EXPLORATION, COLLECTION AND EVALUATION OF BRAHMI (*Bacopa monniera* Wettst.)

By

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THESIS

submitted in partial fulfillment of the requirement
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DECLARATION

I hereby declare that this thesis entitled **Exploration collection and evaluation of Brahmi (*Bacopa monniera* Wettst)** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree diploma, fellowship or other similar title of any other University or Society

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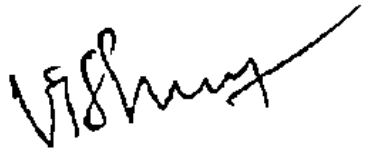

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CERTIFICATE

Certified that this thesis entitled **Exploration, Collection and Evaluation of Brahmi (*Bacopa monniera* Wettst)** is a bonafide record of research work done independently by **Mr Vishnu Vardhan Reddy Banda** under my guidance and supervision and that it has not previously formed the basis for the award of any degree diploma, fellowship or associateship to him.

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Introduction

INTRODUCTION

Kerala is considered as the homeland of ayurvedic medicine Warm humid tropical climate enjoyed by Kerala is endorsed with a rich biodiversity of various medicinal plant species Foot hills of Western Ghats spread over the eastern boundary of Kerala starting from Kanyakumari to Kasargoad Kerala has a rich biodiversity of various medicinal plant species Deforestation and uncontrolled exploitation of various medicinal herbs from forest area has paved way for the extinction of valuable medicinal plant species Many of the medicinal herbs having the required quality are not available due to the above factor and adulteration of collected material is a common phenomena. It is high time to ensure good quality medicinal herb species to be made available to ayurvedic drug manufacturers Identification of medicinal plant having the requisite quality and high productivity is the need for the iyurvedic drug industry as well as the consumers To supplement the dearth of the collected plant herbs now domestication and cultivation is the only alternative for sustaining the supply of the plant material to plant drug industry

The medicinal plant *Bacopa monniera* commonly called Brahmi is one of the important medicinal plant herb which is required in large quantities by the ayurvedic drug industry Brahmi is used as nervetonic to promote mental health and to improve memory and intellect It is a well known memory booster It is used against epilepsy hysteria, asthenia and nerve break down It also assists in feeling a greater sense of well being during period of restlessness anxiety insomnia, fatigue cloudiness of thought and overactive mind It is used as blood cleanser and is considered helpful for many inflammations chronic skin diseases high fevers hair loss and controls blood pressure (Dar and Channa, 1997) The juice of brahmi leaves mixed in milk improves mental ability and memory Brahmine is a major ingredient, used for preparation of large number of ayurvedic hair and massage oils The ayurvedic practioners also use this plant in urine disorders convulsions and in the treatment of asthma Brahmi is an important constituent of brahmighrita, a medicated ghee used in epilepsy

insanity and other low dynamic disorders (Kapoor 1990) Brahmi a succulent, small creeping herb with numerous branches oblong leaves light purple flowers belongs to family scorphulariaceae The chief constituents in this herb are brahmine herpestine alkaloids and saponins The saponins designated as bacoside-A, bacoside B betulic acid D manitol, stigmastanol, stigmasterol and β sitosterol

Preliminary survey indicates that a lot of variability in brahmi for many of the morphological traits With increasing demands of this herb natural variability existed in this herb has been over exploited leading to depletion of this variability Very little work has been done for assessing its natural variability

The present study was initiated with the following objectives

1 To explore and collect the natural variability existing in the different eco geographical regions of Kerala.

2 To evaluate the collected accessions for high biomass and quality especially bacoside A content.

Review of literature

REVIEW OF LITERATURE

Bacopa monnıera also known as Water Hyssop ıs prominently used ın Ayurveda a holıstıc system of medıcıne orıgınatıng from Indıa *Bacopa monnıera* ıs also called brahmı a name derıved from Brahma, the creator God of the Hındu panthem of deıtıes It ıs celebrated for ıts dıversıty of usage It ıs saıd that the use of brahmı for memory enhancement goes back 3000 years or more ın Indıa, when ıt was cıted for ıts medıcnal propertıes especıally the memory enhancıng capacıty ın the vedıc texts Athar Ved Samhıtha of 800 B C and ın Ayurveda

2 1 Orıgın and dıstrıbutıon

Bacopa monnıera a member of the scrophulanaceae famıly ıs a small creepıng herb wıth numerous branches small oblong leaves and lıght purple flowers In Indıa and ın the tropıcs ıt grows naturally ın wet soıl shallow water and marshes The herb can be found at elevatıons from sea level to altıtudes of 4 400 feet and ıs easıly cultıvated ıf adequate water ıs avaılable In the West, brahmı ıs famılıar as a water plant used ın aquarıums It can be easıly grown ın damp areas and can be propagated by seed and also through cuttıngs (Jha *et al* 2005)

2 2 Descrıptıon

Bacopa monnıera ıs a small creepıng some what succulent herb The leaf and flower bearıng stems are 30 70 cm long and arıse from creepıng stems that forms roots at the nodes The growth habıt of brahmı therefore resembles that of peppermınt The leaves are sımple obovate oblong opposıte approxımately 2 x 1 cm wıth entıre margıns Flowers are blue or whıte wıth purple veıns solıtary on long pedıcels ın the leaf axıls The corolla ıs fıve lobed whıte or pınkısh wıth purple blotches The fruıt ıs an up to 5mm capsule whıch develops ın the persıstent calyx (Jha *et al* 2005)

2 3 Chemical constituents

The chief constituents are brahmine herpestine alkaloids and saponins The saponins designated as bacoside A, bacoside B betulic acid D mannitol stigmastanol β sitosterol and stigmasterol have been isolated Bacoside–A, on hydrolysis gives three sugars two of which have been identified as glucose and arabinose (Ahmed *et al* 2000)

2 4 Traditional medicinal uses

Brahmi belongs to a group of medicinal plants classified as medyarasayana in ayurveda these are nervine tonics used to promote mental health and improve memory and intellect In the folklore of Indian medicine several herbs have been used traditionally as brain or nerve tonics One of the most popular of these neurotonics is *Bacopa monniera* a well known memory booster Brahmi has been administered at religious institutions to help students enhance their memory for learning ancient, religious hymns Direct cardiotonic tranquiliser and sedative improves process of learning, restores memory enhances power of speech and imagination diuretic and nervine tonic anti stress for nervous and mental strain, use in insanity epilepsy hysteria, asthenia and nervous break down Taking brahmi can assist in feeling a greater sense of well being during period of restlessness anxiety insomnia fatigue cloudiness of thought overactive mind It is also used as blood cleanser and is considered helpful in treating inflammations chronic skin diseases high fevers and hair loss It also controls blood pressure (Chopra et al 1956 Sharan and Khare 1991 Moharana and Moharana, 1994 Khanna and Ahamed 1992 Dar and Channa 1997)

2 5 Pharmacological activities

The investigations on the adaptogenic property of a standardized extract of *Bacopa monniera* against acute stress and chronic stress in rats has been performed

(Rai *et al* 2003) Studies shown that the standardized extract of *Bacopa monniera* possess a potent adaptogenic activity

Phosphatidylserine acetyl l carnitine vinpocetine *Ginkgo biloba* extract and *Bacopa monniera* extract have anti anxiety anti fatigue and memory enhancing effects These five substances offer interesting contributions to a personalized approach for restoring cognitive function with judicious application of growth factors (Kidd 2002)

Bacopa monniera and *Ginkgo biloba* are well known cognitive enhancers in Indian and Chinese traditional medicine systems Standardized extracts of *Bacopa monniera* and *Ginkgo biloba* were used to evaluate the antidementic and anticholinesterase activities in adult male swiss mice These extracts possess a significant anticholinesterase and antidementic properties which may be useful in the treatment of dementia (Shanker *et al* 2002)

The plant extract of *Bacopa monniera* is known to heal leprosy and cure anemia, epilepsy and has anti cancerous activity (Shanmugasundaram *et al* 1991 Singh and Dhawan, 1997) It is also reported to possess anti cancer and antioxidant properties (Elangovan *et al.* 1995)

The juice of brahmi leaves mixed in milk improves memory and adds to mental ability Brahmine is major ingredient, which helps for preparation of large number of ayurvedic hair and massage oils The ayurvedic practitioners also use this plant in nervous and urine disorders convulsions mental problems and in treatment of asthma The plant is an important constituent of brahmighrita a medicated ghee used in epilepsy insanity asthenia and other low dynamic disorders (Kapoor 1990) *Bacopa* is also effectively used to treat asthma bronchitis insomnia, gastric disorders (including ulcers) and calms children (Channa *et al* 2003)

In India adults and students take brahmi for better mental function and brahmi tea is even given to infants to encourage optimal mental development The

herb is popular among students for improving mental clarity confidence intelligence concentration and memory recall It is especially helpful to students with Attention Deficit Hyperactivity Disorder (ADHD) (Negi *et al* 2000)

In Australian double blind study 46 healthy adults were given either 300mg of *Bacopa* or a placebo After 12 weeks the group that took *Bacopa* had 13 per cent improvement in learning and memory rates But the most striking result was the significant reduction in anxiety in those who received *Bacopa* (Stough *et al* 2001)

Accordmg to the scientists at the Central Drug Research Institute a number of compounds have been identified in *Bacopa* including bacosides A and B two chemicals that improve the transmission of impulses between nerve cells in the brain These bacosides regenerate synapses and repair damaged neurons making it easier to learn and remember new information. *Bacopa* also increases serotonin levels a neurotransmitter that promotes relaxation (Rostagi *et al* 1994)

A recent study at the university of Catania, Italy found that anti oxidants in brahmi have a protective effect on human DNA fibroblasts (connective tissue cells) suggesting that this may be useful in the treatment of disorders in which free radicals play a key role (Russo *et al* 2003)

There is growing evidence that excessive concentration of nitric oxide generated with in overly activated brain cells might be involved in a variety of neurodegenerative diseases such as Alzheimers s disease and epilepsy A rodent study of brain cells exposed to toxic levels of nitric oxide showed that *Bacopa* inhibited the DNA damage that occurs in these diseases suggesting that it may be helpful in preventing or treating the neurodegenerative diseases (Nath *et al.* 2002)

The medicinal properties of the herb are attributed to the saponin bacoside A, which is present in all the parts of the plant (Kawai and Shibata 1978)

2 6 Morphological variability studies

Twenty seven accessions of *Bacopa monniera* collected from semi temperate subtropical and tropical environments at geographically distinct locations showed morphological variability when grown in a semi temperate environment in earthen pans Observations regarding quantitative and qualitative characters showed significant variability between the accessions (Shalini *et al* 2003)

Shalini *et al* (2003) evaluated different accessions of *Bacopa monniera* collected from different geographic locations in India and observed significant difference between the accessions in shoot length, leaf length leaf width, leaf area, internodal length and bacoside A content

Hedge *et al* (2003) conducted a replicated field evaluation trial involving 13 accessions of *Coleus forskohlii* a forskohn yielding herb with fasciculate tuberous roots Significant differences were recorded for all the characters except dry mass of roots The accession IIHR 80 with medium tuber yield and higher forskolin content can be promoted for commercial cultivation

