VARIABILITY IN ASOKA (*Saraca asoca* (Roxb.) DE WILDE)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Variability in Asoka (*Saraca asoca* (Roxb.) De Wilde)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Variability in Asoka (*Saraca asoca* (Roxb.) De Wilde)"** is a record of work done independently by **Mrs. Vidhu Francis Palathingal,** 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 her.

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VIDHU FRANCIS PALATHINGAL

Dedicated to My

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

INTRODUCTION

Asoka is considered as a sacred tree of Hindus and Buddhists. Asoka is a well known medicinal tree literally means 'remover of sorrow'. Besides its medicinal properties, its decorative orange red flowers and evergreen beautiful foliage makes this a favourable tree in gardens, national parks, roadsides, museums, temples and places for tourist attractions. Scientifically, Asoka is known as *Saraca asoca* (Roxb.) de Wilde, belonging to the family Leguminosae, sub family Caesalpiniaceae.

Cultural and scientific descriptions about the utilisation of Asoka are available in Puranas as well as in Ayurveda. As a medicinal tree, the utility of asoka seems to have been mentioned first in Charaka Samhitha as analgesic and antispasmodic. Other classical works, such as Susrutha Samhitha and Ashtangahridaya mention the therapeutical applications of asoka. During the Nighantu period, pharmacological properties as well as pharmacotherapeutics were indicated. In the medicinal and therapeutic texts, pharmaceutical forms made out of asoka and applications in single and poly herbal formulations have been mentioned (Karalam, 2007). Medicinal preparations of asoka are often referred in Indian system of medicines and various traditional medical practices. It is recommended in formulations as analgesic in treatment of uterine disorders (Govindapanickar, 1993).

The bark of this tree rich in tannins and phenols is the primary medicinal part commercially used in ayurvedic preparations. Flowers, seeds, fruits and leaves are the other parts of tree used in medicinal preparations. Asoka is applied not only to cure various diseases but also effectively utilized for its preventive, promotional and corrective properties (Govindapanickar, 1993).

Asoka bark is used in treatment of excessive menstruation as a uterine sedative. It has a stimulating effect on endometrium and ovarian tissue and is useful in menorrhagia. The well-known Ayurvedic preparations of bark are Asokarishtam and Asokaghrutham. Ash of plant is good for external application in rheumarthritis. Bleeding from piles is reduced with the plant. Bark is

refrigerant, astringent to bowel, alterative, anthelmintic, demulcent and emollient. The bark of asoka is useful in dyspepsia, fever, dipsia, burning sensation, colic ulcers and pimples. Leaves are depurative and their juice mixed with cumin seeds is used for treating stomach ache. Flowers pounded and mixed with water are useful in haemorrhage dysentery. Dried flowers are used in diabetes. Flowers are used in the treatment of bleeding piles, scabies in children and other skin diseases. Seeds are reported to be used in the cure of urinary discharges. Seed extract is effective against dermatophytic fungi. The bark, flowers and fruits are prescribed in combination with other drugs for the treatment of snake bite and scorpion stings (Sivarajan and Balachandran, 1994).

Approximately 70 tonnes of asoka bark is used annually by Ayurvedic industries in Kerala for preparing a variety of Ayurvedic medicines. Due to over exploitation, *Saraca asoca* has been almost depleted from its natural habitats in India, particularly from the Western Ghats. International Union for conservation of Nature (IUCN) has categorized this species as 'globally vulnerable'. The rarity has lead to substitution with the bark of a few leguminous as well as non-leguminous trees. This affects the property and quality of the medicinal preparations. Due to its acute short supply compared to its demand, National Medicinal Plant Board has included *Saraca asoca* in the list of 32 medicinal plants identified and prioritized for development at the national level.

Sustainable utilization of asoka is an accepted viable practice for their perpetuation. But when the demand is high, the extraction exceeds the sustainable limits and leads to rarity and even depletion of the resource from the area. Cultivation of asoka is a viable alternative to reduce the pressure on the resources in their natural habitats and also can ensure the quality and genuineness of the drugs. In Kerala, the existing variability in *S. asoca* germplasm has not been exploited so far, as a result not a single known variety is available for commercial cultivation. Also there is a need to assess the variation among *S. asoca* seedlings, so that better accessions can be selected at seedling stage itself, so as to save time and resources used in germplasm evaluation.

Breeding system for improvement of a particular species is devised from the knowledge of its reproductive behaviour. *S. asoca* is an overexploited medicinal species, which is of high commercial value in herbal industry. The bioactivities of this plant are due to the large number of secondary metabolites present in the various parts of the plant. Now it is the present need for the development of better asoka genotypes having higher biochemical constituents and also to popularise its cultivation, to free from its adulteration and minimize the gap between demand and supply. With this view, the present study in asoka was undertaken with the following objectives.

1. Assessment of morphological variations of existing germplasm
2. Studies on reproductive biology
3. Collection and evaluation of seed and seedling characters
4. Evaluation of therapeutical components
5. Molecular characterization

Review of Literature

2. REVIEW OF LITERATURE

A brief review of the literature on various aspects related to *S.asoca* and its related tree or medicinal species is presented under the following heads.

1. Morphological variations of existing germplasm

2. Reproductive biology of *S. asoca*

3. Collection and evaluation of seed and seedling characters

4. Evaluation of therapeutical components of *S. asoca*

5. Molecular characterization

2.1 MORPHOLOGICAL VARIATIONS OF EXISTING GERMPLASM

2.1.1 Genetic variability

S.asoca was indicated as a small evergreen tree about 5-8 m in height. The leaves were equally pinnate and15-20 cm long. The number of leaflets per leaf varied from 6 to 12. Leaflets were oblong-lanceolate, glabrous and 7.5-20.5 x 1.3 cm. The bark had warty surface and was dark brown to grey or almost black (Dhiman, 2003).

S.asoca was pointed out as a medium sized evergreen tree, reaching a height of 15 m with spreading branches according to Singh and Somadey (2005). The leaves were alternate compound upto 32 cm long. Number of leaflets/leaf varied from 6 to 12. Leaflets were oblong or oblong lanceolate, acute to 20 cm long, entire short petioled.

According to Retnam and Martin(2006), 4-6 pairs of leaflets were detected per single leaf in *S.asoca*. Leaves were found to be paripinnately compound. Leaflets were oblong to lanceolate, coriaceous and glabrous above and pale below.

Hiremath and Gopinath (2005) indicated that *S. asoca* was initially slow to establish but eventually developed into a tree with a compact canopy of pinnate leaves composed of lance-shaped leaflets. New foliage was identified as soft, limp and pale green to light pink, becoming stiffer and deeper green as it matured.

Leaflets of *S.asoca* were reported to be ± 25 x 7 cm size, ovate oblong, acute or obtuse, glabrous, base rounded or cuneate, young leaves flesh red (Subramanian, 1995).

In S.asoca, leaves were 25 cm long, leaflets were 4-6 pairs, oblong-lanceolate, base oblique, to 18 x 5 cm, petiole 0.5 cm long, stipules scarious, ovate, 2 cm long (Sasidharan and Sivarajan, 1996).

Bark from old stem of *S.asoca* was dark green in colour, but often marked by bluish and ash white patches of lichens. The outer surface was rough due to the presence of rounded and oval lenticels, the formation of short vertical narrow fissures of varying lengths and partial exfoliation of the outer rind in thin flakes. The thickness of whole bark varied from 5 mm to 1 cm, depending upon the age of the tree. The entire cut surface turned reddish on exposure to air (Kapoor, 2001).

Genotypes of Quince (*Cydonia oblonga* Mill.) recorded a range of tree height from 2.13 to 6.15 m with the mean of 3.82 m and coefficient of variation of 26.06. Genotype SKAUQ-005 was found to be tallest whereas SKAUQ-004 and SKAUQ-010 were the smallest in respect of tree height. The leaf shape of all the genotypes of Quince was ovate oblong type except SKAUQ-23 which had obviate type of leaf shape. The values of tree girth ranged from 11.2 to 82.7 cm with the mean of 34.41 cm (Ahmed *et al.*, 2008).

Botanical descriptions of *Lannea oleosa* (wild grapes), found in the Sudanian zones of West Africa indicated that the tree grows up to a height of 16 m. Leaves were comprised of 1-3 pairs of asymmetrical leaflets, plus the terminal one. The leaflets were ovate-lanceolate, obtuse and unequal at the base. The

range of leaflet length and width in this species was 5.5-13 cm and 2.7-4.5 cm respectively. The bark was observed to be grey white with a spiral twist. Bark was smooth when the tree was young and became splintery with age (Secande, 2007).

Investigations in ber genotypes were undertaken to know the casual components of relationship among various traits. The data recorded on tree height identified a mean value of 2.64 m with the coefficient of variation 7.62 (Saran *et al.*, 2007).

Morphological analysis of South American tropical *Cecropia obtuse* revealed a mean tree height of 13.9 ± 2.0 m and a mean tree diameter of 18.1 ± 1.2 cm, 1.30 m from the ground (Heuret *et al.*, 2002).

Stoney (1997) indicated *Azadirachta indica* as a small to medium-sized tree, with a short, straight bole. Stem was branched at 2-5 m forming a broad, dense, round or oval crown. Total height was recorded as 15-25 m, occasionally reaching up to 30 m. Leaves were identified as alternate, imparippinnately compound, 20-38 cm and bunched at the tip of branches. The stem girth ranged from 30 to 90 cm. Neem had moderately thick, fissured, grey outer bark, with a reddish-brown inner bark.

Joker and Jepsen (2003) reported *Baikiaea plurijuga* (Rhodesian teak) as a medium to large tree, 8-15 m tall, with a large, dense, spreading crown. Leaves were pointed out to be alternate and compound. In each leaf, 4 to 5 pairs of opposite leaflets occured. Each leaflet was upto 7 cm long, sparingly hairy especially on the lower surfaces and midrib, the tip rounded. Bark was identified as smooth and pale at first, but fissured and cracked in older trees.

Pongamia pinnata, as identified by Kundu (2008) was a medium sized glabrous tree, with a short bole and spreading crown.

Sorbus alnifolia attained growth in any position without a problem and eventually achieved heights of 20 m and it had a rounded crown. The tree of *Salix alba* grew to about 20 m and it had orange-brown young branches (Vermeulen, 1997).

Lateral leaflets in neem were opposite, terminal leaflet somewhat larger than lateral ones, leaflets were sessile or subsessile, 2-3.3 cm long and 0.8-1.5 cm broad ovate-lanceolate, acuminate, crenate to unequally serrate, more or less 3-lobed (Tewari, 1992).

Thin, smooth, grey bark of *Erythrina indica* was identified by Chandrasena (2005). The leaves of Butea frondosa were identified as large, leathery, broad and trifoliate.

An investigation was undertaken to assess the relationships between diameter and height with bark thickness of Lebanon oak (*Quercus libani*) and mean bark thickness measured was about 12.6 mm (Valipour *et al.*, 2009).

Bark of *Polyalthia longifolia* was thick and very hard, compact but sometimes segmented into rectangular blocks. *Eucalyptus globulus* had smooth and soft brown bark, thin outer layer peeled off as large papery strips (Ghosh, 2006).

Leaf of *Butea monosperma* consisted of three leaflets, of which two 8-12 cm across were opposite to each other, the third and larger, 12-20 cm across, was some distance away from the other. All the leaflets were leathery and stiff, the terminal one with equal sides at the base the laterals very strongly unequal-sided at the base, all obtuse or rounded at the apex. Leaves in *Cassia fistula* were of a deep green colour, compound in structure, each consisted of 4-8 pairs of leaflets (Santapau, 1966).

Leaves of *Terminalia chebula* were sub-opposite, ovate or elliptic (Singh *et al.*, 1990).

Daniel (2008) pointed out that the leaves of *Albizzia lebbeck* are bipinnate having 8-18 leaflets.

Pongamia pinnata leaves were imparipinnate, but leaflets were opposite, stipellate, ovate to ovate-elliptic, shortly acuminate, glabrous and bright green (Reddy *et al.*, 2007).

In *Sapindus drummondii*, leaves were alternate, even pinnately compound with entire margin, lanceolate to oblong leaflets, deciduous and 2 to 4 inch long leaflets (Gilman, 1997).

Simarouba glauca was reported as a small to medium sized evergreen tree growing upo to 7-15 m in height seldom exceeding 20 to 22 m with tap root system and cylindrical stem. The canopy was spread to about 3-5 m radius from main trunk (Peter, 2007).

Pterocarpus marsupium was recognized as one of the largest trees of deciduous forests. It attained a height of about 30 m and diameter of about 2½ m. The tree had a highly branched spreading crown (Gopikumar *et al.*, 2003).

2.1.2 Heritability, genetic advance and genetic gain

A study was conducted to estimate the variability for catechin content and other phenotypic traits of *Acacia catechu.* Wide range of genetic variability was observed for almost all the characters. Tree height, diameter at breast height, crown width, catechin content and number of seeds per pod had high estimates of genotypic coefficient of variation, heritability, genetic gain and genetic advance. Catechin content showed significant positive relation with diameter at breast height, whereas significant but negative relation was observed for tree height (Singh *et al.*, 2004).

2.1.3 Phenotypic and genotypic correlation

Qualitative characters investigated by Oboh *et al.* (2008) in *Terminalia catappa* showed that leaves were simple with entire margins and reticulate venation in all trees. Leaf length ranged from 8.58 to 17.30 cm with a mean value of 14.27 cm and coefficient of variation of 13.29%. Leaf length and leaf width exhibited significant positive correlations.

A study was conducted to estimate the variability for catechin content and other phenotypic traits of *Acacia catechu.* Catechin content showed significant positive relation with diameter at breast height, whereas significant but negative relation was observed for tree height (Singh *et al.*, 2004). In ber, the association between plant height and stem girth was pointed out by Saran *et al.* (2007).

2.1.4 Path analysis

In *Broussonetia papyrifera* (paper mulberry), allometric models were used to estimate the inner bark yield. Inner bark yield of paper mulberry ranged from 10 to 208 g/m^2 and increased with age. The models using diameter at breast height and stem height or diameter at breast height only as independent variables explained well the variation in inner bark yield (Saito *et al.*, 2009).

2.1.5 Cluster analysis

To assess the genetic diversity in tamarind, multivariate analysis was undertaken by Hanamashetti *et al.* (2000). The first canonical vector, pulp weight accounted 99.231 per cent towards divergence. Forty genotypes of tamarind were grouped into 10 clusters. Cluster VII, VIII and IX were the largest with 5 genotypes each, while cluster V was the smallest with two genotypes. Cluster mean for eight quantitative traits of forty genotypes were calculated. Study indicated that pulp weight was the most important factor, which was responsible for discrimination in the genotypes studies.

Exploration and collection of *Plumbago rosea* carried out in the foot hills of Western Ghats and 25 collected accessions were evaluated for genetic variability in morphological traits. All the 17 accessions were grouped into 5 clusters based on their genetic distance. Evaluation of the clustering pattern of the accessions indicated that the clustering pattern did not follow the geographical distribution (Radhakrishnan *et al.*, 2008b).

Twenty nine accessions of Brahmi collected from different geographical regions of Kerala were evaluated for variability. Significant genetic variability was expressed by all the characters studied. Cluster analysis indicated that there was no parallelism between geographical distribution and clustering pattern of accessions. Accession 29 was identified as better plant for higher biomass yield and Bacoside A content followed by Accession 14 (Radhakrishnan *et al.*, 2008a).

Genetic divergence studies in jackfruit (*Artocarpus heterophyllus*) grouped the 44 genotypes into 13 clusters. Eight genotypes were in cluster VI, 7 genotypes were in cluster III, 5 genotypes were in clusters VII, VIII and X, while rest of the clusters had one genotype each. The clustering pattern of gentoypes showed that genotypes from the same area did not belong to the same cluster (Maiti *et al.*, 2002).

In the evaluation of sixty four genotypes of Isabgol, all the genotypes were grouped into nine clusters using D^2 statistics for ten characters related to seed yield per plot. Cluster I was large and consisted of fifty two genotypes followed by cluster VI (five genotypes) and remaining seven clusters (II, III, IV, V, VII, VIII, IX) were solitary in nature. The geographical origin of genotypes did not show any definite relationship with genetic diversity. However the genotypes FR192, DRP-64, DRP-72 and BNPO genetically appeared to be the most diverse genotypes (Abert *et al.*, 2006).

2.1.6 Discriminant function analysis

Selection indices were constructed by Francies (1998) in cocoa for all possible combinations of the five predictor variables. The discriminant function involving the five traits viz., dry bean weight, pod weight, bean thickness, bean length and efficiency index, recorded a genetic advance of 2.37. H4 and H3 secured the first and second ranks based on index computed using five traits based discriminant functions.

2.2 Reproductive biology of *S. asoca* and related species

2.2.1 Flowering season

Crop	Characteristics	References
Caesalpiniaceae *Saraca asoca*	Flowers in large compact orange-red clusters in February-March	Randhawa, 1983
	Flowering starts from second week of January and it continues for a period of over 100 days	Kumar *et al.*, 2007(a)
Tamarindus indica	Blooms during mid May to mid July	Sasidharan *et al.*, 1999
Papilionaceae *Pterocarpus santalinus*	Flowering during March to May and tree flowers intermittently for three weeks	Rao and Raju, 2002
Mimosaceae *Acacia mangium*	Flower buds appear in early November. Flowering between January and May	Hopkins and Graham, 1989
Erythrina falcate	Flowers during dry season from late August to late October	Etcheverry and Aleman, 2005
Verbenaceae *Tectona grandis*	Flowering during rainy season and trees tend to flower synchronously	Sedgley and Griffin, 1989
Bombacaceae *Bombax ceiba*	Flowers from January to March	Bhattacharya and Mandal, 2000

2.2.2 Inflorescence

Crop	Characteristics	References
Caesalpiniaceae *Saraca asoca*	Flowers are borne in axillary corymbs	Dhiman, 2006
	Dense, corymbose panicles, usually 10 cm broad from the scars of fallen leaves	Vardhana, 2007
Papilionaceae *Pterocarpus macrocarpus*	Inflorescence is raceme with average thirty flowers	Yupa and John, 2002
Butea monosperma	Profusely branched, paniculate raceme in which upto 160 flowers appear that open in acropetal order	Tandon *et al.*, 2003
Verbenaceae *Gmelina arborea*	Flowers produced in paniculate cymes which arise at terminal and lateral shoots	Raju and Rao, 2006
Myrtaceae *Eucalyptus citriodora*	Axillary, corymbose panicle, umbels 3-5 flowered on terate 5-7 mm long peduncles	Shiva *et al.*, 2002

2.2.3 **Floral biology**

Crop	Characteristics	References
Caesalpiniaceae *Saraca asoca*	Stamens 3-8 and free, filaments very slender, anther versatile, ovary stipitate	Kumar *et al.*, 2005
	Flowers are scented at night	Singh and Somadey, 2005
	Flowers orange, changing to vermillion and are fragrant. Calyx tubular, sepals – 4, petaloid, 1.2 cm long, petals absent	Vardhana, 2007
Papilionaceae *Pterocarpus santalinus*	Flowers are zygomorphic, bisexual. Calyx tubular at base and free towards apex, Papilionaceous corolla, stamens 10, in two bundles of five each. Ovary with two ovules.	Rao and Raju, 2002
Simaroubaceae *Balanites aegyptiaca*	Actinomorphic, hermaphrodite, nectariferous flowers with sepals pubescent and corolla glabrous. Ovary holds 5 anatropous ovules in axial placentation	Ndoye *et al.*, 2004
Guttiferae *Garcinia mangostana*	Four sepals and petals, many stamens with filamentous anthers, sessile stigma, corrugated stigma surface	Te-chato, 2007
Bombacaceae *Bombax ceiba*	Calyx leathery, cup-shaped and persistent, petals fleshy, stamens numerous forming 5 bundles, stigma digitate	Bhattacharya and Mandal, 2000
Rutaceae *Aegle marmelos*	Flowers have four recurved, fleshy petals (green outside, yellowish inside) and fifty or more, greenish	Thampman, 1993

	yellow stamens	
Verbenaceae *Gmelina arborea*	Bell shaped, five toothed calyx, bright orange yellow corolla and four stamens in two pairs. Four celled ovary with one ovule in each cell	Stock *et al.*, 2004

2.2.4 Reproductive period traits

Crop	Characteristics	References
Caesalpiniaceae *Bauhinia variegate*	Duration of flowering ranges from 26-49 days. Mean time to flower bud development is 20.88 to 24.92 days	Wani and Chauhan, 2008

2.2.5 Fruit characteristics

Crop	Features	References
Caesalpiniaceae *Saraca asoca*	Pods 12-20 x 4 cm, oblong, coriaceous, flat	Vardhana, 2007
	Pods upto 15 cm long and contain 4-8 seeds	Singh and Somadey,2005
	Pods are flat, oblong, woody and 7.5-25.0 x 3.8-5.0 cm.	Dhiman, 2006
Papilionaceae *Sophora fernandeziana*	Pods are four winged, dry, coriaceous to subligueous, brown and ultimately dehiscent	Bernardello *et al.*, 2004
Pongamia pinnata	Pods are 4.5 cm long and 1.5-2.5 cm wide, broad, pointed at both ends, and one or two seeded	Kumar *et al.*, 2004
Combretaceae *Terminalia arjuna*	Obvoid, oblong, dark brown to reddish, brown fibrous woody indehiscent drupe	Kumar and Kumar, 1994
Anacardiaceae *Lanvea microcarpa*	Ellipsoidal drupe becomes purple-black at maturity	Secande, 2007
Meliaceae *Azadirachta indica*	Fruit is a smooth, ellipsoidal drupe containing one seed	Stoney, 1997

2.2.6 Seed characteristics

Crop	Features	References
Ceasalpiniaceae *Saraca asoca*	Seeds are grey in colour	Singh and Somadey, 2005
	Seeds 4-8, ellipsoid-oblong, compressed	Chatterjee and Prakashi, 1992
Papilionaceae *Sophera fernandeziana*	Seeds are dark brown, dry and hard and number of seeds per fruit ranged from 1 to 4	Bernardello *et al.*, 2004
Pongamia pinnata	Elliptical, reniform, compressed, reddish-brown seeds are with a thin seed coat	Kumar *et al.*, 2004
Baikiaea plurijuga	Hard, oval seeds are with a smooth and shiny brown surface	Joker and Jepsen, 2003
Verbenaceae *Gmelina arborea*	Seeds germinate in 7-21 days	Kijar, 2002

2.2.7 Anthesis and anther dehiscence

Crop	Features	References
Papilionaceae *Pterocarpus santalinus*	Anthesis around 00.30 hr. Anther dehisces asynchronously after anthesis. Two anthers an hour later, three anthers again after one hour and other five again after one and half hour	Rao and Raju, 2002
Butea monosperma	Anthesis occurs between 09.00 and 10.30 hr. Anthers dehisce between 08.30 and 15.00 hr 1 day before anthesis	Tandon *et al.*, 2003
Rutaceae *Aegle marmelos*	Maximum anthesis occurs between 6.00 and 8.00 hr	Srivastava and Singh, 2000

