Exploration, collection, morphological and biochemical evaluation of brahmi (*Bacopa monniera* wettst.)

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SUMMARY

Twenty-nine accessions of *Bacopa monniera* collected from different eco-geographical locations were examined for genetic variability carried in them. The accessions were grown in pots, arranged in completely random block design, replicated twice, and observed for eight quantitative characters and bacoside-A content in the herbage. All the accessions were distinctly different irrespective of the geographical locations from where they were collected. The accessions were grouped into five clusters and the accessions showed no parallelism between clusters and geographical distribution. The bacoside-A content of the herbage was found to be low in the accessions possessing high internodal length and more number of flowers. Positive correlations for bacoside content was observed with shoot length, leaf length, leaf width, leaf area and biomass. The accessions, which flowered late, had good bacoside-A content. The accessions collected from Ernakulam district recorded good bacoside content when compared to accessions from other districts. The accession from Delhi recorded very trace amount of bacoside-A content. Bacoside content and biomass, which are very important for ayurvedic practioners had high heritabilities indicating the consistency of these two irrespective of the effect of the environment. It can be inferred that both the gross agroclimatic environment of the region and microenvironment in the vicinity of the water bodies where *Bacopa monniera* genotypes occur will regulate its growth and the content of bacoside-A.

Key words : Brahmi

The medicinal plant *Bacopa monniera*, commonly called Brahmi or water hyssop, predominantely used in ayurveda, a holistic system of medicine originating from India. *Bacopa monniera* is also known as *Gratiola monniera*, *Herpestis monniera* and *Moniera cuncifolia* (Family Scrophulariaceae). It has been classified and medharasayana i.e medicinal plant, rejuvenating intellect and memory (Hule and Juvekar 2009). It is profusely branched herb with white to violet coloured flowers whose populations spread both vegetatively and by seeds near river banks, ditches (Shalini *et al*., 2003, Jha *et al.*, 2005). Traditionally *Bacopa* was used as brain tonic to enhance memory, used in insanity, epilepsy, hysteria, blood cleanser and in treating inflammations , chronic skin diseases, high fevers, hair loss and high/low blood pressure (Chopra *et al.*, 1956; Sharan and Khare, 1991; Khann and Ahamed, 1992; Moharana and Moharana, 1994; Dar and Channa, 1997). Extracts of *Bacopa* possess a significant anticholinestease and antidementic properties useful in treating of dementia (Shanker *et al.*, 2002) leprosy, anemia, epilepsy and has anti cancerous activity (Shanmugasundaram *et al.*, 1991; Singh and Dhawan 1997) and anti oxidant properties (Elangovan *et al.*, 1995).

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MATERIALS AND METHODS

Accessions of brahmi (*Bacopa monniera*) were collected from different geographical locations of Kerala and these accessions were raised in pots filled with potting mixture. Transplanting of cuttings in the pots was done on 15th October, 2005. A total of 28 accessions were collected from Kerala and one was obtained from Delhi (Table 1). These accessions were replicated twice in Randomized Block Design (RBD). Each pot was planted with three cuttings of 10cm long of the same accession with 10cm distance between them (triangular fashion). All the accessions were initially observed to record the morphological characters. After flowering the accessions were harvested and the harvested material was shade dried for using in biochemical analysis.

Qualitative characters:

The 29 accessions of brahmi were critically observed at different growth stages to note the following morphological variabilities. The morphological characters noted were shoot length, leaf length, leaf width, leaf area,

internodal length, number of leaves and stem colour. Reproductive biology of the plant was also studied for floral biology, anthesis, days to flowering and flower development, pollen viability, seed setting and seed germination.

Quantitative characters:

Biochemical analysis:

Bacoside-A was estimated in all the accessions by High Performance Thin Layer Chromatography (HPTLC) method.

For the analysis samples were shade dried and then finely powdered using grinder. 10g of finely powdered sample from each accession were exhaustively extracted with methanol on a rotary shaker. Extraction was carried out until the solvent became colourless. The extract was concentrated to 25ml by keeping in a water bath at 33^0C. The 25ml methanolic extracted was successively partioned with petroleum ether and chloroform. The extracts (methanolic and chloroform) were taken for the analysis of bacoside-A. From initial screening studies for bacoside-A, it was found that bacosides were present only in methanol fraction. So the methanol fraction was taken for further studies for estimation of bacoside-A by HPTLC.

