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RESEARCH ARTICLE

HIERARCHIAL CLUSTER ANALYSIS IN *BACOPA MONNIERI* AND ITS GENETIC IMPLICATIONS

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SUMMARY

Profound degree of morphological variation exists among the accessions of *Bacopa monnieri* occurring in Kerala. Sixty accessions of the species were subjected to Ward's minimum variance analysis based on forty morphological characters, both quantitative and qualitative, aimed at clustering them into groups with appreciable degree of intracluster discreteness and intercluster distance. The result was presented in a dendrogram, in which the entire assemblage was classified into ten clusters at the similarity level of 33.8%. The number of members in different clusters ranged from 1–16. Distribution of various characters among the clusters showed considerable degree of intracluster similarity (connectedness) and intercluster distance (isolation). The present clustering has significant genetic implications as it provides reliable clues for selecting parental genotypes with fair degree of genetic difference from clusters exhibiting appreciable isolation, for intraspecific hybridization for exploiting hybrid vigour in the crop.

Keywords: *Bacopa monnieri,* hierarchial cluster analysis, genetic implication.

INTRODUCTION

Bacopa monnieri (L.) Wettst. is a perennial herbaceous creeper belonging to the family Plantaginaceae. The plant has multifarious medicinal properties with immense therapeutic value. It is widely used in folk medicine and in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani, and also in Homoeopathy (Singh & Singh 1980). Pharmacological studies have revealed that *B. monnieri* extract posseses an array of therapeutic properties such as antioxidant (Tripathi et al. 1996), anticancer (Elangovan et al. 1995), immuno-modulatory (Dahanukar & Thatte 1997), antistress (Chowdhuri et al. 2002) and adaptogenic activities (Rai et al. 2003). The Planning Commission of India Task Force on conservation and sustainable use of medicinal plants in its report for the year 2000 identified *B. monnieri* as one of the core species in great demand in pharmaceutical industry of Indian traditional systems of medicine. The Export Import Bank of India have placed the species in the second

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position in the priority list of the most important medicinal plants of the country based on evaluation of its medicinal importance, commercial value and potential for further research and development. (Bammidi et al. 2011, Brinckmann 2008, Rajani 2008). *B. monnieri* is one among the 32 medicinal plants identified for cultivation and conservation by the National Medicinal Plants Board. It is estimated that about 10000 to 12000 tons of fresh biomass of the species is collected annually from the wild in India (Karthikeyan et al. 2011). Recently, there are many reports that *B. monnieri* has become a locally endangered species (Bansal et al. 2014, Karthikeyan et al. 2011, Ramesh et al. 2011, Tripathi et al. 2012), and its cultivation has already been undertaken in various parts of India (Sharma 2004).

There have been many studies in intraspecific taxonomy, most of which based on easily recognizable characters of gross morphology. Variation in such characters has been used to establish taxonomically formal/informal diagnostic classifications. Good examples of such effective intraspecific classifications in crop plants may be found in the works of Rogers & Flemming (1973) in the South American cultivars of cassava (*Manihot esculenta*), Martin & Rhodes (1977) in the yams (*Dioscorea alata*), Mathew et al. (2001) in black pepper (*Piper nigrum*), Subramanian & Subbaraman (2010) in maize germplasm and Tomsone et. al. (2012) in horse radish genotypes (*Armoracia rusticana* L.). In a cline showing great similarities between the terminal members, it is possible that similarity between the terminal members could be less than those between the members of the cline and a nonmember (Writh et al. 1966). Obviously there can be intermediate clusters as well. An acceptable clustering technique should be able to discover and define all of them. A variety of methods have been known for establishing taxonomically sound intraspecific clusters, of which the hierarchial clustering method — Ward's minimum variance cluster analysis — is acclaimed as a fairly viable one helpful for recognizing prospective accessions/cultivars in screening for selection and selecting putative parents for intervarietal crossing. This method has been followed in the present study for clustering 60 accessions of *monnieri*, drawn from across the state of Kerala, for sorting them into clusters with fair degree of intracluster similarity and intercluster isolation. In addition to affecting a viable grouping of the accessions, the result is useful also for selecting accessions with appreciable degree of genetic divergence for future genetic improvement programme by hybridization in the species.

