The coefficient of genetic differentiation (G_{st}) varied from 0.1274 (UBC868)–1.0 (UBC867) with an average 0.4372. The level of N_m varied from 0.2955 (UBC866)–3.4247 (UBC868) with mean of 0.6436. Banding patterns of the studied *H. indicus* populations are shown in Fig. 4 with total amplified bands detected in each population, reflecting the overall genetic diversity present. In R and IM, the heterozygosity trend peaks, while reduced genetic variation or lower heterozygosity was observed in G (Fig. 4).

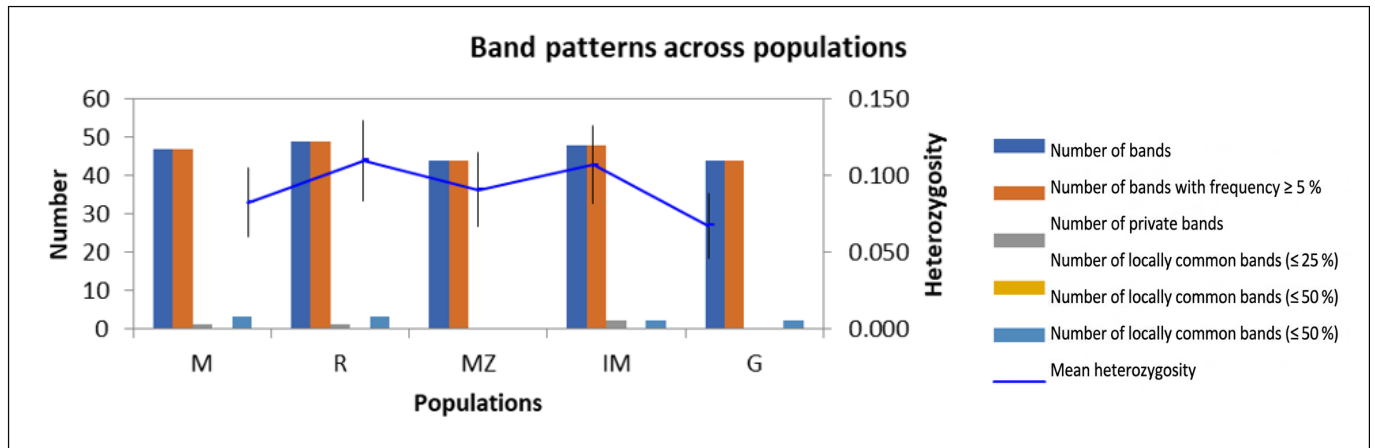


Fig. 4. Band patterns of the inter simple sequence repeat (ISSR) markers across *Hemidesmus indicus* populations.

Table 4. Genetic differentiation as shown by inter simple sequence repeat (ISSR) markers in *Hemidesmus indicus* accessions

Primer	H _t	H _s	Gst	N _m
UBC864	0.1509	0.0826	0.4528	0.6041
UBC865	0.1079	0.0699	0.3529	0.9168
UBC866	0.1029	0.0382	0.6285	0.2955
UBC867	0.24	0	1	0
UBC868	0.1588	0.1386	0.1274	3.4247
UBC873	0.2791	0.1775	0.3641	0.8732
UBC876	0.0443	0.0283	0.3617	0.8822
UBC881	0.2153	0.1077	0.4995	0.5009
UBC885	0.1883	0.1064	0.4347	0.6501
UBC895	0.2014	0.1179	0.4145	0.7063
UBC896	0.0949	0.0746	0.2137	1.8399
UBC897	0.2739	0.1652	0.3968	0.76
Average	0.1714	0.0922	0.4372	0.6436

H_t: total gene diversity; H_s: gene diversity within accessions; Gst: coefficient of gene differentiation; N_m: estimate of gene flow from Gst; N_m: 0.5(1 - Gst)/Gst.