Analysis of bacoside-A in samples:

Keeping the chromatographic parameters constant, the analysis of bacoside-A in the samples was carried out. Ten micro liters from each sample solution was spotted in triplicate on precoated silica gel 60GF254 TLC plate and scanned as mentioned above. The peak areas were recorded and the amount of bacoside-A present in the samples was compared.

Tabulation and statistical analyses:

Observations on the biometric characters and biochemical constituent were tabulated by taking mean of the observation value. The accessions were ranked according to Duncans' Multiple Range Test (DMRT) as suggested by Duncan (1955). Heritability, genetic advance, phenotypic coefficient of variation and genotypic coefficient of variation were calculated by using SPAR1, a statistical program. Clustering was done by using QBASIC program. Genetic parameters like phenotypic variance, genotypic variance, phenotypic and genotypic coefficients of variation, heritability, expected genetic advance and phenotypic and genotypic correlation coefficients were estimated as per the procedure suggested by Burton (1952)

Table 1 : Details of brahmi accessions collected

Acc.No	Place of collection	District/ Region
1	Shornur	Palakkad
2	Changaramkulam	Malappuram
3	Aluva-2	Ernakulam
4	Chuvannamannu-1	Thrissur
5	Peringulam	Trivandrum
6	Kadampuzha	Malappuram
7	Aduvassery-3	Ernakulam
8	Kuttipuram	Malappuram
9	Chuvannamannu-2	Thrissur
10	Vellanikkara-1	Thrissur
11	Vellanikkara-3	Thrissur
12	Aduvassery-2	Ernakulam
13	Ollur	Thrissur
14	Kottakkal	Malappuram
15	Valanchery	Malappuram
16	Pulamanthole	Malappuram
17	Kalpatta	Wayanad
18	Delhi	Delhi
19	Calicut	Calicut
20	Vallikkavu	Kollam
21	Paravur	Ernakulam
22	Aluva-3	Ernakulam
23	Thuruthissery	Ernakulam
24	Ambalavayal	Wayanad
25	Aduvassery-1	Ernakulam
26	Thamarassery	Wayanad
27	Cherpu	Thrissur
28	Vellanikkara-2	Thrissur
29	Aluva-1	Ernakulam

RESULTS AND DISCUSSION

Assesment of the variability available in the plant species is a prerequisite of any breeding programme. Detailed investigation has been carried out in different districts of Kerala and 29 accessions representing various eco-geographical locations have been collected and maintained for the study. The data for various morphological and biochemical traits were statistically analysed and results obtained are presented under various subheads.

Morphological Diversity

The analysed data on vegetative characters namely shoot length, leaf length, leaf width, leaf area, internodal length and stem colour presented in Table 2 revealed significant variations among the accessions. Shoot length in the 29 accessions ranged from 58.42(Accession 9) to 68.63 cm (Accession 18). The accessions 29, 28, 11 and 6 were homogenous with accession 18 with regard to

