PLANT SCIENCE TODAY
Vol 12(sp3): 01–08
https://doi.org/10.14719/pst.9657

eISSN 2348-1900

RESEARCH ARTICLE

Unlocking the genetic diversity of Brahmi (*Bacopa monnieri* L.) genotypes: Insights into morphological, biochemical and molecular traits

Koncha Mounika[1], Sreenivasa V[1], K M Yuvaraj[2], M R Rohini[1], Karthik C S[1], Divya K S[1], V K Rao[3], K V Ravisankar[3], Amaresh[4] & K Hima Bindu[1]*

[1]Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bangalore 560 089, India
[2]College of Horticulture, Anantharajupeta, Dr. Yeduguri Sandinti Rajasekhara Reddy Horticultural University, Annamayya District 516 105, Andhra Pradesh, India
[3]Division of Basic Sciences, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bangalore 560 089, India
[4]ICAR-Sugarcane Breeding Institute, Coimbatore 641 007, Tamil Nadu, India

*Correspondence email - kaipa.himabindu@gmail.com

Received: 27 May 2025; Accepted: 27 August 2025; Available online: Version 1.0: 08 December 2025

Cite this article: Koncha M, Sreenivasa V, Yuvaraj KM, Rohini MR, Karthik CS, Divya KS, Rao VK, Ravisankar KV, Amaresh, Hima BK. Unlocking the genetic diversity of Brahmi (*Bacopa monnieri* L.) genotypes: Insights into morphological, biochemical and molecular traits. Plant Science Today. 2025;12 (sp3):01–08. https://doi.org/10.14719/pst.9657

Abstract

Brahmi (*Bacopa monnieri* L.), a medicinal herb from the family Scrophulariaceae, thrives in humid climates, predominantly in the damp and marshy areas of the subtropical Indian subcontinent. A study was conducted to evaluate the morphological, biochemical and molecular diversity among 50 germplasm accessions of Brahmi, along with two local checks maintained at ICAR-Indian Institute of Horticultural Research, Bengaluru. Analysis of variance revealed significant variations in morphological and biochemical traits. High estimates of phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance were noted for traits like flower diameter, fresh and dry herbage yield and the concentrations of Bacoside A and its components (Bacoside A3, Bacopaside II, Bacopasaponin C and Jujubogenin). The genetic divergence analysis, based on morphological characters, categorized the accessions into five clusters, with the largest inter-cluster distance observed between clusters IV and V (1421.738). Cluster III contained accessions with high herbage yield, including JO26/32, IIHR BM-3, IC 554587, IC 554588 and IC 324774. In terms of total Bacoside A content and its components, the accessions were also divided into five clusters, with the maximum inter-cluster distance between clusters III and IV (2.272). A wide variation in triterpenoid saponins was noted, ranging from 1.13 % to 3.49 %, with the highest Bacoside A content found in IC 554588 followed by IIHR BM-30 (3.40 %). Cluster III predominantly included genotypes with high total Bacoside A content, such as IC 554588, IIHR BM-30 and IIHR BM-21. Molecular characterization using 15 RAPD markers showed that 11 primers exhibited 100 % polymorphism, with PIC values ranging from 0.71 to 0.97 and a mean of 0.92. Dendrograms generated from morphological, biochemical and molecular diversity analyses showed close clustering of genotypes. Genotypes with high herbage yield (IIHR BM 23, JO26/32) and high Bacoside A content (IC 554588, IIHR BM 30) hold potential for future breeding programs. The genetically diverse accessions, IC 426447 and IIHR BM-23, are promising candidates for heterosis breeding to improve the Brahmi crop. This study highlights the extensive variation among Brahmi genotypes in morphological, biochemical and molecular traits, offering significant potential for future crop improvement initiatives.

Keywords: bacosides; biochemical; brahmi; cluster analysis; diversity; molecular; morphological

Introduction

Bacopa monnieri (Linn.) belonging to the family Scrophulariaceae is commonly known as Brahmi. It is a highly valuable medicinal creeper found growing in humid and warmer parts of the world. The genus *Bacopa* comprises about 60 species of aquatic plants distributed throughout the tropical and subtropical regions of the world, most of which are native to America. *B. monnierioides*, *B. paraguariensis*, *B. congesta*, *B. dubia*, *B. hassleriana*, *B. pedersenii*, *B.ranaria*, *B. simulans*, *B. monniera* and *B. floribunda* are some of the important species of the genus. *B. monnieri* (Linn.) Pennell and *B. floribunda* (R.Br.) Wettstein are the two species distributed in India (1, 2).

It is valued as re-energizing herb used by ayurvedic practitioners nearly 3000 years ago (3). The whole plant is used in the indigenous system of medicine as a nerve tonic and for treating epilepsy and insanity (4). The herb is known to improve memory cells and thus is known as “memory booster” or “thinking person’s herb”. In India, Brahmi is used to prepare important ayurvedic preparations like “Brahmighritam” and “Brahmirasayanam” (5). In Siddha system of medicine, the plant is used against painful joints, swelling in joints, peripheral neuritis, constipation and burning urination. *B. monnieri* is classified under Medhya Rasayana and is described in ancient ayurvedic medical encyclopedias, namely, Charaka Samhita, Sushruta Samhita and Astanga Hridaya, as cure for mental disorders and loss of intellect and memory (6). Scientific research conducted previously on brahmi has shown its nootropic ability and role in improving learning and respiratory function (7).

The plant also exhibits many pharmacological activities like antioxidant, anti-inflammatory, antipyretic, antiulcer, cardioprotective, cooling, laxative and adsorbing effects (7). These therapeutic properties of the plant are attributed to the presence of different phytocompounds with saponins being the most important. Pharmacological activity is mainly due to the presence of alkaloids (brahmine and herpestine), sterols (stigmasterol and β-sitosterol), glycosides, flavonoids and characteristic saponins called "bacosides," which are complex mixtures of structurally similar compounds, glycosides of pseudojujubogenin or jujubogenin (8). These compounds play a significant role in neuroprotection by modulating of antioxidant enzymes (namely superoxide dismutase and catalase) in stressed neuronal cells.