The AMOVA analysis showed 73 % of the total genetic variation within populations and 27 % molecular variation among populations (Table 5). Relationships between the *H. indicus* accessions were additionally depicted by a dendrogram employing UPGMA algorithm as per Jaccard's index of similarity (Fig. 5). Generated dendrogram from the ISSR profiles separated the *H. indicus* accessions into two main clusters. Cluster I consisted of four

sub clusters comprising of *H. indicus* accessions exclusively from Mizoram. Cluster II consisted of two sub clusters with the grouping of *H. indicus* accessions from different states-Mizoram, Manipur and Assam. Overall, the dendrogram highlighted the genetic relationships across the accessions. Jaccard's similarity values for all the *H. indicus* accessions ranged from 0.680–0.954 (Table 6). Maximum similarity values were recorded between accessions M4 and M5 while the lowest was observed between M2 and IM1 (Table 6).

A spatial visualisation of the genetic diversity of the *H. indicus* accessions was assessed through PCoA (Fig. 6). The two principal components explained 34.74 % of the total variation, with the first accounting for 21.02 % and the second 13.72 %. The PCoA plot of clustering the accessions supports the result of dendrogram. It showed the congruency with two dimensional plot of the distribution of all *H. indicus* accessions.

Table 5. Analysis of molecular variance (AMOVA) for *Hemidesmus indicus* accessions

Source	df	SS	MS	Est. var.	Variance (%)
Among pops	4	34.550	8.638	1.294	27
Within pops	15	52.900	3.527	3.527	73
Total	19	87.450		4.821	100

df: degree of freedom; SS: sum of squares; MS: mean of squares; Est. var: estimated variation.

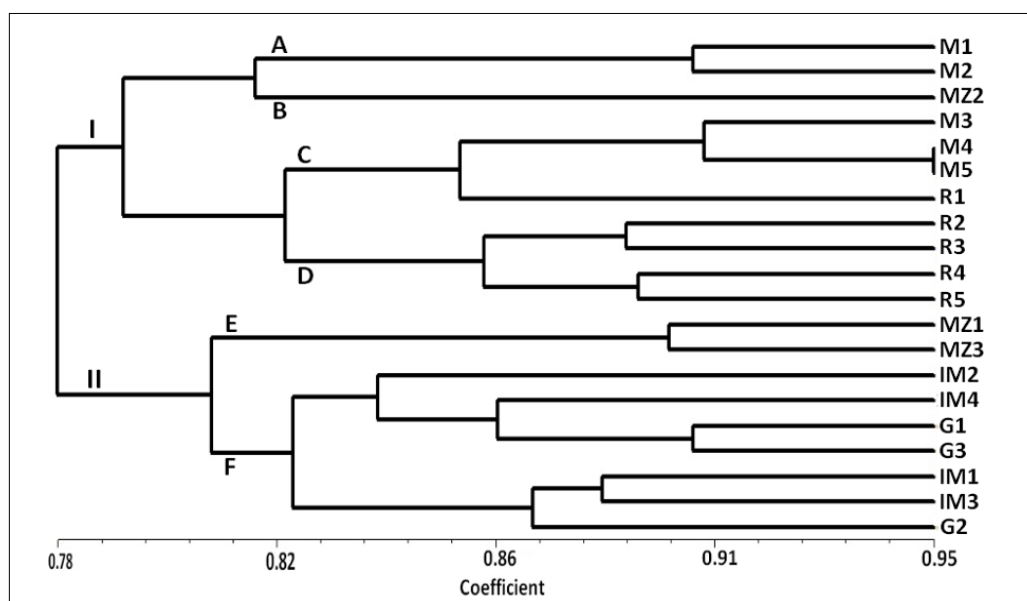
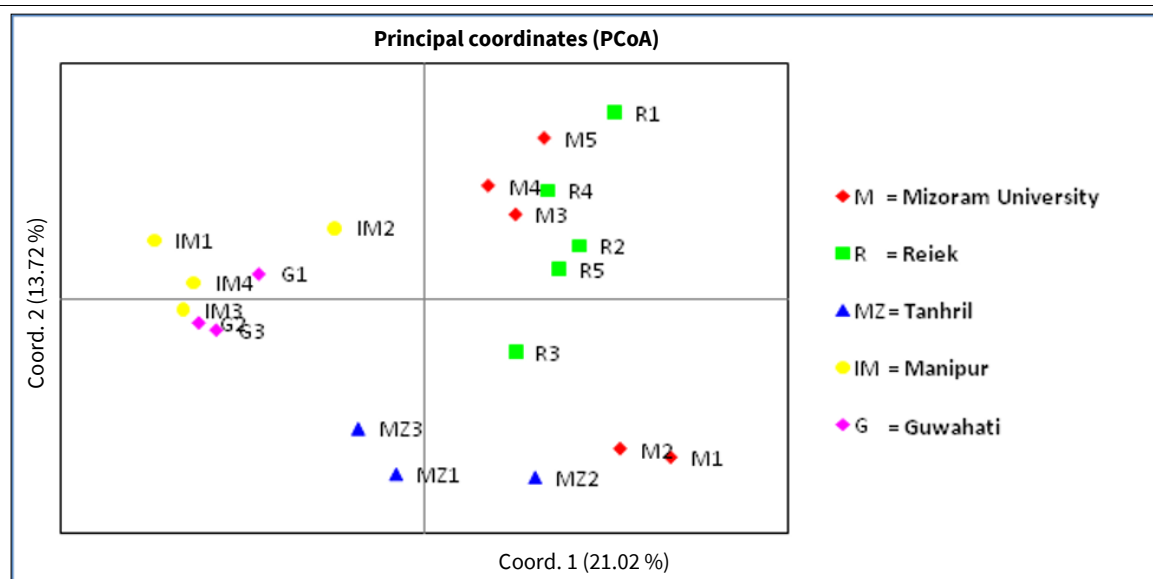