Table 2: Table showing the means of the observations of the morphological traits

Accessions	Shoot length (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	No. of flowers	Internodal length (cm)	No. of leaves	Biomass (g)
1	66.05	2.070	0.94	1.946	19.13	0.88	1786.4	137.5
2	65.68	1.940	0.94	1.824	19.63	0.76	1843.9	130.0
3	62.93	1.795	0.90	1.623	29.75	1.96	2190.0	193.0
4	59.00	1.960	0.92	1.808	20.63	1.31	1919.2	287.5
5	65.10	1.990	0.85	1.695	20.00	1.91	1758.7	187.0
6	66.65	1.945	0.96	1.871	22.00	1.46	1753.5	167.5
7	60.80	1.930	0.90	1.737	27.88	1.76	1803.9	139.5
8	60.63	2.175	1.07	2.328	23.75	1.41	2204.3	126.0
9	58.42	1.915	0.97	1.858	21.38	1.36	2433.6	229.0
10	65.77	1.795	0.73	1.332	17.38	1.89	2095.1	106.5
11	66.72	1.885	0.81	1.537	18.50	2.11	2288.8	128.5
12	64.80	1.695	0.85	1.451	21.88	1.95	1830.4	129.5
13	65.17	1.850	0.73	1.360	18.00	2.18	1826.6	172.6
14	63.15	1.855	0.89	1.666	12.75	1.23	1837.2	210.6
15	65.88	1.930	0.94	1.814	12.00	1.33	1904.7	146.3
16	63.17	1.920	0.85	1.641	22.00	1.31	2187.9	295.0
17	63.30	1.795	0.78	1.411	0.875	1.56	2080.5	140.7
18	68.63	1.445	0.54	0.782	24.63	1.46	2404.0	276.9
19	63.38	1.965	0.95	1.885	22.63	1.55	2228.1	254.1
20	63.08	2.015	0.75	1.512	21.75	1.33	2408.7	337.0
21	62.72	1.385	0.58	0.805	17.00	1.59	2393.4	315.2
22	63.50	1.755	0.87	1.527	23.00	1.69	2134.8	322.5
23	63.18	1.870	0.87	1.627	18.63	1.71	1805.2	207.0
24	61.77	1.980	0.85	1.693	1.750	1.40	2120.0	220.5
25	64.78	2.045	0.97	1.982	13.013	1.60	1614.4	259.3
26	64.78	1.930	0.94	1.814	2.125	1.44	2302.9	217.7
27	65.85	1.880	0.78	1.467	11.38	1.45	2008.0	177.4
28	67.30	2.015	0.80	1.623	16.38	1.82	2196.1	209.8
29	68.22	1.930	1.00	1.930	14.38	1.83	2286.6	308.4
C.D. (P=0.05)	2.05	0.214	0.129	0.383	5.45	0.579	185.68	247.6

Values are mean of two replications

shoot length. The variability in leaf length ranged from 1.385 cm to 2.175 cm. The accession 8 recorded high leaf length (2.175 cm). Lowest leaf length was recorded in accession 21. Accession eight which recorded maximum leaf length also recorded maximum leaf width followed by accessions 29, 25, 9 and 6 with negligible difference between them. The accession which recorded lowest leaf width was 18 followed by 21 and 13. Leaf width in accessions ranged from 0.54 cm to 1.07 cm. Most of the accessions had leaf width between 0.94 cm to 0.78 cm. The calculated leaf area in accession 8 was high compared to other accessions (2.328 cm^2). The leaf area of accession 18 recorded the least (0.782 cm^2). With regard to leaf area accession 8 is homogenous with accessions 25, 1, 2 and 9. Most of the accessions recorded between 1.332 cm^2 and 1.885 cm^2. Number of flowers varied significantly in all the accessions.

Number of flowers in the accessions varied between 0.8750 and 29.75. The accession 3 recorded higher number of flowers and the accession 17 recorded the lowest number of flowers. Accessions 7 and 18 also recorded higher number of flowers. Accessions 8, 24 and 26 also recorded lower flower number. The internodal length in the 29 accessions ranged from 0.766 to 2.18 cm. Maximum internodal length was recorded in the accession 13. Most of the accessions recorded high internodal length (11, 3, 12, 5, 10, 29, 28, 7, 22, 23, 25, 21, 17 and 19). Accessions 1 and 2 had low internodal length with latter having the lowest. Number of leaves per plant ranged from 1614.4 to 2433.6. Accession 9 recorded maximum number of leaves where as accession 25 recorded lower number of leaves. Other accessions which recorded more number of leaves are 20, 18, 21, 26, 11, 29 and 19. Stem colour in all the accessions were green at

maturity. In some accessions shoots showed anthocyanin pigmentation during the early stages of growth. Anthocyanin pigmentation was lost when shoots matured. Biomass produced by accession 20 was found to be high (337 g/plant). Accession 10 recorded lowest biomass (106.5 g). Accessions 29, 22 and 21 also recorded high biomass.

Floral biology:

Flowers were pale violet, axillary, solitary on long pedicels; calyx 5-partite, lobes unequal; corolla gamopetalous, funnel like, white with purple blotches; stamens four, didynamous and ovary was two chambered. The 29 accessions collected from different eco-geographical locations of Kerala did not show much variability in flower colour. Flower colour in the accessions was pale violet with purple blotches on corolla. 18 accessions recorded minimum values for days to flowering (106) while accession 17 recorded the maximum value (143), followed by accession 24 which recorded 136 days to flowering. Accessions 1, 4, 16, 21, 22 and 25 had flowered 121 days after transplanting. Duration of anthesis was almost same in all the accessions *i.e.* flower buds took 48 hours for complete opening. Pollen was viable in all the accessions. The acetocarmine test showed that all the accessions had viable pollens. Seed setting and seed germination studies were not investigated as the crop was harvested before seed setting. To analyse the bacoside-A content in each of the accessions by HPTLC method the crop was not grown till seed setting.