Owing to its inherent potential to enhance memory and vitality, this miracle plant is gaining attention globally for its commercial cultivation. *B. monnieri* was placed second in the priority list of most important medicinal plant in a sector study of Export–Import bank of India (9), evaluated on the basis of their medicinal importance, commercial value and potential for further research and development.

The annual demand or trade of this crop has taken a quantum jump due to its potential use in the pharma industry and ayurvedic system of medicine to treat a variety of ailments. It has been identified among the seven important medicinal plants recommended for research and included in the list of highly endangered medicinal plants of India by the National Medicinal Plants Board (NMPB) and Technology Information, Forecasting & Assessment Council (TIFAC) New Delhi (1). The natural populations of *B. monnieri* are threatened with over exploitation leading to depletion of valuable genetic resources. Much of the significant work has been done on its medicinal importance and only limited work has been conducted to assess its genetic diversity using morphological and molecular markers. Hence, the present study was undertaken to assess the morphological, biochemical and molecular diversity of brahmi. This study is relevant as it will give the estimate of diversity present in this species, which can be utilized for further conservation and crop improvement programmes.

Materials and Methods

The present investigation was carried out at ICAR- Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru during the period from March to December 2021. The experiment was conducted in Augmented block design with 2 checks and 50 accessions as listed in Table 1. The experimental field is located at an altitude of 890 m above mean sea level and at 13° 7´ N latitude, 72° 29´ E longitudes respectively. All recommended practices were followed to raise healthy crop.

Analysis of morphological diversity

The technique of random sampling was adopted for selecting plants for recording the observations on various growth parameters of brahmi. Ten plants in each genotype leaving the border plants were randomly selected and tagged. Various morphological parameters were recorded and data from ten plants from each genotype were averaged and used for statistical analysis. The observations were recorded on number of primary branches per plant, fresh weight of leaf and stem (g), dry weight of leaf and stem (g), fresh herb yield (g per plot), dry herb yield (g per plot), leaf length (cm), leaf width (cm) and flower diameter (cm).

Analysis of biochemical diversity

Ultra-High-performance Liquid Chromatographic (UHPLC) analysis was carried out by following the procedure (Natural Remedies Pvt. Ltd., Bengaluru) with minor modifications to estimate Bacoside-A components viz. Bacisode-A3, Bacopaside-II, Jujubogenin and Bacopasaponin C in 50 genotypes of Brahmi.

Statistical analysis

The experiment was laid out in Augmented Randomized Block Design (10) where whole field was divided into five blocks, each consisting of 14 beds of measuring 1.0 x 1.5 m^2 area. The genotypic (GCV) and phenotypic (PCV) coefficient of variation were calculated (11). Heritability in broad sense [$h^2_{(b)}$] was estimated using another formula (12). From the heritability estimates, the genetic advance

Table 1. List of Brahmi genotypes used in the experimental study

Sl. No.	Designation	Genotypes	Place of collection	Sl. No.	Designation	Genotypes	Place of collection
1	Gen-7	IIHR BM-2	Moothakunnum, Kerala	27	Gen-35	IC 324774	NBPGR, Thrissur
2	Gen-34	IIHR BM-3	Kottuvalikodu, Kerala	28	Gen-42	IC 324777	NBPGR, Thrissur
3	Gen-9	IIHR BM-4	Satara island,Kerala	29	Gen-2	IC 343108	NBPGR, Thrissur
4	Gen-12	IIHR BM-5	Kasbara , West Bengal	30	Gen-19	IC 353203	NBPGR, Thrissur
5	Gen-26	IIHR BM-6	Jamatala, West Bengal	31	Gen-31	IC 353204	NBPGR, Thrissur
6	Gen-4	IIHR BM-8	Shyamnagar, West Bengal	32	Gen-48	IC 370640	NBPGR, Thrissur
7	Gen-13	IIHR BM-9	Bansberia, West Bengal	33	Gen-23	IC 375976	NBPGR, Thrissur
8	Gen-16	IIHR BM-13	Ramanthpur, West Bengal	34	Gen-15	IC 392242	NBPGR, Thrissur
9	Gen-47	IIHR BM-15	Hessaraghatta lake, Karnataka	35	Gen-43	IC 392842	NBPGR, Thrissur
10	Gen-44	IIHR BM-16	Ivarkandapura, Karnataka	36	Gen-29	IC 426442	NBPGR, Thrissur
11	Gen-20	IIHR BM-17	Hessaraghatta, Karnataka	37	Gen-18	IC 426447	NBPGR, Thrissur
12	Gen-49	IIHR BM-18	Biljaji, Karnataka	38	Gen-25	IC 439118	NBPGR, Thrissur
13	Gen-41	IIHR BM-19	Hessaraghatta lake, Karnataka	39	Gen-37	IC 468878	NBPGR, Thrissur
14	Gen-39	IIHR BM-20	Hessaraghatta lake, Karnataka	40	Gen-11	IC 554535	NBPGR, Thrissur
15	Gen-46	IIHR BM-21	Haniyur, Karnataka	41	Gen-45	IC 554585	NBPGR, Thrissur
16	Gen-36	IIHR BM-23	Thippapura, Karnataka	42	Gen-6	IC 554586	NBPGR, Thrissur
17	Gen-38	IIHR BM-27	NBPGR, Thrissur	43	Gen-27	IC 554587	NBPGR, Thrissur
18	Gen-1	IIHR BM-28	NBPGR, Thrissur	44	Gen-50	IC 554588	NBPGR, Thrissur
19	Gen-24	IIHR BM-29	NBPGR, Thrissur	45	Gen-5	IC 565466	NBPGR, Thrissur
20	Gen-3	IIHR BM-30	NBPGR, Thrissur	46	Gen-8	IC 565499	NBPGR, Thrissur
21	Gen-30	IIHR BM-31	NBPGR, Thrissur	47	Gen-14	IC 565503	NBPGR, Thrissur
22	Gen-21	IIHR BM-33	NBPGR, Thrissur	48	Gen-22	IC 565508	NBPGR, Thrissur
23	Gen-33	IIHR BM-35	NBPGR, Thrissur	49	Gen-17	JU2O/34	NBPGR, Thrissur
24	Gen-32	IC 249250	NBPGR, Thrissur	50	Gen-40	JU26/32	NBPGR, Thrissur
25	Gen-10	IC 284992	NBPGR, Thrissur	51		Check 1	Hessaraghatta, Karnataka
26	Gen-28	IC 321278	NBPGR, Thrissur	52		Check 2	Agrahara, Karnataka

was calculated (13). Diversity analysis for morphological traits was done using UPGMA clustering.