Information about germplasm collecting sites may be an important additional data because those conditions are normally associated with the patterns of genetic variability The characterization of germplasm according to origin through GIS (Geographical Information System) is a tool that may help in understanding and accessing the genetic variability of large germplasm collections (Burle *et al* 2003)

A study shows that the Kattunaikka tribe can distinguish 21 taxa of which they consume 19 The local classification is based on morphology and appearance when processed for consumption while the botanical classification is based entirely on morphological characters The key characters adopted by the indigenous community for classifying members of *Dioscorea* are usually the eating quality and morphology of the underground tuber (Balakrishnan *et al* 2003)

Louati *et al* (2003) used enzyme electrophoresis to characterize genetic diversity and population structure in 10 populations of the *Hedysarum carnosum* They found genetic differences among populations caused by habitat fragmentat on from human land use which reduced population size and restricted gene flow

A germplasm collection, comprising 16 accessions of *Centella asiatica* from different locations in India were characterized using multivariate approaches The 16 accessions were found to harbour considerable variation and dispersed into 9 clusters/classes on the basis of principal component and canonical variate analysis of 18 quantitative traits It was hypothesized that the plant s ability to adapt successfully to a wide range of ecological conditions is the main factor underlying the extent of its genetic variability (Mathur *et al.* 2003)

Dwivedi *et al* (1999) studied 15 morphological traits in 26 genotypes of Periwinkle and observed variation in most of the characters including total herbage yield total leaf area, leaf area index and plant height

Fifteen accessions of *Centella asiatica* when studied for growth, herbage yield and active principle content showed significant variation (Singh *et al* 1999)

Field investigation of 41 genotypes of soyabean showed significant variation for days to 50 percent flowering and maturity leaf area/plant, plant height, root dry matter in yield total dry matter/plant and yield/plant (Mehetre *et al* 1998)

Genetic divergence among 37 accessions of Ashwagandha (*Withania somnifera*) were quantified for six characters namely plant height plant canopy leaf area root length, root diameter and dry yield Five accessions were identified as widely divergent from each other (Misra *et al* 1998)

Studies carried out on 52 germplasm collections of *Andrographis paniculata* at TBGRI showed that morphological variation observed in many accessions persisted generation after generation and it was identified as a medicinal plant showing prominent intraspecific variation (Seem *et al* 1998)

Growth analysis on castor cultivars showed significant difference for plant height, number of nodes leaf area index, dry matter production and in yield contributing factors (Reddy *et al* 1997)

Significant variation in plant height, number of leaves fresh weight/plant, dry weight/plant, pod length and number of seeds/pod were observed in different kalmegh accessions (*Anon* 1996)

Balu and Alagesaboopathi (1996) studied the morphometrics of the cuttings of kalmegh and observed variation for initial internodal length and maximum root length when observed 110 days after planting

A collection of 182 *Ocimum* accessions when evaluated for growth characteristics and yield components showed a wide range of variation within the accessions (Hammer *et al* 1996)

Studies on 18 collections of *Swertia chirayita* revealed that the plant height, number of branches/plant, number of leaves/plant, leaf area and root thickness were significantly correlated with herbage yield and bitter content in herb (Rastogi and Srivastava 1995)

Phadnis (1994) studied growth cycle of *Phyllanthus niruri* for 12 weeks and observed that major share of dry matter was concentrated in leaves and stem (75%) and that dry weight/fresh weight ratio varied with plant parts

Morphometric observations of Kalmegh taken from natural habitat in Salem revealed the following variation in plant characters Leaf length varied from 15 1 to 18 0 cm and breadth between 5 0 to 6 0 cm The basal leaf area varied between 452 3 to 1320 2 cm^2 The dry biomass of the individuals varied from 4 8 to 10 1g (Alagesaboopathi 1993)

2 7 Biochemical studies

The herb contains the alkaloids brahmine herpestatine ($C_{34}H_{46}N_2O_6$ m p 116 117°) and a mixture of three bases Brahmine is highly toxic when administered at a dose of 0 5mg/Kg body weight of cat as it produces a fall in the blood pressure In therapeutic doses it resembles strychnine The herb also contains saponins monnierin ($C_5H_{82}O_{21}$ $3H_2O$ m p 63°) hersaponin (m p 232 234°) bacoside A ($C_4H_6O_{13}$ $4H_2O$ m p 200°) and bacoside B ($C_4H_{68}O_3$ $5H_2O$ m p 203°) Monnierin on hydrolysis gives glucose arabinose and aglycone ($C_{30}H_{48}O_4$ m p 235 237°) Where as bacosides A and B give glucose arabinose and bacogenins A_1 A_2 A_3 and A_4 Bacogenins $A_1$and A_2 are epimers and A4 is an ebalin lactone Smith de Mayo degradation of bacoside A gave jujubogenin and pseudojujubogenin. Bacosides A and B possess haemolytic activity

Other constituents present in plant are D mannitol betulic acid b sitosterol stigmasterol and its esters heptacosin, octasane nonacosane triacontane hentriacontane dotriacontane nicotine 3formyl 4 hydroxy 2H pyran ($C_6H_6O_3$) luteolin and its 7 glucosides Presence of a alanine aspartic acid glutamic acid and serine are also reported

Apigenin 7 glucuronide and luteolin 7 glucuronide was isolated from leaves A minor saponin, bacoside A, isolated from leaves was characterized as 3 o a L arabinofuranosyl (1 3) a L arabinosyl jujubogenin, isolation of another saponin bacoside A_3 and its structure elucidated as 3 o b D glucosyl (1 3) o a L arabinofuranosyl (1 2) o b D glucosyl jujubogenin revision of structure of cis isomer of ebeline lactone obtained during acid hydrolysis as another artifact of jujubogenin (Ahmed *et al* 2000)

Alcoholic/hydroalcoholic extracts of the whole plant have been found to possess nootropic activity The major chemical constituents isolated and characterized from the alcoholic extract are dammarane type triterpenoid saponins with jujubogenin and pseudojujubogeninas the aglycones including bacosides A_1 A_3 (Chakravarthy *et al* 2003)

The nootropic activity of the extract has been attributed to the presence of two saponins namely bacoside A and bacoside-B of which the former is more important (Singh and Dhawan, 1997)

2 8 Need for establishing identities of bacoside-A and B

Many products derived from *Bacopa monniera* are available on the international market with labels that claim a specific content of bacosides A and B Surprisingly however the chemical identities of these two saponins have still not been established using modern spectroscopic methods Consequently there is an ambiguity in the quality control of *Bacopa monniera* based on the content of bacosides (Deepak and Amit, 2004)

2 9 Quantitative determination of bacoside-A

Preliminary studies with ^{13}C-NMR indicated that bacoside A was a mixture of saponins in accordance with an earlier report (Kawai and Shibata 1978) and not a single chemical entity as had been proposed (Chatterji *et al.* 1965)

Analytical High Performance Thin Layer Chromatography (HPTLC) of bacoside A gave four major peaks and the compounds corresponding to each peak were subsequently isolated by preparative HPTLC The isolated compounds were characterized as bacoside A_3 bacopaside II, jujubogenin (isomer of bacopasaponin C) and bacopasaponin C by comparison of the ^{13}C NMR spectral data of the isolated compounds with those in the literature values (Li *et al* 1999)

The presence of two common flavonoids namely luteolin and apigenin was confirmed in all samples of *Bacopa monniera* by comparison of retention times and on line UV spectra of the peaks (Greenham *et al* 2003) with those of reference standards

To meet the demands of industry there is need to cultivate *Bacopa monniera* variety that is rich in bacoside A and to standardize a post harvest processing

procedure for recovery of bacoside A in high amounts from harvested material A plant breeding programme to select *Bacopa monniera* variants rich in bacoside A needed a quick, accurate and inexpensive analytical procedure for the estimation of bacoside A in the extract of individual plants Although a spectrophotometric method (Pal and Sarin 1991) has been reported for bacoside A estimation the method lacks precision due to other compounds present in the extract having similar absorbance pattern in the same UV region. A High Performance Thin Layer Chromatography (HPTLC) procedure for the estimation of bacoside A in *Bacopa monniera* was developed for the detection of variation in the bacoside A contents in the *Bacopa monniera* herbage samples dried variously towards determination of an efficient post harvest procedure (Gupta *et al* 1998)

In other crops

Chauhan *et al* (1999) developed a precise and sensitive High Performance thin layer chromatography method for the estimation of andrographolide They found that the sensitivity was 0 1µg and linearity in the range 0 1 to 1 0µg

Farooqi *et al* (1999) reported a maximum of 2 5 percent andrographolide in leaves and a minimum of 2 percent in stem

Analysis of 52 germplasm collections of Kalmegh maintained under uniform conditions at TBGRI showed variation in andrographolide content at a range from 0 5 to 1 5 percent dry weight (Padmesh *et al* 1999)

Investigations carried out on collections of chirata revealed a great deal of variation ranging from 0 75 to 1 14 percent with respect to bitter content in the crop (Dutt *et al* 1999)

Sarma (1998) investigated the chemotaxonomy of 42 taxa of acanthaceae family and observed positive reaction for flavonoids in all taxa

Andrographolide content was variable in each plant part at different stages with maximum recorded in leaves and stem (*Anon* 1998)

Alagesaboopathi and Balu (1996) studied three different species of andrographis and reported the presence of flavonoids in all the species

Materials and Methods

MATERIALS AND METHODS

The present investigation on Exploration, Collection and Evaluation of *Bacopa monniera* (brahmi) was conducted in the Department of Plant Breeding and Genetics College of Horticulture Vellanikkara Thrissur Biochemical analysis using HPTLC was done in Sri Ramakrishna Institute of Paramedical sciences College of Pharmacy Coimbatore Crop was raised during the period from October 2005 to May 2006 The location is situated at an altitude of 40 29m above MSL at 10°31 N latitude and 76°13 E longitude

3 1 Materials

Accessions of brahmi (*Bacopa monniera*) were collected from different geographical locations in different districts 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 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

3 2 Methods

The 29 accessions of brahmi were critically observed at different growth stages to note the following morphological variabilities

3 2 1 Morphological characters

The morphological characters noted were shoot length leaf length, leaf width, leaf area internodal length, number of leaves and stem colour

Table 1 Details of brahmi accessions collected

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

General view of the crop after transplanting

General view of the crop after sixty days of transplanting

Accession from Delhi

3 2 1 1 Shoot length

Length of shoots was recorded at every 15 days interval The length of the shoots was measured by using measuring scale Shoot length was measured from the point where the plant touches the soil in the pot to the tip of the shoot Shoot length was recorded for three plants in a pot for all the accessions and expressed in cm

3 2 1 2 Leaf length

Twenty leaves were selected at random for each plant for taking observations related to leaf length By using a measuring scale leaf length for all the accessions was recorded for every 15 days Maximum length of leaf was taken as the observation and expressed in cm

3 2 1 3 Leaf width

The selected 20 leaves for measuring the leaf length were used for measuring the leaf width Maximum width of leaves was recorded using a measuring scale and expressed in cm

3 2 1 4 Leaf area

Leaf area was calculated for 20 leaves in each accession by using leaf area meter and expressed in cm^2

3 2 1 5 Internodal length

Internodal length for all the accessions was recorded by using a scale Top three internodal lengths were recorded for each plant in the pot. The average of these readings was taken as internodal length for each accession.