Biochemical analysis:

For the 29 accessions chloroform and methanol extracts were prepared. HPTLC analysis showed that the saponins were present only in methanolic extract and not in chloroform extracts (Table 3). The graphs showing no absorbance or peaks for chloroform extracts are given. The important chemical constituent of *Bacopa monniera* is bacoside-A, its presence was seen in methanolic extracts of the plant sample. The HPTLC analysis of the 29 accessions showed significant difference in bacoside-A content. The bacoside-A concentration in the accessions ranged from 0.47% (w/v) to 5.4% (w/v). Accessions which recorded high bacoside-A content were 14 and 29. The accession which recorded lowest was 12. Bacoside to biomass ratio in per cent is given in (Table 3). Accession 14 had high (2.564%) bacoside to biomass ratio followed by accession 6 (2.089%) and 1 (2.036%). The accession which recorded low bacoside to biomass ratio is 20 (0.445%).

Table 3 : Showing the bacoside-A content in % (w/v) and the ratio of bacoside to biomass

Accessions	Days to flowering	Bacoside-A content (% w/v)	Bacoside/Biomass (%)
1	121	2.80	2.036
2	106	2.25	1.730
3	106	3.45	1.787
4	121	2.35	0.817
5	106	0.85	0.454
6	106	3.50	2.089
7	106	1.50	1.075
8	106	1.65	1.309
9	106	1.30	0.567
10	106	1.00	0.938
11	106	1.00	0.778
12	106	0.47	0.366
13	106	1.25	0.724
14	106	5.40	2.564
15	106	1.40	0.956
16	121	1.65	0.559
17	143	2.75	1.954
18	106	1.20	0.443
19	106	3.05	1.200
20	106	1.50	0.445
21	121	2.05	0.650
22	121	3.20	0.992
23	110	3.75	1.811
24	136	1.10	0.498
25	121	3.55	1.369
26	115	2.40	1.102
27	117	1.65	0.930
28	106	2.15	1.024
29	106	5.40	1.750
C.D. (P=0.05)	97.3	0.61	0.061

Values are mean of two replications

Variability studies:

The variability parameters studied for the different characters are presented in Table 4. Among the nine characters studied wide range was noticed for number of leaves followed by biomass and number of flowers. While narrow range was noticed for leaf width, internodal length and leaf area. For all the characters *i.e.* shoot length, leaf length, leaf width, leaf area, number of flowers, internodal length, number of leaves, biomass, and bacoside-A the phenotypic coefficient of variation was greater than genotypic coefficient of variation indicating influence of environment. Maximum phenotypic coefficient of variation was recorded in bacoside-A content (56.50%) followed by number of flowers (41.30%). The phenotypic coefficient of variations for biomass, internodal length,

Table 4 : Showing the variability parameters

Character	Range	P.C.V (%)	G.C.V (%)	Heritability (%)	G.A	G.G (%)
Shoot length	58.42 to 68.63 cm	4.05	3.74	85.62	4.56	7.1
Leaf length	1.38 to 2.17 cm	9.56	7.71	66.09	0.24	12.73
Leaf width	0.54 to 1.07 cm	14.41	12.49	75.93	0.19	22.06
Leaf area	0.78 to 2.32 cm^2	20.71	17.63	73.32	0.51	31.09
No. of flowers	0.87 to 29.75	41.3	38.47	87.17	13.09	73.81
Internodal length	0.76 to 2.18 cm	24.7	16.82	47.74	0.37	23.61
No. of leaves	1614.40 to 2433.60	12.05	11.21	87.04	442.04	21.49
Biomass	337.00 to 106.50 g	33.47	32.96	97.08	139.03	66.87
Bacoside-A	0.47 to 5.4% w/v	56.5	54.93	94.72	2.49	110.12

where- P.C.V and G.C.V indicate phenotypic and Genotypic coefficients of variation
G.A- Genetic advance, G.G- Genetic gain

leaf area, leaf width, number of leaves, leaf length and shoot length were 33.47%, 24.70%, 20.71%, 14.41%, 12.05%, 9.56%, 4.05%, respectively. The genotypic coefficient of variation was maximum for bacoside-A content 54.93%. The genotypic coefficients when ranked showed similar results as that of phenotypic coefficient of variation. High genotypic and phenotypic coefficients were observed in case of bacoside-A, number of flowers, biomass, internodal length and leaf area. Medium/ moderate genotypic and phenotypic coefficients of variation were observed for leaf width and number of leaves. Low genotypic and phenotypic coefficients of variation were observed in case of leaf length and shoot length.