Fig. 5. Dendrogram showing the genetic clustering of 20 *Hemidesmus indicus* accessions generated from inter simple sequence repeat (ISSR) marker profiles.

Table 6. Similarity matrix of 20 accessions of *Hemidesmus indicus* as revealed by Jaccard's similarity method

	M1	M2	M3	M4	M5	R1	R2	R3	R4	R5	MZ1	MZ2	MZ3	IM1	IM2	IM3	IM4	G1	G2	G3
M1	1.000																			
M2	0.905	1.000																		
M3	0.841	0.837	1.000																	
M4	0.804	0.841	0.907	1.000																
M5	0.804	0.800	0.907	0.954	1.000															
R1	0.783	0.778	0.884	0.844	0.844	1.000														
R2	0.787	0.783	0.844	0.809	0.771	0.867	1.000													
R3	0.771	0.766	0.787	0.792	0.755	0.771	0.891	1.000												
R4	0.830	0.787	0.889	0.891	0.891	0.870	0.872	0.854	1.000											
R5	0.771	0.804	0.787	0.830	0.830	0.771	0.851	0.872	0.894	1.000										
MZ1	0.814	0.810	0.791	0.796	0.756	0.696	0.778	0.800	0.822	0.800	1.000									
MZ2	0.818	0.814	0.796	0.761	0.723	0.739	0.783	0.804	0.787	0.766	0.767	1.000								
MZ3	0.818	0.814	0.837	0.841	0.800	0.739	0.783	0.844	0.826	0.766	0.900	0.773	1.000							
IM1	0.680	0.708	0.804	0.809	0.771	0.714	0.755	0.740	0.796	0.776	0.778	0.708	0.783	1.000						
IM2	0.723	0.717	0.818	0.822	0.783	0.800	0.804	0.826	0.848	0.787	0.791	0.756	0.796	0.766	1.000					
IM3	0.723	0.756	0.818	0.822	0.783	0.723	0.766	0.750	0.809	0.787	0.833	0.717	0.837	0.886	0.778	1.000				
IM4	0.714	0.708	0.766	0.771	0.735	0.750	0.755	0.776	0.796	0.740	0.739	0.745	0.822	0.830	0.844	0.804	1.000			
G1	0.739	0.773	0.837	0.841	0.800	0.778	0.822	0.804	0.826	0.766	0.810	0.733	0.857	0.822	0.837	0.881	0.864	1.000		
G2	0.702	0.733	0.796	0.800	0.761	0.739	0.745	0.766	0.787	0.766	0.810	0.773	0.814	0.864	0.837	0.881	0.783	0.814	1.000	
G3	0.783	0.778	0.841	0.844	0.804	0.745	0.750	0.771	0.792	0.735	0.773	0.778	0.861	0.826	0.841	0.884	0.867	0.905	0.861	1.000