3 2 1 6 Number of leaves

Total number of leaves was counted for every 15 days in all the accessions The final leaf count was taken by taking the average of the last reading for all the accessions

3 2 1 7 Stem colour

Stem colour was recorded for all the accessions The stem colour was observed visually to find any anthocyanin pigmentation during the growth stages in all the accessions

3 2 2 Reproductive biology

Reproductive biology of the plant was studied Floral biology and anthesis were studied under reproductive biology

3 2 2 1 Floral biology

Flowering nature type of flowers variation of flower colour and number of flowers were studied throughout the growth of the plant

3 2 3 Anthesis

In anthesis time and duration of anthesis pollen viability seed setting and seed germination were studied

3 2 3 1 Days to flowering and flower development

Number of days taken by the plant to flower was recorded and also the number of days for complete opening of flower bud

3 2 3 2 Pollen viability

Pollen was taken on a glass slide stained with acetocarmine and viewed through microscope The viability was recorded based on the colour and shape of pollen.

3 2 3 3 Seed setting

Accessions were observed for seed setting till the harvest

3 2 3 4 Seed germination

Seed setting was not observed and so seed germination was not studied

3 3 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°C 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

3 3 1 High Performance Thin Layer Chromatography (HPTLC)

HPTLC is a versatile separation technique and is official in most of the pharmacopoeias for determining content, uniformity purity profile assay values and dissolution rates in an unlimited number of monographs It can simultaneously handle several samples even of divergent nature and composition supporting several analysates at a given time It is the most simple separation technique

3 3 1 1 Sample preparation

Proper sample preparation is an pre requisite for success of HPTLC separation. The choice of a suitable solvent for a given analysis is very important Usually application of 0 5 5µl of sample for HPTLC is recommended keeping the size of the starting zones down to a minimum

3 3 1 2 Sample application

Precise volumes and positioning are the two vital factors in HPTLC The sample applicator used in HPTLC was CAMAG LINOMAT IV

3 3 1 3 Chromatogram development chamber

Chamber saturation has pronounced influence on the separation profile So the chamber should be saturated with solvent system for a period of time The chamber used in HPTLC was CAMAG TWIN TROUGH CHAMBER.

3 3 1 4 Visualization

The HPTLC plate after development was visualized in the CAMAG UV cabinet HPTLC is the only chromatogram technique and this cabinet makes the chromatogram visible

In the U V cabinet, at 254nm wavelength, the chromatographed plate appears green and the fractions black. At 366nm the plate appears dark while fractions that have fluorescence appear bright and coloured Fluorescence should be observed in total darkness only

3 3 1 5 Detector

The detector used in HPTLC was the CAMAG TLC scanner 3 Th s can quantify samples by U V light in nanogram/pictogram range as well as record their absorbance spectra Fluorescence is also measured It is fully automatic and has inbuilt programme for impurity quantification and content uniformity test

3 3 1 6 Recorder

The recorder used was CAMAG video densitometer This is a multipurpose instrument that can store the plate image as well as quantify fractions at UV 254nm 366nm and 540nm It was a video head for data acquisition.

3 3 2 Procedure for estimation

A sensitive HPTLC method was used to analyse the level of bacoside A content in the plant extract of *Bacopa monniera* accessions collected from different locations of various districts in Kerala The stationary phase was precoated silica gel 60GF254 (20 x 10 cm Aluminium sheet) It was developed using the mobile phase chloroform methanol water (18 9 0 6) The wavelength selected for analysis was 254nm

A CAMAG HPTLC system comprising of linomatV automatic sample applicator and CAMAG TLC scanner 3 with CAT s version 4 0 software were used for sample application and quantitative evaluation respectively

3 3 3 Bacoside A standard

Bacoside A standard was obtained from Natural Remedies Pvt Ltd Veerasandra, Husoor Road Banglore

3 3 4 Estimation of bacoside-A in the extracts

The TLC fingerprint profile of the extracts was established by using HPTLC Suitable diluted stock solution of methanolic extract was spotted on a precoated silica gel 60GF254 TLC plate (E Merck) using CAMAG LinomatV automatic sample spotter and the plate was developed in a solvent system of toluene ethylacetate glacial acetic acid (12 5 7 5 0 5v/v) and chloroform methanol water (18 9 0 6) good resolution was obtained for second solvent system The scanning was done using CAMAG TLC scanner 3 at UV 254nm and Rf values spectral wavelength and peak area of the resolved bands were recorded Relative percentage area of each band was calculated from peak areas

The standard bacoside A solution (1mg in 10ml) was prepared in methanol in a volumetric flask From this stock solution standard solutions of 10 70µg/ml were prepared by transferring aliquots (1 to 7ml) of stock solution to 10ml volumetric flask and adjusted the volume to 10ml with methanol 10µl of each of standard solution was applied on precoated silica gel 60GF254 TLC plate (E Merck) using

CAMAG LinomatV automatic sample spotter The plate was developed in the solvent system of chloroform Methanol water(18 9 0 6) in twin triangle chamber to a distance of 7 9cm plates were dried in air for 15 minutes and scanned at 254nm Data of peak area of each band was recorded It was also scanned at 540nm The standard curve for bacoside A in the range of 10-60µg/ml was generated by plotting the peak area against concentration of bacoside A

3 3 5 Analysis of bacoside-A in samples

Keeping the above 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

3 4 Tabulation and Statistical Analyses

Observations on the biometric characters and biochemical constituent were tabulated by taking means 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

Estimation of genetic parameters

The variance components were estimated as per the procedure suggested by Burton (1952)

3 4 1(a) Phenotypic variance

Phenotypic variance (Vp) – Vg + Ve

where (Vg) – Genotypic variance

(Ve) – Environmental variance

3 4 1(b) Genotypic variance

$$\text{Genotypic variance (Vg)} = \frac{VT \quad VE}{N}$$

where VT – Mean sum of squares due to treatments
VE – Mean sum of squares due to error
N Number of replications

Environmental variance Ve = VE

where VE – Mean sum of squares due to error

3 4 1(c) Phenotypic and genotypic coefficients of variation

The phenotypic and genotypic coefficients of variation were calculated by the formula suggested by Burton and Devane (1953)

$$\text{Phenotypic coefficient of variation (PCV)} \quad \frac{\sqrt{Vp}}{\bar{X}} \times 100$$

where Vp – Phenotypic variance

X – Mean of the character under study

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{Vg}}{X} \times 100$$

where Vg – Genotypic variance

$\bar{X}$ Mean of the character under study

The estimates of PCV and GCV were classified as

<10 per cent	Low
10 20 per cent	Moderate
>20 per cent	High

3 4 1(d) Heritability

Heritability in the broad sense was estimated by following the formula suggested by Burton and Devane (1953)

$$\text{Heritability (H)} \quad \frac{Vg}{Vp} \times 100$$

where Vg – Genotypic variance

Vp – Phenotypic variance

The heritability was categorised as

60 100 per cent	High
30 60 per cent	Moderate
>30 per cent	Low

3 4 1(e) Expected genetic advance

The expected genetic advance of the cultures was measured by the formula suggested by Lush (1949) Johnson *et al* (1955a) at five per cent selection intensity using the constant K as 2 06 given by Allard (1960)

$$\text{Expected genetic advance (GA)} \quad \frac{Vg}{\sqrt{Vp}} \times K$$

where Vg Genotypic variance

Vp – Phenotypic variance

K – Selection differential

Genetic gain (Genetic advance as percentage of mean)

Genetic advance (GA) calculated in the above method was used for estimation of genetic gain.

$$\text{Genetic gain (GG)} \quad \frac{GA}{\bar{X}} \times 100$$

$\bar{X}$ Mean of the character under study

Genetic gain was categorised as

>20 per cent	High
10 20 per cent	Moderate
<10 per cent	Low

3 4 1(f) Phenotypic and genotypic correlation coefficients

The phenotypic and genotypic covariances were worked out in the same way as the variances were calculated Mean product expectations of the covariance analyses are analogous to the mean square expectation of the analyses of variance The different covariance estimates were calculated by the method suggested by Fisher (1954)

Phenotypic covariance between two characters 1 and 2 (CoVp12) CoVg12 + CoVe12

CoVg12 Genotypic covariance between characters 1 and 2
CoVe12 Environmental covariance between 1 and 2

Genotypic covariance between two characters 1 and 2

$$\text{CoVg12} \quad \frac{Mt12 \quad Me\ 12}{N}$$

where

Mt12 Mean sum of product due to treatment between characters 1 and 2

Me12 – Mean sum of product due to error between characters 1 and 2

N Number of replications

The phenotypic and genotypic correlation coefficients among the various characters were worked out in all possible combinations according to the formula suggested by Johnson *et al.* (1955b)

Phenotypic correlation coefficient between two characters 1 and 2

$$(r_p12) - \frac{CoVp12}{\sqrt{Vp1\ Vp2}}$$

where

CoVp12 – phenotypic covariance between characters 1 and 2
Vp1 – Phenotypic variance of character 1
Vp2 – Phenotypic variance of character 2

Gebotypic correlation coefficient between two characters 1 and 2

$$(r_g12) - \frac{CoVg12}{\sqrt{Vg1\ Vg2}}$$

where

CoVg12 – Genotypic covariance between characters 1 and 2
Vg1 Genotypic variance of character 1
Vg2 – Genotypic variance of character 2

Results

Table 2 Mean performance of the accessions for 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
CD at 5%	2 05	0 214	0 129	0 383	5 45	0 579	185 68	247 6

Results

RESULTS

A knowledge about the variability existing 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

Variability in morphological characters

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 to 68 63 cm Accession 18 recorded maximum shoot length (68 63 cm) where as accession 9 recorded the minimum (58 42 cm) The other accessions which are homogenous with accession 18 with regard to shoot length are 29 28 11 and 6

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 1 070 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 7825 cm^2) With regard to leaf area accession eight 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