Molecular diversity analysis

Random amplified polymorphic DNA (RAPD) markers were employed to characterize and to estimate the genetic diversity among the germplasms. The modified Cetyl trimethylammonium bromide (CTAB) method was used for DNA isolation (14). DNA concentration in the sample was estimated by recording absorbance at 260 nm using UV/VIS spectrophotometer. DNA quality was checked by Agarose gel electrophoresis protocol and documented with a gel documentation system (Model Fire Reader V10, Uvitech Cambridge). Fifteen polymorphic amplifications were performed with thermal cycler (BAIORAD T100 thermal cycler) (15). The list of RAPD primers used in study is presented in Table 2. Amplification results from 15 primers across 50 genotypes of Brahmi were scored for presence (1) or absence (0) of bands and the binary data were converted into binary matrix for analysis using Cervus (ver. 3.0.7). The data were used to generate distance matrix based on Euclidean distance and the dendrogram (cluster diagram) was generated using Ward's minimum variance method.

Table 2. List of RAPD primer used in diversity analysis of Brahmi genotypes

Sl. No.	RAPD primers	Sequence (5'- 3')
1	OPA-09	GGGTAACGCC
2	OPB-12	CCTTGACGCA
3	OPB-20	GGACCCTTAC
4	OPAB-08	GTTACGGACC
5	OPAB-12	CCTGTACCGA
6	OPAB-17	TCGCATCCAG
7	OPAB-19	ACACCGATGG
8	OPAC-20	ACGGAAGTGG
9	OPI-10	ACAACGCGAG
10	OPI-19	AATGCGGGAC
11	OPM-01	GTTGGTGGCT
12	OPM-02	ACAACGCCTC
13	OPM-20	AGGTCTTGGG
14	OPN-10	ACAACTGGGG
15	OPN-17	CATTGGGGAG

Results and Discussion

Morphological diversity

The analysis of variance revealed that mean sum of squares due to blocks was significant for traits such as number of primary branches per plant, leaf width, flower diameter, fresh herbage yield, dry herbage yield and leaf/stem ratio, when analysis was done by ignoring the treatments (Table 3). The mean sum of squares due to treatments was significant for fresh and dry herbage yield while the mean sum of squares due to checks and varieties was significant for leaf length and dry herbage yield. Number of primary branches per plant, leaf length and leaf width recorded moderate PCV and GCV along with higher heritability and higher genetic advance as percent of mean (Table 4). Flower diameter, fresh herbage yield and dry herbage yield recorded higher PCV, GCV, heritability and genetic advance as percent of mean. The leaf/stem ratio recorded moderate PCV and GCV, higher heritability and moderate genetic advance as percentage of mean (Table 4). The Brahmi genotypes were assessed using percent Euclidean distance based on seven morphological characters and were grouped into five clusters. Among the five, Cluster I was the largest, comprising 24 genotypes followed by Cluster II with 11 genotypes, Cluster III with 15 genotypes, Cluster IV with single genotype (IC 426447) and Cluster V with one genotype (IIHR BM-23) (Table 5, Fig. 1).

Assessment of biochemical diversity

The mean sum of squares due to checks and varieties were significant for all the characters under study indicating a considerable amount of genetic variability present among treatments (Table 6). The results were presented separately for total Bacoside A and its components. Bacoside A3, Bacopaside II Jujubogenin and Bacopasaponin C. Bacoside A and its components registered higher PCV, GCV, heritability and genetic advance as percent of mean (Table 7). The distribution of Brahmi accessions for triterpenoid saponins contents into five different clusters was noted (Table 8, Fig. 2). Among the five, cluster I was the largest, comprising 19 genotypes followed by Cluster II with 15 genotypes. The third cluster contained 5 genotypes followed by IV with 8 genotypes. Cluster V had 5 genotypes and no specific grouping based on saponin content was noticed, Cluster III consisted of genotypes (IC 554588, IIHR BM 30, IIHR BM 21) with high Bacoside A content.

Table 3. Analysis of variance of Augmented Block design for Brahmi genotypes

Source of variation	d. f	No. of primary branches	Leaf length (cm)	Leaf width (cm)	Flower diameter (cm)	Fresh weight (g plot^{-1})	Dry weight (g plot^{-1})	Leaf/Stem ratio
Block (ignoring treatments)	4	20.47 *	0.12	0.03 **	0.25**	201044.10**	8535.76 **	0.02 *
Treatment (eliminating blocks)	51	7.07	0.08	0.00	0.05	70036.42*	2831.32 *	0.00 *
Block (eliminating check + var.)	4	1.63	0.01	0.00	0.05	20965.19	736.74	0.01
Entries (ignoring blocks)	51	8.55	0.09	0.01*	0.06	84160.25*	3443.01*	0.01*
Checks	1	0.48	0.02	0.00	0.04	7155.62	189.65	0.00
Varieties	49	8.71	0.08	0.01 *	0.07	86091.00 *	3484.40 *	0.01
Checks vs Varieties	1	8.48	0.42 *	0.00	0.00	665558.3	4668.51*	0
Error	4	1.91	0.03	0.00	0.01	11206.71	371.23	0.00

* Significant at 5 % level, ** Significant at 1 % level.