**Fig. 6.** Two-dimensional representations of the first two axes of the principal coordinate analysis (PCoA) from the matrix of genetic distances of 20 in *Hemidesmus indicus* accessions.

Bayesian analysis of ISSR profiles, carried out in STRUCTURE using the admixture model with delta K approach of Evanno method, showed a prominent peak at $K = 4$ (Fig. 7) indicating the classification of the 20 *H. indicus* accessions into four groups as shown in the bar plot (Fig. 8). STRUCTURE generated a Q-matrix (QST), listing the estimated membership coefficients for each accession for every value of K (Table 7). Accessions were assigned to a certain K subgroup with a maximum value ≥ 0.85 to specific subgroup while those with lower values were classified as admixed (23).

Discussion

With the rapid exploitation coupled with declining population of *H. indicus*, comprehensive knowledge of the species genetic diversity is pivotal for its sustainable use, management and conservation plans. Assessment of the genetic variability in the local population is fundamental for establishing effective breeding approach to the development of new varieties (24). The present work focused on understanding the genetic diversity among 20 *H. indicus* accessions, sampled from the north-eastern states-Assam, Manipur and Mizoram of India. The present investigation revealed the polymorphic variation for *H. indicus* was high as compared to the results from 12 *H. indicus* samples using random

amplified polymorphic DNA (RAPD) markers (7). Compared to other dominant markers like RAPD, which anneals within the temperature range of 34–37 °C, ISSR markers exhibit a wider range of annealing temperatures, between 40–55 °C (25, 26). The findings of this study regarding annealing temperature align with previous reports on *Calotropis procera* (27). Primers with PIC values exceeding 0.5 are considered highly indicative in terms of polymorphic information content, while values ranging from 0.25–0.5 are moderate and those below 0.25 are deemed poorly informative (28). Consequently, the primers assessed in the study were considered moderately to highly informative with PIC values varying from 0.24–0.55. The moderate to high PIC values observed, suggest that the ISSR primers employed in the present study were sufficiently informative for detecting genetic variation among the accessions. Likewise, the corresponding MI values further reflected the efficiency of these primers in resolving polymorphism, with primers such as UBC897 exhibiting comparatively greater discriminatory capacity.

Understanding the diversity of vulnerable species is vital for formulating effective conservation strategies. Polymorphism can be assessed through various metrics such as I , H , average heterozygosity (H_s), total heterozygosity (H_t), N_m and G_{st} (29). The primer UBC873 exhibited the maximum genetic diversity ($H = 0.305$, $I = 0.446$), closely followed by UBC897 ($H = 0.278$, $I = 0.415$), indicating