Table 2 Mean performance of the accessions for 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
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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
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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
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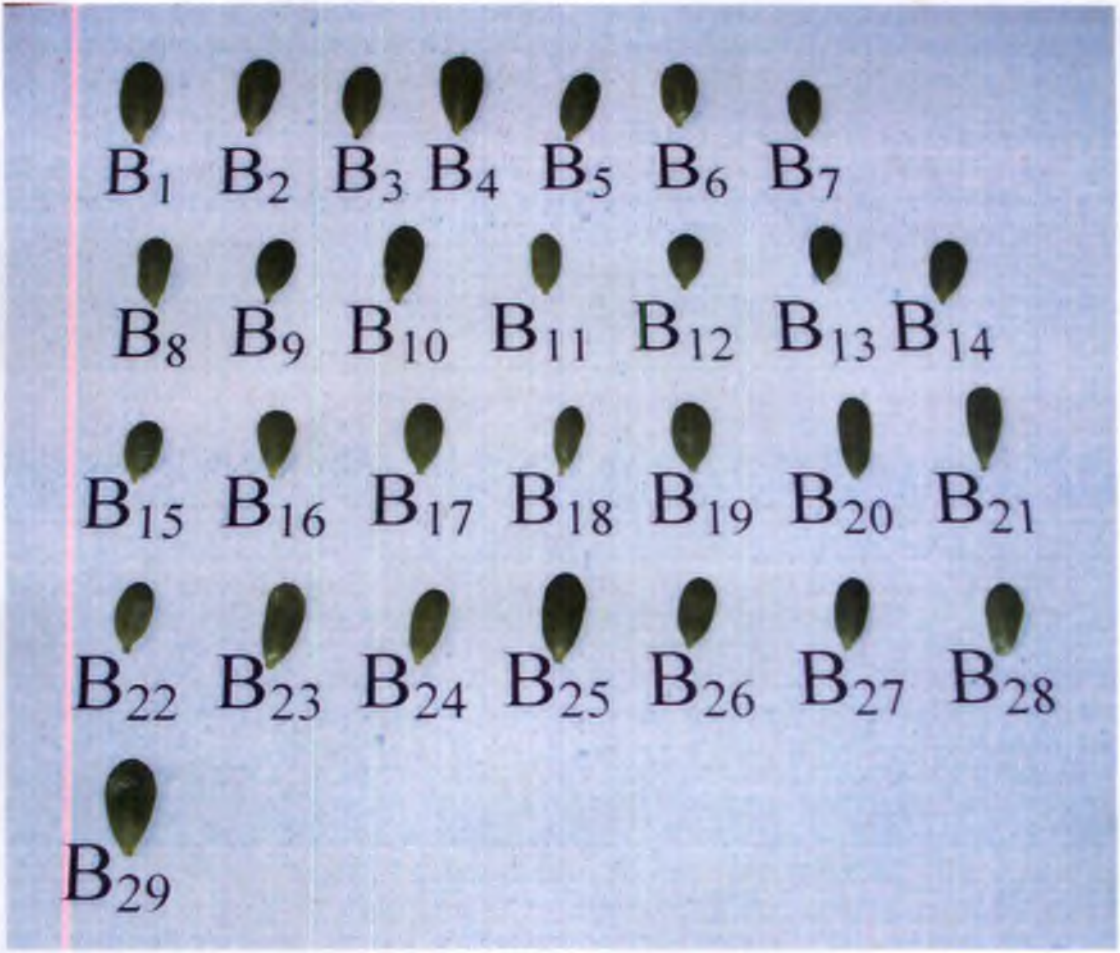
28	67 30	2 015	0 80	1 623	16 38	1 82	2196 1	209 8
29	68 22	1 930	1 00	1 930	14 38	1 83	2286 6	308 4
CD at 5%	2 05	0 214	0 129	0 383	5 45	0 579	185 68	247 6

Stem colorations during different growth stages in some accessions

Morphological variability between Delhi and Pulamanthole accessions

Leaf variability in all the accessions



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 recorde l 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) Accession 10 recorded lowest biomass (106 5 g) Accessions 29 22 and 21 also recorded high biomass

Floral biology

Flowers are 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 is 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 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

Table 3 Mean performance of accessions for days to flowering, bacoside A content and bacoside biomass ratio

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
CD 5%	97 3	0 61	0 061

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 Variation

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

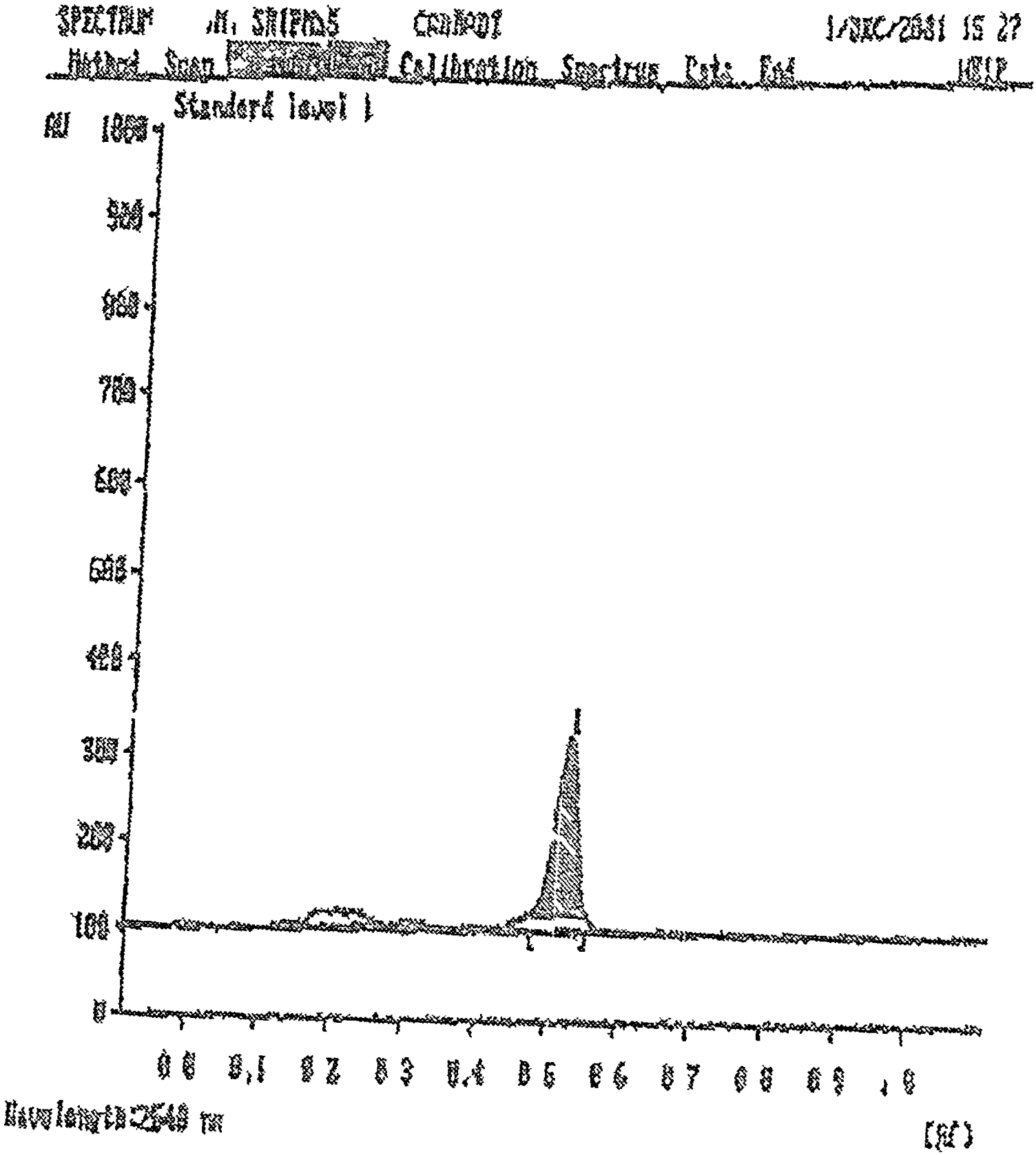
Bacoside to biomass ratio in percent is given in [Table 3] Accession 14 has high (2 564%) bacoside to biomass ratio followed by accession 6 (2 089%) and 1 (2 036%) The accession which recorded low bacoside to biomass ratio is 20 (0 445%)

Variability studies

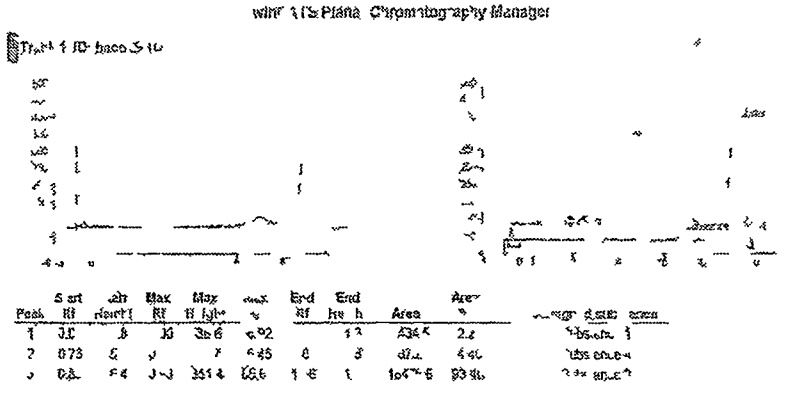
The variability parameters studied for the different characters are presented in [Table 4] Among the nine characters studied wide range was noticed for number of leaves followed by biomass and number of flowers While narrow range was noticed for leaf width, internodal length and leaf area.

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 if environment Maximum phenotypic coefficient of variation was recorded in bacoside A content (56 50%) followed by number of

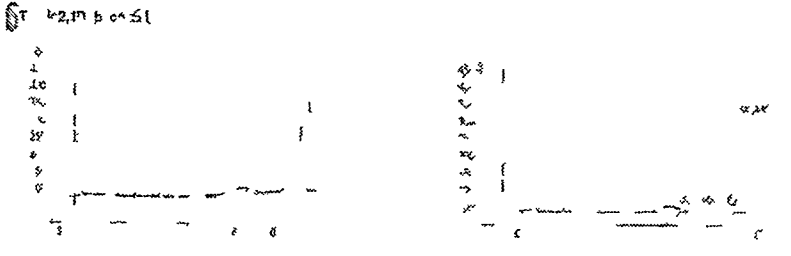
Chromatogram of standard bacoside A at 254 nm RF value 0 57