Heritability was maximum for biomass (97.08%) followed by bacoside-A (94.72%). Heritabilities for number of leaves, shoot length, leaf width, leaf area and leaf length were 87.04%, 85.62%, 75.93%, 73.32%, 66.09%, respectively. Heritability for the character internodal length was low (47.74%) when compared with other characters. The characters having heritability values between 60-100% are regarded to have high heritability. Here biomass, bacoside-A content, number of leaves, shoot length, leaf width, leaf area and leaf length have high heritability. Where as medium heritability was recorded for internodal length *i.e.* between 30-60%. Genetic advance when expressed in per cent is genetic gain. High genetic gain was observed for bacoside-A content (110.12%) and was more when compared to other characters. The other characters which had high genetic gain were number of flowers (73.81%), biomass (66.87%), leaf area (31.09%), internodal length (23.61%), leaf width (22.06%), number of leaves (21.49%). However, moderate genetic gain was observed for leaf length (12.73%) and low genetic gain for shoot length (7.10%).

Cluster analysis:

Based on the vegetative characters *viz*., shoot length, leaf length, leaf width, leaf area, number of flowers, internodal length, number of leaves, biomass and biochemical evaluation the 29 accessions were grouped into five clusters (Table 6). Cluster I had the maximum number of accessions (15) followed by cluster V (6). Clusters II and III have three accessions each. Cluster IV has two accessions.

The cluster means of the characters are given in Table 7. The inter and intra cluster distances are given in Table 8. The intra cluster distance in cluster I was 69.25, in cluster II was 70.21, in cluster III was 40.84, in cluster IV was 70.19 and in cluster V was 157.15. The inter cluster distance between I and II was 321.82, between I and III was 227.24, between I and IV was 574.28, between I and V was 170.91, between II and III was 485.18, between II and IV was 822.87, between II and V was 349.99, between III and IV was 760.06, between III and V was 321.91 and between IV and V was 699.40. The average intra cluster distance was 103.39.

Correlation studies:

The phenotypic and genotypic correlations between the characters and the bacoside-A content are given in (Table 5). Only few characters showed significant correlation between them. In all the significant correlations, genotypic correlations were higher than phenotypic correlations. The significant phenotypic correlations were between leaf length and leaf width (0.720), leaf length and leaf area (0.881), leaf width and leaf area (0.957) and between number of leaves and biomass (0.426). The significant genotypic correlations were also between the same characters but with higher values. Between leaf length and leaf width it was 0.809, leaf length and leaf area is 0.910, leaf width and leaf area was 0.975, and between number of leaves and

Table 5: Phenotypic and genotypic correlations for bacoside-A yield

	Shoot length	Leaf length	Leaf width	Leaf area	No.of. flowers	Internodal length	No.of leaves	Biomass	Bacoside-A
Shoot length		-0.125	-0.236	-0.208	-0.136	0.157	-0.088	-0.135	0.09
Leaf length	-0.234		0.720*	0.881*	-0.055	-0.195	-0.323	-0.203	0.106
Leaf width	-0.321	0.809*		0.957*	0.016	-0.251	-0.342	-0.189	0.369
Leaf area	-0.314	0.910*	0.975*		0	-0.268	-0.347	-0.203	0.29
No.of. flowers	-0.11	-0.115	0.019	-0.021		0.14	-0.003	0.078	-0.086
Internodal length	0.223	-0.311	-0.226	-0.303	0.144		0.082	-0.061	-0.118
No.of leaves	-0.067	-0.282	-0.329	-0.306	0.029	0.026		0.426*	-0.151
Biomass	-0.151	-0.266	-0.226	-0.248	0.066	-0.095	0.457*		0.293
Bacoside-A	0.09	0.102	0.404	0.308	-0.103	-0.181	-0.155	0.306	