MATERIALS AND METHODS

Field surveys were conducted throughout Kerala, and 60 accessions of *B. monnieri* were located and collected from different parts of the State (Table 1). Planting materials of the accessions collected during the field surveys were planted in a Nursery at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram, and on establishment, the accessions were transplanted to the Field Gene Bank (FGB) at the institute. The accessions were maintained in the FGB, and propagated vegetatively for four successive generations. Five-noded stem cuttings of the fourth generation vegetatively propagated plants of the accessions collected from the FGB were planted in an Experimental Plot (EP) in three replications, in which the accessions were grown under uniform environmental conditions. The data on the 40 characters, 15 qualitative (Table 2) and 25 quantitative (Table 3) collected from the 60 accessions were pooled together, standardized and subjected to Ward's minimum variance cluster analysis (Szekely & Rizzo 2005), applying the SAS. The variability with respect to 15 qualitative characters was associated based on the number of character states in which a particular is expressed. A similarity matrix was prepared by calculating the PROC (Programmed Random Occurrence) distance between pairs of accessions, and a dendrogram was constructed illustrating the interrelationships between and among the accessions.

TABLE 1: Details of collection localities of the 60 accessions of *B. monnieri.*

Acc. No.	Place of collection	District	Acc. No.	Place of collection	District
Bm 1	Viraly	Thiruvananthapuram	Bm 31	Pattambi	Palakkad
Bm 2	Kadakkavoor	Thiruvananthapuram	Bm 32	Kambram	Palakkad
Bm 3	Karikkakom	Thiruvananthapuram	Bm 33	Pulakkad	Palakkad
Bm 4	Aakkulam	Thiruvananthapuram	Bm 34	Varakulam	Malappuram
Bm 5	Vettamukku	Kollam	Bm 35	Paravanna	Malappuram
Bm 6	Panayam	Kollam	Bm 36	Kannoorkettu	Kozhikode
Bm 7	Kochuplamoodu	Kollam	Bm 37	Musankandi	Kozhikode
Bm 8	Dalavapuram	Kollam	Bm 38	Kakkoor	Kozhikode
Bm 9	Padukkottukala	Pathanamthitta	Bm 39	Pathinonnammile	Kozhikode
Bm 10	Thakazhi	Alappuzha	Bm 40	Chelapram	Kozhikode
Bm 11	Pattaniyidukku	Alappuzha	Bm 41	Manicherry hills	Kozhikode
Bm 12	Pathirapally	Alappuzha	Bm 42	Payyannur	Kannur
Bm 13	Vattayil	Alappuzha	Bm 43	Muttukatti	Kannur
Bm 14	Nerekadavu	Kottayam	Bm 44	Kakkad	Kannur
Bm 15	Koduppadom	Kottayam	Bm 45	Andalloorkavu	Kannur
Bm 16	Kumarakom	Kottayam	Bm 46	Sasimala	Wayanad
Bm 17	Vazhikkadavu	Kottayam	Bm 47	Channothukolli	Wayanad
Bm 18	Kumbalam	Ernakulam	Bm 48	Seethamount	Wayanad
Bm 19	Thripunithura	Ernakulam	Bm 49	Nadavayal	Wayanad
Bm 20	Charthedam	Ernakulam	Bm 50	Meppadi	Wayanad
Bm 21	Near Aarch dam	Idukki	Bm 51	Padannakkadu	Kasaragod
Bm 22	Vellayamkudi	Idukki	Bm 52	Kumbla	Kasaragod
Bm 23	Munnar	Idukki	Bm 53	Edayilekkadu	Kasaragod
Bm 24	Chellarkovil	Idukki	Bm 54	Madakkara	Kasaragod
Bm 25	Kumily	Idukki	Bm 55	Manakkakadavu	Kottayam
Bm 26	Chelakkara	Thrissur	Bm 56	Channikkadavu	Alappuzha
Bm 27	Karuppadannapalam	Thrissur	Bm 57	Pedikkattuthuruthu	Ernakulam
Bm 28	SN beach	Thrissur	Bm 58	Cheriyathuruthu	Ernakulam
Bm 29	Undaikadavu	Thrissur	Bm 59	Chungam	Alappuzha
Bm 30	Kodakara	Thrissur	Bm 60	Kinanoor	Kasaragod

TABLE 2: Character states of the 15 qualitative characters observed in the 60 accessions of *B. monnieri.*

Sl. No.	Character	Character states
1	Disposition of primary branches	Erect, Sub-erect, Prostrate
2	Stem colour	Green, Pale green, Yellowish green
3	Node-centric anthocyanin pigmentation	Absent (NAP), Low (LAP), Medium (MAP), High (HAP)
4	Leaf colour	Dark green, Green, Light green
5	Leaf shape	Obovate, Oblong, Oblanceolate
6	Leaf tip	Rounded, Obtuse, Retuse
7	Corolla lobe	Unequal, Equal

(Continued)

TABLE 2: (Concluded)