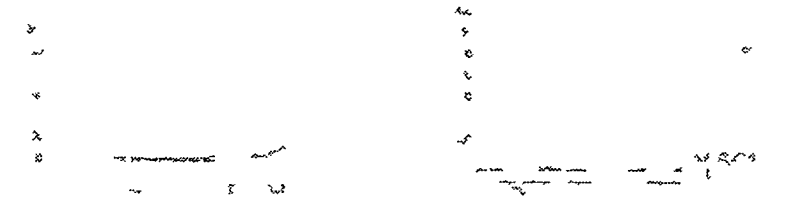
Chloroform extract graphs of samples



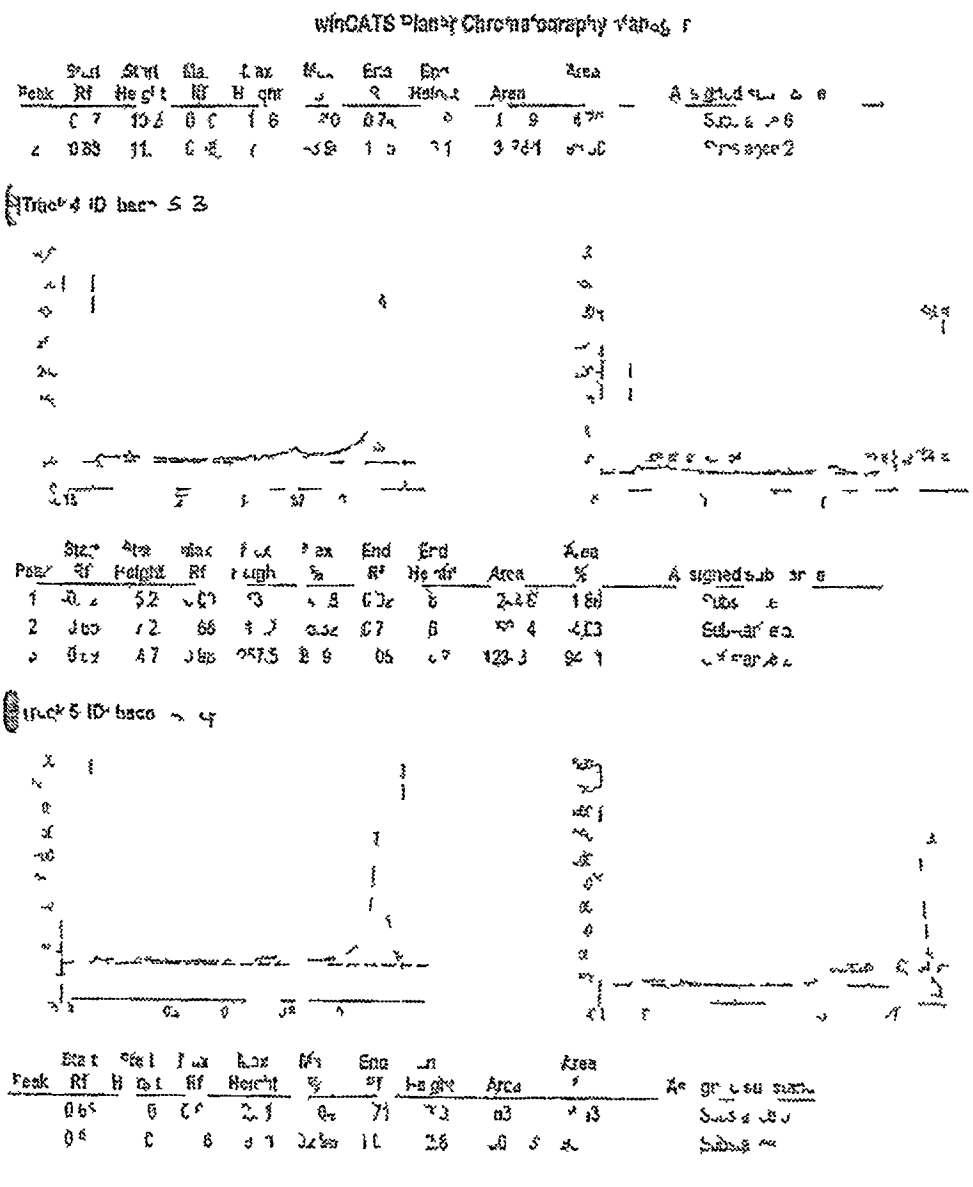
Peak	Start RT	Peak RT	Max RT	Max Height	Area	End RT	End Height	Area	Area %	Assigned Substance
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Unknown 1
2	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Unknown 2
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Unknown 3



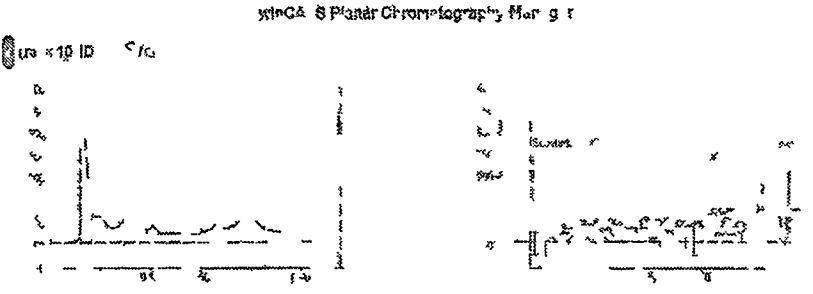
Peak	Start RT	Peak RT	Max Pf	Max Height	Area	End RT	End Height	Area	Area %	Assigned Substance
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Unknown 1
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Unknown 2



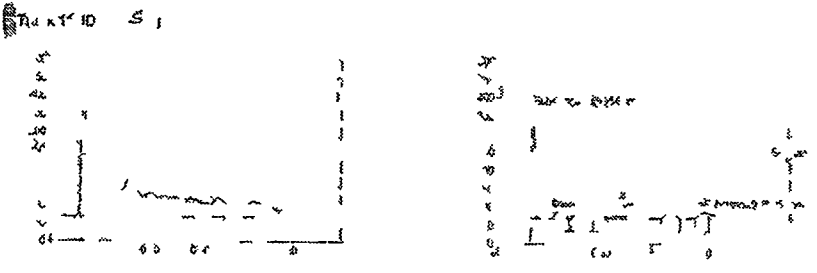
Chloroform extract graphs of samples



Methanol extract graphs of samples

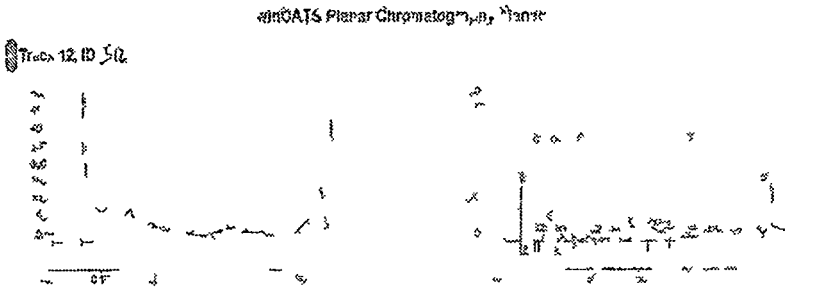


Peak	Start R	Start Height	Peak Pr	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
	0		0 3		3	0			2	
2	0									
	0		0							
4	0	4	0							
	0		0				0			
7										
8										
9										

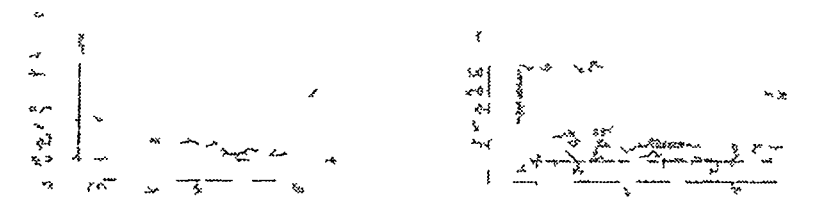


Peak	Start R	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
2	0									
3	0									
6										

Methanol extract graphs of samples

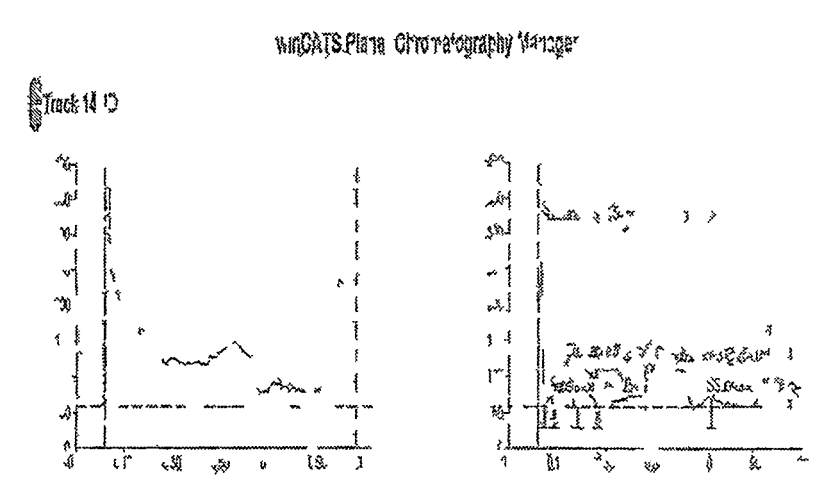


Peak	R	Start Height	Max Rf	ax	End Rf	End Height	Area		Assignment Substance
1									
2									
3									
4									
5									
6									
7									



Peak	Rf	Height	Max Pk	Max Height	ax	End Rf	End Height	Area		Assignment Substance
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Methanol extract graphs of samples



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assignment
1	0.02	0.0	0.04	2.1	5.6	0.0		26.2	0.7	Spot 1
2	0.06	0.1	0.08	4.0	2.6	0.1	0.0	9.9	1.0	Spot 2
3	0.14	0.0	0.1	6.1	12.3	0.16	0.1	0.0	1.6	Spot 3
4	0.19	0.0	0.22	3.5	6.6	0.27	0.3	10.0	7.6	Spot 4
5	0.2	0.0	0.3	2.9	2.3	0.3	7.0	0.0	1.0	Spot 5
6	0.4	0.0	0.4	3.0	4.2	0.4	0.0	6.6	3.1	Spot 6
7	0.5	0.0	0.5	2.0	0.4	0.5	0.0	0.0	0.0	Spot 7
8	0.6	0.0	0.6	0.0	2.0	0.6	0.0	0.0	2.0	Spot 8
9	0.74	0.0	0.7	4.7	30.2	0.76	0.0	0.0	0.0	Spot 9

Table 4 Estimates of range variability heritability and genetic advance

Character	Range	P C V (%)	G C V (%)	Heritability (%)	G A	G G (%)
Shoot length	58 42 to 68 63	4 05	3 74	85 62	4 56	7 10
Leaf length	I 38 to 2 17	9 56	7 71	66 09	0 24	12 73
Leaf width	0 54 to 1 07	14 41	12 49	75 93	0 19	22 06
Leaf area	0 78 to 2 32	20 71	17 63	73 32	0 51	31 09
No of flowers	0 87 to 29 75	41 30	38 47	87 17	13 09	73 81
Internodal length	0 76 to 2 18	24 70	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	33 47	32 96	97 08	139 03	66 87
Bacoside A	0 47 to 5 4%	56 50	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

flowers (41 30%) The phenotypic coefficient of variations for biomass internodal length, 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 percent 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 have high genetic gain are 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 constituent the 29 accessions were grouped into five clusters

Table 6 grouping of accessions 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

Table 7 Mean performance of access ons in different clusters

	Cluster I	Cluster II	Cluster III	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 80
Leaf width	0 90	0 91	0 72	0 90	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 50	1 46	1 89	1 52	1 60
Number of leaves	2073 40	1908 50	1992 90	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

Table 8 Mean intra and inter cluster distances among five clusters formed by 29 accessions of brahmi (*Bacopa monniera*) based on expression of eight morphological and one biochemical characters

Cluster	I	II	III	IV	V
I	69 25				
II	321 82	70 21			
III	227 24	485 18	40 84		
IV	574 28	822 87	760 06	70 19	
V	170 91	394 99	321 91	699 40	157 15

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 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

Cluster I had the accessions collected from Palakkad Thrissur Ernakulam, Malappuram, Wayanad Calicut and Kollam. Cluster II had accessions from Ernakulam and Malappuram. Cluster III had accessions from Thrissur Ernakulam and Trivandrum. Cluster IV had accessions from Ernakulam and Malappuram. Cluster V had accessions from Thrissur Ernakulam, Malappuram, Wayanad and accession from Delhi The accessions collected from different districts were in one cluster instead of different clusters Cluster I had accessions from all the districts from where the accessions were collected except Trivandrum and Delhi This shows there is no parallelism with in geographic regions from where the accessions were collected