* indicates significance of value at P=0.05

Values on the upper diagonal represent phenotypic correlations and genotypic correlations on lower diagonal

Table 6 : Accessions grouped in to clusters

	Accessions	Total number of accessions
Cluster I	1, 4, 7, 8, 9, 12, 16, 17, 19, 20, 24, 25, 27, 28 and 29	15
Cluster II	6, 14, and 22	3
Cluster III	5, 13 and 21	3
Cluster IV	15 and 23	2
Cluster V	2, 3, 10, 11, 18 and 26	6

biomass it was 0.457. Leaf area showed significant positive correlation with leaf width in phenotypic and genotypic correlations followed by leaf length. Bacoside-A content though not significant, but it was positively correlated with biomass in both phenotypic and genotypic correlations.

In any breeding programme, assessment of variability existing in the germplasm is the prerequisite for the genetic improvement of species under domestication. 29 accessions of brahmi (*Bacopa monniera*) collected from different eco-geographical locations of Kerala were assessed for the genetic variability for therapeutically important morphological characters and its biochemical constituents. The variability observed for eight morphological characters has been shown quantitatively and that for biochemical constituent, which was measured quantitatively by using HPTLC method is also discussed. The variability with regard to shoot length found in the accessions is similar to the observations made by Rajesh (1994) and Jamwal and Kaul (1997) in *Andrographis paniculata* accessions collected from different districts in Tamil Nadu. The variability found in leaf characteristics such as leaf length, leaf width and leaf area are in accordance with the findings of Datta and Mukerji (1952) in *Andrographis paniculata*.

The accessions, which recorded high bacoside-A content had medium shoot length, medium leaf length, high leaf width, less internodal length, medium number of flowers and more number of leaves. From the above observations it can be said that the internodal length should be minimum and the leaf width should be on higher side for the plant to have good bacoside-A content. Stem colour was observed during different growth stages of the plants in all the accessions collected. Anthocyanin pigmentation was observed on the stems in all the accessions a few days after transplanting. However, the pigmentation disappeared in the later stage of crop growth. Such changes in colouration was also reported by Paul (2000) in *Andrographis paniculata*. In Indian system of medicine the whole plant of *Bacopa monniera* is often harvested and used (Farooqi *et al*., 1999; Aiyar and Kolammal, 1962). In the present study, the total biomass obtained from each accession was recorded because the Ayurvedic practioners use freshly harvested whole plant for the preparation of the drug by extracting the important chemical constituent *i.e*. bacoside-A. The bacoside content in a quickly dried material was found to be higher as compared to material dried by the traditional method in some preliminary experiments in laboratory..

Flowers in the accessions were pale violet, axillary, solitary on long pedicels, calyx 5, gamosepalous, lobes unequal: corolla gamopetalous, funnel like, white with purple blotches; stamens four, didynamous, with ovary

Table 7: Cluster means of the characters

	Cluster I	Cluster II	ClusterIII	Cluster IV	Cluster V
Shoot length	63.38	64.43	64.33	64.53	65.75
Leaf length	1.96	1.85	1.74	1.9	1.8
Leaf width	0.9	0.91	0.72	0.9	0.81
Leaf area	1.76	1.69	1.29	1.72	1.49
Number of flowers	18.42	19.25	18.33	15.31	18.67
Internodal length	1.5	1.46	1.89	1.52	1.6
Number of leaves	2073.4	1908.5	1992.9	1854.95	2187.45
Biomass	222.18	233.53	224.93	176.65	175.43
Bacoside-A	2.15	2.83	1.38	2.57	1.88

two chambered as described by Datta and Mukerji (1952). However, the purple patches in the upper lip of corolla showed little variation.

Days to flowering varied among the accessions. This variation in flowering habit may be attributed to the eco-geographical separation. Moreover, in some cases, the accessions collected from the same district showed difference in days to flowering. This may be attributed to the climatic conditions prevailing in their respective locations.

The important chemical constituents isolated and characterized from the alcoholic extract are saponins with jujubogenin and pseudojujubogenin as the aglycones, including bacoside-A (Jain and Kulshreshtha, 1993; Rastogi *et al*., 1994; Rastogi and Kulshreshtha, 1999).