2.2.8 Stigma receptivity

Crop	Features	References
Caesalpiniaceae *Tamarindus indica*	Stigma becomes receptive one day prior to anthesis, and characterizes as bulged and turgid	Mandal and Gibson, 1998
Papilionaceae *Pterocarpus santalinus*	Stigma become receptive when first two anthers dehisce and remain until late evening of same day	Rao and Raju, 2002
Rutaceae *Citrus sp.*	Stigma is receptive 2-3 days earlier to anthesis and loss receptivity 4 days after anthesis with maximum receptivity on the day of anthesis.	Shukla *et al.*, 2004
Verbenaceae *Tectona grandis*	Papillate stigma of teak is wet type with a hollow style and has a short receptive period (19.00-13.00 hr)	Tangmitcharroen and Owens, 1997
Rosaceae *Prunus dulcis*	Pistil is receiptive for 48 hrs after anthesis	Egea *et al.*, 2004

2.2.9 Pollen morphology

Crop	Features	References
Caesalpiniaceae *Bauhinia sp.*	Tricolporate pollens	Zou *et al.*, 2003
Papilionaceae *Pterocarpus santalinus*	Pollen grains are fertile, yellow, spherical, tricolpate, smooth-walled and 21 micrometre in size	Rao and Raju, 2002
Dalbergia latifolia	3-zonocolporate, size 19.17 x 16.17 μm, exine surface psilate	Kuriakose, 2005
Uraria crinita	Tricolporate and two celled at the time of shedding	Chin *et al.*, 1999
Bombacaecae *Bombax ceiba*	3-colporate, polar outline triangular, equational outline elliptic, exine thick, reticulate	Bhattacharya and Mandal, 2000
Rosaceae *Prunus armeniaca*	Pollen aperture is tritem, pore shapers are colpate, large pollen of size 50.18 to 60.83 μm	Asma, 2008
Rhamnaceae *Ziziphus sp.*	Tricolporate, psilate or faintly reticulate exine ornamentation	Gupta *et al.*, 2002
Bignoniaceae *Millingtonia hortensis*	Tricolporate and tectum of exine is reticulate	Aftab and Perveen, 2006
Convolvulaceae *Convolvulus sp.*	3-zonocolpate pollen grains	Menemen and Jury, 2002

2.2.10 Pollen viability

Crop	Features	References
Punicaceae *Punica granatum*	Absolute pollen viability ranges from 41.75 to 89.74	Ali *et al.*, 1998
Pinaceae *Abies pinsapo*	Pollen viability in fertilization period is 100%, but lost after 6 months of storage from shedding	Arista and Talavera, 1994
Simaroubaceae *Balanites aegyptiaca*	Viability rate is 92	Ndoye *et al.*, 2004
Rosaceae *Prunus avium*	Pollen viability values ranges from 12.4% to 93%	Davarynejad *et al.*, 2008

2.2.11 Pollination system

Crop	Features	References
Caesalpiniaceae *Saraca asoca*	Fruit set following geitnogamous selfing by hand pollinationis very low (5.2%) compared to manual cross pollination (44.12%).	Kumar *et al.*, 2007(a)
Papilionaceae *Baikiaea plurijuga*	Insect cross pollinate the flowers	Joker and Jepsen, 2003
Verbenaceae *G. arborea*	Per cent fruit set of 16.5 in open pollination. Common pollinators are birds, honeybees, *Xylocopa*, ants, beetles and small insects	George, 2007
Meliaceae *Azadirachta indica*	Highly cross pollinated	Farooqui *et al.*, 1998
Bombacaceae *Bombax ceiba*	Allogamy by flower visitors like *Sturnus, Nectarinia, Acridotheres, Pycnonotus*	Bhattacharya and Mandal, 2000
Simaroubaceae *Balanites aegyptiaca*	Ratio of allopollination is 37%. Wind and insects (Hymenopterae) favours crosspollination	Ndoye *et al.*, 2004
Acanthaceae *Adhatoda vasica*	Species is self incompatible but over 50% of cross pollinated flowers set fruits. Pollination efficiency under field conditions is high (95%)	Shivanna, 2009

2.2.12 Evaluation of reproductive biology traits

2.2.12.1 Correlation among reproductive biology traits

Carpenter et al. (2003) assessed the reproductive traits of tropical rain-forest tree in New Caledonia. Seed size was significantly correlated with fruit size. Other correlations, between flower size and fruit size, between seed size and seed number were significant.

2.2.13 Influence of weather parameters on reproductive biology traits

2.2.13.1 Genetic variability

Flowering behaviour of 15-20 years old *S. asoca* trees revealed that under Thrissur conditions, maximum flower production occurred during February – March, while maximum mature pods and number of seeds per pod was seen during March-April and February-April respectively (Maiti, 2008).

A study was undertaken in teak to assess the extent of variation for floral traits. Interclonal variation was significant for all the floral traits, while within the clone variation was negligible. The broad sense heritability estimated based on clonal means of floral traits were higher, suggesting a strong genetic control, hence selection could yield beneficial results (Vasudeva *et al.*, 2004).

2.2.13.2 Genetic correlation

In sour cherry cultivars, flowering period took approximately 8-19 days. Davaryneiad et al. (2008) observed that the floral traits changes with temperature.

Krishnamani (2002) examined the nature and extent of spatial variation in the species diversity of woody plants in the rainforest of the Western Ghats. Flowering phenology of these trees were inversely correlated with rainfall and positively associated with the day time maximum temperature whereas the fruiting was highly correlated with night time minimum temperatures.

A study was conducted by Wolgast and Stout (1977) to determine the influence of relative humidity at the time of flowering on fruit set in bear Oak (*Quercus ilicifolia*) trees. The effect of humidity greater than 61% was found to greatly reduce fruit set.

2.3 COLLECTION AND EVALUATION OF SEED AND SEEDLING CHARACTERS

2.3.1 Genetic variability

Kumar *et al.* (2007b) studied the seed biology of *S. asoca*. They produced large recalcitrant seeds, typical to wet evergreen forest tree species with critical moisture content of around 35 per cent. Seeds germinated without any pre-germination treatment. The germination continued upto 80 days after sowing. Hundred per cent germination was achieved within a span of around 80 days.

S. asoca seeds took 28 days for germination to initiate and was completed in 42 days. Seeds sown in standard nursery bed gave 82% germination. Asoka seedlings attained an average height of 32 cm after 3 months growth. At this stage, seedlings had 5-7 pinnately compound leaves and a rachis length of 6.9 – 9.3 cm (Nayagam and Varghese, 2007).

A study of seeds of *Pongamia pinnata* from different locations indicated significant difference among seed sources for seed and seedling traits. Seed length amongst the different sources varied from 7.70 to 11.33 mm and seed width from 6.95 to 9.22 mm. S_1 source excelled other seed sources for seed length and width. Mean values of height of *P. pinuata* seedlings indicated significant differences between seed sources. Shivanna *et al.* (2007) reported a range of 5.75 to 13.95 cm for the seedling height. The collar diameter was in the range of 0.14 to 0.33 cm. Collar diameter was found maximum and minimum for seed sources S_1 and S_6 respectively. A range of 9.65 to 11.19 for number of leaves was recorded for seedlings from different seed source.

Loha *et al.* (2008) quantified the variations in seed size, germination and seedling growth of *Milletia ferruginea* based on seeds collected from six sites in Ethiopia. All the seed and seedling traits exhibited highly significant differences among seed sources and the magnitude of genetic variation was substantially higher (77-99%).

Barmukh and Nikam (2008) observed that natural resurgence in *Pterocarpus marsupium* was poor and the nursery germination was unpredictable. Joker and Jepsen (2003) revealed that the seeds of *Baikiaca plunjuga* showed high viability and germination percentage of 80-90 within 7-25 days.

Selected populations of the service tree (*Sorbus domestica* L.) were evaluated in terms of fruit and seed characteristics and field germination rate. Average field germination rate ranged from 7.14 to 83.3%, depending on the place of collection Growth intensity of one year old *Sorbus domestica* L. seedlings were evaluated by Miko and Gazo (2004). The average height of annual seedlings ranged from 60 to 210 cm.

Seeds of *Erythrina indica* were collected from ripe pods and sown in the nursery. They attained a height of 10-15 cm in one year and were used for field planting (Gupta, 1993).

Leaves of *Terminalia arjuna* were found clustered at the end of branches. First two leaves were opposite, others alternate, simple, green, elliptic or elliptic-obovate, petiolate, obtuse or apiculate at the apex, narrowed at the base, slightly wary at margin. In *Polyalthia longifolia,* first two leaves were alternate, simple, short petiolate, greenish, acuminate, entire or slightly wavy at margin, acute at the base whereas subsequent leaves were alternate, simple and stipulate. Seedlings of *Mimusops elengii* had alternate, simple, oval-elliptic, green, entire, acuminate, petiolate leaves. Seedlings of *Gmelina arborea* were raised from seeds collected from different habitats in Guwahati. They indicated internode as straight, long, light green and length of first, second internodes were 6.8 and 1.8 cm respectively. Internodes of *Mimusops elengii* were short, 9-1.4 cm long, straight slightly curved green. In *Polyalthia longifolia,* internodes were long green, straight or slightly curved (Saha *et al.*, 1998).

Source variation study of *Sapindus mukorossi* was undertaken to identify the superior seed sources for production of quality seedling. Thirteen seed sources were sampled from different locations in Himachal Pradesh. On average,

the population of Deothal, Majheen and Naina, Tikker were found to be the best on the basis of weight, germination per cent and vigour index of seed as an important criterion for delineation of the superior seed source (Bahar and Singh, 2007).

In order to assess natural variation in three species *Emblica officinalis, Syzygium cumini, Sapindus mukorossi*, a study was carried out in Himachal Pradesh. A wide range of variation was observed in fruit and seed characters of these species. Bilaspur 3 seed source was rated as the best in *E. officinalis* as it registered maximum fruit diameter, dry fruit and pulp weights. In *Sapindus mukorossi*, maximum seed diameter was recorded in Bhunter and Rehri seed sources whereas highest seed weight was recorded in Machian seed source. In *Syzygium cumini*, seed length varied from 1.18 cm to 2.12 cm (Thakur *et al.*, 2008).

In *Santalum album*, as per Veerendra *et al.* (1991), the difference in the time taken for emergence and completion of germination was highly variable, suggesting that the germination was entirely dependent on the genetic factors. The 110-140 mg light weight seeds and 141-160 mg medium weighed seeds exhibited a highly variable period of germination and the germination percentages were high. Heavier seed range showed almost uniformity in the germination period but the germination period was too longer, with low percentage of emergence (36.14%).

Kumaran *et al.* (1996) studied the variation of traits of seeds collected from 28-one-parent families of neem in seven agroclimatic zones of Tamil Nadu. Results showed significant differences among families for all seed parameters studied. The seed parameters like length, breadth, length-breadth ratio recorded moderate genotypic coefficient of variation.

2.3.2 Genetic correlation

In the different tropical tree species in Southern India, investigations proved a strong correlation of seed size with days to germination. Smaller seeds was found to germinate faster than larger seeds and species which flowered during the rainy reason had lighter seeds. It was found that seed size and viability of seeds were related to season of fruiting. Species which fruit during rainy season had heavier seeds and shorter viability than species which fruit during the dry season (Murali, 1997).

Seed size was examined as a possible explanation for variation in the size of pine seedlings. Larger seeds were found to germinate more quickly. Size differences among seedlings resulted from differences in the rate of germination unique to each seed size class. Consequently, seedling size and possibly uniformity of growth were considered to be a function of germination patterns which were strongly influenced by seed size (Dunlap and Barnett, 1983).

Results of study of *Pongamia pinnata* seeds revealed wide range of variation in germination per cent. S_2 attained highest germination per cent (89.20) and significantly differed from rest of the seed sources. Seed sources with heavier seeds possessed higher germination per cent than that of smaller and lighter seeds, may be because of more stored food in endosperm (Shivanna *et al.*, 2007).

In *Holostemma ada-kodien*, sowing of seeds at 1.0 cm depth gave higher germination (83.6%) and seedling vigour in terms of root length (4.5 cm), shoot length (4.5 cm) and vigour index (752) (George *et al.*, 2004).

A study was conducted by Kumar (2007) to determine the effect of various grades of Neem seeds on germination and seedling performance. Larger size fresh seeds showed significantly higher mean germination time as compared to medium and small size seeds. Small seeds showed maximum number of leaves, vigour index, maximum volume index followed by medium and larger size seeds.

In *Gmelina arborea*, analysis of juvenile data showed that, of the two juvenile traits measured (basal diameter and total height), total height at 9 months of age was the best predictor of performance at 72 months with genetic correlations ranging from 0.50 to 0.90. These results suggested that early selection at 9 months in gmelina clonal plantings is feasible and is a practical means of reducing the breeding and testing cycles (Padua, 2004).

2.4 Evaluation of therapeutical components of *S. asoca* and related species

Five lignan glycosides, lyoniside, nudiposide, 5-methoxy-9-β-xylopyranosyl-(−)-isolariciresinol, icariside E_3, and schizandriside, and three flavonoids, (−)-epicatechin, epiafzelechin-(4β→8)-epicatechin and procyanidin B_2, together with β-sitosterol glucoside, were isolated from a methyl alcohol (MeOH) extract of *Saraca asoca* dried bark. Their structures were determined by 1D and 2D nuclear magnetic resonance (NMR) and mass spectroscopic analysis. Antioxidant activities were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay (Sadhu *et al.*, 2007).

Flowers of asoka give β-sitosterol, flavonoids and flavone glycosides-quercetin, kaempferol-3-O-β-Dglucoside, quercetin-3-O-β-D-glucoside. The anthocyanins present are pelargonidin-3, 5-diglucoside and cyanadin-3, 5-diglucoside. Bark yields catechol and sterols-(24ζ)-24-methyl cholest-5-en-3β-ol, (22E, 24ζ)-24-ethylcholesta-5, 22-dien-3 β-ol and (24ζ)-24-ethyl cholest-5-en-3β-ol, a wax containing n-alkanes, esters and free primary alcohols. Alcoholic extract and glycoside P2 from stem bark is oxytoxic. Aerial part is CNS active, hypothermic, CNS depressant and diuretic. Stem bark is anticancerous, has spasmodic action on rabbit intestine and cardiotonic action in frog and dog. Seed is antifungal. Stem bark is astringent, antileucorrhoeic, antibilious and uterine sedative. Flower is uterine tonic, antidiabetic and antisyphilitic. Stem bark and flower is antibilious (Joy *et al.*, 1998).

The response of asoka in the treatment of menorrhagia is due to presence of ketosterol, which seem to be androgenic in nature and the organic calcium salt, which exert their effects on the functional disorder rather than any direct effect on uterine muscles. The pharmacological response observed seemed to be due to presence of the steroidal fraction and calcium salt (Sen, 1963).

Bark of asoka tree contain tannin, catechin, an organic compound of calcium, hemotoxyline and a ketosterol. The alcoholic extract, which was mostly soluble in hot water, showed the presence of fair amount of tannin and probably an organic substance containing iron. Reports indicates the isolation of ketosterol, a glycosidal fraction, a saponin and an organic calcium compound from whole plant (Kapoor, 2001).

The asoka bark yields tannin (6%), catechol, volatile oil, haematoxylin, a ketosterol and saponins (Daniel, 2005).

Asoka bark contain tannin and catechol and an active phenolic glycoside depending upon the availability place, time of collection and storage condition. Besides, the bark also contains hematoxylin, an iron and other similar substances (Dhiman, 2006).

Alcoholic extract of dry powdered bark of *S. asoca*, which was mostly soluble in hot water, showed the presence of a fair amount of tannin and probably an organic substance containing iron. Asoka bark contained a fair amount of tannin and catachin. Leucopelargonidin - 3-O-β-D-glucoside, leucopelargonidin and leucocyanidin along with β-sitosterol were isolated from stem bark as reported by Singh and Panda (2005).

George and John (2007) isolated a lectin called saracin from seed integument of *S. asoca.* Five lignan glycosides viz. lyoniside, nudiposide, icariside E_3, 5-methoxy-9-β-xylopyranosyl-(-) isolariciresinol and schizandriside and three flavonoids viz. (-) epicatechin, epiafzelechin-(4-β→B)-epicatechin and procyanidin B_2, together with β-sitosterol glucoside were isolated from a methyl alcohol extract of *S. asoca* dried bark. Quercetin, kaempferol-3- β-D-glucoside, quercetin-3- β-D-glucoside and β-sitosterol had been isolated from the petroleum ether and methanol extract of the plant. Leucopelargonidin-3-0- β-D-glucoside, leucopelargonidin and leucocyanidin had been isolated and identified from the acetone extract of the stem bark.

Flowers of *S. asoca* constituted fatty acids and gallic acid, apigenin - 7-O-β-D-glucoside, cyanidin – 3, 5 – diglucoside, kaempferol - 3-O-β-D-glucoside, pelargonidin – 3, 5 – diglucoside, quercetin and its 3-O-β-D-glucoside and sitosterol. Bark yielded alkanes esters and primary alcohols, n-octacosanol, tannin, catachin, (+) catechol, (-) – epicatechin, (-) epicatechol, leucocyanidin, leucopelargonidin, procyanidin B_2, 11-desoxy procyanidin B, (24ζ) – 24-methylcholest-5en-3β-o1, (24ζ) -24 ethylcholest-5en-3β-ol, (22B, 24ζ) – 24 ethylcholest-5, 22-dien-3β-ol (Chatterjee and Prakashi, 1992).

The presence of haematoxylin in asoka was identified by the scientist Abet in 1887. Bark has large proportion of tannin and catachin (Madukakuzhi, 1993).

Biochemical constituents of asoka bark were indicated as 6% tannin, catechol, glycosides like ketosterol, haematoxylin and saponin. Bark, also reported to contain calcium and steroids (Nesamony, 1995).

S. asoca plant was identified to be widely used in Indian medicines for the treatment of uterine disorders. The activity of the drug was due to the presence of steroidal component and calcium salt. Bark also contained tannins (Maiti, 2008).

S. asoca bark was reported to have tannin 6%, catechol, sterol. Flower had β-sitosterol, flavonoids, flavone glucosides like quercetin, kaempferol – 3-O- β-D glucosides, quercetin -3-O- β-D glucosides. Biochemical constituents of asoka flowers also included pelargonidin – 3, 5 – diglucoside and cyanidin – 3, 5-diglucoside (Skaria *et al.*, 2009).

24 methylcholest – 5-3n-3B-ol, 24-ethylcholesta-5, 22-dien-3B-ol and 24-ethylcholest-5en-3B-ol were isolated from asoka bark. Wax from the bark contained n-alkanes. Palmitic, steraric, linoleic and linolenic acid were identified in fixed oil from flowers. β-sitosterol, quercetin, kaempterol quercetin were isolated from *S. asoca* flowers (Joshi, 2000).

From different *Andrographis species*, the major classes of compounds isolated were flavonoids, xanthonoids and terpenoids. About 22 flavones, 13 flavone glycosides, 7 flavanones, one flavanone glycoside along with 2 chalcones and one chalcone glycoside had been isolated from various *Andrographis species.* Secondary metabolites like labdane diterpenes and their glycosides, flavones, flavone glycosides and sterol etc. were present in genus *Andrographis* as complex mixtures (Govil *et al.*, 2007).

Bark of *Pterocarpus marsupium* was identified to contain l-epicatechin. Heartwood yielded flavanoids liquiritigenin, isoliquiritigenin and a number of C-glycosides like pterocarposide, pteroisoauroside, marsuposide, benzoturanone C-glucoside, pteroside, vijayosin and a glucoside of 2,6-dihydroxybenzene (Daniel, 2008).

In the experiments conducted by Lincy (2007) it was revealed that Gymnema and *Costus pictus* recorded four similar compounds of phenolic origin. This similarity might have been contributed by enzymes of glycoprotein origin. The presence of hydrolysable tannins in *C.pictus* and Gymnema attracted the medicinal use of these plants as antidiabetic plants. Secondary metabolites such as phenols, tannin, flavonoids, quinones, terpenoids, saponins, carotenoids, alkaloids and plant acid were also expressed in these plants.

As per Shyamkumar *et al.* (2007), the important medicinal tree *Terminalia chebula* contained hydrolysable type of tannins. Maximum amount of tannin was present in the fruit pulp of *T. chebula*, known as myrobalan. The dried pericarp of seed contained tannin and 30-35% of astringent substances. Tannins such as chebulagic acid, chebulinic acid, tannic acid and gallic acid were extensively used for medicinal purposes and *T. chebula* was called the 'King of medicines'.

Monomeric and dimeric indole alkaloids were characteristic of *Alstonia scholaris.* A study by Thomas *et al.* (2008) first reported the presence of a new secoiridoid glucoside, named alstonoside 1, together with two isoflavone apioglucosides isolated from the stems of *A. scholaris.*

Leaves and bark of fifteen species of Mexican Anacardiaceae species were analyzed for the presence of toxic phenols such as catechols, resorcinols and biflavonoids. Based on the results, it was indicated that toxic phenols are present in fifty two species belonging to twenty-seven genera in Anacardiaceae. The majority of species contained toxic catechols, a few species contain toxic resorcinols and sixteen species contain biflavonoids (Ortigoza *et al.*, 2003).

Phytochenical screening of medicinal plant *Mallotus oppositifolius* revealed the presence of secondary metabolites such as alkaloids, phenols flavonoids, anthroquinones and cardenolides. A higher concentration of these residues was observed in the leaves than in the root. Five hydrolysable tannins and cytotoxic phloroglucinol had been reported from the bark of *M. japonicus,* another *Mallotus* species (Okigbo *et al.*, 2009).

Tannic composition was studied in reproduction cork samples from three different trees of Spanish *Quercus suber* by Cadahia *et al.* (1998). The ellagitannins, roburins A and E, grandinin, vescalagin and castalagin, were identified and quantified. The group of hydrolysable tannins was the most abundant in the tannic extract in all samples. Among them, castalagin was the main component, followed by vescalagin, grandinin, roburin E, and to a much lesser extent, roburin A.

Leaves, flowers and fruits of *Caesalpinia pulcherrima* constituted tannins, gums, resin, benzoic acid. Presence of cyanidin-3, 5-diglucoside was also reported from the flowers, hydrocyanic acid from the leaves. The root contained caesalpin type diterpenoids along with sitosterol (Khare, 2004).

Alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin and cardiac glycoside distribution in medicinal plants such as *Cleome nutidosperma, Emilia coccinea, Euphorbia, heterophylla, Physalis angolata, Richardia bransitensis, Scoparia dulcis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis* and *Tridax procumbens* were investigated. All the plants were found to contain alkaloids, tannins and flavonoids except for the

absence of tannins in *S. acuta* and flavonoids in S. cayennsis respectively (Edeoga *et al.*, 2005).

In *Myrothannus flabellifolia,* as per Pizzi and Cameron (1986), the resuscitation behaviour expressed when subjected to drought and subsequent rehydration was due to absence of cell wall cracking. One of the reasons attributed to this was the high polyflavonoid tannins, which increased under drought-induced stress. The helicoidal tridimensional structures of these tannins functioned as springs disallowing cracking of the cell walls and rendered possible the resuscitation characteristics of *M. flabellifolia.* This explained that many trees, originally of Southern Africa, a drought area, were high producers of tannins.