8	Flower colour	Violet, Pale violet, White
9	Corolla throat colour	Dark purple, Purple, Light purple, White
10	Corolla base colour	Yellow, Yellowish green, Light yellowish green, White
11	Stigma colour	Dark green, Green, Light green
12	Style colour	Green, Light green, Pale yellowish green, White
13	Ovary colour	Green, Light green, Pale yellowish green
14	Capsule shape	Obovate, Oblong
15	Seed colour	Dark brown, Brown, Light brown

TABLE 3: Measures of central tendency (Mean) and dispersion (Range) of the 25 quantitative characters of the 60 accessions of *B. monnieri.*

Sl. No.	Character	Grand mean	Standard error	Range	Critical difference 1%	5%
1	Internode length	1.55 cm	0.05	0.84–2.76	0.2641	0.2010
2	Leaf length	1.98 cm	0.02	1.50–2.80	0.2127	0.1618
3	Leaf width	0.73 cm	0.01	0.50–1.1	0.1297	0.0986
4	Leaf area	96.88 mm^2	2.80	48–159	24.0397	18.2910
5	Leaf thickness	0.04 mm	0.002	0.02–0.06	0.0337	0.0257
6	No. of dentations/leaf	2.31	0.25	0–6	0.6976	0.5308
7	Stem thickness	0.16 mm	0.003	0.10–0.25	0.0287	0.0218
8	Pedicel length	2.35 cm	0.07	1.10–3.80	0.2795	0.2126
9	Flower diameter	1.30 cm	0.03	0.90–2.00	0.1356	0.1031
10	Bracteole length	0.24 cm	0.01	0.10–0.45	0.0494	0.0376
11	First sepal length	0.56 cm	0.01	0.40–0.75	0.0601	0.0458
12	First sepal width	0.34 cm	0.01	0.25–0.45	0.0501	0.0381
13	Second sepal length	0.54 cm	0.01	0.40–0.75	0.0600	0.0456
14	Third sepal length	0.53 cm	0.01	0.40–0.70	0.0429	0.0326
15	Fourth sepal length	0.49 cm	0.01	0.30–0.60	0.0458	0.0349
16	Corolla length	1.06 cm	0.02	0.85–1.8	0.1376	0.1047
17	Corolla width	1.75 cm	0.03	1.0–2.15	0.1848	0.1406
18	Short stamen length	0.20 cm	0.001	0.15–0.25	0.0137	0.0104
19	Long stamen length	0.40 cm	0.00	0.40–0.40	0.000	0.000
20	Style length	0.52 cm	0.01	0.40–0.60	0.0385	0.0293
21	Ovary length	0.18 cm	0.003	0.10–0.20	0.0210	0.0160
22	Fruit length	0.40 cm	0.01	0.23–0.52	0.0562	0.0428
23	Fruit breadth	0.18 cm	0.004	0.11–0.30	0.0352	0.0268
24	No. of seeds/capsule	72.23	5.13	09–198	12.0085	9.1369
25	Biomass yield (%)	8.37	0.16	6.2–11.98	1.0910	0.8301

OBSERVATIONS

The result of hierarchial clustering of 60 accessions is presented in a dendrogram (Fig. 1). Clusters were recognized arbitrarily against the phenetic value (similarity level) of 33.8%, and 10 clusters are formed at this level (Table 4). These clusters constitute maximal connected subgroups. The number of members belonging to different clusters ranged from 1 (Cluster IV) to 16 (Cluster X).