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

Table 5 Estimates of Phenotypic and genotypic correlation coefficients among characters 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	0 090
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*		0 000	0 268	0 347	0 203	0 290
No of flowers	0 110	0 115	0 019	0 021		0 140	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	0 090	0 102	0 404	0 308	0 103	0 181	0 155	0 306	

* significance at 5% level

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

0 809 leaf length and leaf area is 0 910 leaf width and leaf area was 0 975 and between number of leaves and biomass it was 0 457 Leaf area was highly positively correlated 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

Discussion

DISCUSSION

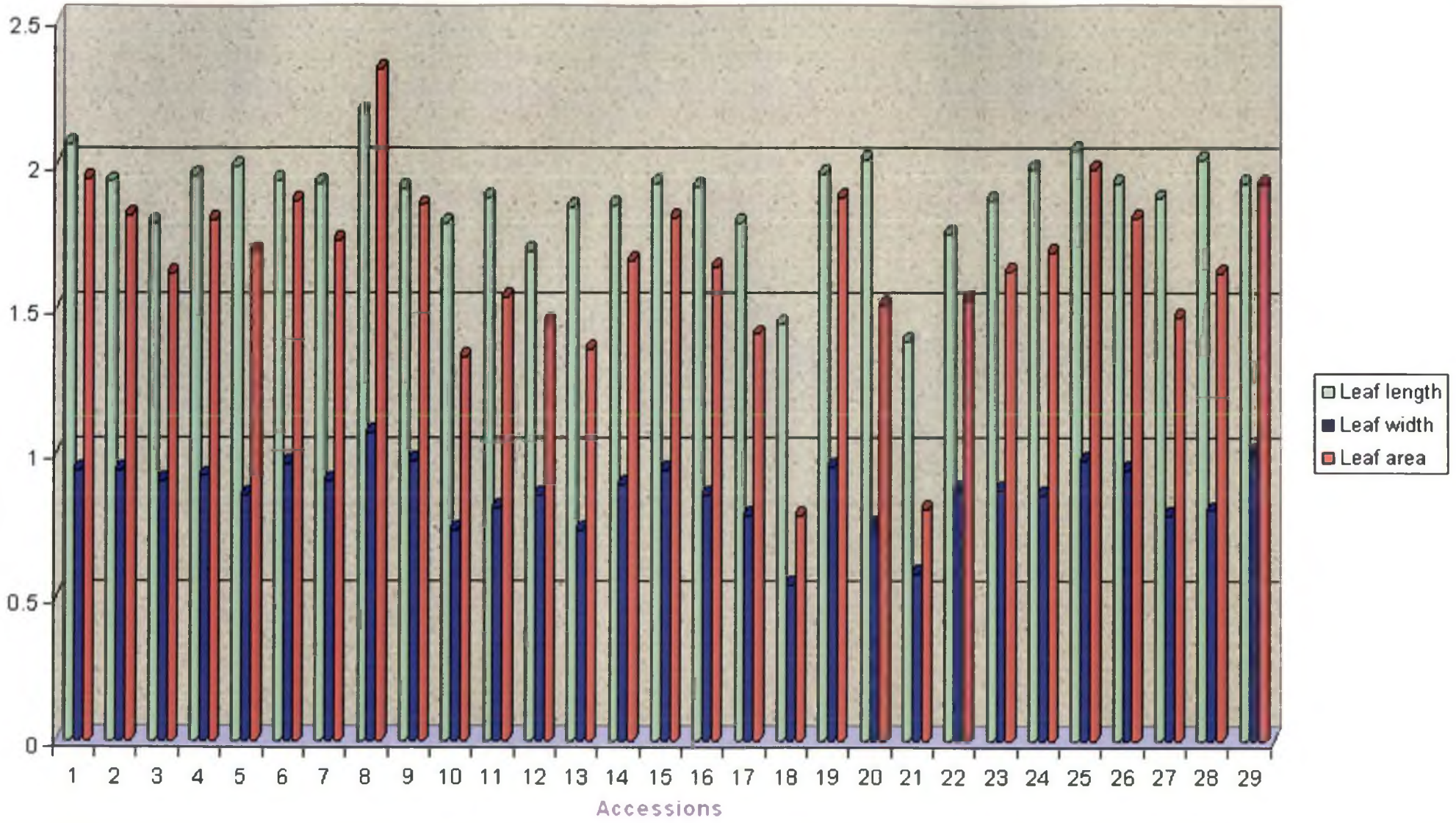
In any breeding programme assessment of variability existing in the germplasm is the prerequisite for the genetic improvement of species under domestication. 29 accessions of brahmi (*Bacopa monniera*) collected from different eco geographical locations of Kerala were assessed for the genetic variability for therapeutically important morphological characters and its biochemical constituents

The variability observed in 29 accessions of *Bacopa monniera* collected from different eco geographical locations in Kerala is summarized below 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 observed for shoot length leaf length leaf width, leaf area, number of flowers internodal length number of leaves and biomass has been shown quantitatively in table 2 and that for traits days to flowering, bacoside A content and bacoside/biomass measured quantitatively is presented in table 3 It will be seen from table 2 that the accessions had variability in all the traits mentioned in the table The variability among the accessions for the observed traits was found to be significant statistically It will be further seen from table 3the variability among the accessions for days to flowering bacoside A content and bacoside/biomass ratio were also statistically significant The above observations demonstrated that the collection of accessions perhaps consisted of many ecotypes carrying distinctive characteristics

The accession collected from Delhi had good shoot length, internodal length and had more number of leaves For other characters observed it recorded medium to low values The remaining accessions collected from Kerala varied among themselves The variability among accessions collected from the same district may be attributed to the conditions prevailing there For all the characters observed the accessions collected from different places in Ernakulam district were good The

Mean performance of accessions for leaf length, leaf width and leaf area



experiment by Shalini *et al* (2003) also recorded the variability among the accessions The only difference is that the accessions collected by them were from different states in India, where as this experiment had the accessions from different districts in Kerala except one accession from Delhi

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 is 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* where he noticed changes in leaf colour at maturity

In Indian system of medicine the whole plant of *Bacopa monniera* is often harvested and used [Farooqi *et al* (1999) and 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

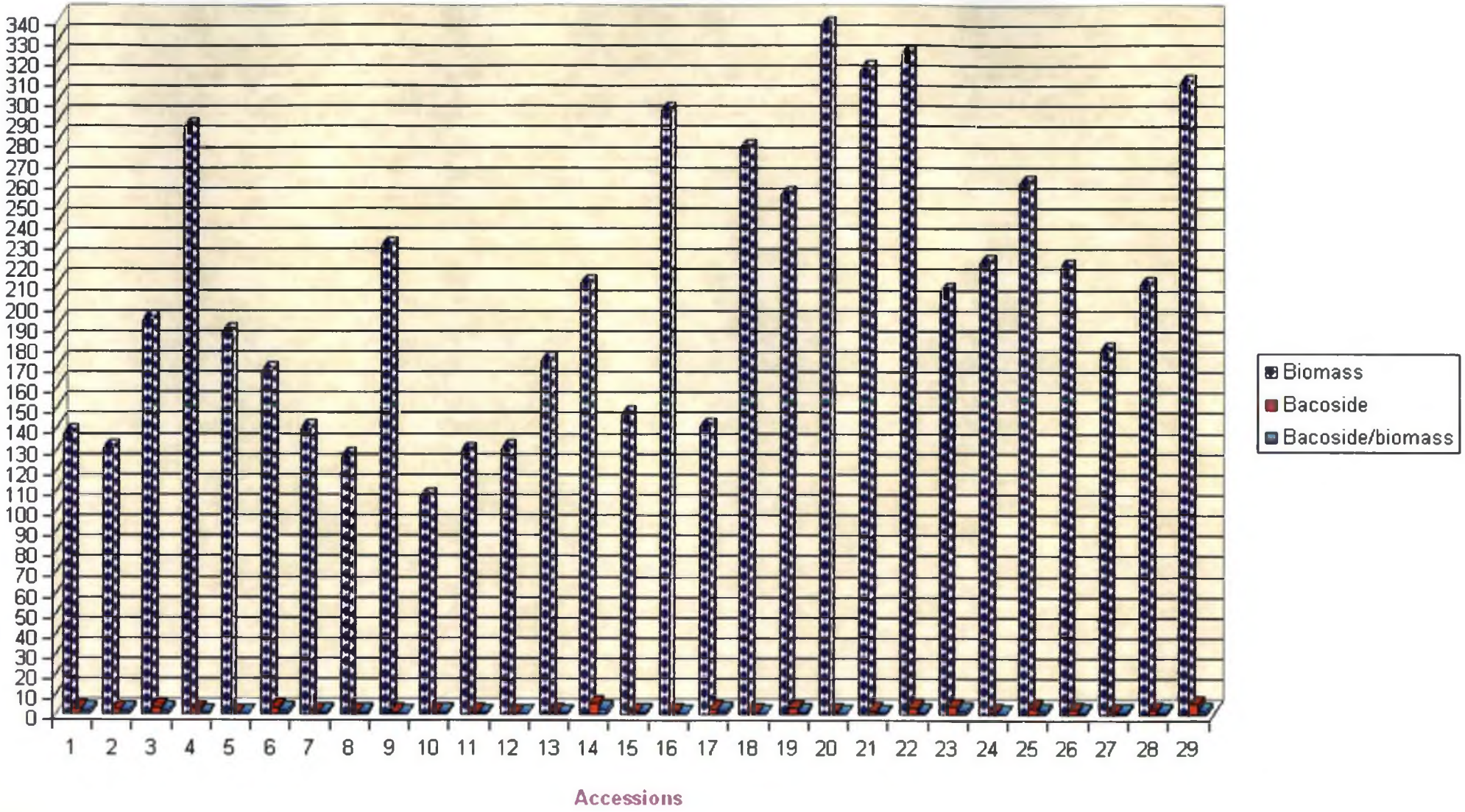
Traditionally the *Bacopa monniera* shoot is harvested and air dried in shade to produce the raw material for the commercial brahmi drug Since the plant is native to high humidity areas the process of drying the herb is usually slow and takes a few weeks time The samples of brahmi have variable bacoside A content Besides the differences in origin and from the places they were collected the slow drying process may permit variation in bacoside A contents of the herbage 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 by Gupta *et al* (1998)

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 two chambered as described by Bentley and Trimen (1890) Hooker (1892) and 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 Some times the accessions collected from the same district showed difference in days to flowering This may be attributed to the climatic conditions 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) Bacoside A was isolated from the methanol extract of *Bacopa monniera* accessions as a major component in a yield ranging between 0 47 to 5 4% on w/v from raw material The accession 12 collected from Aduvassery (Ernakulam) recorded the least chemical constituent Accessions from Kottakkal (Malappuram) and from Aluva 1 (Ernakulam) recorded the highest chemical constituent in them The other accessions 3 23 and 25 also recorded good bacoside

Mean performance of accessions for biomass, bacoside content and bacoside/biomass