Reports by Saxena *et al.* (2008) indicated that various species of genus *Gentiana* were widely used as appetite stimulant, febrifuge, poison antidote, antiplatelet and antipsychotic in the traditional systems of medicine. Chemical investigation of genus *Gentiana* resulted in the isolation and characterization of a variety of constituents viz. anthocyanins, secoiridoids, iridoids, flavonoids, xanthones and terpenoids.

An analysis of the flavonoid complexes in *Inula helenium*, revealed the presence of quercetin and kaempferol glycosides at a total flavonoid content of 3.74%. Pollen and flowers contained pentamethyl quercetin. Pollen also contained isoquercitrin, quercetin-3-O-glucosyl-galactoside, and kaempferol – 3-O-glucoside (Kolesnikov and Gins, 2001).

2.5 Molecular characterisation

2.5.1 RAPD markers

Padmalatha and Prasad (2006) presented the optimized procedure of DNA isolation and PCR conditions for RAPD analysis of selected medicinal and aromatic plants containing high levels of polysaccharides, polyphenols and secondary metabolites including *Saraca asoca*. The modified conditions used in RAPD analysis, consistently amplified DNA fragments of these plant species belonging to different genera, which are highly recalcitrant.

RAPD markers were reported to be cheaper and faster to produce when compared to RFLP, but they were dominant markers and cannot be used to differentiate homozygous and heterozygous individuals and sometimes not reproducible between laboratories (Govil *et al.*, 2004).

Genetic diversity and relationships among 10 genotypes in *Andrographis paniculata* were evaluated using RAPD by Sharma *et al.* (2009). A total of 37 bands were generated from 10 primers, of which 26 were polymorphic. Number of RAPD bands ranged from 2-7 per primer and the percentage of polymorphism as revealed by individual primer varied from 33.3 to 100. Dendrogram indicated that the DNA analysis of genotypes grouped them broadly into two main clusters, comprising 3 and 7 genotypes respectively.

In *Costus speciosus*, RAPD-PCR analysis involving 12 decamer random primers were used to assess the quantum of genetic variation at genomic level. Despite morphological identity, a great deal of polymorphism was observed among the accessions. The dendrogram separated the accessions collected from 14 different locations into two major groups, having 62% similarity. The study confirmed the suitability of RAPD as a reliable, simple, easy to handle and elegant tool in molecular diagnosis of different accessions of this species (Mandal *et al.*, 2007).

RAPD analysis was carried out by Padmesh *et al.* (1999) to determine intraspecific variability in 15 accessions of *Andrographis paniculata.* Accessions collected from parts of India and South-East Asia on molecular analysis revealed moderate variation within the species. Similarity measurement using UPGMA followed by cluster analysis resulted in 5 major groups based on geographical distribution. Results indicated that RAPD could be effectively used for genetic diversity analysis in wild species of prospective value, as it is reliable, rapid and superior to those based on pedigree information.

Joy and Maridass (2008) estimated the genetic inter-relationship of nine *Cinnamomum* species using RAPD markers. Fifteen selected RAPD primers were used out of which, 2 primers were amplified in all cinnamon species. *Cinnamomum verum* indicated close similarity (87%) with *C. citronella* and another two species of *C. camphora* and *C. glucens.*

A RAPD analysis employing 39 RAPD markers used to assess the patterns of genetic variation in *Prunus Africana* in Cameroon and Kenya revealed that significantly more variation partitioned among Kenyan populations than in Cameroon, with a clear genetic disjunction between Kenyan stands (Muchugi *et al.*, 2005).

Genetic diversity was assessed by RAPD markers in *Oroxylum indicum* collected from eight locations in Andhra Pradesh. High level of genetic similarity was observed in the collected accessions as reported by Jayaram and Prasad (2008). Forty random primers used generated a polymorphism of 49.61%. Cluster analysis based on Dice coefficient showed two major groups indicating that the genetic diversity of this species is low.

The assessment of genetic diversity at DNA level among 15 elite accessions of *Mentha spicata* identified that all the accessions were differentiable from each other with the combination of primers. The polymorphism analysis showed that 68 out of 80 random primers responded in terms of amplified DNA bands and 61 of these responding primers produced polymorphic bands among the

accessions. A total of 341 polymorphic bands were observed from the 450 bands scored indicating wider application at RAPD profiling in *Mentha spicata* (Shasany *et al.*, 2002).

Anand *et al.* (2007) reported that the RAPD primers showed distinct polymorphism within the neem accessions that amounted to about 50% of the bands detected through electrophoresis. The dendrogram profile clearly demarcated the accessions into two main groups.

To identify the extent of genetic variation in neem, the powerful RAPD technique had been employed. RAPD profiles of 34 accessions of neem were generated with 200 decamer random primers, of which the data from the 49 primers, that resulted in reproducible amplification products, were considered for analysis. The similarities in RAPD profiles amongst the different DNAs suggested that neem may be having a narrow genetic base (Farooqui *et al.*, 1998).

Padmalatha et al., (2007) reported the molecular variations in accessions of *Pterocarpus santalinus* collected from Kerala, Karnataka and Andhra Pradesh. Molecular investigations by RAPD markers revealed that, out of the 40 primers screened, 26 primers selected for the data analysis generated a total of 217 scorable markers, all of which were polymorphic. This high proportion of polymorphism was found with 53 unique markers.

Rossetto *et al.* (1997) finger-printed some *Eucalyptus* species using RAPD markers. Their studies strongly supported that *Eucalyptus grandicola* as a hybrid of *E. rudis* and *E. drummondii* because of the additive effect of the bands from the parents in the hybrid. The dendrogram produced formed two clusters and revealed that *E. grandicola* was more similar to *E. rudis* than *E. drummondii*.

In an effort by Raja *et al.* (2005) to characterize twelve ber genotypes using RAPD markers, 17 out of twenty primers generated polymorphic bands. The primers that amplified all the 12 genotypes were OPC-20, OPD-1, OPD-3 and OPD-4. Out of 109 bands obtained 85 bands were polymorphic, while 24 bands

were monomorphic. For the cultivars tested an average of 5.45 bands per primer were obtained. The highest number of bands were generated by OPA-4 and OPD-3. With un-weighted group method using arithmetic mean (UPGMA) cluster analysis, the twelve ber genotypes fell into four major clusters.

For assessing genetic diversity in wild populations of *Bunium Persicum* an important medicinal spice, RAPD markers were used. Majeed *et al.* (2008) collected germplasm accessions from parts of Himachal Pradesh and Jammu and Kashmir to assess genetic diversity. Out of thirty seven decamer primers used, six primers revealed fair amount of variability. Similarity measurement using UPGMA followed by dendrogram analysis resulted in two major clusters.

Studies were undertaken for identification and genetic relationships in six tree species of *Acacia* through RAPD markers. A total of 253 distinct DNA bands were amplified by using selected 17 random 10-mer primers. Genetic similarity was conducted on the basis of presence or absence of bands which revealed a wide range of variability within the species. The cluster analysis clearly showed that there was high degree of diversity, about 70%, within the six tree species of *Acacia* and three major clusters were obtained belonging to 6 species of *Acacia.* RAPD markers were helpful in tree breeding programs and provide an important input into conservation biology (Nanda *et al.*, 2004).

Among the 40 random primers tested in *Bacopa monnieri* accessions at CIMAP, 29 primers generated one or more polymorphic bands. The number of polymorphic bands generated was primer dependent, ranging from 2 to maximum of 8. From similarity matrices and dendrograms constructed, all the accessions were found to be in the range of 0.8-1.0 of similarity, which was indicative of a narrow genetic base among the various accessions with a medium level of polymorphism (Darokar *et al.*, 2001).

Genomic DNAs from Ephedra plants were studied by RAPD analysis by Takeuchi et al. (2003). Dendrogram revealed that 9 samples were grouped into 3 clusters, cluster A, cluster B and cluster C.

Genetic diversity among 20 genotypes of *Psidium guajava* and two species *P. friedrichsthalianum* and *P. cattleianum* were analyzed using random amplified polymorphic DNA. All the 41 primers used discriminated between two and more genotypes. In the dendrogram, all the 22 genotypes were grouped into two major clusters. RAPD markers detected a high level of polymorphism and could be used for breeding of improved guava varieties as per Sharma *et al.* (2005).

Jain *et al.* (2003) determined the extent of genetic variability in a collection of *Phyllanthus amarus* through RAPD analysis. RAPD profiling of 33 collections from different locations were generated. Analysis through UPGMA revealed upto 65% variation among these accessions. Intrapopulation variation was found to be much larger in the accession from the Southern part of the country. RAPD profiles of *P. amarus* displayed vast genetic variation indicative of the evolving nature of the taxa.

Using RAPD analysis in basil, 11 primers generated 98 polymorphic bands, ranging from 300 to 2000 base pairs, discriminated among 37 accessions across nine *Ocimum* spp. Means of genetic similarities with *Ocimum* spp. showed that the domesticated species, *O. americanum, O. gratissimum* showed the lowest similarity (Vieira, 2003).

RAPD analysis of *Santalum album* and its closely related *Osyris wightiana* were used for distinguishing sandalwood pieces from Osyris, an adulterant. Out of total of 42 loci, 35 were found to be polymorphic. On the basis of genetic similarity coefficient, the UPGMA dendrogram was constructed. Two main clusters were obtained in the dendrogram, one containing S. album samples and the other cluster containing Osyris sample (Parvathy *et al.*, 2009).

Nine medicinal *Dendrobium* species were distinguished from each other by the banding pattern generated by the sixteen 10-mer digonucleotide primers in the random amplified polymorphic DNA reaction, RAPD analysis was also applied to estimate the genetic relationship among the nine species. The

dendrogram constructed clearly distinguished the mine species into four groups (Zha *et al.*, 2009).

Twenty one pomegranate accessions were studied using DNA markers to reveal their relatedness. Of the random primers used, fourteen primers showed good amplification and polymorphism in these samples. Estimates of genetic similarity, using Jaccard's similarity coefficient, ranged from 0.13 to 1.0 using the RAPD data. This study showed high level of similarity between pomegranate accessions (Sarkhosh *et al.*, 2009).

Materials and Methods

3. MATERIALS AND METHODS

The present investigation was carried out under the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period 2006-2009. The experiments were laid out in different age groups of *S. asoca.* The seed and seedling study was conducted in the accessions collected from different districts of Kerala. Morphological variations were assessed in the germplasm accessions maintained at AICRP on Medicinal and Aromatic Plants, College of Horticulture. The studies on reproductive biology of *S. asoca* were undertaken in the eight trees of different age groups maintainted at Kerala Agricultural University campus. The seedlings, germplasm accessions and the trees were utilised for the evaluation of therapeutical components of *S. asoca.* Molecular characterisation of *S. asoca* germplasm accessions were conducted at the Centre for Plant Biotechnology and Molecular Biology, College of Horticulture.

3.1 Experiment 1

Morphological variations of existing germplasm

3.1.1 Materials

Forty three indigenous collections (IC) of germplasm maintained at AICRP on M&AP, College of Horticulture, Vellanikkara were used in the experiment. IC number and place of collection of the accessions are given in Table 1. The accessions were collected from different districts of Kerala and were raised in the field in 2004. Field view of accessions are given in Plate1.

3.1.2 Methodology

The study was carried out during April 2007 to March 2009. Observations on morphological characters were taken monthly from the 43 accessions. Bark thickness was measured once in a year and bark yield was recorded at quarterly intervals.

Table 1. Details of indigenous collections (IC) of *S.asoca*

Sl.No.	IC No.	Place of Collection	District
1	IC 566463	Thrissur	Thrissur
2	IC 566465	Thrissur	Thrissur
3	IC 566466	Thrissur	Thrissur
4	IC 566467	Thrissur	Thrissur
5	IC 566468	Thrissur	Thrissur
6	IC 566469	Vellanikkara	Thrissur
7	IC 566464	Thrissur	Thrissur
8	IC 566456	Thenjipalam	Kozhikode
9	IC 566457	Vellanikkara	Thrissur
10	IC 566458	Thenjipalam	Kozhikode
11	IC 566459	Vellanikkara	Thrissur
12	IC 566460	Kozhikode	Kozhikode
13	IC 566461	Kottarakara	Kollam
14	IC 566462	Kottarakara	Kollam
15	IC 566485	Thrissur	Thrissur
16	IC 566484	Thrissur	Thrissur
17	IC 566479	Thrissur	Thrissur
18	IC 566478	Thrissur	Thrissur
19	IC 566471	Thrissur	Thrissur
20	IC 566470	Kadampuzha	Malappuram
21	IC 566475	Thrissur	Thrissur
22	IC 566498	Kuttipuram	Malappuram
23	IC 566497	Changaramkulam	Malappuram
24	IC 566496	Kottakkal	Malappuram
25	IC 566495	Vellanikkara	Thrissur
26	IC 566494	Valanchery	Malappuram
27	IC 566492	Thiruvananthapuram	Thiruvananthapuram
28	IC 566489	Thiruvananthapuram	Thiruvananthapuram

Sl.No.	IC No.	Place of Collection	District
29	IC 566488	Thiruvananthapuram	Thiruvananthapuram
30	IC 566480	Thrissur	Thrissur
31	IC 566477	Thrissur	Thrissur
32	IC 566474	Thuruthissery	Ernakulam
33	IC 566473	Thrissur	Thrissur
34	IC 566472	Kadampuzha	Malappuram
35	IC 566483	Thrissur	Thrissur
36	IC 566493	Peringamala	Thiruvananthapuram
37	IC 566491	Peringamala	Thiruvananthapuram
38	IC 566490	Peringamala	Thiruvananthapuram
39	IC 566487	Thuruthissery	Ernakulam
40	IC 566486	Thrissur	Thrissur
41	IC 566482	Thrissur	Thrissur
42	IC 566476	Thuruthissery	Ernakulam
43	IC 566481	Thrissur	Thrissur

Plate 1. Field view of germplasm accessions at third and fourth year after planting

3.1.3 Characters

1. Height of plant

The height of plant from ground level to the tip of the last leaf was measured and expressed in centimeters.

2. Number of leaves

The total number of leaves were counted monthly in each accession.

3. Leaflet length

Length of leaflet was measured from base to the tip of the leaflet. Observations were recorded in 10 selected leaves which were tagged in each accession.

4. Leaflet breadth

Breadth of leaflet was measured in 10 selected leaves which were tagged in each accession.

5. Colour of leaves

Colour of both the tender and mature leaves were recorded.

6. Number of leaflets per leaf

In each accession, number of leaflets was counted in ten randomly selected leaves.

7. Internodal length

Length of five selected internodes were observed and expressed as mean length in centimeters.

8. Stem girth

Girth of the stem was measured at 65 cm intervals from 5 cm above the ground level and mean values worked out in centimeters.

9. Bark thickness

Thickness of the bark was measured at 65 cm intervals from 5 cm above the ground level. Bark thickness was estimated by modified bark gauge method (West, 2009). Thickness was noted at 3 triangularly opposite points on the bark and the mean value worked out as bark thickness. In modified bark gauge method, a puncher was pushed through the bark, until the resistance of underlying wood was felt. Then a copper wire is inserted through hole to touch the wood. A bend was made in copper wire at the outside end of it. The length of wire inserted was measured by placing wire on a graph. Length of wire upto bend end was measured as bark thickness in millimeters.

10. Bark yield

Bark yield as extractable bark volume (cm^3) was derived from the height of tree, stem girth and bark thickness (Athai *et al.*, 2005).

3.1.4 Statistical Analysis

3.1.4.1 Estimation of Selection Parameters

a) Variability

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

Phenotypic variance (Vp) = Vg + Ve

where (Vg) = Genotypic variance

(Ve) = Environmental variance

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

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

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

where Vp = Phenotypic variance
$\bar{X}$ = Mean of the character under study

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

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

b) Heritability

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

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

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

c) Genetic advance

The expected genetic advance of the genotypes 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).

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

where Vg = Genotypic variance

Vp = Phenotypic variance

K = Selection differential

d) Genetic gain (Genetic advance as percentage of mean)

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

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

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

Genetic gain was categorised as

>20 per cent	- High
10-20 per cent	- Moderate

<10 per cent - Low

e) 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

$$CoVg12 = \frac{Mt12 - Me\ 12}{N}$$

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

Genotypic 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

f) Direct and indirect effects of yield attributes on bark yield through path analysis.

Path coefficient analysis suggested by Wright (1923) was applied to study the cause and effect relationship of bark yield and other morphological traits. The direct and indirect effects were classified based on the scale given by Lenka and Mishra (1973).

Very high	-	>1.0
High	-	0.30 – 0.99
Moderate	-	0.20 – 0.29
Low	-	0.10 – 0.19
Negligible	-	0.00 – 0.09

g) Evolving a selection index using discriminant function (Hazel, 1943)

For selecting superior accessions from the indigenous collection, the selection should be based on the minimum number of characters. An estimation of discriminant function based on most reliable and effective characters is valuable tool for breeders. Thus discriminant function would ensure a maximum concentration of the desired genes in the accessions selected. Discriminant function analysis was conducted using the statistical package SPAR 2.0.

h) Cluster analysis

In plant breeding, genetic diversity plays an important role, as it provides the basis for selection of superior genotypes. One of the potent techniques of measuring genetic divergence in various breeding materials is the cluster analysis (Nadarajan and Gunasekaran, 2005). Analysis and clustering of the accessions were carried out using the statistical package SPSS 11.5.

3.2 Experiment 2

Reproductive biology of *S. asoca*

3.2.1 Materials

Eight trees of different age groups in the Kerala Agricultural University Campus constituted the material for the study. Details of these trees are given in Table 2.

3.2.2 Methods

The data were recorded monthly on eight trees for the following reproductive biology traits from June 2007 to May 2008.

Table 2. Details of *S. asoca* trees used for the experiment

Sl. No.	Accession number of the trees	Age of the tree (years)
1	KAU 1	15
2	KAU 2	15
3	KAU 3	15
4	KAU 4	9
5	KAU 5	19
6	KAU 6	25
7	KAU 7	25
8	KAU 8	31

3.2.3 Observations recorded

1. Number of inflorescence per tree

In each tree, the total number of inflorescence putforth in a month was counted.

2. Number of flowers per inflorescence

Number of flowers in an inflorescence was noted. Ten inflorescence per tree was utilized for this purpose.

3. Number of pods per tree

The total number of pods in a tree were taken in each month of the year.

4. Number of seeds per pod

In each pod, the number of seeds were determined. The observations were taken on 10 pods per tree.

5. Flower length

Length of flower from 10 selected inflorescence in a tree was measured in centimeters from base of flower to the tip.

6. Flower breadth

From 10 selected inflorescence in a tree, breadth of flower was measured in centimeters.

7. Pod length

Length of ten randomly selected pods from each tree was measured in cm and mean values worked out.

8. Pod breadth

Breadth of ten randomly selected pods from each tree was measured in cm and mean values worked out.

9. Seed length

In ten randomly selected pods from a tree, the length of the seed was measured in centimeters.

10. Seed breadth

In ten randomly selected pods from a tree, the breadth of the seed was measured in centimeters.

11. Seed volume

The volume of the seeds from 10 pods from each tree was determined by water displacement using a measuring cylinder.

12. Duration of inflorescence development

The time taken for full development of inflorescence from visual observation of bud was studied by tagging ten flowering shoots in eight trees.

13. Duration of blooming

The time taken for the entire inflorescence to bloom was determined from the opening of first flower in the inflorescence to the last flower in the same inflorescence.

14. Days for flower to pod

The duration in days for the flower to form an immature pod was determined in ten selected inflorescence in each tree.

15. Days for pods to mature

In each tree, the days taken for the immature pods to become mature pods were studied in ten randomly selected pods.

16. Total reproductive period

The total reproductive period in days from visual bud initiation to the fully mature pods was determined.

17. Colour and shape of pods

The colour and shape of the pods were recorded from ten randomly selected pods in each of 8 trees studied.

18. Colour and shape of seeds

The colour and shape of seeds were recorded from ten randomly selected pods in each of eight trees studied.

19. Floral morphology and biology

Ten fresh flower buds and flowers were collected. Hand sections, both L.S. and T.S. were taken and examined under microscope and description of features like colour and number of floral parts, androecium and gynoecium were undertaken.

20. Anthesis

A preliminary study revealed that there was no flower opening between 6 am and 6 pm. Therefore ten inflorescence having mature buds along with leaves and small piece of branch were collected previous day evening from each of the eight trees and placed in water and flower buds were observed at one hour interval between 6 pm and 6 am the next day. The time of anthesis was recorded.

21. Time of anther dehiscence and stigma receptivity

The colour and appearance of anthers were examined with hand lens at one hour interval from 6 pm to 9 am the next day in fully mature flower buds of each inflorescence to find out the time of anther dehiscence in a flower. The stigmatic surface was also observed for any change in colour or appearance in the same buds, at same interval of time to find out the stigma receptivity.

22. Pollen morphology

For determining morphology, pollen samples were taken from fully opened new flowers and acetolysed as per Menemen and Jury (2002). Then the sculpturing on the exine was examined under the microscope. Pollen size was measured using an ocular micrometer, after calibration.

23. Pollen fertility and viability

Fertility of pollen was assessed on the basis of stainability of pollen grains in acetocarmine glycerine mixture. Pollen grains were collected from newly opened flowers and stained in a drop of acetocarmine mixture on a clean slide and kept aside for one hour. All the pollen grains that were well filled and stained were counted as fertile and others as sterile. Two fields of five slides were prepared and observed under microscope and the values expressed as percentage.

Pollen germination was assessed on the basis of germination of pollen grains in sucrose media. Different doses of sucrose 1%, 5%, 10% were used as media. Germination rate was same in all the sucrose concentrations. For testing pollen germination, pollen grains were collected from newly opened flowers. Saturated petridishes method was used to determine germination rate of pollen. Pollen germination was considered to occur when a pollen tube formed was equal to or greater than the diameter of pollen grain. Two fields of five slides were observed and number of germinated pollen was expressed as percentage of total number of pollen grains.

Absolute pollen viability or effective germination capacity was determined as per Ali *et al.* (1998) as

$$\text{Absolute pollen viability} = \frac{\%\text{ fertile pollen x }\%\text{ germinated pollen}}{100}$$

24. Pollination system and pollinating agents

Twenty flowers were covered with butter paper a day before anthesis and observed for self pollination. Percentage of pod set was noted.

Twenty flowers were emasculated a day before anthesis and observed for cross pollination. Pod set percentage was recorded.

Ten inflorescence were removed of ants and covered with polythene cover and observed for pod set.

25. Influence of weather parameters on reproductive biology traits

Weather parameters given below were collected from Department of Meteorology, College of Horticulture

1. Rainfall
2. Relative humidity
3. Maximum temperature
4. Minimum temperature
5. Sunshine hours

3.2.4 Statistical analysis

3.2.4.1 Estimation of variability

Variability for various reproductive biology traits among the different months of the year were estimated.

3.2.4.2 Estimation of correlation

a. Phenotypic and genotypic correlation coefficients among various reproductive biology traits were estimated.

b. Simple correlation among the weather parameters and reproductive biology traits were worked out.

3.3 Experiment 3

Collection and evaluation of seed and seedling characters

3.3.1 Materials

Eighty accessions collected from different districts of Kerala were used in the experiment. Details of accessions are given in Table 3 and Plate 2.