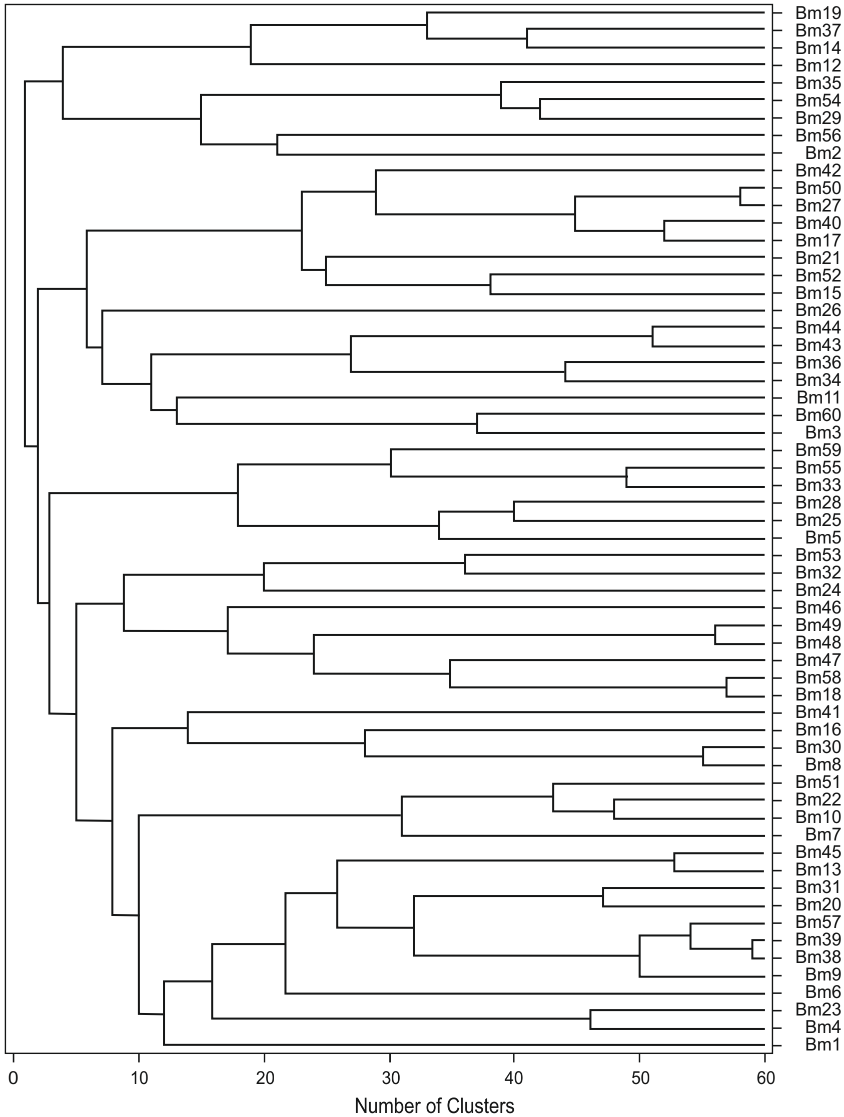

Fig. 1: Dendrogram showing ten clusters of accessions of *B. monnieri* formed by hierarchial cluster analysis at 33.8% similarity level.

TABLE 4: Composition of the clusters of the 60 accessions of *B.monnieri* based on hierarchial cluster analysis.

Cluster No.	No. of accessions	Accessions
I	4	Bm 19, Bm 37, Bm 14, Bm 12
II	5	Bm 35, Bm 54, Bm 29, Bm 56, Bm 2
III	8	Bm 42, Bm 50, Bm 27, Bm 40, Bm 17, Bm 21, Bm 52, Bm 15
IV	1	Bm 26
V	7	Bm 44, Bm 43, Bm 36, Bm 34, Bm 11, Bm 60, Bm 3
VI	6	Bm 59, Bm 55, Bm 33, Bm 28, Bm 25, Bm 5
VII	3	Bm 53, Bm 32, Bm 24
VIII	6	Bm 46, Bm 49, Bm 48, Bm 47, Bm 58, Bm 18
IX	4	Bm 41, Bm 16, Bm 30, Bm 8
X	16	Bm 51, Bm 22, Bm 10, Bm 7, Bm 45, Bm 13, Bm 31, Bm 20, Bm 57, Bm 39, Bm 38, Bm 9, Bm 6, Bm 23, Bm 4, Bm 1

DISCUSSION

Among the 10 clusters formed at the similarity level of 33.8%, one is large (X) holding 16 members, five medium holding 5–8 members and four small holding 1–4 members of which one is smallest. The clusters obtained are fairly well defined as their members possessing appreciable discontinuity, and such clusters cannot be subdivided. It is claimed that the clusters appropriating this property may be considered as maximal connected subgroups (Mathew et al. 2001). Distribution of various characters among the accessions showed considerable degree of solidarity existing in each of the clusters. From the clusters formed by hierarchial clustering, it is not possible to make decisions about the taxonomic level of any cluster, and hence no definitions of hierarchial categories arise, because there are no known rules by which to apply such definitions to the clusters of biological objective, and *ipso facto,* among the clusters of accessions of *B. monnieri* presented here, no heirarchial order may be expected. In cases of intraspecific classification of crop species in which cultivated forms display reticulate relationships, multiple referable associations have been felt to be a problem (Rogers & Fleming 1973). This is particularly so in *B. monnieri* in which the multiplicity of accessions exhibit enormous degree of morphological variation. The clustering effected in the accessions of this species may be considered important for choosing parents for intraspecific crossing exercise, especially in view of the extent of homogeneity apparent in the different clusters in terms of observed intracluster similarity and the magnitude of intercluster distance (isolation).The present clustering, therefore, is significant in as much as it provides reliable clues for selecting parental genotypes with the desired degree of genetic divergence from fairly distant and discrete clusters for future hybridization programmes in the species.