Table 4. Estimates of variability and genetic parameters for morphological traits in Brahmi genotypes

Sl. No.	Character	Variance		Coefficient of variation (%)		Heritability (Broad sense) (%)	Genetic advance as percent of mean (%)
		Genotypic	Phenotypic	Genotypic	Phenotypic		
1	No. of primary branches	6.06	7.97	13.09	15.01	76.03	23.51
2	Leaf length (cm)	0.05	0.08	13.52	17.11	62.39	22
3	Leaf width (cm)	0.00	0.01	15.79	17.39	82.47	29.54
4	Flower diameter (cm)	0.04	0.06	19.22	21.92	76.89	34.72
5	Fresh weight (gm)	66715.08	77921.803	33.61	36.33	85.62	64.08
6	Dry weight (gm)	2773.54	3144.78	47.80	50.90	88.2	92.48
7	Leaf/ stem ratio	0.00	0.01	10.26	11.03	86.62	19.68

Table 5. Cluster composition of 50 genotypes of Brahmi based on morphological traits

Sl. No.	Cluster	No. of genotypes included	Genotypes
1	I	24	IIHR BM-2, IC 343108, IIHR BM-30, IIHR BM-8, IC 565466, IC 554586, IIHR BM-28, IC 565499, IIHR BM-5, IC 565503, IC 392242, JU20/34, IC 353203, IIHR BM-33, IIHR BM-29, IC 439118, IIHR BM-06, IC 321278, IIHR BM-15, IIHR BM-35, IIHR BM-20, IIHR BM-18, Check 2, Check 1
2	II	11	IIHR BM-19, IIHR BM-4, IIHR BM-13, IIHR BM-16, IC 565508, IC 375976, IC 426442, IIHR BM-31, IC 353204, IC 370640, IIHR BM-27
3	III	15	IC 324777, IC 284992, IC 554535, IIHR BM-9, IC 392842, IIHR BM-17, IC 554585, IIHR BM-21, IC 554587, IC 249250, IIHR BM-3, IC 324774, IC 468878, JO26/32, IC 554588
4	IV	01	IC 426447
5	V	01	IIHR BM-23

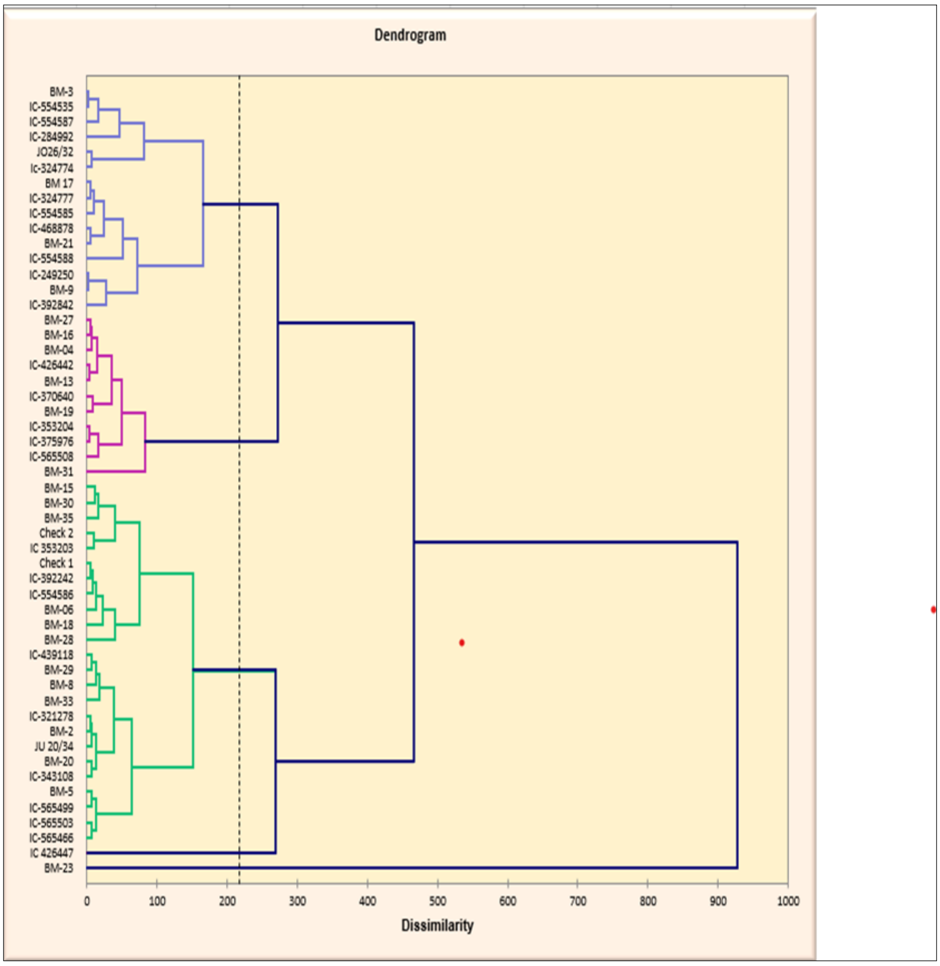

Fig. 1. UPGMA dendrogram of Brahmi genotypes based on morphological traits.