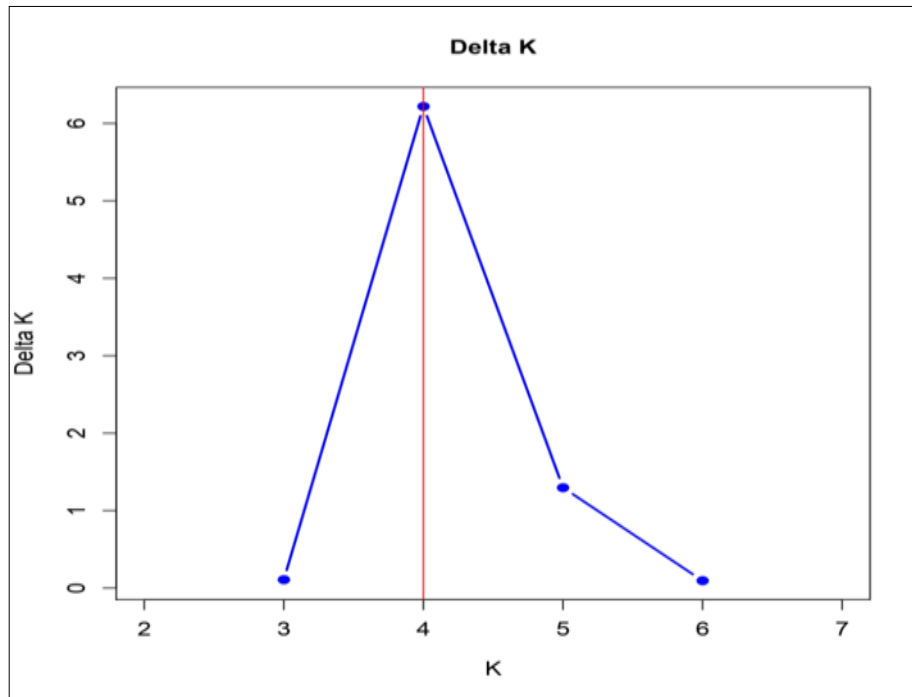


Fig. 7. Graphical representation population structure based on ISSR profile. The bayesian assignment analysis depiction of the maximum peak value (delta K) at 4.

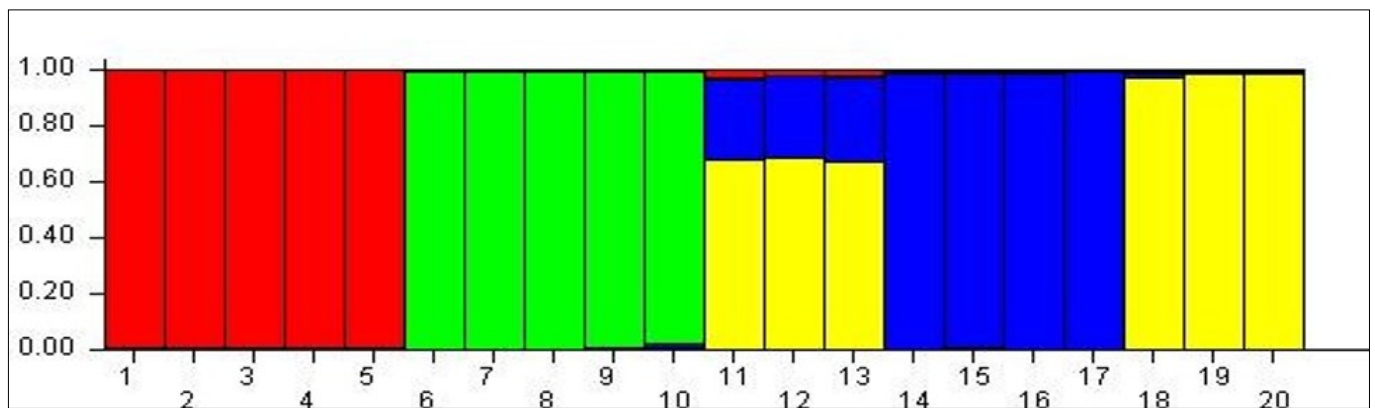


Fig. 8. Bar plot representation of genetic cluster for 20 *Hemidesmus indicus* accessions.

Table 7. Population structure group of genotypes based on inferred ancestry values