3.3.2 Methodology

The study was carried out during May 2008 to April 2009. The accessions collected were evaluated for seed and seedling characters. Observations on seed traits were recorded from each of the 80 accessions collected. Seedling trait observations were taken monthly from each of the accessions. Seeds of accessions collected were sown in sand for germination and two weeks old seedlings were transplanted to polythene cover.

3.3.3 Characters

3.3.3.1 Seed traits

1. Seed length

The length of seeds was recorded in centimeters.

2. Seed breadth

The breadth of seeds was taken at the widest point of seed in centimeters.

Table 3. Details of *S.asoca* accessions collected

Sl.No.	Accession No.	Place of Collection	District
1	KMK 1	Kumarakom	Kottayam
2	KMK 2	Kumarakom	Kottayam
3	KMK 3	Kumarakom	Kottayam
4	KMK 4	Kumarakom	Kottayam
5	OKL 1	Odakkali	Ernakulam
6	OKL 2	Odakkali	Ernakulam
7	OKL 3	Odakkali	Ernakulam
8	OKL 4	Odakkali	Ernakulam
9	KKL 1	Kottakkal	Malappuram
10	KKL 2	Kottakkal	Malappuram
11	KKL 3	Kottakkal	Malappuram
12	KKL 4	Kottakkal	Malappuram
13	CKD 1	Chalakudy	Thrissur
14	CKD 2	Chalakudy	Thrissur
15	TTR 1	Tripunithura	Ernakulam
16	TTR 2	Tripunithura	Ernakulam
17	TTR 3	Tripunithura	Ernakulam
18	TTR 4	Tripunithura	Ernakulam
19	VTR 1	Vettichira	Malappuram
20	VTR 2	Vettichira	Malappuram
21	VTR 3	Vettichira	Malappuram
22	VTR 4	Vettichira	Malappuram
23	VTR 5	Vettichira	Malappuram
24	VTR 6	Vettichira	Malappuram
25	VKA 1	Vellanikkara	Thrissur
26	VKA 2	Vellanikkara	Thrissur
27	VKA 3	Vellanikkara	Thrissur
28	VKA 4	Vellanikkara	Thrissur

Sl.No.	Accession No.	Place of Collection	District
29	VKA 5	Vellanikkara	Thrissur
30	VKA 6	Vellanikkara	Thrissur
31	VKA 7	Vellanikkara	Thrissur
32	VKA 8	Vellanikkara	Thrissur
33	KDR 1	Kodakara	Thrissur
34	KDR 2	Kodakara	Thrissur
35	KDR 3	Kodakara	Thrissur
36	KRT 1	Koratty	Thrissur
37	KRT 2	Koratty	Thrissur
38	PBR 1	Perumbavoor	Ernakulam
39	PBR 2	Perumbavoor	Ernakulam
40	PTB 1	Pattambi	Palakkad
41	PTB 2	Pattambi	Palakkad
42	CTA 1	Chettupuzha	Thrissur
43	CTA 2	Chettupuzha	Thrissur
44	CTA 3	Chettupuzha	Thrissur
45	MNT 1	Mannuthy	Thrissur
46	MNT 2	Mannuthy	Thrissur
47	MNT 3	Mannuthy	Thrissur
48	ALV 1	Aluva	Ernakulam
49	ALV 2	Aluva	Ernakulam
50	ALV 3	Aluva	Ernakulam
51	CBG 1	Thenjipalam	Kozhikode
52	CBG 2	Thenjipalam	Kozhikode
53	CBG 3	Thenjipalam	Kozhikode
54	CBG 4	Thenjipalam	Kozhikode
55	TVM 1	Thiruvananthapuram	Thiruvananthapuram
56	TVM 2	Thiruvananthapuram	Thiruvananthapuram
57	TVM 3	Thiruvananthapuram	Thiruvananthapuram

Sl.No.	Accession No.	Place of Collection	District
58	KRA 1	Kottaraka	Kollam
59	KRA 2	Kottarakara	Kollam
60	PMA 1	Peringamala	Thiruvananthapuram
61	PMA 2	Peringamala	Thiruvananthapuram
62	PMA 3	Peringamala	Thiruvananthapuram
63	TTY 1	Thuruthissery	Ernakulam
64	TTY 2	Thuruthissery	Ernakulam
65	TTY 3	Thuruthissery	Ernakulam
66	KPM 1	Kuttipuram	Malapuram
67	KPM 2	Kuttipuram	Malapuram
68	KPM 3	Kuttipuram	Malapuram
69	KLA 1	Kalpatta	Wayanad
70	KLA 2	Kalpatta	Wayanad
71	CKM 1	Changaramkulam	Malappuram
72	CKM 2	Changaramkulam	Malappuram
73	CKM 3	Changaramkulam	Malappuram
74	CKM 4	Changaramkulam	Malappuram
75	VCY 1	Valancherry	Malappuram
76	VCY 2	Valancherry	Malappuram
77	VCY 3	Valancherry	Malappuram
78	KPA 1	Kadampuzha	Malappuram
79	KPA 2	Kadampuzha	Malappuram
80	KPA 3	Kadampuzha	Malappuram

Plate 2. Plot view of seedlings raised from seeds collected

3. Seed volume

The volume of the seeds was determined by water displacement using a measuring cylinder.

4. Number of days for germination

Number of days was counted from sowing of seeds to the time of germination.

5. Germination percentage

To assess germination percentage, seeds were sown in sand. Germinated seeds were counted at regular intervals in each accession.

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

3.3.3.2 Seedling traits

1. Seedling vigour

Seedling vigour was calculated for each of the 80 accessions collected.

According to Faluyi *et al.* (1986), emergence percentage (L%) and seedling height (SH) were more reliable indices for seedling vigour, since they were more heritable and less influenced by the environment.

Seedling vigour = germination percentage (L%) x seedling height (SH)

2. Height of seedling

The height of seedling was measured monthly from the soil level to the tip of the last leaf and expressed in centimeters.

3. Number of leaves

The total number of leaves was counted monthly in each accessions.

4. Leaflet length

Length of the leaflet was measured from base to the tip of the leaflet.

5. Leaflet breadth

Breadth of leaflet was measured in centimeters in each accession.

6. Number of leaflets/leaf

In each accession, number of leaflets in each leaf was counted.

7. Internodal length

Length of two selected internodes were observed and expressed as mean length in centimeters.

8. Stem girth

Girth of the stem was measured in centimeters.

3.3.4 Statistical analysis

3.3.4.1 Estimation of variability

Variability existing among the eighty accessions for the various characters under observation were estimated.

3.3.4.2 Estimation of correlation

Simple correlation of the seed traits and seedling traits with seedling vigour were worked out.

3.4 Experiment 4

Evaluation of therapeutical components

Tannin and phenol are important biochemical constituents of *S. asoca*. The evaluation of therapeutical components were carried out in different age groups of asoka. Phenol and tannin content were estimated in bark, leaves and flowers of KAU campus trees (9 to 31 years old), bark and leaves of germplasm accessions (3 to 4 years old) and leaves of seedlings (1 year old) collected.

3.4.1 Tannin content

0.5 g dried powder of bark, leaf or flower was taken. 75 ml water was added to the sample and boiled gently for 30 minutes. The sample was centrifuged at 2000 rpm for 20 minutes and the supernatant collected was made upto 100 ml. 1 ml of this sample extract was transferred to 75 ml water. 5 ml of Folin-Denis reagent and 10 ml of sodium carbonate solution was added to this. Then the volume was made upto 100 ml with water. The colour developed was read at 700 nm using spectrophotometer. 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 ml of standard tannic acid solution (1mg/ml) was taken and colour developed as in the case of sample. Using the standard graph the amount of tannin present in the sample was calculated. 1 ml of water with reagents added was used as a blank (Sadasivam and Manickam, 1992).

Concentration of tannin in the sample (mg/g) =

$$\frac{\text{Concentration of Standard tannic acid}}{\text{Absorbance of standard tannic acid}} \times \frac{\text{Absorbance of test sample}}{\text{Volume of test sample extract}} \times \frac{\text{Volume made up of test sample}}{\text{Weight of sample}}$$

3.4.2 Phenol content

0.5 g dried powder of bark, leaf or flower was taken. The powder was ground in 10-time volume of 80% ethanol. The homogenate was centrifuged at

10,000 rpm for 20 minutes and the supernatant was collected. The procedure was repeated and the supernatants collected were pooled and evaporated to dryness. Residue was dissolved in 5 ml of distilled water. From this, 0.2, 0.5, 0.8, 1.1,1.4, 1.7, 2.0 ml were taken and made upto 3 ml with water. To this 0.5 ml of Folin-Ciocalteau reagent was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added. The solution was placed in boiling water for one minute and cooled. The colour developed was read at 650 nm using spectophotometer. 0.2, 0.5, 0.8, 1.1, 1.4, 1.7, 2 ml of standard catechol solution (1mg/ml) was taken and colour developed as in the case of sample. Using the standard graph the concentration of phenol present in the sample was calculated. 3 ml of water with reagents added was used as a blank (Sadasivam and Manickam, 1992).

Concentration of phenol (mg/g) =

$$\frac{\text{Concentration of standard catechol}}{\text{Absorbance of standard catechol}} \times \frac{\text{Absorbance of test sample}}{\text{Volume of test sample}} \times \frac{\text{Volume made up of test sample}}{\text{Weight of sample}}$$

3.5 Experiment 5

Molecular characterisation

3.5.1 Materials

Ten germplasm accessions indicating six districts and maintained at AICRP on Medicinal and Aromatic Plants, College of Horticulture, Vellanikkara were used in the experiment. Details of the accessions are given in Table 4.

Table 4. Details of *S. asoca* accessions used in molecular characterisation

Sl.No.	IC No.	Place of Collection	District
1	IC 566463	Thrissur	Thrissur
2	IC 566461	Kottarakara	Kollam
3	IC 566488	Thiruvananthapuram	Thiruvananthapuram
4	IC 566493	Peringamala	Thiruvananthapuram
5	IC 566456	Thenjipalam	Kozhikode
6	IC 566497	Changaramkulam	Malappuram
7	IC 566498	Kuttipuram	Malappuram
8	IC 566496	Kottakkal	Malappuram
9	IC 566474	Thuruthissery	Ernakulam
10	IC 566495	Vellanikkara	Thrissur

3.5.2 Methodology

Genomic DNA was isolated from each of the accessions and RAPD analysis was adopted for molecular characterization.

3.5.2.1Isolation of genomic DNA

Modified CTAB method (Padmalatha and Prasad, 2006) was used to isolate genomic DNA. Tender leaves were taken from the selected plants using sterile blades. The leaf samples were collected on ice and then wiped with cotton soaked in 70 per cent alcohol and immediately used for extraction.

a.Reagents used

For the isolation of genomic DNA from *S. asoca*, the following stock solutions were used.

1.Extraction buffer

a. 100 mM Tris (pH 8.0)

b.20 mM EDTA (pH 8.0)

c.1400 mM NaCl

d.2% CTAB

2. Sodium metabisulphite

3.β-mercaptoethanol

4.Chloroform : Isoamylalcohol (24:1) mixture

5.Isopropanol

6.Ethanol (70% and 100%)

b.Procedure

In the ten *S. asoca* accessions, leaflets of the 3rd node leaf was collected on ice and cut into pieces with a sterile blade. 1 g of this sample was transferred to a mortar. To this leaf sample, a pinch of sodium metabisulphite and 50 µl β-mercaptoethanol were added. Then the leaves were ground into fine powder using liquid nitrogen. 5 ml prewarmed extraction buffer was added to the powder. The extraction buffer along with the powder was transferred to a centrifuge tube. These tubes containing mixture were incubated at 65°C for 20 minutes with periodic gentle inversions of tube. After the incubation, 5 ml of chloroform : isoamylalcohol (24:1) mixture was added into the mixture and was mixed gently by inversion of tube. The mixture was centrifuged at 10000 rpm at 4°C for 15 minutes. To the supernatent in a fresh centrifuge tube, equal volume of chloroform : isoamylalcohol (24:1) mixture was added and mixed by gentle inversion of tube. The mixture was then centrifuged at 10000 rpm at 4°C for 15 minutes. The supernatent was transferred to a fresh centrifuge tube. To the supernatant, ice cold isopropanol was added at the rate of 0.6 volume of the supernatent and was incubated at –20°C for 1 hour. After incubation, the mixture was centrifuged at 8000 rpm at 4°C for 10 minutes to pellet the DNA. The supernatent was discarded and the DNA in the pellet form was washed with 70

per cent ethanol by centrifuging at 10,000 rpm for 5 minutes and air dried. The pellet was then resuspended in 50 µl sterile water and transferred to a eppendorff tube. To this 50 µl DNA, 0.5 µl of RNase was added and incubated at 37°C for 20 minutes. The dissolved DNA was extracted with equal volumes of chloroform : isoamylalcohol (24:1) mixture at 10000 rpm for 10 minutes. The supernatent was transferred to a fresh centrifuge tube. To the supernatent ice cold isopropanol was added at the rate of 0.6 volume of the supernatent and was incubated at –20°C for 1 hour. After incubation, the mixture was centrifuged at 8000 rpm at 4°C for 10 minutes to pellet the DNA. The supernatent was discarded and the DNA in the pellet form was washed with 70 per cent ethanol by centrifuging at 10,000 rpm for 5 minutes and air dried. The pellet was then resuspended in 50 µl sterile water and transferred to a eppendorff tube.

3.5.2.2 Agarose gel electrophoresis of DNA samples

a.Materials and equipments used

The following were the materials and equipments used

1.Agarose

2.TAE buffer 50x

a. Tris base – 242 g

b. Glacial acetic acid – 57.1 ml

c. EDTA (0.5 M) – 100 ml (pH 8.0)

Made up with distilled water to 1 litre

3.Gel loading dye 2x (100 ml)

a. Glycerol	-	40 ml
b. 4 x TAE buffer	-	50 ml
c. Bromophenol	-	0.5%

4.Ethidium bromide solution (0.1%)

5.Electrophoresis unit, power supply unit, casting tray and comb

b.Procedure

Gel buffer (TAE 1X) was taken in a conical flask (100 ml for large gel and 30 ml for small). Agarose (0.7% for DNA and 1.5% for RAPD samples) was weighed, added to the flask, stirred and boiled with frequent stirring till the agarose dissolved completely. Ethidium bromide was added into the flask and it was allowed to cool to 65°C. The open end of the gel casting tray was sealed with cello tape and placed on a horizontal surface and the comb was placed properly on the tray. The dissolved agarose was poured gently into the tray. The gel was allowed to solidify for 30 minutes and then the comb was removed carefully. The gel was then placed in the electrophoresis unit (Genei) with the well side directed towards cathode. IX TAE buffer was added to cover the gel with a few mm of buffer. 3 µl of DNA sample (20 µl in case of RAPD products) was pipetted out onto a parafilm and mixed well with 3 µl of loading dye. The samples were then loaded carefully into the well by using micropipette. Lambda DNA EcoR1-Hind 111 double digest used as molecular marker was added in one well. The cathode and anode of the electrophoresis unit were then connected to the power supply and the gel was run at constant voltage (60 mA). The power supply was turned off when the loading dye moved to more than half distance of gel (2 to 3 hours).

3.5.2.3 Gel documentation

The gel was taken from electrophoresis unit and viewed under UV light in a UV transilluminator. The ethidium bromide stain intercalates between the nitrogen bases of DNA and fluoresces in orange colour under UV light. The image of the gel was monitored and stored in a gel documentation system (Alpha Imager-2000, Alpha Infotech, USA).

3.5.2.4 Quantification of DNA

The quantity of isolated DNA was evaluated using a Nanodrop (Spectrometer). 1 µl of the sample was diluted 2.5 times using sterile water and the optical density was determined by reading the absorbance at two specific wave length viz., 260 nm and 280 nm. The 260/280 ratio was then calculated to check the purity. Pure DNA gives the ratio 1.8. The quantity of DNA in the pure sample was calculated in nanogram/µl.

3.5.2.5 RAPD analysis

RAPD is a technique in which a single short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a template DNA. The number of amplified products in RAPD depends on the length of primer and the size of the target genome, and is based on the probability that a given DNA sequence (complementary to that of the primer) will occur in the genome on opposite strands of the DNA, in opposite orientation within a distance readily amplifiable by PCR. The variation in RAPD profile in the form of presence or absence of band results from variation in primer binding sites. The products can be easily separated by standard electrophoresis techniques and visualized by ultraviolet illumination of ethidium bromide stained gels. PCR amplification process involves repeated thermal cycles.

The procedure reported by Padmalatha and Prasad (2006) was slightly modified and used for amplification of *S. asoca* DNA.

The thermal cycles included

Step No.	Temperature (°C)	Duration	Steps involved	Number of cycles
1	94	3 min	Initial denaturation	1
2	94	45 sec	Denaturation	30
3	37	1 min	Annealing	
4	72	1 min	Extension	
5	72	7 min	Final extension	1

The reaction mixture (20 µl) consisted the following

1. Taq PCR buffer A - 2.0 µl
2. dNTPs mix - 0.5 µl
3. Taq DNA polymerase - 0.3 unit
4. Primer - 1.5 µl
5. Template DNA - 3.0 to 3.3 µl (50 ng)
6. Sterile Milli-Q water - 12.4 to 12.7 µl

A master mix for ten samples without the template DNA and sterile water was prepared using the reaction mixture for the required number of reactions. From this master mix, 4.3 µl was pipetted into each PCR tube and 15.7 µl of template DNA and sterile water was added. The PCR tubes were loaded in the thermal cycler (PTC 200, MJ Research, USA) and the programme was run. The programme was completed in 3 hours. The amplified products were electrophoresed on 1.5 per cent agarose gel. The gel was viewed under UV light and documented.

3.5.2.6 Screening of random primers for RAPD

A total of 10 decamer primers (Table 5) under Operon series were screened for amplification of genomic DNA extracted from Asoka samples, using

the Thermal Cycler mentioned under RAPD. These included 7 primers under OPRN, OPRY, OPAH, OPF and 3 primers viz., OPA8, OPA21, OPA24. Out of the primers, OPA8 was already reported to be efficient in amplification of genomic DNA from *S. asoca* (Padmalatha and Prasad, 2006). From these, 4 primers that gave good amplification were selected and utilized for further characterization of 10 accessions. The total number of bands along with the number of polymorphic bands obtained in all ten genotypes with each of the four primers tried were recorded.

Table 5. List of primers used for screening

Sl. No.	Primer code	Primer sequence
1	OPA8	GTGACGTAGG
2	OPA21	CAGGCCCTTC
3	OPA24	AATCGGGCTG
4	OPRN8	ACCTCAGCTC
5	OPRN9	TGCCGGCTTG
6	OPRN5	ACTGAACGCC
7	OPF3	CCTGATCACC
8	OPAH9	AGAACCGAGG
9	OPAH12	TCCAACGGCT
10	OPRY3	ACAGCCTGCT

3.5.2.7 Data Analysis

The pattern of DNA amplification for the 4 primers was scored as 1 or 0 by the presence or absence of bands respectively and the data was fed to the NTSYS PC 2.0 software package.

The DNA fingerprint data were used to construct dendrogram by employing Unweighted Pair Group Method of Arithmetic Averages (UPGMA) using NYSTS PC Version 2.01 programme using Jaccard's coefficient.

Results

4. RESULTS

The results obtained in the study are grouped into five major heads.

1. Morphological variations of existing germplasm
2. Reproductive biology of *S. asoca*
3. Collection and evaluation of seed and seedling characters
4. Evaluation of therapeutical components of *S.asoca*
5. Molecular characterization

4.1 Morphological variations of existing germplasm

Forty three indigenous collections of *S. asoca* were evaluated for various morphological characters from April 2007 to March 2009. The first year data, second year data and pooled data were subjected to statistical analysis and the results are presented below.

4.1.1 Morphological variations studied from April 2007-March 2008

4.1.1.1 Genetic variability

In the present study, the extent of genetic variability with respect to various characters was estimated. Abstract of analysis of variance of the characters is given in Table 6. In this analysis, the quarterly data of a year were taken as replications and the forty three accessions as treatments. Results of analysis of variance revealed significant differences among the accessions for the eight characters studied. Characters like height of plant, number of leaves, stem girth, bark yield have higher variability due to treatment than replication which indicates the higher magnitude of its genetic material in the expression of that characters. Other characters like leaflet length, leaflet breadth, number of leaflets per leaf and intermodal length have higher magnitude of variability due to replication indicating its environmental impact in the expression of that character.

Table 6. ANOVA for morphological characters studied in *S. asoca* indigenous collections from April 2007-March 2008

Source of variation	Degrees of freedom	Mean sum of squares							
		Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/ leaf	Internodal length	Stem girth	Bark yield
Replication	3	16747.74**	369.40**	37.38**	2.26**	4.00**	24.26**	25.22**	8548.21**
Treatment	42	25263.02**	674.40**	27.93**	1.74**	2.04**	2.25**	31.72**	71292.79**
Error	126	128.51	1.66	0.05	0.005	0.015	0.19	0.25	331.99

**Significant at 1% level

4.1.1.2 Phenotypic coefficient of variation (PCV) and genotypic coefficients of variation (GCV)

The estimates of PCV, GCV, heritability, genetic advance and genetic gain are given in Table 7. Among the different characters studied, bark yield (87.08, 85.81), number of leaves (58.75, 58.46), stem girth (57.02, 56.11), height of plant (54.53,53.98) leaflet length (22.55, 22.46) and leaflet breadth (21.30, 21.18) recorded high magnitudes of PCV and GCV. But internodal length (19.80, 16.89) and number of leaflets per leaf (11.95, 11.95) showed moderate values. PCV in general was higher for all the characters except for number of leaflets per leaf where PCV and GCV were equal.

4.1.1.3 Heritability

Among the different characters studied, heritability (broad sense) estimates varied from 72.78 (internodal length) to 99.70 (number of leaflets per leaf).

Table 7. Estimation of genetic parameters for morphological characters studied from April 2007-March 2008

Sl. No.	Characters	PCV (%)	GCV (%)	Heritability (%)	Genetic advance (GA)	Genetic gain (GG)
1.	Height of plant	54.53	53.98	97.99	160.39	109.23
2.	Number of leaves	58.75	58.46	99.01	26.57	119.79
3.	Leaflet length	22.55	22.46	99.20	5.43	46.21
4.	Leaflet breadth	21.30	21.18	98.84	1.35	43.40
5.	No. of leaflets/leaf	11.95	11.95	99.70	2.06	24.61
6.	Internodal length	19.80	16.89	72.78	1.26	29.71
7.	Stem girth	57.02	56.11	96.84	5.66	113.40
8.	Bark yield	87.08	85.81	98.16	271.83	277.20

High heritability in the broad sense was estimated for the characters, height of plant (97.99), number of leaves (99.01), leaflet length (99.20), leaflet breadth (98.84), number of leaflets per leaf (99.70), internodal length (72.78), stem girth (96.84) and bark yield (98.16).

4.1.1.4 Genetic advance and genetic gain

Genetic advance expressed as percentage of mean was maximum (277.20) for bark yield and the minimum (24.61) for number of leaflets per leaf. High estimates of heritability coupled with high genetic gain was noticed for height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth and bark yield (Table 7).