A content in them and these are collected from Aluva, Thiruthissery and Aduvassery All these accessions (3 23 25 and 29) were collected from same district, Ernakulam These accessions recorded good amount of bacosides in them The accessions from Malappuram district recorded low bacoside A content, except the accession from Kadamphuza and Kottakkal which recorded the highest along with one of the accessions from Aluva (Acc no 29) The accessions from Thrissur district did not record much high bacoside content except the one from Chuvannamannu The sole accession from Trivandrum recorded second lowest bacoside-A content in it Except the accession from Ambalavayal from Wayanad the other two from Kalpatta and Thamarassery recorded good quantity of bacoside A Accession from Calicut has high bacoside content. The accession from Vallikkavu (Kollam) recorded only trace amount of bacoside content

Bacoside/biomass ratio was calculated to find out which accession will give high bacoside A content from the total biomass produced by the same accession Accessions 14 and 29 collected from Kottakkal and Aluva, recorded high amount of bacoside A content However the biomass produced by these accessions differed *i e* the amount of bacoside that can be obtained from the total biomass produced by the accession also differed The ratio when expressed in percent showed that accession 14 collected from Kottakkal (Malappuram) had 2 564 % whereas the ratio was 1 75% in accession 29 collected from Aluva Even though these two accessions had similar bacoside content they differ with respect to bacoside/biomass ratio Accessions 1 and 6 collected from Shornur (Palakkad) and Kadamphuza (Malappuram) also recorded good bacoside/biomass ratio following the accession 14 from Kottakkal (Malappuram) The accession collected from Kollam Delhi Trivandrum recorded very poor bacoside/biomass ratio These accessions did not have good amount of bacoside content, and it was reflected in the 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 has good bacoside content but the biomass production is 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 genotypic coefficient of variation and phenotypic coefficient of variation are calculated for the observed traits The phenotypic coefficient of variation for the observed traits is greater than genotypic coefficient of variation for the same traits Phenotypic coefficient of variation is highest for the bacoside A followed by number of flowers and biomass Phenotypic coefficient of variation is very low for shoot length, leaf length number of leaves and leaf width Same is 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)

For all the characters the phenotypic coefficient of variation is higher than genotypic coefficient of variation, so selection for improvement of these traits may mislead because the variation in these traits is not only due to genotypes but also due to environmental effects

Heritability is an index of the transmission of characters from parents to their offspring The heritability values indicate broad sense heritability which is the percentage ratio of genotypic variance to the phenotypic variance expressed in percentage 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% *Bacopa monniera* is used in Ayurvedic medicine due to the

important chemical constituent, bacoside A (Deepak et al 2005) As whole plant is used for the extraction of the important chemical constituent biomass will also become an important criterion.

The other characters with good heritability values are number of leaves (87 04%) number of flowers (87 17%) shoot length (85 62%) leaf width (75 93%) and leaf area (73 32%) The heritability values for leaf length(66 09%) and internodal length (47 74%) are lower when compared with the above characters heritabilities

If 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 are not influenced by environmental effects Selection for improvement of these characters may not be useful because of the total genetic variance which includes fixable and non fixable variances

The broad sense heritability value for internodal length (47 74%) is veryless when 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) Internodal length is highly influenced by environmental effects and its improvement by selection will be difficult

The characters having heritability values greater than 65% are not that much influenced by environmental effects as that of internodal length Number of leaves number of flowers and shoot length have considerably high heritability values

which indicate less environmental effects on them But the selection for improvement of these characters is not possible because of total genetic variance

Leaf width, leaf area and leaf length have medium heritability values The environmental effects on these characters will be high when compared to those characters which had heritabilities greater than 85%

Genetic advance is improvement in the mean genotypic value of selected plants over the parental population (Allard 1960) The genetic advance for the characters under study are presented in the table (4) In the table the genetic advance values are high for number of leaves (442 04) and biomass (139 03) remaining characters have very low genetic advance values When genetic advance is expressed as genetic gain then bacoside A content has high (110 12%) genetic gain value when compared to all other characters under study B icoside A is follwed by number of flowers (73 81%) and biomass (66 87%) For all other characters the genetic gain values lie between 7 24%

If 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) According to this characters bacoside A, number of flowers and biomass are governed by additive genes and selection will be rewarding for improvement of these characters

If the genetic gain values are low it shows that the characters are governed by non additive genes and heterosis may be useful The characters with genetic gain values between 7 24% are governed by non additive genes and for improvement of these characters heterosis breeding will be useful

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) According to this the high heritability value for bacoside A content is mostly due to the additive genes and selection may be

effective The accession with good bacoside content can be selected for further improvement

Biomass also has high heritability along with considerably high genetic gain value which indicates the heritability value may not be completely due to additive genes so the selection for biomass may not be that effective as that of bacoside content

If high heritability is accompanied with low genetic gain It is indicative of non additive gene action The high heritability is being exhibited due to favourable influence of environment rather than genotype and selection for such traits may not be rewarding (Singh and Narayanan 2000) Number of leaves number of flowers shoot length leaf width and leaf area are the characters with high heritability values and with low genetic gain values The high heritabilities for these characters is due to the favourable influence of environment rather than genotype Selection for these traits will not be rewarding

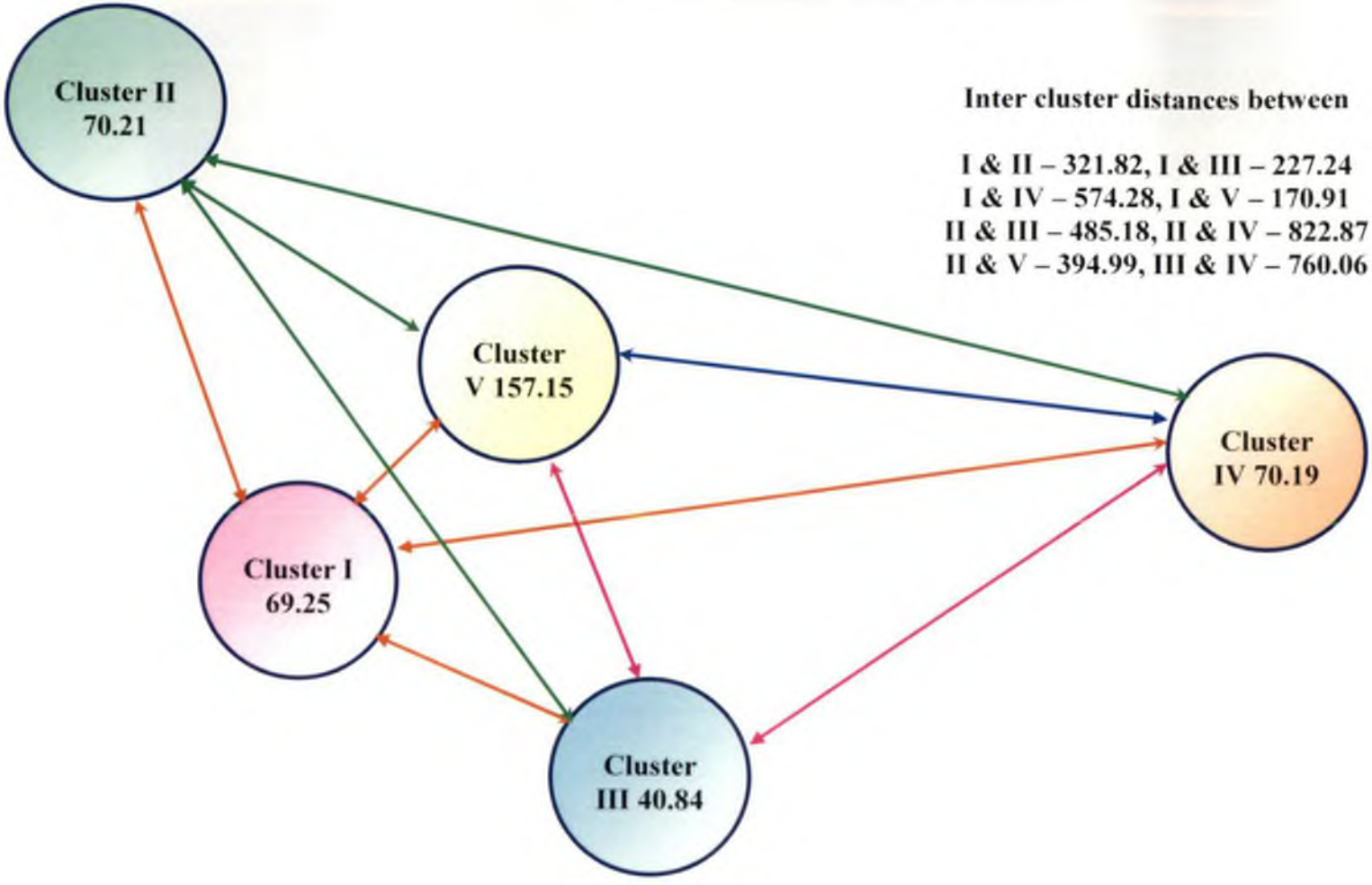
Low heritability accompanied with low genetic gain values It indicates that the character is highly influenced by environmental effects and selection would be ineffective Here internodal length has low heritability and low genetic gain values this shows that internodal length is highly influenced by environment and selection will be in effective

Cluster analysis

Based on the observations of shoot length leaf length leaf width leaf area number of flowers internodal length number of leaves biomass and biochemical trait the collected 29 accessions were grouped into five clusters and comparison was done among the clusters as it was found to be convenient and reliable

The character means for the five clusters show that, Cluster I comprising of 15 accessions (1 4 7 8 9 12 16 17 19 20 24 25 27 28 and 29) had high mean values for leaf length, leaf width, leaf area and number of leaves Medium values

Intra and inter-cluster distances



were obtained for number of flowers internodal length, biomass and bacoside A content The accessions in this cluster were characterized by high leaf area, leaf length leaf width medium biomass and bacoside A content

Cluster II included three accessions (6 14 and 22) which have medium values for shoot length leaf area number of leaves and leaf length High values were obtained for bacoside A content, leaf width, number of flowers and biomass Low values were obtained for internodal length The accessions included in this cluster are characterized by high biomass bacoside A content leaf width and number of flowers and also by very low inte nodal length

Cluster III included three accessions (5 13 and 21) which had medium values for biomass number of leaves number of flowers and shoot length High values were obtained for internodal length Low values were obtained for leaf length, leaf width, leaf area and bacoside A content This cluster is characterized by low bacoside content, leaf area leaf width and leaf length and the accessions in this cluster have high internodal length

Accessions 15 and 23 are included in cluster IV These accessions have high values for leaf length, leaf width leaf area and bacoside A content Low values were obtained for number of flowers biomass and number of leaves Medium values were obtained for shoot length and inte nodal length

Accessions 2 3 10 11 18 and 26 are in cluster V which had medium values for leaf length, leaf width, leaf area number of flowers internodal length and bacoside A content These accessions also had high values for shoot length and number of leaves and low values for biomass

From inter and intra cluster distances it will be seen that the intra cluster distances are 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 are very distinct from each other Similar kind of clustering was done in brahmi by Shalini *et al* (2003)