These accessions did not have good amount of bacoside content, as indicated by bacoside/biomass ratio. The accessions where the biomass production is less and the bacoside content is not very high, bacoside/biomass ratio gives a false impression of that accession. The accessions 1 and 6 had good bacoside content but the biomass production was not good. Hence, the bacoside/ biomass ratio is on higher side. Even if the bacoside content in the accession is low, accession should be able to give more biomass which will compensate the low bacoside –A content.

Variability studies:

The phenotypic coefficient of variation for the observed traits is greater than genotypic coefficient of variation for the same traits. Phenotypic coefficient of variation was highest for the bacoside-A followed by number of flowers and biomass. Phenotypic coefficient of variation was very low for shoot length, leaf length, number of leaves and leaf width. Same was the case with genotypic coefficient of variation.

If the phenotypic coefficient of variation is higher than genotypic coefficient of variation, it means that the apparent variation is not only due to genotypes but also due to the influence of environment. Selection for such traits sometimes may be misleading (Falconer, 1989).

Heritabilities for characters like shoot length, leaf length, leaf width, leaf area, number of flowers, internodal length, number of leaves, biomass and bacoside-A show that, Biomass with 97.08% heritability stand first and closely followed by bacoside-A with 94.72% (Deepak *et al*., 2005).

The value of heritability in broad sense is high, it indicates that though the character is least influenced by the environmental effects, the selection for improvement of such character may not be useful, because broad sense heritability is based on the total genetic variance which includes both fixable (additive) and non fixable (dominant and epistatic) variances (Singh and Narayanan., 2000). In the same lines, biomass and bacoside-A content, which have high heritabilities were not influenced by environmental effects.

Broad sense heritability value for internodal length (47.74%) was very less as compared to other characters. If broad sense heritability is low, it reveals that the character is highly influenced by environmental effects and genetic improvement through selection will be difficult due to masking effects of the environment on the genotypic effects (Singh and Narayanan, 2000).

Genetic advance is improvement in the mean genotypic value of selected plants over the parental population (Allard, 1960). For all other characters the genetic gain values ranged between 7-24%.

The value of genetic gain is high, it shows that the character is governed by additive genes and selection will be rewarding for improvement of such trait (Singh and Narayanan, 2000).

When a character has high heritability and genetic gain values, it indicates that most likely the heritability is due to additive genes and selection may be effective (Singh and Narayanan, 2000).

Cluster analysis:

From inter and intra cluster distances, it was observed that the intra cluster distances were usually smaller than inter cluster distances. The intra cluster in cluster III was minimum and in cluster V it was maximum. The inter cluster distances are a measure of genetic distinctness among the clusters based on observed variation in all the characters studied: Larger the distance, more the difference between the clusters. Maximum inter cluster distance was found between cluster II and cluster IV closely followed by distance between cluster III and cluster IV and cluster IV and cluster V. This indicates that members of these groups were very distinct from each other. Similar kind of clustering was done in brahmi by Shalini *et al*. (2003). The accessions collected from different districts were in one cluster instead of different clusters. Cluster I has accessions from all the districts from where the accessions were collected except Trivandrum and Delhi. This shows that there is no parallelism between geographic regions from where the accessions were collected and in its clustering pattern.

Correlation studies:

In brahmi the selection of accessions for cultivation is done based on the bacoside-A content, as it is the main biochemical constituent, used in ayurvedic preparations, which cures many disorders. The characters, which are positively correlated to the bacoside-A content, should be observed for further improvement in bacoside content. Of the various characters observed, shoot length, leaf length, leaf width, leaf area and biomass were positively correlated with bacoside-A content. Out of these characters only biomass had high positive correlation with bacoside-A content when compared to the correlations of other characters that were positively correlated with bacoside-A content.

Characters like internodal length, number of flowers and number of leaves were negatively correlated to bacoside-A content. Of these, number of flowers and internodal length were on higher side.

The accession should have good shoot length, leaf length, leaf width, leaf area and should give good biomass if it has to have good bacoside-A content. These characters mentioned should be good, at the same time the accession should have minimum internodal length and should have minimum number of flowers if it has to have more bacoside-A content, since these characters were negatively correlated to bacoside content. For the brahmi plant to have higher bacoside-A content, ideal plant type for increased bacoside content *i.e*., with less internodal length, big sized fleshy leaves and with late flowering habit. A search for seed setting and artificial production of seeds through hybridization for increasing the genetic variability can be suggested for future line of work.

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