4.1.1.5 Mean performance

The mean observations of various morphological characters studied in forty three accessions from April 2007 to March 2008 are presented in Table 8.

Plant characters such as plant height was highest for IC 566463 (Thrissur), IC566489 (Thiruvananthapuram) while IC566477 (Thrissur) recorded the lowest. IC566488 and IC566489 collected from Thiruvananthapuram, IC566463 (Thrissur) had the highest number of leaves and IC566477 (Thrissur) showed the minimum number. In the case of accessions IC566499, IC566457, IC566458, IC566479, IC566498, IC566494, IC566492, IC566489, IC566480 and IC566482, the number of leaflets recorded was ten per leaf. A minimum of six leaflets per leaf was noted for IC566478 and IC566487. Among the 43 accessions, IC566489 and IC466488 had the longest leaflets. Leaflets were comparatively small for IC566477 and IC566486. Wider leaflets were observed in IC566489 and IC566488. New leaflets were identified as soft, coppery red coloured, becoming stiffer and deeper green as it is matured.

Longer internodes was recorded for IC566489 followed by IC566488, whereas IC566477 had the shortest internode. Among the stem characters,

Table 8. Mean performance of *S. asoca* indigenous collections for morphological traits from April 2007-March 2008

Indigenous collection	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflet/leaf	Internodal length (cm)	Stem girth (cm)	Bark yield (cm^3)
IC 566463	390.27	45.75	13.05	2.92	8	4.9	12.07	470.92
IC 566465	97.75	17.50	14.67	3.57	8	4.37	4.77	62.07
IC 566466	70.22	15.75	10.05	2.40	8	2.95	3.32	23.27
IC 566467	142.22	21.00	13.95	3.92	8	4.42	5.67	102.15
IC 566468	117.27	17.25	10.65	3.25	8	3.70	3.82	49.72
IC 566469	99.97	15.50	13.20	3.07	10	4.27	2.92	19.02
IC 566464	118.52	17.50	13.17	2.90	8	4.50	6.95	55.60
IC 566456	147.25	20.00	9.87	2.77	8	4.37	5.67	51.07
IC 566457	151.97	32.00	10.92	2.90	10	4.17	5.25	49.87
IC 566458	175.15	25.50	8.42	2.80	10	3.52	6.10	126.85
IC 566459	195.35	30.00	14.90	3.75	8	4.07	5.27	68.57
IC 566460	93.25	8.50	12.90	3.25	8	2.75	2.37	15.45
IC 566461	118.65	19.50	9.35	2.20	8	4.55	4.12	37.12
IC 566462	212.42	26.50	12.32	3.40	8	4.57	5.00	160.00
IC 566485	78.12	14.75	10.85	2.90	8	4.37	2.70	16.20
IC 566484	130.27	15.75	10.55	2.80	8	4.60	2.67	40.12
IC 566479	105.97	14.50	9.05	2.90	10	4.35	3.17	38.10
IC 566478	61.05	6.75	9.00	2.20	6	2.97	1.57	6.30
IC 566471	52.12	10.00	9.57	2.40	8	3.35	2.87	8.92
IC 566470	74.32	24.50	11.15	2.55	8	4.37	3.45	17.25
IC 566475	212.05	30.00	11.02	2.90	8	4.50	7.65	260.10
IC 566498	99.82	15.00	12.50	2.97	10	4.42	2.72	26.57
IC 566497	119.90	15.00	12.60	2.80	8	4.52	2.85	22.80
IC 566496	120.10	30.00	10.55	2.97	8	4.42	3.87	31.00

Contd.

Table 8 continued.

Indigenous collection	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflet/leaf	Internodal length (cm)	Stem girth (cm)	Bark yield (cm^3)
IC 566495	102.10	18.50	9.57	3.07	8	4.22	2.75	17.87
IC 566494	169.25	21.00	15.00	3.75	10	4.32	4.52	54.30
IC 566492	153.90	26.50	14.80	3.55	10	4.42	5.65	110.20
IC 566489	353.00	56.50	19.02	5.37	10	5.97	15.17	591.82
IC 566488	273.27	73.50	18.82	5.00	8	5.77	9.05	352.95
IC 566480	216.07	29.00	10.25	2.87	10	4.62	4.90	78.40
IC 566477	28.62	2.75	6.95	2.40	8	2.15	2.27	1.71
IC 566474	67.20	9.00	9.77	2.80	8	3.32	1.42	3.60
IC 566473	139.80	17.50	11.37	3.45	8	4.27	6.12	33.70
IC 566472	87.82	17.25	10.57	3.17	8	4.32	3.70	14.80
IC 566483	135.97	19.00	13.05	3.17	8	4.42	5.25	94.50
IC 566493	279.62	38.50	10.02	2.37	8	4.17	8.57	334.42
IC 566491	108.85	10.25	10.95	3.07	8	4.72	4.75	38.00
IC 566490	108.95	14.75	10.25	3.25	8	3.60	2.70	43.20
IC 566487	171.80	26.50	13.45	3.45	6	4.32	5.02	84.42
IC 566486	65.77	14.75	7.30	2.00	8	3.60	2.67	21.40
IC 566482	285.42	33.50	10.25	2.97	10	4.47	10.57	371.17
IC 566476	137.10	15.50	13.70	3.77	8	4.52	5.37	64.50
IC 566481	245.40	21.00	15.92	4.02	8	4.27	7.52	146.72
CD (1%)	20.64	2.35	0.43	0.12	0.42	0.79	0.92	33.18
CD (5%)	15.71	1.78	0.32	0.09	0.21	0.60	0.70	25.25

IC566489 followed by IC566463 showed the maximum stem girth, but IC566474, IC566478 and IC566477 showed the minimum girth. The maximum extractable bark yield was for IC566489 followed by IC566463, IC566482, IC566488. IC566477 recorded the minimum value for bark yield.

4.1.1.6 Correlation

The genotypic and phenotypic correlation coefficient among different morphological characters studied are given in Table 9.

Bark yield was found to be positively and significantly correlated both at genotypic and phenotypic levels with height of plant (0.916, 0.906), number of leaves (0.823, 0.818), leaflet length (0.480, 0.472), leaflet breadth (0.493, 0.489), number of leaflets per leaf (0.241, 0.232), internodal length (0.525, 0.479) and stem girth (0.935, 0.931).

Among the various bark yield components, plant height was found to be positively and significantly correlated at both levels with number of leaves (0.828, 0.818), leaflet length (0.527, 0.517), leaflet breadth (0.515, 0.510), number of leaflets per leaf (0.241, 0.239), internodal length (0.641, 0.580) and stem girth (0.911, 0.900).

Number of leaves indicated positive and significant association at both levels with leaflet length (0.579, 0.574), leaflet breadth (0.581, 0.575), number of leaflets per leaf (0.213, 0.200), internodal length (0.710, 0.622), stem girth (0.797, 0.786).

Leaflet length showed positive significant correlation with all characters except number of leaflets per leaf. Leaflet length and breadth exhibited positive significant association with internodal length (0.689, 0.638) and stem girth (0.546, 0.552). In contrast to leaflet length, leaflet breadth identified positive significant association with number of leaflets per leaf also. All the morphological traits were

Table 9. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between bark yield and other morphological traits from April 2007-March 2008

	Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Bark yield
Height of plant	1	0.828**	0.527**	0.515**	0.241**	0.641**	0.911**	0.916**
No. of leaves	0.818**	1	0.579**	0.581**	0.213*	0.710**	0.797**	0.823**
Leaflet length	0.517**	0.574**	1	0.886**	0.125	0.689**	0.546**	0.480**
Leaflet breadth	0.510**	0.575**	0.878**	1	0.185*	0.638**	0.552**	0.493**
No. of leaflets/ leaf	0.239**	0.200*	0.124	0.184*	1	0.219*	0.244**	0.241**
Internodal length	0.580**	0.622**	0.581**	0.548**	0.186*	1	0.612**	0.525**
Stem girth	0.900**	0.786**	0.533**	0.545**	0.240**	0.544**	1	0.935**
Bark yield	0.906**	0.818**	0.472**	0.489**	0.232**	0.479**	0.931**	1

**Significant at 1% level
* Significant at 5% level

found to be positively and significantly correlated with number of leaflets per leaf except the leaflet length.

Internodal length indicated significant correlation with stem girth at both genotypic (0.612) and phenotypic (0.544) levels. Internodal length and stem girth reported positive significant association with all the morphological characters studied.

4.1.1.7 Path analysis

Path analysis was carried out using significant genotypic correlation of seven morphological characters viz. height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length and stem girth. Abstract of the results are given in Table 10.

The residual effect was found to be 0.2735. The highest positive direct effect was exhibited by stem girth (0.567) on bark yield. This was followed by height of plant (0.328). Moderate positive direct effect was identified for number of leaves (0.249). Moderate negative direct effect on bark yield was obtained for internodal length (-0.203). Number of leaflets per leaf (-0.016) and leaflet length (-0.015) also exhibited negative direct effect on bark yield. Number of leaflets per leaf exhibited low direct effect and the indirect effect through other characters were also found to be negligible. Leaflet length and leaflet breadth also showed low direct effect but they had high indirect effect on bark yield through stem girth (0.310, 0.313). This was indicated by their high significant correlation with bark yield. The highest positive indirect effect was observed for height of plant via stem girth (0.517) and number of leaves through stem girth (0.452).This was followed by internodal length (0.347), leaflet length and leaflet breadth through stem girth. Stem girth via height of plant (0.299), internodal length through height of plant (0.210) and number of leaves through height of plant (0.272) recorded moderate indirect effect on bark yield..

Table 10. Direct and indirect effects of various morphological traits on bark yield (April 2007-March 2008)

	Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Correlation coefficient with bark yield
Height of plant	**0.328**	0.206	-0.008	0.006	-0.003	-0.130	0.517	0.916
No. of leaves	0.272	**0.249**	-0.009	0.007	-0.003	-0.144	0.452	0.823
Leaflet length	0.173	0.144	**-0.015**	0.010	-0.002	-0.140	0.310	0.480
Leaflet breadth	0.169	0.145	-0.014	**0.012**	-0.003	-0.129	0.313	0.493
No. of leaflets/ leaf	0.079	0.053	-0.002	0.002	**-0.016**	-0.044	0.168	0.241
Internodal length	0.210	0.177	-0.010	0.007	-0.003	**-0.203**	0.347	0.525
Stem girth	0.299	0.199	-0.008	0.006	-0.004	-0.124	**0.567**	0.935

Bold figures indicate direct effect, residual effect = 0.2735

4.1.2 Morphological variations studied from April 2008-March 2009

4.1.2.1 Genetic variability

The extent of genetic variability with respect to different morphological traits in forty three indigenous collections of *S. asoca* was estimated. The abstract of analysis of variance of different characters is given in Table 11. In this analysis, the quarterly data of a year were taken as replications and the forty three accessions as treatments. Results from analysis of variance revealed highly significant difference among the 43 accession for the morphological characters studied. The characters include height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth and bark yield. In the second year of morphological study, the pace of growth of various accessions of asoka were not uniform which reflected in the variability due to replication.

Variability parameters like phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) computed for characters are presented in Table 12. Among the morphological traits, bark yield recorded the highest PCV (88.78) and GCV (86.65). High magnitudes of PCV and GCV was also indicated by stem girth (43.58, 42.31), number of leaves (45.77, 45.52), height of plant (41.40, 40.10) and internodal length (23.52, 22.87). But leaflet length (18.90, 18.76), leaflet breadth (16.63, 15.65) and number of leaflets per leaf (10.03, 10.03) showed moderate values. PCV in general was higher for all the characters except for number of leaflets per leaf where PCV and GCV were equal.

4.1.2.2 Heritability, genetic advance and genetic gain

Genetic parameters like heritability, genetic advance and genetic gain estimated for various morphological traits are presented in Table 12.

Table 11. ANOVA for morphological characters studied in *S. asoca* indigenous collections from April 2008-March 2009

Source of variation	Degrees of freedom	Mean sum of squares							
		Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/ leaf	Internodal length	Stem girth	Bark yield
Replication	3	262173.02**	594.30**	77.48**	24.90**	42.52**	14.30**	388.83**	242813.30**
Treatment	42	51947.81**	896.00**	33.80**	1.99**	4.25**	8.35**	77.50**	225178.70**
Error	126	844.95	2.49	0.12	0.06	0.20	0.11	1.16	2771.98

**Significant at 1% level

Table 12. Estimation of genetic parameters for morphological characters studied from April 2008-March 2009

Sl. No.	Characters	PCV (%)	GCV (%)	Heritability (%)	Genetic advance (GA)	Genetic gain (GG)
1.	Height of plant	41.40	40.10	93.79	225.50	80.00
2.	Number of leaves	45.77	45.52	98.89	30.63	93.29
3.	Leaflet length	18.90	18.76	98.55	5.93	38.35
4.	Leaflet breadth	16.63	15.65	88.57	1.34	30.24
5.	No. of leaflets/leaf	10.03	10.03	99.88	2.12	20.64
6.	Internodal length	23.52	22.87	94.51	2.86	45.61
7.	Stem girth	43.58	42.31	94.25	8.73	84.59
8.	Bark yield	88.78	86.65	95.25	474.06	174.20

High estimates of heritability (broad sense) were noticed for most of the traits studied. Maximum heritability of 99.88 per cent was noticed in the number of leaflets per leaf and the minimum of 88.57 per cent in the case of leaflet breadth. High heritability was estimated for the characters height of plant (93.79), number of leaves (98.89), leaflet length (98.55), leaflet breadth (88.57), number of leaflets per leaf (99.88) internodal length (94.51), stem girth (94.25) and bark yield (95.25).

Highest genetic gain value of 174.2 was observed for bark yield and the lowest of 20.64 for number of leaflets per leaf. High heritability and high genetic gain values were exhibited by height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth and bark yield.

4.1.2.3 Mean performance

The mean values of various accessions for morphological traits studied from April 2008 to March 2009 are presented in Table 13.

Among the morphological traits observed, plant height was maximum for IC566463 while IC566477 recorded the minimum height. IC466488 and IC566489 collected from Thiruvananthapuram had the highest number of leaves and IC566477 (Thrissur) showed the minimum number. The accessions IC566469, IC566457, IC566479, IC566498, IC566494, IC566492, IC566489, IC566480 and IC566482 indicated twelve leaflets per leaf. A minimum of eight leaflets per leaf was noted for IC566478, IC566490 and IC566487. Among the 43 accessions, IC566489 and IC566488 had the longest leaflets. Leaflets were comparatively small for IC566477 and IC566486. Wider leaflets were observed in IC566489 and IC566488.

Internodal length was maximum for IC566463 and IC566489 followed by IC566488, whereas IC566477 had the shortest internode. IC566489 and IC566463 indicated high stem girth values but IC566474 and IC566477 showed

Table 13. Mean performance of *S. asoca* indigenous collections for morphological traits from April 2008-March 2009

Indigenous collection	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflet/leaf	Internodal length (cm)	Stem girth (cm)	Bark yield (cm^3)
IC 566463	539.92	79.00	16.70	4.30	10	9.70	20.00	780.00
IC 566465	206.97	31.75	19.42	4.87	10	5.37	10.02	200.50
IC 566466	166.60	27.50	14.62	3.22	10	5.77	7.47	89.70
IC 566467	271.95	32.25	18.65	5.30	10	6.22	11.57	324.10
IC 566468	236.90	27.50	15.12	4.52	10	5.52	9.40	135.35
IC 566469	237.60	27.25	17.65	4.40	12	7.75	9.00	129.60
IC 566464	270.15	27.50	17.55	4.25	10	6.05	11.60	208.80
IC 566456	320.22	31.50	13.80	3.45	10	7.85	11.62	226.72
IC 566457	333.55	40.00	14.77	4.55	12	7.65	12.70	247.67
IC 566458	298.45	34.00	12.45	4.05	10	7.05	11.45	370.97
IC 566459	374.02	38.00	18.40	5.37	10	6.92	14.05	274.02
IC 566460	141.55	16.75	16.67	4.35	10	5.40	5.90	59.00
IC 566461	262.10	33.75	12.85	3.45	10	6.30	8.50	110.50
IC 566462	386.92	35.00	15.95	4.67	10	8.05	11.90	464.10
IC 566485	208.45	26.50	15.32	4.25	10	5.37	6.75	72.87
IC 566484	279.02	27.50	15.02	4.05	10	6.15	6.75	189.00
IC 566479	232.80	22.50	12.60	4.25	12	5.37	6.92	138.50
IC 566478	99.05	15.75	11.15	3.45	8	4.35	4.02	33.90
IC 566471	85.60	18.50	13.50	3.65	10	4.40	5.15	26.77
IC 566470	208.42	33.00	14.97	3.95	10	5.37	9.02	105.62
IC 566475	362.47	38.00	14.87	4.25	10	6.05	13.92	624.47
IC 566498	262.07	23.75	16.10	4.30	12	5.72	6.45	92.20
IC 566497	256.47	23.75	16.20	4.05	10	5.82	7.32	128.57
IC 566496	273.70	38.00	15.02	4.30	10	5.72	8.37	125.30
IC 566495	210.70	32.75	13.50	4.40	10	5.52	6.10	91.22

Contd.

Table 13 continued

Indigenous collection	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflet/leaf	Internodal length (cm)	Stem girth (cm)	Bark yield (cm^3)
IC 566494	326.92	32.25	18.50	5.37	12	5.62	12.12	307.30
IC 566492	299.17	35.00	18.30	5.17	12	5.72	11.72	358.80
IC 566489	516.90	67.25	23.57	6.15	12	9.70	24.07	1079.72
IC 566488	497.90	84.00	21.05	6.02	10	9.50	16.00	655.17
IC 566480	350.02	37.00	13.77	4.47	12	5.92	13.12	281.52
IC 566477	33.77	3.25	8.07	3.65	10	2.65	2.87	18.75
IC 566474	87.50	17.50	13.70	4.05	10	4.35	3.15	20.50
IC 566473	283.95	31.75	14.82	5.27	10	7.75	11.37	147.87
IC 566472	243.90	27.50	14.05	4.87	10	5.62	9.37	146.25
IC 566483	311.02	30.50	17.00	4.42	10	5.72	13.27	549.57
IC 566493	465.72	52.25	12.00	3.42	10	7.65	15.90	620.10
IC 566491	239.70	17.00	15.42	4.40	10	6.02	7.60	125.42
IC 566490	210.50	26.50	13.77	4.35	8	5.42	6.75	162.00
IC 566487	314.87	35.00	18.17	5.07	8	5.62	12.05	352.45
IC 566486	201.82	26.50	9.82	3.25	10	5.42	5.87	82.25
IC 566482	470.40	47.25	13.77	4.30	12	7.95	17.90	698.10
IC 566476	323.87	27.25	17.07	5.25	10	5.82	11.32	441.67
IC 566481	415.65	32.25	19.02	5.45	10	7.75	13.47	394.12
CD (1%)	52.94	2.87	0.64	0.45	0.61	0.62	1.96	95.90
CD (5%)	40.28	2.19	0.48	0.34	0.44	0.47	1.49	72.96

the minimum girth. The extractable bark yield was maximum for IC566489 followed by IC566463, IC566482, IC566488. IC566477 recorded the minimum value for bark yield.

4.1.2.4 Correlation

The genotypic and phenotypic correlation coefficient among different morphological traits studied are given in Table 14.

Bark yield was positively and significantly correlated both at genotypic and phenotypic levels with height of plant (0.881, 0.861), number of leaves (0.808, 0.787), leaflet length (0.506, 0.493), leaflet breadth (0.499, 0.442), number of leaflets per leaf (0.235, 0.231), internodal length (0.737, 0.719) and stem girth (0.928, 0.919).

The various bark yield components studied indicated that plant height was positively and significantly correlated at both levels with number of leaves (0.867, 0.843), leaflet length (0.517, 0.508), leaflet breadth (0.517, 0.470), number of leaflets per leaf (0.291, 0.282) internodal length (0.881, 0.850), stem girth (0.933, 0.914).

Number of leaves showed positive significant association at both levels with leaflet length (0.494, 0.488), leaflet breadth (0.446, 0.415), number of leaflets per leaf (0.179, 0.168), internodal length (0.835, 0.820) and stem girth (0.844, 0.819).

Leaflet length, leaflet breadth, number of leaflets per leaf recorded positive significant correlation with all the other morphological traits. Leaflet length (0.823, 0.772) was positively and significantly associated with leaflet breadth at both levels. Number of leaflets per leaf reported significant association with leaflet length (0.209, 0.208) and leaflet breadth (0.232, 0.218) at genotypic and phenotypic level. Leaflet length (0.581), leaf breadth (0.559) and number of leaflet per leaf (0.309) showed significant positive correlation with stem girth.

Table 14. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between bark yield and other morphological traits from April 2008-March 2009

	Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Bark yield
Height of plant	1	0.867**	0.517**	0.517**	0.291**	0.881**	0.933**	0.881**
No. of leaves	0.843**	1	0.494**	0.446**	0.179*	0.835**	0.844**	0.808**
Leaflet length	0.508**	0.488**	1	0.823**	0.209*	0.512**	0.581**	0.506**
Leaflet breadth	0.470**	0.415**	0.772**	1	0.232**	0.444**	0.559**	0.499**
No. of leaflets/ leaf	0.282**	0.168*	0.208*	0.218*	1	0.269**	0.309**	0.235**
Internodal length	0.850**	0.820**	0.495**	0.381**	0.262**	1	0.831**	0.737**
Stem girth	0.914**	0.819**	0.568**	0.503**	0.300**	0.800**	1	0.928**
Bark yield	0.861**	0.787**	0.493**	0.442**	0.231**	0.719**	0.919**	1

**Significant at 1% level
* Significant at 5% level

Internodal length indicated significant correlation with stem girth at both genotypic (0.831) and phenotypic (0.800) levels. Internodal length and stem girth reported positive significant association with all the morphological traits studied.

4.1.2.5 Path analysis

Analysis of direct and indirect effects of seven morphological traits on bark yield was carried out and the results are presented in Table 15.

The residual effect was 0.3462. Highest positive direct effect on bark yield was identified for stem girth (0.839). Moderate value was identified for height of plant (0.239). Negative direct effect on bark yield was indicated moderate by internodal length (-0.239). Number of leaflets per leaf (-0.053), leaflet length (-0.021) and leaflet breadth (-0.01) also exhibited negative direct effect on bark yield. Number of leaflets per leaf exhibited low direct effect and the indirect effect through other characters was also found to be negligible. Leaflet length and leaflet breadth also showed low direct effect but they had high indirect effect on bark yield through stem girth (0.488, 0.469). Maximum indirect effect on bark yield was observed for height of plant via stem girth (0.783), and number of leaves through stem girth (0.708). High indirect effect was also indicated by internodal length (0.697), leaflet length and leaflet breadth through stem girth. Stem girth (0.223) and internodal length (0.210) exhibited moderate indirect effect through height of plant.