Table 6. Analysis of variance of total Bacoside A content in Brahmi genotypes

Source of variation	d. f	Bacoside A3	Bacopasaponin II	Jujubogenin	Bacopasaponin	Bacoside A
Block (ignoring treatments)	4	0.02 *	0.19 **	0.06 **	0.03 **	0.53 **
Treatment (eliminating blocks)	51	0.04 **	0.09 **	0.04 **	0.05 **	0.53**
Block (eliminating check + var.)	4	0	0.00	0	0	0.00
Entries (ignoring blocks)	51	0.04 **	0.11 **	0.53 **	0.05 **	0.57 **
Checks	1	0. 00 *	0.00	0	0	0
Varieties	49	0.04 **	0.10 **	0.03 **	0.03 **	0.36 **
Checks vs varieties	1	0.47 **	0.40 **	0.98 **	1.17 **	11.56 **
Error	4	0	0	0	0	0.002

***** Significant at 5 % level**, **** Significant at 1 % level.

Table 7. Estimates of variability and genetic parameters for total Bacoside A content and its components in genotypes of Brahmi

Sl. No	Character	Variance		Coefficient of variation (%)		Heritability (Broad sense) (%)	Genetic advance as percent of mean (%)
		Genotypic	Phenotypic	Genotypic	Phenotypic		
1	Bacoside A3	0.03	0.03	34.18	34.23	99.69	70.30
2	Bacopaside II	0.09	0.09	34.96	35.03	99.57	71.87
3	Jujobogenin	0.03	0.03	40.78	40.81	99.89	83.97
4	Bacopasaponin C	0.03	0.03	31.01	31.11	99.41	63.71
5	Bacoside A	0.32	0.32	23.28	23.36	99.31	47.80

Table 8. Cluster composition of 50 genotypes of Brahmi based on total Bacoside A content (Bacoside A3, Bacopaside II, Jujubogenin, Bacopasaponin C)

Sl. No.	Cluster	No. of genotypes	Genotypes
1	I	19	IIHR BM-2, IIHR BM-8, IIHR BM-19, IC 324777, IC 565503, IIHR BM-16, IC 426447, IC 353203, IIHR BM 17, IC 565508, IC 439118, IIHR BM-6, IIHR BM-31, IC 249250, IIHR BM-35, IIHR BM-3, IIHR BM-23, IC 468878, IIHR BM-18
2	II	15	IC 343108, IIHR BM-28, IIHR BM-04, IC 284992, IC 554535, IIHR BM-5, IIHR BM-9, IC 392242, IIHR BM 13, IC 392842, JU20/34, IC 554585, IC 554587, IC 321278, IIHR BM 20
3	III	05	IIHR BM-30, IIHR BM-21, IC 324774, IIHR BM-27, IC 554588
4	IV	08	IC 565466, IC 554586, IC 375976, IC 426442, IIHR BM-15, JO26/32, Check 2, Check 1
5	V	05	IC 565499, IIHR BM-33, IIHR BM-29, IC 353204, IC 370640

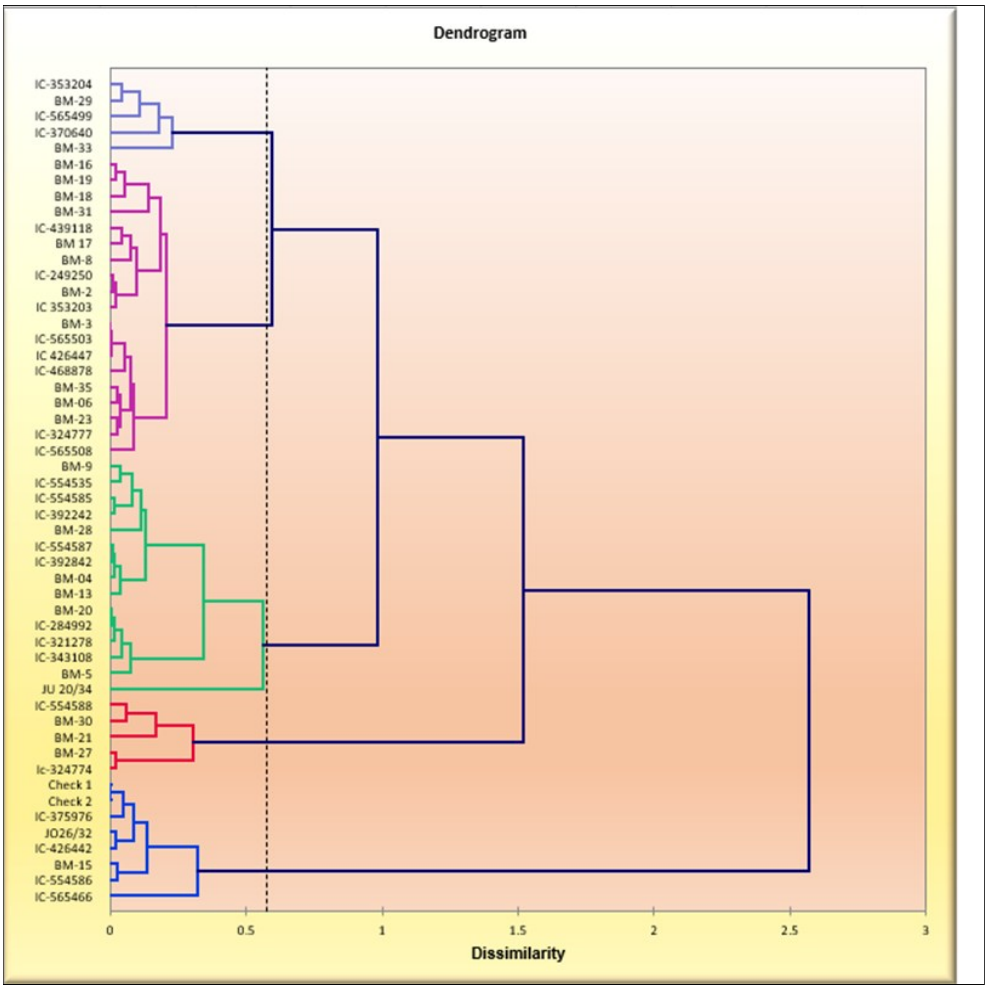

Fig. 2. UPGMA dendrogram of Brahmi germplasm based on total Bacoside content and it's component.