Sl. No.	Sample ID	Inferred ancestry				Structure group
		Q1	Q2	Q3	Q4	
1	TRS-M1	0.991	0.003	0.003	0.003	SG1
2	TRS-M2	0.991	0.004	0.003	0.003	SG1
3	TRS-M3	0.991	0.004	0.002	0.002	SG1
4	TRS-M4	0.991	0.004	0.002	0.002	SG1
5	TRS-M5	0.990	0.005	0.003	0.002	SG1
6	TRS-R1	0.004	0.989	0.003	0.003	SG2
7	TRS-R2	0.004	0.990	0.003	0.003	SG2
8	TRS-R3	0.006	0.989	0.003	0.002	SG2
9	TRS-R4	0.004	0.988	0.005	0.003	SG2
10	TRS-R5	0.004	0.968	0.020	0.008	SG2
11	TRS-MZ1	0.022	0.007	0.286	0.684	AD
12	TRS-MZ2	0.015	0.005	0.288	0.692	AD
13	TRS-MZ3	0.017	0.006	0.300	0.678	AD
14	TRS-IM1	0.003	0.006	0.987	0.004	SG3
15	TRS-IM2	0.004	0.006	0.981	0.009	SG3
16	TRS-IM3	0.003	0.005	0.986	0.006	SG3
17	TRS-IM4	0.003	0.004	0.989	0.005	SG3
18	TRS-G1	0.004	0.005	0.014	0.977	SG4
19	TRS-G2	0.003	0.003	0.007	0.988	SG4
20	TRS-G3	0.002	0.004	0.004	0.990	SG4

appreciable levels of polymorphism among the studied accessions. Rather than representing absolute measures on a diversity, H and I values are interpreted comparatively and are influenced by species biology, population structure and the marker systems employed (30). The diversity estimates obtained in this study were comparable with those reported for other medicinal and wild plant species analysed using ISSR markers, including *Myracrodruon urundeuva* ($H = 0.270$; $I = 0.420$) and *Copernicia prunifera* ($H = 0.327$; $I = 0.470$), indicating a moderate level of genetic diversity in *H. indicus* (31, 32). The observed level of diversity may be associated with the species' reproductive behaviour, ecological adaptations, as well as the effects of habitat fragmentation and anthropogenic pressures, all of which are recognised as important factors influencing genetic structuring in medicinal plants (33).

The assessment of genetic variation using ISSR primers showed a level of N_m with a mean value of 0.6436 and G_{st} mean of 0.4372. The G_{st} values are designated as low (< 0.05), intermediate (0.05–0.15) and high (> 0.15), thereby allowing differentiation in the extent of genetic structuring among the populations (34). Additionally, the threshold value at which a considerable amount of population differentiation occurs is represented by the value of N_m less than 1, indicating less than one migrant into a population per generation (35). It is also suggested that genetic drift could reduce the genetic diversity maintained in the population where N_m is found to be less than one (36). The combination of a high G_{st} value of 0.4372 coupled with the low N_m value (0.6436) reflects pronounced genetic differentiation among the *H. indicus* accessions which is consistent with previous reports (34). The observed low values of N_m could possibly suggest genetic drift within the population thereby promoting the genetic variations in the *H. indicus* accessions studied (37). The restricted gene flow implied by the low N_m value may enhance the effects of genetic drift, thereby promoting local differentiation among populations. In medicinal plants, such patterns are often associated with fragmented population distribution, geographic isolation, restricted pollen and seed dispersal and species-specific reproductive constraints. Furthermore, anthropogenic pressures such as overexploitation for medicinal use, habitat degradation and land-use changes may further reduce population connectivity and contribute to increased genetic structuring. These factors collectively may explain the observed differentiation among *H. indicus* accessions and the observed variation in diversity values may also be attributed to differences in sample size and the choice of primers (38). Similarly, ISSR primers have also shown efficacy in assessing genetic diversity, in studies involving forest species of the Caatinga region (30). Jaccard's similarity coefficient values were found to range from 0.78–0.95. A similar outcome was reported in *Swertia chirayita*, with Jaccard's coefficient varying from 0.68–0.97 (39).

The band pattern analysis shows expected heterozygosity, a measure of genetic diversity based on allele frequencies. The comparatively higher heterozygosity of populations R and IM suggests more genetic variation and potentially higher evolutionary resilience. Lower heterozygosity in populations like G suggests reduced variation, which could be due to bottlenecks or inbreeding (40). The similar total band counts but varying heterozygosity indicates, that allele distribution patterns vary among populations despite the overall genetic richness being similar. The low private band count points that most genetic variation is shared across populations, possibly due to historical gene flow (41).