4.1.3 Morphological variations studied from April 2007-March 2009

Morphological characters in forty three S. asoca accessions assessed in two years (April 2007 to March 2008 and April 2008 to March 2009) were analysed and presented in the previous sections. To derive a precise information of morphological variability of the accessions, the morphological data in both the years were pooled and analysed. In order to assess the relationship of biochemical traits and morphological traits, biochemical constituents like tannin content and phenol content assessed in these accessions were also considered for pooled

Table 15. Direct and indirect effects of various morphological traits on bark yield (April 2008-March 2009)

	Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Correlation coefficient with bark yield
Height of plant	**0.239**	0.102	-0.011	-0.005	-0.015	-0.211	0.783	0.881
No. of leaves	0.207	**0.117**	-0.010	-0.004	-0.009	-0.200	0.708	0.808
Leaflet length	0.123	0.058	**-0.021**	-0.008	-0.011	-0.122	0.488	0.506
Leaflet breadth	0.123	0.052	-0.017	**-0.010**	-0.012	-0.106	0.469	0.499
No. of leaflets/ leaf	0.079	0.021	-0.004	-0.002	**-0.053**	-0.064	0.259	0.235
Internodal length	0.210	0.098	-0.010	-0.004	-0.014	**-0.239**	0.697	0.737
Stem girth	0.223	0.099	-0.012	-0.006	-0.016	-0.199	**0.839**	0.928

Bold figures indicate direct effect, residual effect = 0.3462

analysis. The results of this analysis based on mean values of two years are presented below.

4.1.3.1 Genetic variability

The genetic variability was estimated with respect to different morphological and biochemical traits in forty three indigenous collections of *S. asoca*. Abstract of analysis of variance are presented in Table 16. Results from analysis of variance revealed highly significant difference among the 43 accession for the morphological and biochemical characters studied. The characters include height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth, bark yield, tannin content and phenol content. In pooled analysis, only bark yield has shown higher variability due to treatment compared to replication which indicates that bark yield is the reliable character for assessing genetic variability of the treatment.

The parameters of variability such as phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) computed for characters are presented in Table 17. Among the morphological traits of indigenous collections, high magnitude of PCV and GCV were observed for bark yield (94.47, 93.23), number of leaves (50.93, 49.68), stem girth (48.22, 44.70) and height of plant (45.83, 43.16). Other morphological traits like leaflet length also indicated high PCV (20.41) and GCV (20.01). Internodal length exhibited a higher PCV (21.88) compared to moderate GCV (16.10). The level of PCV and GCV were moderate for leaflet breadth and number of leaflets per leaf. With respect to biochemical traits, PCV and GCV were high and equal for tannin content, while phenol content recorded moderate values of PCV and GCV.

4.1.3.2 Heritability, genetic advance and genetic gain

The estimates of heritability, genetic advance and genetic gain are presented in Table 17.

Table 16. ANOVA for morphological and biochemical traits studied in *S. asoca* indigenous collections from April 2007-March 2009

Source of variation	Degrees of freedom	Mean sum of squares									
		Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/ leaf	Internodal length	Stem girth	Bark yield	Tannin content	Phenol content
Replication	7	391898.25**	2439.11**	295.72**	37.21**	78.18**	88.31**	610.04**	651332.08**	894.90**	723.89**
Treatment	42	18211.8**	383.05**	15.13**	0.907**	1.97**	0.04**	25.38**	668140.62**	104.46**	18.77**
Error	294	1090.90	9.54	0.30	0.02	0.09	0.60	1.91	7277.25	0.24	0.19

**Significant at 1% level

High heritability estimates were recorded for morphological characters leaflet length (96.06 per cent), number of leaves (95.13 per cent), leaflet breadth (94.20), number of leaf per leaf (91.20 per cent), height of plant (88.69 per cent), stem girth (85.93 per cent) and bark yield (80.36 per cent). Internodal length exhibited moderate level of broad sense heritability (54.15 per cent). Biochemical traits viz. tannin content and phenol content indicated high heritability values (99.52 per cent, 97.92 per cent).

Genetic gain values ranged from 20.38 for number of leaflets per leaf to 172.18 in the case of bark yield. High heritability values along with high genetic gain was indicated by the characters height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, stem girth, bark yield, tannin content and phenol content. Internodal length exhibited moderate heritability but high genetic gain (24.38).

Table 17. Estimation of genetic parameters for morphological and biochemical characters studied from April 2007-March 2009

Sl. No.	Characters	PCV (%)	GCV (%)	Heritability (%)	Genetic advance (GA)	Genetic gain (GG)
1.	Height of plant	45.83	43.16	88.69	179.48	83.73
2.	Number of leaves	50.93	49.68	95.13	27.43	99.74
3.	Leaflet length	20.41	20.01	96.06	5.50	40.44
4.	Leaflet breadth	18.10	17.57	94.20	1.32	35.01
5.	No. of leaflets/leaf	10.89	10.40	91.20	1.90	20.38
6.	Internodal length	21.88	16.10	54.15	1.28	24.38
7.	Stem girth	48.22	44.70	85.93	6.53	85.24
8.	Bark yield	94.47	93.23	80.36	318.70	172.18
9.	Tannin content	24.52	24.46	99.52	14.85	50.33
10.	Phenol content	18.26	18.07	97.92	6.20	36.77

4.1.3.3 Mean performance

The mean performance of forty three indigenous collections of *S. asoca* for morphological and biochemical traits from April 2007 to March 2009 are presented in Table 18.

Height of plant when assessed in different accessions, maximum height was reported for IC566463 (465.1 cm) followed by IC566489 (434.95 cm) and IC566488 (385.61 cm), while IC566477 (31.2 cm) had the minimum plant height. IC566488 (78.75), IC566463 (62.37) and IC566489 (61.87) had the highest number of leaves and IC566477 (3) showed the minimum number. The accessions IC566469, IC566457, IC566479, IC566498, IC566494, IC566492, IC566489, IC566480 and IC566482 recorded a maximum of 11 leaflets per leaf. Among the accessions, IC566489 had the longest leaflets followed by IC566488. Leaflets were comparatively shorter for IC566477 and IC566486. Maximum width for the leaflets were observed in the accessions IC566489 and IC566488.

Internodes were long in the case of the accessions IC566489 (7.83 cm), IC566488 (7.63) and IC566463 (7.3 cm). The accession IC566477 had the shortest internode (2.4 cm). Stem girth recorded indicated that the accession IC566489 followed by IC566463 had the maximum value. The minimum stem girth was observed for IC566474, IC566477 and IC566478. The maximum extractable bark yield was for IC566489 followed by IC566463, IC566482 and IC566488. IC566477 recorded the minimum value for bark yield.

In the biochemical traits analysed, high tannin content was observed in accessions IC566476 (40.37 mg/g), IC566474 (40.28 mg/g) and IC566467 (39.72 mg/g). Tannin content was low in the accessions IC566460 (18.11 mg/g) and IC566478 (18.14 mg/g). Phenol content was high in IC566474 (22.38 mg/g) and IC566467 (22.22 mg/g), whereas low in IC566470 (12.03 mg/g) and IC566460 (12.16 mg/g).

Table 18. Mean performance of *S. asoca* indigenous collections for morphological and biochemical traits from April 2007-March 2009

Indigenous collection	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflet/leaf	Internodal length (cm)	Stem girth (cm)	Bark yield (cm^3)	Tannin content (mg/g)	Phenol content (mg/g)
IC 566463	465.10	62.37	14.87	3.61	9	7.30	16.03	625.46	32.87	17.55
IC 566465	152.36	24.62	17.05	4.20	9	4.87	7.40	131.28	31.02	18.14
IC 566466	118.41	21.62	12.33	2.81	9	4.36	5.40	56.48	31.90	17.58
IC 566467	207.08	26.62	16.30	4.61	9	5.32	8.62	213.12	39.72	22.22
IC 566468	177.08	22.37	12.88	3.88	9	4.61	6.61	92.53	31.60	17.89
IC 566469	168.78	21.37	15.42	3.73	11	6.01	5.96	74.31	31.25	17.98
IC 566464	194.33	22.50	15.36	3.57	9	5.27	9.27	132.20	27.96	17.32
IC 566456	233.68	25.75	11.83	3.11	9	6.11	8.65	138.90	25.78	16.35
IC 566457	242.76	36.00	12.85	3.72	11	5.91	8.97	148.77	19.07	13.42
IC 566458	236.80	29.75	10.43	3.42	10	5.28	8.77	248.91	28.33	16.36
IC 566459	284.68	34.00	16.65	4.56	9	6.50	9.66	171.30	22.08	13.03
IC 566460	117.41	12.62	14.78	3.80	9	4.07	4.13	37.22	18.11	12.16
IC 566461	190.37	26.62	11.10	2.82	9	5.42	6.31	73.81	37.98	21.14
IC 566462	299.67	30.75	14.13	4.03	9	6.31	8.45	312.05	36.67	19.45
IC 566485	143.28	20.62	13.08	3.57	9	4.87	4.72	44.53	32.59	17.12
IC 566484	204.65	21.62	12.78	3.42	9	5.37	4.71	114.56	37.17	19.94
IC 566479	169.38	18.50	10.82	3.57	11	4.86	5.05	88.30	32.63	16.87
IC 566478	80.05	11.25	10.07	2.82	7	3.66	2.80	20.10	18.14	12.75
IC 566471	68.86	14.25	11.53	3.02	9	3.87	4.01	17.85	18.84	12.31
IC 566470	141.37	28.75	13.06	3.25	9	4.87	6.23	61.43	19.76	12.03
IC 566475	287.26	34.00	12.95	3.57	9	5.27	10.78	442.28	37.31	18.16
IC 566498	180.95	19.37	14.30	3.63	11	5.07	4.58	59.38	34.38	18.18
IC 566497	188.18	19.37	14.40	3.42	9	5.17	5.08	75.68	29.66	16.77
IC 566496	196.90	34.00	12.78	3.63	9	5.07	6.12	78.15	36.34	19.32

Contd.

Table 18.Continued

Indigenous collection	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflet/leaf	Internodal length (cm)	Stem girth (cm)	Bark yield (cm^3)	Tannin content (mg/g)	Phenol content (mg/g)
IC 566495	156.40	25.62	11.53	3.73	9	4.87	4.42	54.55	18.84	12.26
IC 566494	248.08	26.62	16.75	4.56	11	4.97	8.32	180.80	33.50	17.44
IC 566492	226.53	30.75	16.55	4.36	11	5.07	8.68	234.50	36.94	20.34
IC 566489	434.95	61.87	21.30	5.76	11	7.83	19.63	835.77	33.05	17.71
IC 566488	385.61	78.75	19.93	5.51	9	7.63	12.52	504.06	18.33	12.88
IC 566480	283.05	33.00	12.01	3.67	11	5.27	9.01	179.96	30.46	17.39
IC 566477	31.20	3.00	7.51	3.02	9	2.40	2.57	10.23	19.91	12.42
IC 566474	77.35	13.25	1.73	3.42	9	3.83	2.28	12.05	40.28	22.38
IC 566473	211.87	24.62	13.10	4.36	9	6.01	8.75	90.78	28.01	16.07
IC 566472	165.86	22.37	12.31	4.02	9	4.97	6.53	80.52	19.26	12.75
IC 566483	223.50	24.75	15.02	3.80	9	5.07	9.26	322.03	33.61	18.18
IC 566493	372.67	45.37	11.01	2.90	9	5.91	12.23	477.26	37.13	20.86
IC 566491	174.27	13.62	13.18	3.73	9	5.37	6.17	81.71	33.17	19.08
IC 566490	159.72	20.62	12.01	3.80	8	4.51	4.72	102.6	32.56	17.84
IC 566487	243.33	30.75	15.81	4.26	7	4.97	8.53	218.43	35.19	18.88
IC 566486	133.80	20.62	8.56	2.60	9	4.51	4.27	51.82	19.70	12.42
IC 566482	377.91	40.37	12.01	3.63	11	6.21	14.23	534.63	27.27	18.09
IC 566476	230.48	21.37	15.38	4.51	9	5.17	8.35	253.08	40.37	21.38
IC 566481	330.52	26.62	17.47	4.73	9	6.01	10.50	270.42	19.89	12.53
CD (1%)	89.31	8.35	1.49	0.44	0.81	2.10	3.74	230.66	1.34	1.20
CD (5%)	66.75	6.24	1.11	0.33	0.60	1.57	2.80	172.40	1.01	0.89

4.1.3.4 Correlation

The genotypic and phenotypic correlation coefficients between morphological traits and biochemical traits are presented in Table 19. The genotypic correlations were found to be higher than phenotypic correlations for all the characters. Direction of genotypic and phenotypic correlations were the same.

Bark yield was found to positively and significantly correlated at genotypic and phenotypic levels with stem girth (0.934, 0.920), height of plant (0.893, 0.864), number of leaves (0.826, 0.783), internodal length (0.658, 0.605), leaflet length (0.514, 0.485), leaflet breadth (0.509, 0.442), number of leaflets per leaf (0.210, 0.201). Biochemical traits tannin content (0.222, 0.204) and phenol content (0.218, 0.211) exhibited significant positive correlation with bark yield at both levels.

Correlation coefficients among the component characters showed that height of plant was significantly correlated with number of leaves (0.876), leaflet length (0.527), leaflet breadth (0.523), number of leaflets per leaf (0.288), internodal length (0.907), stem girth (0.937), tannin content (0.212) and phenol content (0.189).

Number of leaves was positively and significantly correlated with leaflet length (0.514), leaflet breadth (0.478), number of leaflets per leaf (0.189), internodal length (0.852) and stem girth (0.850).

Leaflet length indicated positive significant correlation with all characters except number of leaflets per leaf. Leaflet length and breadth exhibited positive significant association with internodal length (0.565, 0.509) and stem girth (0.585, 0.564). In contrast to leaflet length, leaflet breadth identified positive significant association with number of leaflets per leaf also. All the morphological traits were found to be positively and significantly correlated with number of leaflets per leaf except the leaflet length.

Table 19. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between morphological traits and biochemical traits from April 2007-March 2009

	Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Bark yield	Tannin content	Phenol content
Height of plant	1	0.876**	0.527**	0.523**	0.288**	0.907**	0.937**	0.893**	0.212*	0.189*
No. of leaves	0.838**	1	0.514**	0.478**	0.189*	0.852**	0.850**	0.826**	0.066	0.045
Leaflet length	0.508**	0.503**	1	0.847**	0.161	0.565**	0.585**	0.514**	0.160	0.152
Leaflet breadth	0.474**	0.442**	0.791**	1	0.225*	0.509**	0.564**	0.509**	0.072	0.041
No. of leaflets/ leaf	0.274**	0.184*	0.159	0.212*	1	0.292**	0.306**	0.210*	0.122	0.114
Internodal length	0.829**	0.796**	0.504**	0.398**	0.215*	1	0.852**	0.658**	0.082	0.094
Stem girth	0.913**	0.811**	0.561**	0.505**	0.289**	0.783**	1	0.934**	0.161	0.155
Bark yield	0.864**	0.783**	0.485**	0.442**	0.201*	0.605**	0.920**	1	0.222*	0.218*
Tannin content	0.201*	0.067	0.157	0.065	0.120	0.079	0.158	0.204*	1	0.974**
Phenol content	0.177*	0.043	0.147	0.049	0.110	0.073	0.148	0.211*	0.960**	1

**Significant at 1% level
* Significant at 5% level

Internodal length indicated significant association with stem girth at both genotypic (0.852) and phenotypic (0.783) levels. Internodal length and stem girth reported positive significant association with all the morphological characters studied.

Biochemical traits tannin content and phenol content did not exhibit correlation with most of the morphological traits except bark yield and height of plant. Tannin content reported positive significant association with phenol content both at genotypic (0.974) and phenotypic (0.960) levels.

4.1.3.5 Path analysis

The significant genotypic correlations of nine traits of indigenous collections namely height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth, tannin content and phenol content with bark yield were included in path analysis. The estimates of direct and indirect effects of these nine component characters on bark yield are given in Table 20.

The residual effect of path analysis was found to be 0.2968. It was observed that the characters viz. stem girth, height of plant, tannin content, number of leaves and leaflet breadth exerted positive direct effect on bark yield. On the other hand intermodal length, phenol content, leaflet length and number of leaflets per leaf recorded negative direct effects on bark yield. Stem girth had the highest positive direct effect (0.871). This was followed by height of plant (0.211) and tannin content (0.200) which indicated moderate direct effects. Low negative direct effect was observed for intermodal length (-0.197) and phenol content (-0.157). The path coefficient of leaflet breadth was the least (0.044). Number of leaflets per leaf exhibited low direct effect and the indirect effect through other characters were negligible except stem girth.

The highest positive indirect effect with bark yield was exhibited by height of plant (0.716) through stem girth, internodal length (0.643) and number of

Table 20. Direct and indirect effects of morphological and biochemical traits on bark yield (April 2007-March 2009)

	Height of plant	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Tannin content	Phenol content	Correlation coeff. with bark yield
Height of plant	**0.211**	0.154	-0.047	0.023	-0.014	-0.179	0.716	0.096	-0.067	0.893
No. of leaves	0.197	**0.176**	-0.045	0.021	-0.009	-0.168	0.641	0.030	-0.016	0.826
Leaflet length	0.058	0.090	**-0.089**	0.037	-0.009	-0.111	0.510	0.082	-0.054	0.514
Leaflet breadth	0.058	0.084	-0.075	**0.044**	-0.011	-0.100	0.491	0.033	-0.014	0.509
No. of leaflets/ leaf	0.032	0.033	-0.017	0.010	**-0.049**	-0.057	0.267	0.034	-0.041	0.210
Internodal length	0.101	0.150	-0.050	0.022	-0.014	**-0.197**	0.643	0.037	-0.033	0.658
Stem girth	0.104	0.149	-0.052	0.025	-0.015	-0.168	**0.871**	0.074	-0.055	0.934
Tannin content	0.023	0.021	-0.016	0.015	-0.016	-0.063	0.184	**0.200**	-0.128	0.222
Phenol content	0.011	0.015	-0.013	0.001	-0.005	-0.018	0.145	0.240	**-0.157**	0.218

Bold figures indicate direct effect, residual effect = 0.2968

leaves (0.641) via stem girth, leaflet length (0.510) and leaflet breadth (0.491) through stem girth.

4.1.3.6 Cluster analysis

Hierarchical cluster analysis in 43 *S. asoca* accessions was conducted in pooled data from April 2007 to March 2009. The dendrogram (Fig.1) of hierarchial cluster analysis based on average linkage distance between clusters indicated the grouping of various accessions into two major clusters.

At an average linkage distance of 1, the 43 accessions formed 8 clusters, one large cluster with 20 accessions, one cluster with 9 accessions, one cluster with 4 accessions, 2 clusters with 3 accessions, 1 cluster with 2 accessions and 2 small clusters with one accession each. At an average linkage distance of 2, the clusters with 9 accessions and 3 accessions merged to form a single cluster. In the same way, a single cluster was formed by the clusters with 3 accession and 1 accession. Based on this, it was identified that 43 accessions formed 6 clusters at an average linkage distance of 2. At an average linkage distance of 3, the accessions formed 4 clusters, one large cluster with 24 accessions, one cluster with 12 accessions, one cluster with 6 accessions and a small cluster with one accession. A large cluster with 24 accessions was formed by merging of a large cluster with 20 accessions and a small cluster with 4 accessions. At an average linkage distance of 6, the cluster with 24 accessions and the cluster with 12 accessions formed a larger cluster of 36 accessions. At this stage, the total number of clusters became reduced to three. At an average linkage distance of 14 between clusters, the 43 accessions were grouped into 2 clusters, one large cluster with 36 accessions and a small cluster of 7 accessions. The two clusters were resulted when a single cluster with one accession merged with the cluster with 6 accessions.

The accessions in each of 6 clusters formed at an average linkage distance of 2 is presented in Table 21. Evaluation of the clustering pattern of all the 43 accessions indicated that the clustering pattern did not follow the

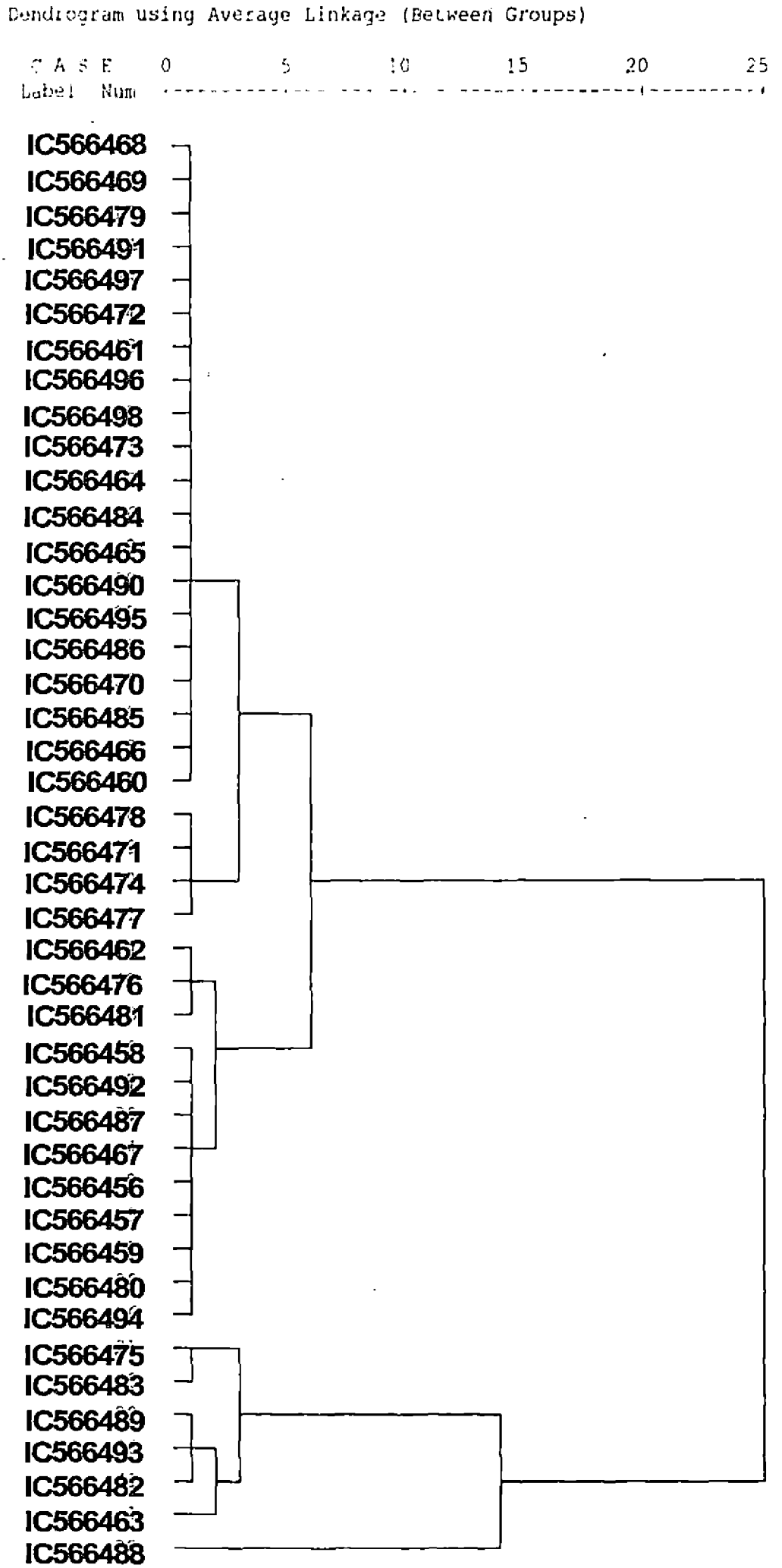


Fig.1 Cluster analysis dendrogram

geographical distributions. Cluster 3 was represented by all the six districts whereas cluster 1 by Thrissur, Malappuram, Thiruvananthapuram, Kollam and Kozhikode. Cluster 2 was indicated by Thrissur, Ernakulam while cluster 5 by Thrissur, Thiruvananthapuram. Cluster 4 and cluster 6 was pointed out by Thrissur and Thiruvananthapuram respectively.