Molecular diversity analysis

Brahmi genotypes were initially screened using 60 RAPD primers using bulk DNA. Among 60 primers, 15 primers exhibited good amplification with clear, sharp, reproducible bands. The primers generated a total of 426 bands, out of which 411 were polymorphic and 15 were monomorphic across the accessions. This indicated that most of the selected primers were polymorphic across accessions. The number of bands generated by each primer varied from 44 (OPM-01) to 9 (OPI-19) with an average of 28.4 per primer. The polymorphism ranged from 66.6 to 100 % among the primers which could be due to primer sequence. Out of 15 primers, OPM-01 produced the maximum number [44] of bands with 100 % polymorphism. Primers, OPAB-08, OPAB-12, OPAB-19, OPB-20, OPB-12, OPI-10, OPI-19, OPM-01, OPN-17, OPA-09, OPM-20 also recorded 100 % polymorphism. The primer, OPM-02 showed least polymorphic with 66.6 %. The genetic divergence in the genotypes was estimated by subjecting them to distance analysis, using Euclidian distance (9). Among the six clusters generated, Cluster-I was the largest, comprising 25 genotypes followed by Cluster II with 19 genotypes. Cluster III consisted of 2 genotypes (IC 426442, IIHR BM-35) followed by Cluster IV with 1 genotype (IIHR BM-31). Cluster V with 2 genotypes (IC 353204, IC 249250) and Cluster VI with 1 genotype (IC 468878) (Table 9, Fig. 3).

Genetic variability is crucial for crop improvement, aiding selection and crossbreeding. Plant breeders must assess germplasm to determine genomic variability and traits like resistance and quality. Understanding genetic distances helps maintain diverse accessions and prevent duplication, ensuring effective breeding strategies. The mean sum of squares due to checks and varieties were significant for leaf length and dry herbage yield under study indicating a considerable amount of genetic variability present among Brahmi accessions. Significant values of mean sum of squares due to blocks for the characters indicated that the presence of sufficient genetic variability and scope for improvement of these characters through selection and hybridization.

Table 9. Cluster composition of 50 Brahmi genotypes based on molecular marker data

Sl. No.	Cluster	No. of genotypes	Genotypes
1	I	25	IIHR BM-28, IC 343108, IIHR BM-30, IIHR BM-8, IC 565466, IC 554586, IIHR BM-2, IC 565499, IIHR BM-04, IC 284992, IC 554535, IIHR BM-5, IIHR BM-9, IC 565503, IC 392242, IIHR BM-13, JU20/34, IC 426447, IC 353203, IIHR BM-17, IIHR BM-33, IC 565508, IC 375976, IIHR BM-29, IC 439118
2	II	19	IIHR BM-06, IC 554587, IC 321278, IIHR BM-3, IC 324774, IIHR BM-23, IIHR BM-27, IIHR BM-20, JO26/32, IIHR BM-19, IC 324777, IC 392842, IIHR BM -16, IC 554585, IIHR BM-21, IIHR BM-15, IC 370640, IIHR BM-18, IC 554588
3	III	02	IC 426442, IIHR BM-35
4	IV	01	IIHR BM-31
5	V	02	IC 353204, IC 249250
6	VI	01	IC 468878

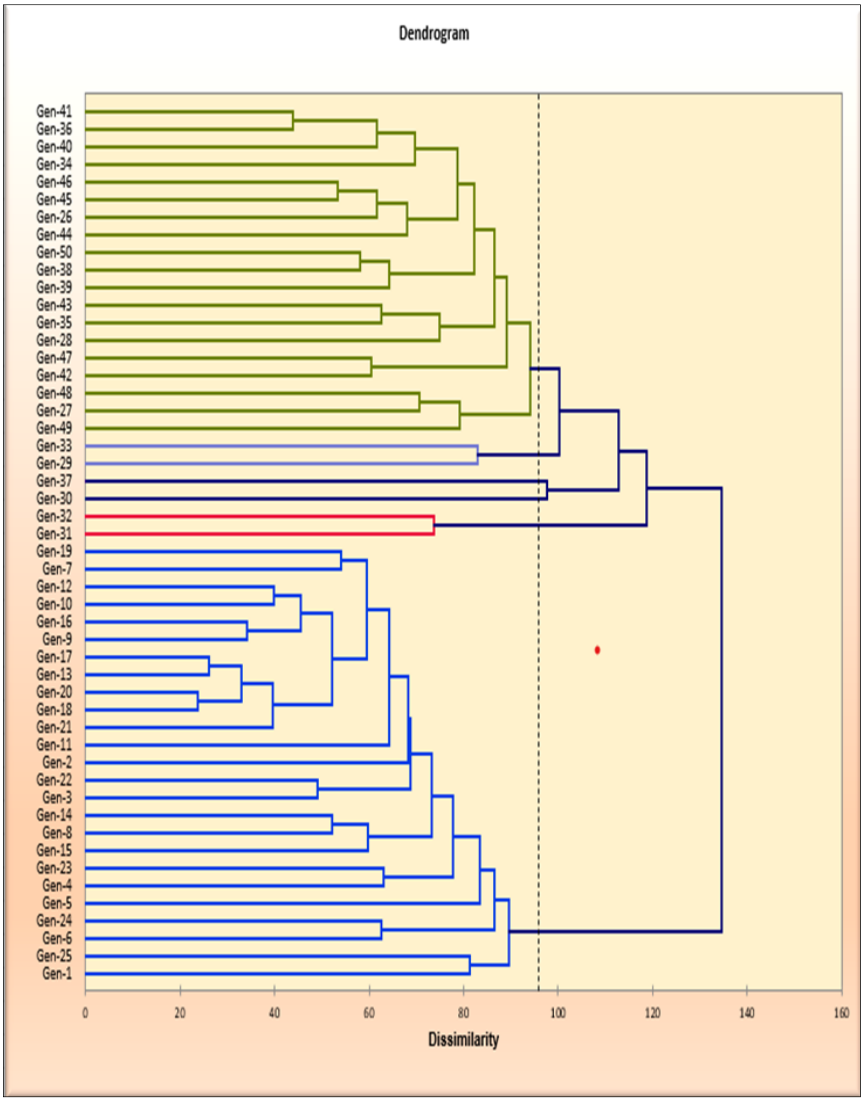

Fig. 3. Dendrogram showing cluster pattern (UPGMA method) Brahmi germplasm lines based on RAPD markers.