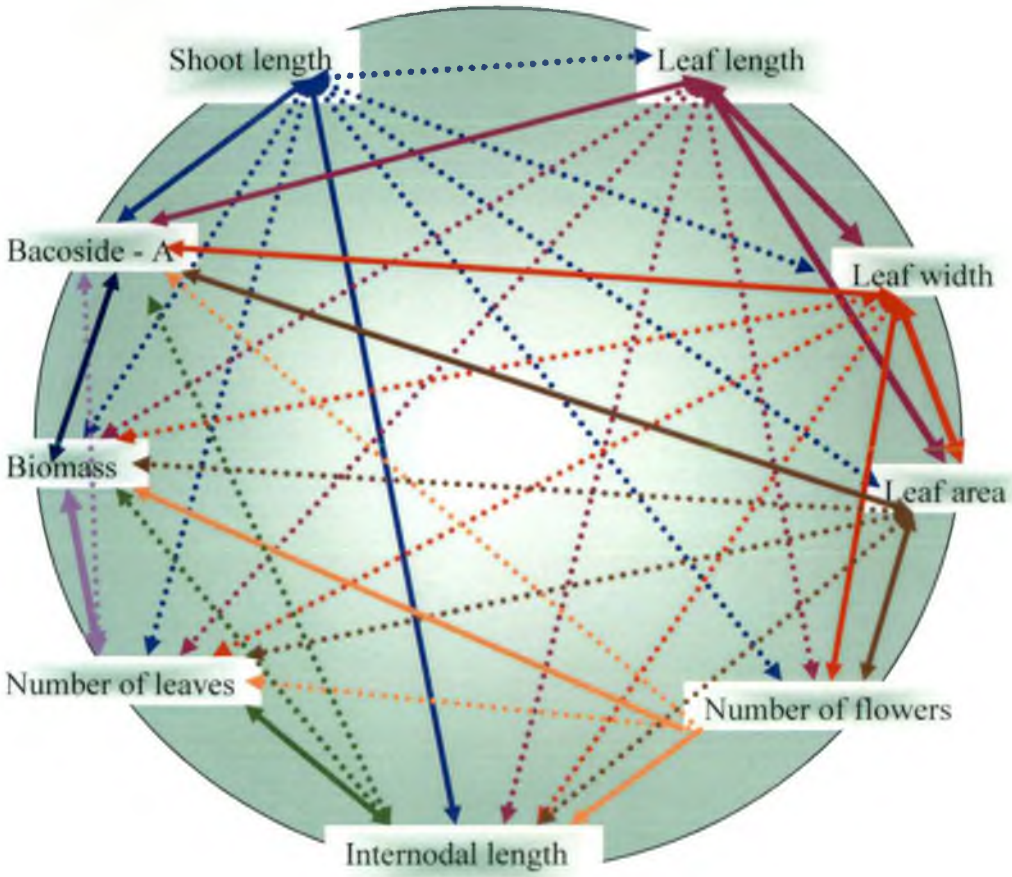
Cluster I has the accessions collected from Palakkad Thrissur Ernakulam Malappuram Wayanad Calicut and Kollam Cluster II has accessions from Ernakulam and Malappuram Cluster III has accessions from Thrissur Ernakulam and Trivandrum Cluster IV has accessions from Ernakulam and Malappuram Cluster V has accessions from Thrissur Ernakulam Malappuram, Wayanad and accession from Delhi The accessions collected from different districts are 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

The association between the two variables which can be directly observed is termed as phenotypic correlation It includes both genotypic and environmental effects and therefore differs under different environmental conditions The inherent or heritable association between variables is known as genotypic correlation (Falconer 1989)

If the correlation value is positive and significant, the association between two characters is very high and if the correlation value bears a negative sign it means that the increase in one character will lead to decrease in second character and vice versa Similarly if it bears the positive sign it means that increase in one variable will cause increase in the other and vice versa

Phenotypic correlations



Negatively correlated

Positively correlated

Significant and positively correlated

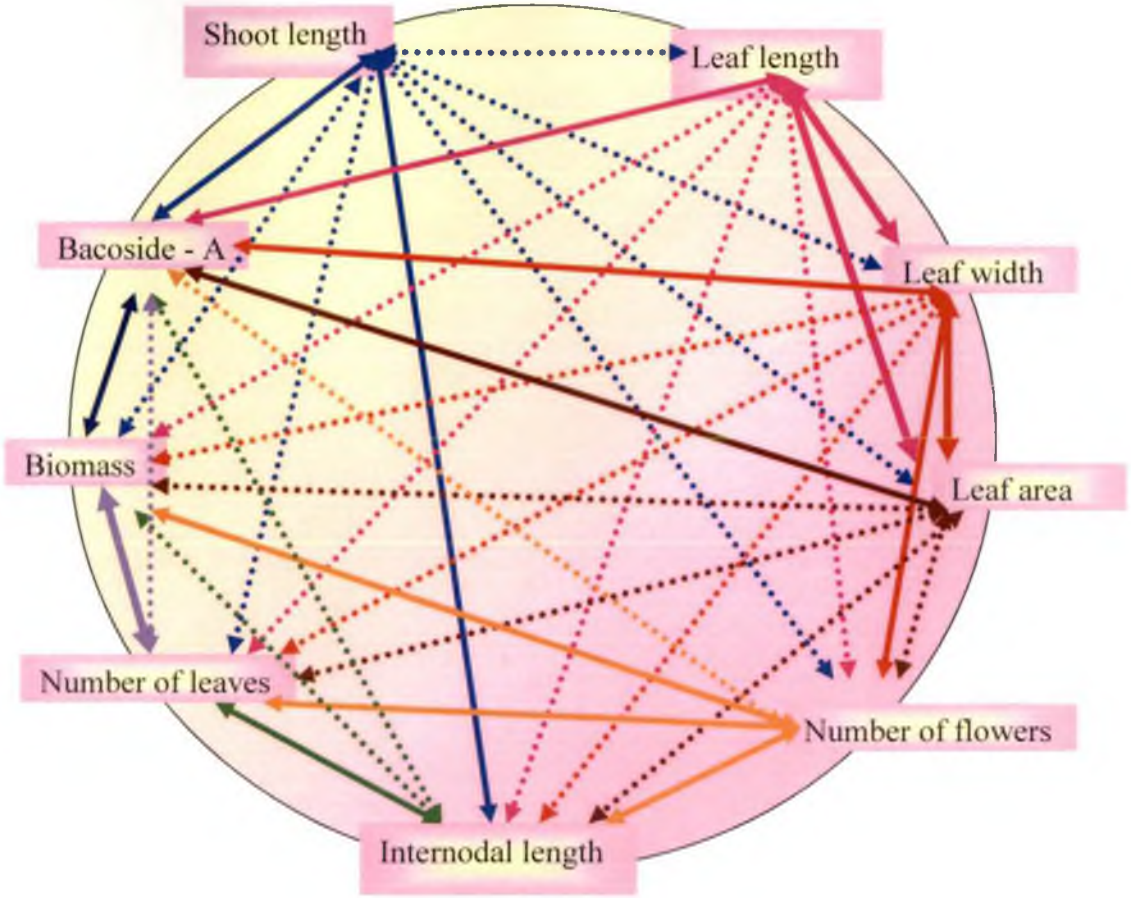
It is evident that the genotypic correlation values are higher than phenotypic correlation values If the values of genotypic correlations are higher than phenotypic correlations it means that there is a strong association between these two characters genetically but the phenotypic value is lessened by the significant interaction of environment (Falconer 1989)

From the diagram showing phenotypic correlations it is evident that, shoot length is negatively correlated with leaf length leaf width, leaf area number of flowers number of leaves and biomass This means that with the improvement in the shoot length, the improvement in all these characters will be on negative side Shoot length is positively correlated with internodal length and bacoside A content With the improvement in shoot length, internodal length and bacoside content can also be improved

Leaf length is negatively correlated with number of flowers internodal length, number of leaves and biomass It is positively correlated with bacoside A content and is positive and significantly correlated with leaf width and leaf area Here there is positive and significant correlation between the characters This type of correlation helps in the improvement two or more characters simultaneously by improving one character Here by improving leaf length leaf width leaf area can be improved

Leaf width is positively correlated with internodal length, number of leaves and biomass Leaf area is positively correlated with number of flowers and bacoside –A content and is negatively correlated with internodal length, number of leaves and biomass Number of flowers is positively correlated with internodal length and biomass It is negatively correlated with number of leaves and bacoside A content Internodal length is positively correlated with number of leaves but negatively correlated with biomass and bacoside A content Number of leaves is positively correlated with biomass and negatively correlated to bacoside A content Biomass is

Genotypic correlations



Negatively correlated

Positively correlated

Significant and positively correlated

positively correlated with bacoside A content. Though it is not significant, the value is high when compared to all other positive correlations leaving the significant ones

In brahmi the selection of accessions for cultivation is done based on the bacoside A content as it is the main chemical constituent, 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 are positively correlated with bacoside A content Out of these characters only biomass has high positive correlation with bacoside A content when compared to the correlations of other characters that are positively correlated with bacoside A content

Characters like internodal length, number of flowers and number of leaves are negatively correlated to bacoside A content Of these number of flowers and internodal length are 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 are negatively correlated to bacoside content For the brahmi plant to have higher bacoside A content plant should be compact *i e* with less internodal length big sized fleshy leaves and with late flowering habit

Summary

SUMMARY

A study has been undertaken at college of Horticulture during the year 2004 2006 with the objective of exploring and collecting the natural variability present in the germplasm of brahmi (*Bacopa monniera*) existing in the different eco geographical regions of Kerala and also to evaluate the accessions for high biomass and its therapeutically important constituents The summary of the work done and the silent findings are listed below

1 Twenty nine accessions of brahmi were collected representing various eco geographically distinct regions of Kerala

2 Significant genetic variability was observed for eight morphological characters as well as the main pharmaceutically important constituent i e bacoside A

3 The estimation of variability among the germplasm for various economic traits indicated that many ecotypes were distinctly different irrespective of their geographical origin

4 Accession from Delhi stood apart for its economic traits namely shoot length, Internodal length, number of leaves and bacoside A content

5 Among the accessions of Kerala accessions collected from Ernakulam district were good for pharmaceutically important chemical constituent i e bacoside A

6 A wide variability was observed in the days to flowering among the accessions ranging from very early to very late

7 Biomass and bacoside A showed maximum heritability indicating its consistency in performance irrespective if the environment

8 All the accessions were grouped into five clusters and there is no parallelism in clustering pattern and geographical distribution

9 An association of morphological traits as well as qualitative attributes indicated that the bacoside A content has positive association with shoot

length leaf length, leaf width and biomass indicating that search for these traits will have corresponding increase for the quality also

Non flowering or late flowering types will also give higher bacoside content than flowering and early flowering types

The plant architecture of brahmi (*Bacopa monniera*) for high biomass and bacoside A content can be as follows

The accession should have good shoot length leaf length, leaf width leaf area and either with late flowering or non 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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Abstract

EXPLORATION, COLLECTION AND EVALUATION OF BRAHMI (*Bacopa monniera* Wettst.)

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ABSTRACT OF THE THESIS

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ABSTRACT

The study on 'Exploration, Collection and Evaluation of brami (*Bacopa monniera* wettst) was undertaken during 2004 2006 at the Department of Plant Breeding and Genetics College of Horticulture Vellanikkara Kerala Agricultural University for evaluating the collected accessions from different eco geographical locations of Kerala based on the morphological characters and the pharmaceutically important constituent i e bacoside A.

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