The AMOVA results revealed that 73 % of the overall variance was attributed within the population and 27 % distributed among populations from ISSR profiles which indicates that genetic differentiation has occurred among the *H. indicus* accessions (42). These results correspond to the findings on genetic diversity and population structure (43).

According to the STRUCTURE analysis, most accessions exhibited high membership coefficients ($Q > 0.98$) for a single genetic cluster, suggesting distinct genetic differentiation and limited admixture. Accessions from SG1, SG2, SG3 and SG4 can be considered genetically pure representatives of their respective clusters based on the generally used threshold ($Q \geq 0.80$ –0.85) for confident assignment (44, 45). Given their high Q -values, these accessions reflect unique genetic lineages with minimal contemporary gene flow. In contrast, the coefficients of TRS-MZ1, TRS-MZ2 and TRS-MZ3 were intermediate (0.68–0.69 in Q4 and 0.28–0.30 in Q3), indicating substantial admixture and falling below the threshold for pure assignment. Such admixture could be the result of ongoing gene flow or historical hybridisation (46).

Population structure studies have shown similar trends with, genetically diverse but geographically close populations exchanging alleles over time (22). These results point to a well-structured population with distinct genetic groups, interrupted by a small number of admixed individuals, most likely representing transitional genetic backgrounds. Such admixture patterns are pivotal for directing germplasm conservation, understanding evolutionary processes and planning in breeding strategies (47). Based on the genetic structure analysis, genetically distinct populations should be regarded as priority targets for *in situ* conservation to maintain unique gene pools and preserve evolutionary potential. Conversely, admixed accessions, which may represent regions of genetic exchange, could be considered suitable candidates for *ex situ* conservation through germplasm repositories or field gene banks (11–13). Conserving these accessions outside their natural habitats can help to capture a broader spectrum of genetic variability. Such integrated conservation strategies will not only safeguard valuable genetic resources but also support future breeding, restoration and sustainable utilisation programmes.

Genetic diversity within medicinal plants increases the likelihood of particular genotypes with features that provide tolerance or resistance under various environmental conditions, allowing populations to endure biotic and abiotic stress. The loss of this diversity could make a species more susceptible to diseases, pests and climate extremes, which would lower its ecological resilience and jeopardise the long-term supply of bioactive chemicals needed for medicinal and pharmaceutical uses (48, 49). The application of ISSR markers as a robust approach for assessing genetic relatedness among *H. indicus* accessions could offer reliable, environment-independent data to guide selection, crossing tactics and germplasm management for breeders and conservationists. Although no direct studies have been conducted on *H. indicus*, investigations on other endemic and medicinal plants highlight the general utility of ISSR markers and show their effectiveness in assessing diversity (13). Consequently, employing ISSR analysis on *H. indicus* would improve knowledge of its genetic variation, facilitating breeding programs with diverse germplasm and eventually aiding in conservation and adaptive improvement for future challenges.

Conclusion

Hemidesmus indicus is regarded as a very valuable medicinal herb. To the best of our understanding, this study is the first documented effort to assess the genetic diversity of *H. indicus* from Northeast India. This study underscores the feasibility of ISSR markers for delineating genetic diversity within and among *H. indicus* accessions and generates baseline insights into the genetic diversity and population structure of the species. These insights may provide useful information for conservation planning, germplasm management and sustainable utilisation of this medicinally important species. Future investigations employing different ISSR primers and encompassing diverse *H. indicus* accessions could aid in the identification of valuable plant specimens. Additionally, comparative analyses involving various molecular and morphological markers would be beneficial. Considering the medicinal importance of the species, conservation efforts are imperative to safeguard its gene pool against overexploitation and population decline. The present study will be helpful in the identification and collection of *H. indicus* individuals for future improvement and preservation programmes.

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Authors' contributions

PST contributed to sample collection, PCR amplification, statistical analysis and manuscript preparation. BA assisted with the statistical analysis. RS, ST and DSL contributed to the discussion for the enrichment of the manuscript. LH and RT supervised the study, designed the experiments and improved and finalised the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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