A study screened fourteen collections of Brahmi, obtained from various places of Bihar for their growth and herbage yield traits under north Bihar condition. The pooled data of three years result revealed that there were significant variations among the genotypes with respect to vegetative growth and herbage yield (16). A study evaluated morphological, chemical and genetic parameters of five elite accessions and found significant variation for the traits (17). From this study it was observed that the traits flower diameter, fresh herbage yield, dry herbage yield had higher GCV, PCV, heritability and genetic advance as percent of mean. These traits were amenable for selection and could be effectively used in genetic improvement. Similar findings were reported in fennel (18), mandukaparni (19), *Ocimum gratissimum* L. (20) and Brahmi (21).

Cluster analysis in the current study indicated that many genotypes were grouped in the same cluster from different geographical origin. High herbage yield genotypes (JO26/32, IIHR BM-3, IC 554587, IC 554588, IC 324774) were observed in cluster II. It was also observed that IIHR BM-23 and IC 426447 were genetically diverse from all other genotypes.

The mean sum of squares due to checks and varieties was significant for all the biochemical characters under study, indicating a considerable amount of genetic variability present among treatments and scope for improvement of these characters through hybridization and selection. Bacoside A3, Bacopaside II, Jujubogenin, Bacopasaponin C and Bacoside A recorded higher PCV, GCV, heritability and genetic advance as percent of mean which was attributed to additive genes, hence selection could be effective. Although, the Bacoside A content was an inherent quality character specific to a particular genotype, its production might have been influenced by prevailing external environmental conditions. This may have been due to genetic character of the genotype and the influence of environment. A study reported significant variations in chemical characters of different Kalmegh accessions because of their different geographical locations with genetic makeup (22). Similar conclusions were drawn for bacoside in brahmi (21), for asiaticoside in *Centella* (23), for andrographalide in Kalmegh (24), for withanolide- A in Ashwagandha (25), for stevioside content in Stevia (26, 27).

Traditionally, genetic diversity was assessed through various morphological features. However, in Brahmi, morphological trait variability was limited. These traits might not have adequately classified germplasm or accurately reflected genetic divergence. The pattern of distribution of genotypes into different clusters was at random through molecular analysis in the current study. Genotypes belonging to same geographic origin were included in different clusters. Differences in genetic constitution and the presence of unabated influence of environmental factors might have contributed to this clustering pattern (28). In addition, the clustering pattern in the present study indicated that genetic diversity was not necessarily related to geographical distribution. In a report by 48 % polymorphism was reported among distinct accessions of *B. monnieri* using RAPD markers. It was concluded that the vegetative propagation of *B. monnieri* species could have contributed to narrow genetic base. The lack of RAPD variations could be attributed to heterozygosity of plants (29). In another study, genetic diversity in *B. monnieri* was established using RAPD and ISSR markers. A high level of polymorphism was found, which made RAPD markers as an effective tool for genetic diversity analysis (5). In a study with the 10 RAPD primers, 110 distinct bands were generated, of which only 14 (12.72 %) were polymorphic. A low level of genetic variation (12.72 %) was reported between randomly collected *B. monnieri* accessions from four different Southern Indian states and *in vitro* micropropagated plants originally derived from a Kerala accession (30).

Conclusion

Based on the results, traits like fresh and dry herbage yield, Bacoside A could be selected for crop improvement programme of Brahmi, as they exhibited higher GCV, PCV, heritability and genetic advance as percent of mean. The genotypes IIHR BM-23 and IC 426447 were identified through morphological analysis as high diverse lines. The diverse genotypes IIHR BM-31 and IC 468878 were identified through molecular diversity analysis. The genotypes IIHR BM-30, IC 565466 and IC 554586 were grouped together in cluster analysis based on morphological, biochemical and molecular data, indicating high similarity. The elite lines identified for herbage yield (IIHR BM 23, JO26/32) and total Bacoside A content (IC 554588, IIHR BM 30) need to be evaluated further for their stability in herbage yield and triterpenoid saponin content. Morphological, biochemical and molecular analysis revealed huge variation exists in Brahmi genotypes, which can be further exploited for identifying donors for crop improvement programme.

Acknowledgements

The authors are highly thankful to the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India for providing facilities and support.

Authors' contributions

KM, KMY, VKR, KVR and KHB conceived and designed the experiments. KM, KCS, DKS, VKR, KVR and KHB laid out the trials and collected data on morphological and biochemical traits. KM, KCS, DKS and KVR carried out the molecular studies. KM, SV, MRR and KHB processed and analyzed the data. KM, VS, A, KMY and KHB prepared the first draft of the manuscript. All authors discussed, edited, read and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

References

1. Sudhakaran MV. Botanical pharmacognosy of *Bacopa monnieri* (Linn.) Pennell. Pharmacogn J. 2020;12(6):1559-72. https://doi.org/10.5530/pj.2020.12.214
2. Praveen N, Naik PM, Manohar SH, Nayeem A, Murthy HN. *In vitro* regeneration of brahmi shoots using semisolid and liquid cultures and quantitative analysis of bacoside A. Acta Physiol Plant. 2009;31:723-28. https://doi.org/10.1007/s11738-009-0284-5
3. Gohil KJ, Patel JA. A review on *Bacopa monniera*: current research and future prospects. Int J Green Pharm. 2010;4:1-9. https://doi.org/10.22377/ijgp.v4i1.111
4. Balkrishna A, Misra LN. Ayurvedic plants in brain disorders: The Herbal Hope. J Tradit Med Clin Natur. 2017;6:221. https://doi.org/10.4172/2573-4555.1000221
5. Tripathi N, Saini N, Tiwari S. Morphological and molecular characterization of endangered medicinal plant species *Coleus forskohlii* collected from central India. J Crop Sci Biotechnol. 2013;16:253-61. https://doi.org/10.1007/s12892-013-0083-9
6. Kongkeaw C, Dilokthornsakul P, Thanarangsarit P, Limpeanchob N, Scholfield CN. Meta-analysis of randomized controlled trials on cognitive effects of *Bacopa monnieri* extract. J Ethnopharmacol. 2014;151(1):528-35. https://doi.org/10.1016/j.jep.2013.11.008
7. Nemetchek MD, Stierle AA, Stierle DB, Lurie DI. The Ayurvedic plant *Bacopa monnieri* inhibits inflammatory pathways in the brain. J Ethnopharmacol. 2017;197:92-100. https://doi.org/10.1016/j.jep.2016.07.073
8. Charoenphon N, Anandsongvit N, Kosai P, Sirisidthi K, Kangwanrangsan N, Jiraungkoorskul W. Brahmi (*Bacopa monnieri*): Up to date of memory boosting medicinal plant: A review. Indian J Agric Res. 2016;50(1):1-7. https://doi.org/10.18805/ijare.v50i1.8582
9. Rajani M. *Bacopa monnieri*, a nootropic drug. Bioact Mol Med Plants. 2008;175-95. https://doi.org/10.1007/978-3-540-74603-4_9
10. Federer WT. Augmented (or hoonuiaku) designs. Hawaiian Planters Rec. 1956. https://doi.org/10.2307/2529707
11. Burton GW. Quantitative inheritance in grasses. Proc Sixth Int Grassland Congr. 1952;1:277-83.
12. Lush JL. Inter size correlation and regression of offspring on dams as a method of estimating heritability of characters. Proc Am Soc Anim Prod. 1940;33:293-01.
13. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybean. Agron J. 1955;47:314-18. https://doi.org/10.2134/agronj1955.00021962004700070009x
14. Doyl JJ, Doyl JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13-5.