REFERENCES

BAMMIDI S R, VOLLURI S S, CHIPPADA S C, AVANIGADDA S & VANGALAPATI M 2011 A review on pharmacological studies of *Bacopa monnieri* *J Chem Bio Phy Sci* **1** 250–259

BANSAL M, KUMAR A & REDDY M S 2014 Diversity among wild accessions of *Bacopa monnieri* (L)Wettst and their morphogenetic potential *Acta Physiol Plan* **36** 1177–1186

BRINCKMANN J 2008 *Summary of report - Support to sustainable export development of Indian natural medicinal products: A needs assessment study* Government of India: Ministry of health and family welfare

CHOWDHURI D K, PARMAR D, KAKKAR P, SHUKLA R, SETH P K & SRIMAL R C 2002 Antistress effects of bacosides of *Bacopa monnieri*: modulation of Hsp70 expression superoxide dismutase and cytochrome P450 activity in rat brain *Phytother Res* **16** 639–645

DAHANUKAR S A & THATTE U M 1997 Current status of Ayurveda in phytomedicine *Phytomedicine* **4** 359 –368

ELANGOVAN V, GOVINDASAMY S, RAMAMOORTHY N & BALASUBRAMANIAN K 1995 *In vitro* studies on the anticancer activity of *Bacopa monniera* *Fitoterapia* **66** 211–215

KARTHIKEYAN A, MADHANRAJ A, PANDIAN S K & RAMESH M 2011 Genetic variation among highly endangered *Bacopa monnieri* (L) Pennell from southern India as detected using RAPD analysis *Genet Resour Crop Ev* **58** 769–782.

MARTIN F W & RHODES A M 1977 Intraspecific classification of *Dioscorea alata* *Trop Agri* **54** 1–13

MATHEW P J, MATHEW P M & KUMAR V 2001 Graph clustering of *Piper nigrum* L *Euphytica* **118** 257–264

RAI D, BHATIA G, PALIT G, PAL R, SINGH S & SINGH H K 2003 Adaptogenic effect of *Bacopa monniera* (Brahmi) *Pharmacol Biochem Be* **75** 823–830

RAJANI M 2008 *Bacopa monnieri* a nootropic drug *In* Ramawat K G & Merillon J M (eds) *Bioactive molecules and medicinal plants* Berlin Heidelberg: Springer-Verlag pp 175–195

RAMESH M, VIJAYAKUMAR K P, KARTHIKEYAN A & PANDIAN S K 2011 RAPD based genetic stability analysis among micropropagated synthetic seed derived and hardened plants of *Bacopa monnieri* (L): threatened Indian medicinal herb *Acta Physiol Plant* **33** 163–171

ROGERS D J & FLEMING H S 1973 A monograph of *Manihot esculenta* with an explanation of the taximetrics methods used *Econ Bot* **27** 1–113

SHARMA R 2004 *Agro-techniques of medicinal plants* Daya Publishing House New Delhi

SINGH R H & SINGH L 1980 Studies on the anti-anxiety effect of the medyha rasayana drug Brahmi (*Bacopa monniera* Wettst) *Res Ayur Siddha* **1** 133–148

SUBRAMANIAN A & SUBBARAMAN N 2010 Hierarchial cluster analysis of genetic diversity in maize germplasm *Electronic J Plant Breed* **1** 431–436

SZEKELY G J & RIZZO M L 2005 Hierarchical clustering via joint between-within distances: Extending Ward's minimum variance method *J Classif* **22** 151–183

TOMSONE L, KRUMA Z, ALSINA I & LEPSE L 2012 The application of Hierarchial cluster analysis for classifying horseradish genotype (*Armoracia rusticana* L) roots *Chemine Technol* **4** 52 –56

TRIPATHI N, CHOUHAN D S, SAINI N & TIWARI S 2012 Assessment of genetic variations among highly endangered medicinal plant *Bacopa monnieri* (L) from central India using RAPD and ISSR analysis *Biotech* **2** 327–336

TRIPATHI Y B, CHAURASIA S, TRIPATHI E, UPADHYAY A & DUBEY G P 1996 *Bacopa monniera* Linn as an antioxidant: mechanism of action *Ind J Exp Biol* **34** 523–526

WRITH M, EASTABROOK G F & ROGERS D J 1966 A Graph theory model for systematic biology with an example for the Oncidiinae (Orchidaceae) *Syst Zool* **15** 59–69