Table 21. Grouping of accessions into clusters

Cluster	Accessions	Total number of accessions	Source of collection
Cluster 1	IC566468, IC566479, IC566469, IC566473, IC566464, IC566484, IC566465, IC566495, IC566486, IC566485, IC566466, IC566497, IC566472, IC566496, IC566498, IC566470, IC566491, IC566490, IC566461, IC566460	20	Thrissur, Malappuram, Thiruvananthapuram, Kollam, Kozhikode
Cluster 2	IC566478, IC566471, IC566474, IC566477	4	Thrissur, Ernakulam
Cluster 3	IC566462, IC566476, IC566481, IC566458, IC566492, IC566487, IC566467, IC566456, IC566457, IC566459, IC566480, IC566494	12	Thrissur, Kozhikode, Malappuram, Ernakulam, Thiruvananthapuram, Kollam
Cluster 4	IC566475, IC566493	2	Thrissur
Cluster 5	IC566488, IC566493, IC566482, IC566463	4	Thrissur, Thiruvananthapuram
Cluster 6	IC566489	1	Thiruvananthapuram

4.1.3.7 Discriminant function analysis

Selection index

A selection model for making selection based on several characters simultaneously was developed. The correlation analysis of the mean values of morphological and biochemical traits in *S. asoca* germplasm accessions were studied from April 2007 to March 2009. This indicated that the bark yield was significantly and highly associated with height of plant, number of leaves, leaflet length, leaflet breadth, internodal length and stem girth. All possible combinations of these six characters were formulated and models with maximum expected genetic advance were selected from models with different character combinations. Six models were thus developed and are presented in Table 22.

Table 22. Discriminant function analysis for different bark yield components in *S. asoca* indigenous collection and expected genetic advance

Sl. No.	Combination	Discriminant function	Expected genetic advance
1.	Y, X_1, X_2, X_3, X_4, X_6, X_7	0.21 X_1 + 0.14 X_2 + ¯0.02 X_3 + ¯0.01 X_4 + ¯0.22 X_6 + 0.81 X_7	i (11.29)
2.	Y, X_1, X_2, X_3, X_4, X_6	0.83 X_1 + 0.24 X_2 + 0.07 X_3 + ¯0.01 X_4 + ¯0.23 X_6	i (44.48)
3.	Y, X_1, X_2, X_3, X_4	0.68 X_1 + 0.18 X_2 + 0.03 X_3 + 0.02 X_4	i (36.54)
4.	Y, X_1, X_2, X_3	0.69 X_1 + 0.17 X_2 + 0.05 X_3	i (36.91)
5.	Y, X_1, X_2	0.71 X_1 + 0.18 X_2	i (37.98)
6.	Y, X_1	0.87 X_1	i (46.33)

Y = Mean Grain yield

X_1 (Mean Height of Plant) = 281.84

X_2 (Mean number of leaves) = 32.8

X_3 (Mean leaflet length) = 15.46

X_4 (Mean leaflet breadth) = 4.43

X_6 (Mean internodal length) = 6.27

X_7 (Mean stem girth) = 10.32

i = Selection differential

Maximum expected genetic advance i (46.33) was noted when bark yield and the yield component – height of plant (X_1) was used in the selection index. An almost equal expected genetic advance i (44.48) resulted when bark yield and five yield components were used in the selection index. The characters included in the index were height of plant (X_1), number of leaves (X_2), leaflet length (X_3), leaflet breadth (X_4) and internodal length (X_6). From the proposed six models, both these selection indices, one having single character (selection index 1) and other with character combination including five yield components (selection index 2) gave maximum expected genetic advance over the other models. Of the selection indices, selection index 1 was selected as only a single trait height of plant is used to select better accessions. The 43 accessions were ranked by this selection index. Estimates of selection index using the above mentioned model and ranking according to index and bark yield are given in Table 23. According to selection index 1 involving the character height of plant, first 10 ranks were obtained for the accessions viz., IC566463, IC566489, IC566488, IC566482, IC566493, IC566481, IC566462, IC566459, IC566475, IC566480. Based on bark yield, first ten ranks were for the accessions IC566489, IC566463, IC566482, IC566488, IC566475, IC566493, IC566483, IC566462, IC566476, IC566481. Among these two groups of accessions, eight accessions were same , but there was difference in the ranking order. The best ten accessions identified by selection index 1 may be used in further breeding programmes.

Table 23. Estimates of selection indices for *S. asoca* accessions using height of plant (X_1)

Sl. No.	Accessions	Selection index 1 (X_1)	Rank according to	
			Selection index 1	Bark yield
1.	IC566463	472.97	1	2
2.	IC566489	452.80	2	1
3.	IC566488	436.20	3	4
4.	IC566482	412.07	4	3
5.	IC566493	407.97	5	6
6.	IC566481	364.10	6	10
7.	IC566462	338.94	7	8
8.	IC566459	327.64	8	17
9.	IC566475	317.52	9	5
10.	IC566480	306.62	10	16
11.	IC566457	292.18	11	18
12.	IC566494	286.38	12	15
13.	IC566476	283.71	13	9
14.	IC566456	280.51	14	19
15.	IC566487	275.83	15	13
16.	IC566483	272.45	16	7
17.	IC566492	262.07	17	12
18.	IC566458	261.44	18	11
19.	IC566473	248.74	19	24
20.	IC566484	244.42	20	22
21.	IC566496	239.76	21	31
22.	IC566467	238.22	22	14
23.	IC566464	236.65	23	20
24.	IC566461	229.59	24	32
25.	IC566498	229.57	25	34
26.	IC566497	224.67	26	29

Sl. No.	Accessions	Selection index 1 (X_1)	Rank according to	
			Selection index 1	Bark yield
27.	IC566472	213.65	27	25
28.	IC566491	209.97	28	30
29.	IC566469	208.13	29	28
30.	IC566468	207.52	30	27
31.	IC566479	203.93	31	26
32.	IC566495	184.57	32	35
33.	IC566490	184.39	33	23
34.	IC566485	182.60	34	38
35.	IC566470	182.58	35	33
36.	IC566465	181.31	36	21
37.	IC566486	176.79	37	37
38.	IC566466	145.94	38	36
39.	IC566460	123.99	39	39
40.	IC566478	86.76	40	40
41.	IC566474	76.65	41	43
42.	IC566471	74.98	42	42
43.	IC566477	29.58	43	41

4.1.3.8 Bark thickness

Bark thickness was measured twice in March 2008 and March 2009 during the study of morphological traits from April 2007 to March 2009. Bark thickness did not show significant variation in the two observations taken. Bark thickness of different accessions are presented in Table 24.

Maximum bark thickness was observed in accession IC566463 (2.7 mm). Accessions IC566489, IC566483, IC566465, IC566462, IC566475 recorded a bark thickness of 2.5 mm and IC566488 had 2.4 mm bark thickness. Observations on bark thickness recorded for these accessions were high in both the years. Among the accessions, bark thickness was least in IC566477 (0.5 mm). The accessions IC566471, IC566474 and IC566473 also recorded low bark thickness value of 1 mm in March 2009.

Table 24. Bark thickness of different indigenous collection of *S. asoca*

Indigenous collection	Bark thickness (millimeters)	
	March 2008	March 2009
IC 566463	1.9	2.7
IC 566465	1.8	2.5
IC 566466	1.5	2.0
IC 566467	1.5	2.0
IC 566468	1.2	1.7
IC 566469	1.1	1.6
IC 566464	1.1	1.6
IC 566456	1.2	1.6
IC 566457	1.2	1.6
IC 566458	1.7	2.2
IC 566459	1.2	1.6
IC 566460	1.1	1.5
IC 566461	1.2	1.5
IC 566462	1.8	2.5
IC 566485	1.2	1.7
IC 566484	1.5	2.0
IC 566479	1.6	2.0
IC 566478	1.0	1.5
IC 566471	0.7	1.0
IC 566470	1.1	1.5
IC 566475	1.8	2.5

Indigenous collection	Bark thickness (millimeters)	
	March 2008	March 2009
IC 566498	0.9	1.3
IC 566497	1.1	1.5
IC 566496	1.0	1.3
IC 566495	1.0	1.3
IC 566494	1.2	1.5
IC 566492	1.6	2.0
IC 566489	1.8	2.5
IC 566488	1.8	2.4
IC 566480	0.9	1.3
IC 566477	0.4	0.5
IC 566474	0.7	1.0
IC 566473	0.7	1.0
IC 566472	1.1	1.5
IC 566483	1.8	2.5
IC 566493	1.7	2.3
IC 566491	1.2	1.7
IC 566490	1.5	2.0
IC 566487	1.2	1.7
IC 566486	1.5	2.0
IC 566482	1.7	2.2
IC 566476	1.5	2.0
IC 566481	1.2	1.6

4.2 Reproductive biology of *S. asoca*

The knowledge of reproductive biology is a prerequisite in plant breeding and for obtaining better yield. Variability is controlled by the breeding system, of which pollination mechanism forms an integral component. An adequate knowledge about the reproductive biology of S.asoca is lacking. Therefore the present investigation was undertaken to study the reproductive biology of this species. The reproductive biology aspects studied in eight asoka trees in KAU campus included flowering season, inflorescence and flower traits, pod and seed traits, reproductive period traits. The other components of reproductive biology assessed were anthesis, pollen and pollination studies. The correlation among the reproductive biology traits were analysed. Effect of weather parameters on reproductive biology traits were also checked. The results are presented below.

4.2.1 Season of flowering

Flowering was seen throughout the year, but most profuse flowering was noticed from January to May.

4.2.2 Inflorescence characters

Inflorescence is an axillary corymb. Blooming in an inflorescence is in an irregular manner. The number of inflorescence per tree ranged from 15 to 185 and on an average 30-65 flowers were present in each inflorescence (Table 25). Of the trees studied, maximum number of inflorescence per tree was in KAU7, KAU6 and KAU8. KAU3 and KAU6 produced maximum number of flowers per tree. Larger flowers were observed in KAU3, KAU4, KAU6 and KAU8.

4.2.3 Floral morphology and biology

Flowers are short stalked, fragrant, bisexual and 5.0-6.8 cm long, 1.8-4.6 cm wide (Table 25). Initially flowers are orange or orange-yellow and eventually turns to vermillion.

Table 25. Characters of flowers and pods

Accession no.	No.of inflorescence per tree	No.of flowers per inflorescence	No.of pods per tree	No.of seeds per pod	Mean flower		Mean pod size		Mean seed size		Mean seed volume (ml)
					Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	
KAU1	30	47	50	2	5.5	3.0	12.0	4.5	2.2	2.0	8
KAU2	15	30	41	2	5.0	1.9	11.5	4.5	2.7	2.2	7
KAU3	130	65	210	2	6.8	3.0	13.5	4.3	4.4	2.6	10
KAU4	95	55	182	5	5.8	4.6	13.3	4.0	3.8	2.2	10
KAU5	72	35	110	3	5.6	1.8	11.3	3.5	2.4	2.0	10
KAU6	165	65	285	2	6.1	3.1	10.2	4.1	3.9	2.3	10
KAU7	185	55	371	3	5.6	3.0	10.8	4.4	3.9	2.5	10
KAU8	145	30	480	5	5.8	3.2	14.1	3.6	4.4	2.6	12
Mean	104.6	47.75	216.12	3	5.77	2.95	12.08	4.11	3.46	2.3	9.62
SE	±62.26	±14.6	±155.42	±1.3	±0.52	±0.86	±1.39	±0.39	±0.89	±0.24	±1.5

Calyx is long tubular which open out into four lobes. Petals are absent. Stamens are seven, filaments slender, anthers elliptic-oblong. Anthers are dithecous, dorsifixed and dehisce by longitudinal slits. Ovary superior, unilocular, usually ten to eleven ovuled in marginal placentation. Style long, slender, ending in a capitate stigma (Table 26). After anthesis, calyx tube and style remains with ovary for two weeks.

Table 26. Floral parts

1. Calyx	Orange-yellow and tubular with four lobes
2. Corolla	Absent
3. Androecium	Stamen-seven Filaments slender Anthers elliptic-oblong and dithecous
4. Gynoecium	Long slender style Capitate stigma Ovary unilocular Ovules-ten to eleven

4.2.4 Pod and seed characters

Pods are flat, oblong, woody. Size of pods ranged from 10.2 x 3.5 cm to 14.1 x 4.5 cm. Seed number per pod varied from 2 to 5 (Table 25). Number of pods per tree ranged from 41 to 480. Immature pods are initially dark brown and later turned to coppery red coloured when pods attained full size with immature seeds. On attaining full maturity, pods are green in colour.

Seeds are dark brown, ellipsoid-oblong, compressed. Average seed size was 3.4 cm x 2.3 cm. Seed volume ranged from 7 ml to 12 ml by water displacement method (Table 25).

Among the trees studied, KAU8 had maximum number of pods per tree and seeds per pod. Bigger pods and seeds were produced by KAU3, KAU4 and KAU8 whereas maximum seed volume was observed in KAU8.

4.2.5 Reproductive period

Mature buds are formed about 20-26 days after the appearance of visible buds. Duration of blooming in an inflorescence varied from 12 to 16 days. After anthesis, flowers required 30 to 34 days to form an immature pod. Fully matured pods dehisce by longitudinal slits after 62-69 days from formation of immature pods. In the eight trees studied, the total reproductive period from initiation of flower bud to seed shedding ranged from 126 days to 137 days (Table 27).

4.2.6 Anthesis

A preliminary study revealed that there was no flower opening between 6 am and 6 pm. Therefore inflorescence having mature buds along with leaves and small piece of branch were collected previous day evening from each of the eight trees and placed in water. Hourly observations on flower opening were taken from 6 pm to 6 am the next day. The stages of flower opening at periodic intervals is presented in Table 28. In the inflorescence from different trees studied, the fully mature flower buds showed anthesis between 5.00-6.00 am (Table 27). This indicated that the anthesis occurred in *S. asoca* between 5-6 am irrespective of the genotype and age of the tree.

4.2.7 Anther dehiscence and stigma receptivity

Fully mature flower buds were observed for anther dehiscence and stigma receptivity at periodic intervals (one hour) from 6 pm to 9 am the next day.

Anthers dehisced at the time of anthesis, between 5-6 am. Anthers dehisced by longitudinal slits. Stigma was receptive one hour after anthesis and continued to be receptive for that same full day (Table 27). Receptivity of stigma was identified as bulged, turgid stigma with shiny red appearance.

Table 27: Reproductive traits

Accession no.	Age of tree (years)	Duration of inflorescence development (days)	Duration of blooming (days)	Time of anthesis	Time of anther dehiscence	Time of stigma receptivity	Days for flower to pod	Days for pod to mature	Reproductive period (days)
KAU1	15	26	16	5-6am	5-6am	6-7am to 12midnight	33	62	137
KAU2	15	20	12	5-6am	5-6am	6-7am to12 midnight	34	67	133
KAU3	15	20	11	5-6am	5-6am	6-7am to12 midnight	32	63	126
KAU4	9	23	12	5-6am	5-6am	6-7am to12 midnight	33	66	134
KAU5	19	22	12	5-6am	5-6am	6-7am to12 midnight	31	69	134
KAU6	25	23	13	5-6am	5-6am	6-7am to12 midnight	30	68	134
KAU7	25	22	12	5-6am	5-6am	6-7am to12 midnight	32	69	135
KAU8	31	23	13	5-6am	5-6am	6-7am to12 midnight	30	69	135
Mean		22.37	12.62				31.87	66.62	133.5
SE		±1.92	±1.50				±1.45	±2.77	±3.25

Table 28. Sequence of anthesis

Time	Stage of anthesis
9 pm – 12 midnight	Buds are round and plump
12 midnight – 3 am	Slits appear at tip of buds
3 am – 5 am	Flower starts to open, stamens project out but in folded stage
5 am – 6 am	Flowers opened completely, stamen, style fully project out

4.2.8 Pollen morphology and viability

The morphology of pollen collected from fully mature buds were studied by acetolysis method. Pollen is creamy white, tricolpate and round with smooth exine. Average size of pollen is 31.30 µm length and 30.39 µm breadth (Table 29).

The fertility of pollen collected from fully mature buds was assessed by acetocarmine staining, which revealed hundred per cent fertility (Table 30).

The germination of pollen was assessed in sucrose solution. Mean pollen germination recorded in1%, 5% and 10% sucrose solution was 45.25 % (Table 31).

Absolute pollen viability as assessed from the fertile pollen and germinated pollen indicated a value of 45.25

Table 29. Pollen size

Sl. No.	Length (micrometer)	Breadth (micrometer)
1.	31.23	30.12
2	32.69	30.23
3	30.16	30.26
4	32.42	31.79
5	29.06	29.06
6	30.42	29.42
7	32.69	31.16
8	31.79	31.09
Mean	31.30	30.39
SE	±1.33	±0.91

Table 30. Pollen fertility

Sl. No.	No. of pollen	No. of stained pollen	Pollen fertility (%)
1	52	52	100
2	50	50	100
3	52	52	100
4	54	54	100
5	50	50	100
6	54	54	100
7	52	52	100
8	50	50	100
Mean	51.75	51.75	100
SE	1.66	1.66	0

Table 31. Pollen germination

Sl. No.	No. of pollen	% sucrose solution	No. of pollen germinated	% germinated pollen
1	55	1	26	47
2	52	1	25	48
3	50	1	22	44
4	53	1	25	47
5	52	1	24	46
6	54	1	25	46
7	50	1	20	40
8	52	1	23	44
1	52	5	25	48
2	54	5	25	46
3	53	5	25	47
4	52	5	24	46
5	50	5	22	44
6	52	5	23	44
7	55	5	26	47
8	50	5	20	40
1	53	10	25	47
2	54	10	25	46
3	52	10	23	44
4	50	10	20	40
5	52	10	24	46
6	55	10	26	47
7	50	10	22	44
8	52	10	25	48
Mean	52.25	-	23.75	45.25
SE	1.67	-	1.89	2.43

4.2.9 Pollination system and pollinating agents

Twenty flowers in each of the 8 trees were covered with butter paper a day before starting of anthesis and observed for self pollination. Percentage pod set observed under selfing was 4.37%. This indicated the occurrence of self pollination to a very small extent in *S. asoca.*

In natural condition, trees were identified to be cross pollinated. Pod set observed under open pollination was 42.5% (Table 32).

To identify the pollinating agents, preliminary observations of flower visitors were undertaken. This indicated the presence of ants only. To confirm this, inflorescence were removed of ants and covered with polythene cover and observed for pod set. Absence of pod set in these inflorescence indicated that ants are the major pollinators.

4.2.10 Evaluation of reproductive biology traits

Eight trees of *S. asoca* were evaluated for various reproductive biology traits. Data were subjected to statistical analysis and the results are presented below.

4.2.10.1 Correlation among reproductive biology traits

The genotypic and phenotypic correlation coefficient among different reproductive biology traits are given in Table 33.

The number of inflorescence per tree was found to be positively and significantly correlated with all the reproductive biology traits. Number of flowers per inflorescence indicated correlation with most of the reproductive biology traits except with days for flower to pod (-0.094) and total reproductive period (-0.116). Number of flowers per inflorescence indicated high and significant positive association with number of pods per tree (1.07), number of seeds per pod (1.01),

Table 32. Nature of pollination

Accession No.	Selfing			Crossing		
	No. of flowers	No.of mature pods	Percentage pod set	No. of flowers	No.of mature pods	Percentage pod set
KAU1	20	1	5	20	9	45
KAU2	20	1	5	20	10	50
KAU3	20	1	5	20	8	40
KAU4	20	0	0	20	9	45
KAU5	20	1	5	20	8	40
KAU6	20	1	5	20	8	40
KAU7	20	1	5	20	8	40
KAU8	20	1	5	20	8	40
			4.375			42.5

Table 33. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between reproductive biology traits in *S. asoca*

Sl. No.	No. of inflore-scence/ tree 1	No. of flowers/ inflore-scence 2	No. of pods/ tree 3	No. of seeds/ pod 4	Flower length 5	Flower breadth 6	Pod length 7	Pod breadth 8	Seed length 9	Seed breadth 10	Seed volume 11	Duration of inflore-scence develop-ment 12	Duration of blooming 13	Days for flower to pod 14	Days for pod to mature 15	Repro-ductive period 16
1	1	0.957**	1.041**	0.751**	0.599**	0.794**	0.732**	0.773**	0.863**	0.746**	0.791**	0.262**	0.193*	-0.218*	-1.206**	-0.175*
2	0.872**	1	1.07**	1.01**	0.817**	0.919**	0.826**	0.895**	1.02**	0.926**	0.991**	0.280**	0.242*	-0.094	-1.28**	-0.116
3	0.828**	0.710**	1	0.882**	0.740**	0.885**	0.815**	0.889**	0.947**	0.804**	0.888**	0.345**	0.231*	-0.210*	-1.417**	-0.176*
4	0.325**	0.352**	0.347**	1	1.061**	0.974**	0.956**	1.082**	1.115**	1.013**	1.03**	0.610**	0.606**	0.178*	-0.885**	0.204*
5	0.395**	0.467**	0.410**	0.433**	1	0.987**	1.043**	0.950**	1.022**	0.968**	1.015**	0.827**	0.789**	0.420**	-0.522**	0.445**
6	0.302**	0.392**	0.337**	0.430**	0.615**	1	1.051**	1.021**	1.019**	0.911**	1.016**	0.447**	0.525**	0.184**	-0.692**	0.184*
7	0.172	0.305**	0.161	0.246**	0.356**	0.407**	1	0.895**	1.019**	1.165**	1.093**	0.870**	0.838**	0.637**	-0.582**	0.552**
8	0.404**	0.420**	0.335**	0.236*	0.564**	0.471**	0.417**	1	1.177**	0.966**	0.943**	0.916**	0.683**	0.357**	-0.375**	0.433**
9	0.279**	0.382**	0.299**	0.394**	0.426**	0.498**	0.284**	0.306**	1	1.105**	1.155**	0.574**	0.360*	0.390**	-0.397**	0.308**
10	0.271**	0.339**	0.268**	0.517**	0.437**	0.363**	0.254**	0.304**	0.372**	1	0.938**	1.273**	0.837**	0.612**	-0.502**	0.633**
11	0.362**	0.433**	0.383**	0.389**	0.376**	0.399**	0.242*	0.363**	0.389**	0.479**	1	0.313**	0.297**	0.461**	-0.894**	0.204**
12	-0.108	-0.011	-0.124	0.175*	0.183*	0.233*	0.244**	0.131	0.116	-0.045	0.122	1	1.441**	0.976**	0.741**	1.045**
13	-0.001	0.047	0.003	0.963**	0.256**	0.238*	0.278**	0.062	0.381**	0.190**	0.046	0.385**	1	0.886**	1.464**	1.101**
14	-0.182**	-0.061	-0.179*	0.136	0.133	0.191*	0.229*	-0.072	0.105	0.139	0.119	0.252**	0.137	1	0.819**	0.944**
15	-0.271**	-0.180	-0.233*	0.009	-0.222*	-0.169	0.087	-0.133	-0.077	0.032	-0.119	0.125	0.006	0.396**	1	0.965**
16	-0.228*	-0.088	-0.215*	0.157	0.118	0.175*	0.319**	-0.016	0.190	0.123	0.058	0.655**	0.561**	0.714**	0.634**	1

* Significant at 5% level
** Significant at 1% level

seed length (1.02), number of inflorescence per tree (0.957), seed breadth (0.926), flower breadth (0.919) and seed volume (0.991).