15. Pratima S, Tejovathi G. Analysis of genetic variation among the accessions of *Bacopa monnieri* collected from regions of Madhya Pradesh using RAPD. Int J Adv Innov Res. 2019;6:63-5.
16. Kumar U. Evaluation of Brahmi (*Bacopa monnieri* L.) genotypes for growth and herbage yield under North Bihar agroecological conditions. J Pharmacogn Phytochem. 2017;6(6S):427-29.
17. Saran PL, Damor HI, Lodaya DH, Suthar MK, Kalariya KA, Roy S. Identification of potential accessions of *Bacopa monnieri* L. for herbage yield and bacosides A content. Ind Crops Prod. 2022;176:114348. https://doi.org/10.1016/j.indcrop.2021.114348
18. Hadli AA, Yuvaraj KM, Srinivasulu B, Jayaprada M, Kiran YD, Vali DM. Genetic variability studies in fennel (*Foeniculum vulgare* Mill.) under Rayalaseema Region of Andhra Pradesh. J Pharmacogn Phytochem. 2021;10:1755-60. https://doi.org/10.22271/phyto.2021.v10.i1y.13605
19. Singh LJ. Characterization and evaluation of mandukaparni (*Centella asiatica* L.) germplasm. [MSc thesis]. Univ Hortic Sci, Bagalkot. Krishikosh. 2016.
20. Edet OU. Agro-morphological evaluation and analysis of genetic parameters in African basil (*Ocimum gratissimum* L.) in Southeastern Nigeria. In Crop Sci Niger: Proc 2nd Natl Annu Conf Univ Niger. Nsukka, Enugu State, Nigeria. 2013.
21. Vishnuvardhan RB, Radhakrishnan VV, Mini S, Bastian D, Latha A, Vishnuvardhan. Exploration, collection, morphological and biochemical evaluation of brahmi (*Bacopa monniera* Wettst.). Int J Plant Sci (Muzaffarnagar). 2010;5(2):473-481.
22. Bhan MK, Dhar AK, Khan S, Lattoo SK, Gupta KK, Choudhary DK. Screening and optimization of *Andrographis paniculata* (Burm. f.) Nees for total andrographolide content, yield and its components. Sci Hortic. 2005;107:386-91. https://doi.org/10.1016/j.scienta.2005.09.001
23. Dissanayake TDCK, Abeysekera AM, Salim N, Chandrika UG, Padumadasa C. Evaluation of *Centella asiatica* morphotypes for high yields of asiaticoside. J Pharmacogn Phytochem. 2016;5(6):451-54.
24. Jai Kumar, Animesh, Ras BS, Hiranmayee N, Diwakar PN. Evaluation of (*Andrographis paniculata* Nees.) germplasm for higher biochemical constituents production under agro-climatic conditions of Jharkhand. J Pharmacogn Phytochem. 2018;1:561-67. https://doi.org/10.2139/ssrn.4190971
25. Surya C, Arunabh J, Rohit J, Devendra J. Estimation of Withanolide A in diverse genotypes of Ashwaganda, *Withania somnifera* (L.) Dunal. Indian J Exp Biol. 2019;57:212-17.
26. Cheryl AP, Clinton CS, Michael Q. Dry leaf and steviol glycoside productivity of *Stevia rebaudiana* in the Western United States. Hort Sci. 2016;51(10):1220-27. https://doi.org/10.21273/HORTSCI11149-16
27. Hastoy CC, Patrick C, Sebastien B, Phillippe W, Pierre R, Dominique SL, Valerie. Deciphering performances of fifteen genotypes of *Stevia rebaudiana* in southwestern France through dry biomass and steviol glycoside evaluation. Ind Crops Prod. 2019;128:22-9. https://doi.org/10.1016/j.indcrop.2018.09.053
28. Rahaman M, Hossain M, Das ND. Genetic divergence in Betelvine (*Piper betle* L.). J Plant Crops. 1997;25:57-61.
29. Darokar MP, Khanuja SPS, Kumar Shasany A. Low levels of genetic diversity detected by RAPD analysis in geographically distinct accessions of *Bacopa monnieri*. Genet Resour Crop Evol. 2001;48(6):555-58. https://doi.org/10.1023/A:1013800101604
30. Karthikeyan A, Madhanraj A, Pandian SK, Ramesh M. Genetic variation among highly endangered *Bacopa monnieri* (L.) Pennell from Southern India as detected using RAPD analysis. Genet Resour Crop Evol. 2011;58:769-82. https://doi.org/10.1007/s10722-011-9695-4

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etcSee https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.