The pod traits studied viz., number of pods per tree, pod length and breadth recorded significant genotypic correlation with all the reproductive biology traits. Number of pods per tree exhibited high positive correlation at genotypic and phenotypic level with number of inflorescence per tree (1.041, 0.828) and number of flowers per inflorescence (1.07, 0.710). Pod length (-0.582) and pod breadth (-0.375) recorded correlations in opposite directions with days for pod to mature.

Among the seed traits, number of seeds per pod indicated a high positive genotypic correlation with number of flowers per inflorescence (1.01), flower length (1.061), pod breadth (1.082), seed length (1.115), seed breadth (1.013) and seed volume (1.03). Seed length, seed breadth and seed volume had positive genotypic correlation with most of reproductive biology traits except with days for pod to mature.

Floral traits like flower length and flower breadth exhibited a high and positive association with pod length (1.043, 1.051), seed length (1.022, 1.019), and seed volume (1.015, 1.016). Flower breadth (1.021) exhibited high positive correlation with pod breadth.

Duration of inflorescence development (1.045), duration of blooming (1.101), days for flower to pod (0.944) and days for pod to mature (0.965) were found to be positively and significantly associated with the total reproductive period. At genotypic level, the total reproductive period exhibited a high and significant correlation with pod length (0.552) and seed breadth (0.633).

4.2.11 Influence of weather parameters on reproductive biology traits

The reproductive biology traits were studied in relation to weather parameters from June 2007 to May 2008. The variability among the characters

and their correlation with weather parameters like maximum temperature, minimum temperature, sunshine hours, relative humidity and rainfall were analysed.

4.2.11.1 Mean performance of *S. asoca* in various months

Mean performance of *S. asoca* for different reproductive biology traits over months from June 2007 to May 2008 is presented in Table 34. Among the different months of the year, maximum number of inflorescence per tree was 90 in February while a minimum of 2 inflorescence per tree in September. Number of flowers per inflorescence also followed a similar trend producing more flowers in February and March while the least flowers in the month of September and October. Maximum number of pods per tree were observed in May followed by June. Seeds per pod recorded maximum number in March and April.

Among flower traits, flower length varied from 5.67 cm in September, October to 5.86 in March. A range of flower breadth from 2.86 cm in September, October to 3.01 cm in March was identified in asoka. In the pod traits, maximum pod length of 12.2 cm was observed in March while maximum pod breadth of 4.13 cm was noted in January and March. Seed traits like seed length was recorded maximum in April while minimum in September and October. Seed breadth and seed volume was found to be high in the months from February to May.

Within the reproductive period traits, maximum number of days was taken for bud to develop to a inflorescence in the months of June and December. In *S. asoca*, more duration was observed for blooming in December, January and April. During the month of September, October, flowers developed to pod within the minimum of 31.37 days. Pods matured in a least period of 66.37 days in March. The total reproductive period was shortest (132.62 days) in September-October while longest (135.62 days) in the month of December.

Table 34. Mean performance of *S. asoca* for different reproductive biology traits over months during 2007 June to 2008 May

	June 2007	July	August	September	October	November	December	January 2008	February	March	April	May	
1	15.5	16.5	17	2	2.87	7.5	9.75	31.5	90	54.75	36	10.5	CD=25.80(5%) 34.31(1%)
2	20.37	20.62	21.25	17.37	17.75	19.62	19.37	24.75	38.87	37.25	31.75	24.12	CD=7.155(5%) 9.51 (1%)
3	112.75	17.5	18.62	3.75	4.75	8.75	11.87	18.75	17.5	59.75	71.12	143.87	CD=59.32(5%) 78.89(1%)
4	2.75	2.75	2.62	2.37	2.37	2.5	2.75	2.87	3.12	3.87	3.62	3.12	CD=0.51 (5%) 0.67(1%)
5	5.75	5.7	5.737	5.675	5.675	5.712	5.762	5.8	5.762	5.862	5.825	5.787	CD=0.050(5%) 0.66(1%)
6	2.887	2.9	2.937	2.862	2.862	2.875	2.937	2.975	2.975	3.012	2.962	2.975	CD=0.049(5%) 0.065(1%)
7	12.025	12.012	12.062	11.85	11.987	12.075	12.15	12.112	12.137	12.2	12.112	12.137	CD=0.147(5%) 0.195(1%)
8	4.075	4.087	4.025	4	4.012	4.012	4.062	4.137	4.125	4.137	4.125	4.125	CD=0.075(5%) 0.100(1%)
9	3.412	3.412	3.412	3.35	3.35	3.387	3.4	3.437	3.487	3.487	3.512	3.487	CD=0.074(5%) 0.098(1%)
10	0.287	2.275	2.262	2.212	2.212	2.275	2.275	2.25	2.3	2.337	2.3	2.3	CD=0.058(5%) 0.077(1%)
11	9.187	9	9.187	9.062	9.062	9.25	9.312	9.312	9.625	9.625	9.625	9.625	CD=0.333(5%) 0.443(1%)
12	23	22.875	22.375	22.25	22.25	22.75	23	22.875	22.5	22.875	22.75	22.625	CD=0.576(5%) 0.767(1%)
13	12.875	12.875	12.875	12.25	12.25	12.875	13	13	12.5	12.875	13	12.5	CD=0.584(5%) 0.777(1%)
14	32.25	31.75	31.75	31.375	31.375	32.625	32.5	32	31.625	31.75	32.25	32.625	CD=0.544(5%) 0.723(1%)
15	66.875	67.375	67	66.75	66.75	67.125	67.125	66.875	66.5	66.375	67.125	67	CD=0.709(5%) 0.943 (1%)
16	135	134.875	134	132.625	132.625	135.375	135.625	134.75	133.125	133.875	135.125	134.75	CD=1.414(5%) 1.881 (1%)

Contd.

Table 34 . Continued

1. Number of inflorescence/tree
2. Number of flowers/inflorescence
3. Number of pods/tree
4. Number of seeds/pod
5. Flower length (cm)
6. Flower breadth (cm)
7. Pod length (cm)
8. Pod breadth (cm)
9. Seed length (cm)
10. Seed breadth (cm)
11. Seed volume (ml)
12. Duration of inflorescence development (days)
13. Duration of blooming (days)
14. Days for flower to pod
15. Days for pod to mature
16. Total reproductive period (days)

4.2.11.2 Variability of reproductive biology traits over months

In the present study, the extent of variability of various reproductive biology traits with respect to the various months from 2007 June to 2008 May was estimated. Mean observations of these traits on 8 trees were taken as replication and mean trait values over 12 months of a year were taken as treatments in this analysis of variance. Abstract of analysis of variance of the characters is given in Table 35. Results of analysis of variance revealed significant differences among the 12 months for all the characters studied except flower breadth, duration of inflorescence development, duration of blooming and days for pod to mature. The eight accessions or trees also exhibited a significant variation for all the characters studied.

Phenotypic and genotypic coefficient of variation, heritability estimated for the various reproductive biology traits are presented in Table 36. Most of the reproductive biology traits like number of pods per tree, number of inflorescence per tree, number of flowers per inflorescence had the highest PCV and GCV. Number of seeds per pod exhibited high PCV while GCV was moderate. Among the reproductive biology traits, the flower traits, pod traits, seed traits and reproductive period traits indicated low PCV and GCV.

Heritability estimates were moderate for the traits namely, number of inflorescence per tree, number of flowers per inflorescence, number of pods per tree, number of seeds per pod, flower length, flower breadth and days for flower to pod. The reproductive period traits except the days for flower to pod exhibited low heritability. The values of heritability were low for the pod and seed traits.

4.2.11.3 Correlation of weather parameters with reproductive biology traits

The reproductive biology traits were studied in relation to weather parameters namely, rainfall, relative humidity, maximum temperature, minimum temperature and sunshine hours. Abstract of monthly weather data from June 2007 to May 2008 is given in Table 37.

Table 35. ANOVA for reproductive biology traits in *S. asoca*

Source of variation	Degrees of freedom	Mean sum of squares							
		No. of inflore-scence/tree	No. of flowers/ inflore-scence	No. of pods/ tree	No. of seeds/pod	Flower length	Flower breadth	Pod length	Pod breadth
Replication	7	2140.03**	568.55**	8459.85*	9.06**	3.27**	8.94**	22.72**	1.788**
Treatment	11	5283.10**	444.26**	17221.68**	1.76**	0.027**	0.021	0.07**	0.022**
Error	77	665.65	51.20	3519.14	0.26	0.002	0.002	0.021	0.005

Source of variation	Degrees of freedom	Mean sum of squares							
		Seed length	Seed breadth	Seed volume	Duration of inflorescence development	Duration of blooming	Days for flower to pod	Days for pod to mature	Total reproductive period
Replication	7	9.53**	0.686**	24.96**	40.67**	23.29**	19.438**	100.91**	113.70**
Treatment	11	0.024**	0.010**	0.470**	0.601	0.646	1.646**	0.639	8.784**
Error	77	0.005	0.003	0.111	0.332	0.341	0.296	0.502	2.00

* Significant at 5% level
** Significant at 1% level

Table 36. Estimation of genetic parameters for reproductive biology traits in *S. asoca*

Sl. No.	Characters	PCV (%)	GCV (%)	Heritability (%)
1.	Number of inflorescence/tree	143.95	98.10	46.44
2	Number of flowers/inflorescence	41.00	28.69	48.96
3	Number of pods per tree	177.50	101.56	32.73
4	Number of seeds/pod	23.13	14.99	42.01
5	Flower length	1.30	0.97	55.17
6	Flower breadth	2.35	1.65	48.98
7	Pod length	1.38	0.65	21.84
8	Pod breadth	2.17	1.14	27.46
9	Seed length	2.58	1.41	29.61
10	Seed breadth	2.88	1.31	20.87
11	Seed volume	4.24	2.27	28.74
12	Duration of inflorescence development	2.67	0.81	9.15
13	Duration of blooming	4.83	1.53	10.05
14	Days for flower to pod	2.13	1.28	36.31
15	Days for pod to mature	1.08	0.19	3.27
16	Total reproductive period	1.26	0.69	29.97

Table 37. Monthly weather data of College of Horticulture, Vellanikkara during June 2007 – May 2008

Month	Rainfall (mm)	Relative humidity (%)	Maximum temperature (°C)	Minimum temperature (°C)	Sunshine (hours)
June 2007	826.5	84	30.1	23.5	105.5
July	1131.9	88	28.4	22.9	22.1
August	549.7	84	29.0	22.8	100.5
September	765.9	86	29.4	22.9	75.1
October	383.8	79	30.5	22.5	135.2
November	24.8	67	31.7	21.6	239.2
December	8.7	56	31.6	22.7	207.1
January 2008	0.0	59	32.3	21.7	292.9
February	29.7	61	33.6	22.9	236.9
March	205.3	64	33.2	23.4	212.9
April	65.6	75	33.2	24.9	189.9
May	11.5	73	33.0	24.7	188.6

Source: Department of Agricultural Meteorology, COH, Vellanikkara

The monthly weather parameters and the reproductive biology traits over months were subjected to correlation studies and the results are presented in Table 38. The results revealed that maximum temperature had a positive significant correlation with most of the reproductive biology traits except the reproductive period traits. Minimum temperature exhibited a positive significant association with number of seeds per pod (0.596), seed length (0.623) and seed volume (0.542). Number of pods per tree (0.502), flower length (0.586), flower breadth (0.586), pod length (0.689) and seed volume (0.650) showed a positive association with sunshine hours.

Rainfall and relative humidity recorded a negative significant influence on the flower length (-0.545, -0.548), flower breadth (-0.556, -0.586), pod length (-0.690, -0.740) and seed volume (-0.720), -0.585). Relative humidity also exhibited a negative significant correlation with number of pods per tree (-0.506).

The reproductive period traits such as duration of inflorescence development, duration of blooming, days for flower to pod, days for pod to mature and the total reproductive period did not exhibit a significant correlation with the weather parameters.

4.3 Collection and evaluation of seed and seedling traits

Asoka seeds collected from 80 trees in different districts of Kerala were evaluated for seed traits. Seedlings from these collected seed accessions were assessed for various seedling traits. Seedling study was conducted for one year from May 2008 to April 2009. The results of investigation of seed traits and seedling traits of the collected eighty accessions are presented below.

4.3.1 Mean performance

The mean performance of eighty asoka seedlings for various seed and seedling traits from May 2008 to April 2009 is presented in Tables 39 and 40.

Table 38. Correlation of weather parameters with reproductive biology traits

Weather parameters	No. of inflore-scence/ tree	No. of flowers inflore-scence	No. of pods/ tree	No. of seeds/ pod	Flower length	Flower breadth	Pod length	Pod breadth	Seed length	Seed breadth	Seed volume	Duration of inflore-scence develop-ment	Duration of blooming	Days for flower to pod	Days for pod to mature	Repro-ductive period
Rainfall	-0.339	-0.432	-0.398	-0.385	-0.545*	-0.556*	-0.690*	-0.372	-0.461	-0.308	-0.720**	-0.102	-0.147	-0.461	0.226	-0.205
Relative humidity	-0.468	-0.463	-0.506*	-0.367	-0.548*	-0.586*	-0.740**	-0.457	-0.371	-0.363	-0.585*	-0.382	-0.309	-0.363	0.289	-0.263
Maximum temperature	0.612*	0.740**	0.685**	0.701**	0.740**	0.712**	0.743**	0.673**	0.737**	0.586*	0.916**	0.225	0.113	0.364	-0.419	0.134
Minimum temperature	0.119	0.341	0.162	0.596*	0.442	0.362	0.195	0.436	0.623*	0.480	0.542*	0.049	-0.038	0.206	0.027	0.099
Sunshine hours	0.443	0.479	0.502*	0.384	0.586*	0.586*	0.689**	0.466	0.440	0.326	0.650*	0.270	0.269	0.408	-0.315	0.235

* Significant at 5% level
** Significant at 1% level

Table 39. Mean performance of *S. asoca* accessions collected for seed traits

Seedling accessions	Seed length (cm)	Seed breadth (cm)	Seed volume (ml)	No. of days for germination	Germination %
KMK 1	4.4	2.2	10	18	80.0
KMK 2	4.4	2.4	10	16	81.8
KMK 3	4.4	2.3	10	20	80.0
KMK 4	4.4	2.2	10	22	80.0
OKL 1	4.2	3.0	12	30	84.6
OKL 2	4.4	3.1	13	25	85.7
OKL 3	4.2	3.0	12	31	84.6
OKL 4	4.3	3.2	13	30	85.7
KKL 1	4.1	3.0	12	25	81.8
KKL 2	4.3	3.0	13	21	83.3
KKL 3	4.0	3.0	12	23	81.8
KKL 4	4.0	3.0	12	27	80.0
CKD 1	2.6	1.4	4	31	33.3
CKD 2	2.5	2.0	5	30	42.8
TTR 1	4.3	2.5	10	28	80.0
TTR 2	4.3	2.4	10	32	78.5
TTR 3	4.2	2.6	10	25	81.8
TTR 4	4.2	2.5	10	30	78.5
VTR 1	2.8	2.3	6	26	62.5
VTR 2	2.7	2.2	5	31	57.1
VTR 3	2.8	2.2	6	25	66.6
VTR 4	2.8	2.3	6	26	66.6
VTR 5	2.8	2.3	6	30	62.5
VTR 6	2.8	2.1	5	32	57.1
VKA 1	3	2.4	7	30	77.7
VKA 2	3.7	2.3	8	30	77.7
VKA 3	4.5	2.5	12	28	78.5
VKA 4	3.8	2.3	8	32	76.9

Contd.

Table 39 Continued

Seedling accessions	Seed length (cm)	Seed breadth (cm)	Seed volume (ml)	No. of days for germination	Germination %
VKA 5	4.3	2.5	10	28	78.5
VKA 6	4.4	2.4	10	25	80.0
VKA 7	4.5	2.7	12	2	80.0
VKA 8	4.5	2.6	12	26	80.0
KDR 1	4.2	2.5	10	29	76.9
KDR 2	4.2	2.6	10	20	80.0
KDR 3	4.1	2.6	10	24	78.5
KRT 1	3.5	2.7	9	20	66.6
KRT 2	3.5	2.6	9	24	62.5
PBR 1	3.9	2.4	9	35	75.0
PBR 2	3.9	2.5	9	24	81.8
PTB 1	3.6	2.6	9	40	70.0
PTB 2	3.6	2.5	9	45	62.5
CTA 1	4.4	2.4	10	28	75.0
CTA 2	4.3	2.5	10	25	80.0
CTA 3	4.3	2.4	10	32	72.7
MNT 1	4.2	2.6	10	35	76.9
MNT 2	4.3	2.5	10	36	72.7
MNT 3	4.3	2.4	10	38	70.0
ALV 1	4.0	2.0	8	31	57.1
ALV 2	3.9	2.2	8	26	66.6
ALV 3	3.9	2.1	8	28	62.5
CBG 1	3.7	2.2	8	31	70.0
CBG 2	3.7	2.3	8	25	80.0
CBG 3	3.9	2.1	8	29	72.7
CBG 4	3.8	2.2	8	27	75.0
TVM 1	4.1	3.0	12	32	77.7
TVM 2	4.3	3.0	13	29	78.5
TVM 3	4.0	3.0	12	34	75.0

Contd.

Table 39 Continued

Seedling accessions	Seed length (cm)	Seed breadth (cm)	Seed volume (ml)	No. of days for germination	Germination %
KRA 1	4.4	2.4	10	30	78.5
KRA 2	4.3	2.4	10	34	72.7
PMA 1	3.7	2.6	9	31	62.5
PMA 2	3.8	2.6	9	28	66.6
PMA 3	3.7	2.5	9	34	57.1
TTY 1	4.2	2.5	10	35	62.5
TTY 2	4.3	2.4	10	33	66.6
TTY 3	4.3	2.5	10	30	75.0
KPM 1	4.4	2.5	12	31	75.0
KPM 2	4.4	2.6	12	28	78.5
KPM 3	4.5	2.5	11	33	70.0
KLA 1	4.2	2.4	10	32	75.0
KLA 2	4.2	2.5	10	27	77.7
CKM 1	4.2	2.5	10	32	72.7
CKM 2	4.1	2.6	10	27	78.5
CKM 3	4.2	2.4	10	34	66.6
CKM 4	4.2	2.6	10	29	76.9
VCY 1	3.8	2.4	9	30	62.5
VCY 2	3.9	2.5	9	27	66.6
VCY 3	3.9	2.4	9	33	57.1
KPA 1	3.8	2.1	8	35	50.0
KPA 2	3.8	2.3	8	29	62.5
KPA 3	3.7	2.3	8	32	54.5
Mean	3.95	2.47	9.51	28.7	72.09
SE	±0.50	±0.29	±1.98	±5.69	±10.05

Table 40. Mean performance of *S. asoca* accessions collected for seedling traits

Seedling accessions	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflets /leaf	Internodal length (cm)	Stem girth (cm)	Seedling vigour
KMK 1	25.5	3	8.5	3.0	4	2.5	0.87	1240.0
KMK 2	29.3	3	10.1	3.6	6	2.9	0.85	1905.9
KMK 3	25.6	3	9.6	3.4	4	2.9	0.87	1392.0
KMK 4	26.2	3	9.4	3.4	4	2.6	0.86	1396.0
OKL 1	34.4	3	8.8	5.3	4	2.7	0.87	2334.9
OKL 2	37.4	4	8.3	5.2	4	2.8	0.91	2790.5
OKL 3	31.4	3	7.2	4.9	4	2.6	0.84	2157.3
OKL 4	37.6	4	10	5.6	4	2.6	0.93	2690.9
KKL 1	34.5	4	10.2	2.5	4	2.7	0.87	2462.2
KKL 2	36.3	3	11.5	2.9	6	2.9	0.91	2511.01
KKL 3	33.4	5	10.9	2.7	4	2.5	0.84	2388.5
KKL 4	30.3	4	9.8	2.2	4	2.4	0.85	2040.0
CKD 1	13.7	3	4.2	0.98	4	1.8	0.54	273.6
CKD 2	16.3	3	5.1	1.3	4	1.7	0.56	466.5
TTR 1	31.7	2	10.1	5.3	4	2.5	0.85	1880.0
TTR 2	28.3	2	8.2	5.5	4	2.4	0.87	1585.7
TTR 3	33.1	1	11	5.9	6	2.7	0.83	2085.9
TTR 4	27.6	2	9.3	4.9	4	2.4	0.86	1601.4
VTR 1	20.8	3	7.8	2.2	4	1.8	0.62	893.7
VTR 2	16.4	3	6.7	2.0	4	1.8	0.57	650.9
VTR 3	22.1	3	9.3	2.4	4	2.4	0.7	1105.5
VTR 4	21.7	4	8.2	2.7	4	2.3	0.68	1058.9
VTR 5	18.5	2	6.4	1.9	4	1.8	0.62	818.7
VTR 6	17.4	2	7.3	2.1	4	1.9	0.61	713.7
VKA 1	25.7	4	7.2	0.98	4	1.9	0.69	1219.8
VKA 2	22.1	3	6.4	1	4	1.8	0.67	1289.8
VKA 3	25.3	4	7.7	1	4	1.9	0.71	1444.4
VKA 4	23.3	3	5.9	1.3	4	1.9	0.62	1361.1

Contd.

Table 40 Continued

Seedling accessions	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflets /leaf	Internodal length (cm)	Stem girth (cm)	Seedling vigour
VKA 5	26.2	5	8.4	1.3	4	1.9	0.7	1570.0
VKA 6	25.3	3	8.8	1.3	6	2.0	0.8	1368.0
VKA 7	27.9	3	9.4	1.3	6	2.0	0.8	1664.0
VKA 8	28.3	3	9.5	1.4	6	2.1	0.86	1616.0
KDR 1	25.5	2	8.8	4.5	4	1.8	0.79	1330.3
KDR 2	29.5	3	10.0	5.2	4	2.3	0.82	1880.0
KDR 3	27.6	3	8.3	4.1	4	2.2	0.8	1609.2
KRT 1	23.7	2	7.7	3.8	4	1.8	0.71	1205.4
KRT 2	21.9	3	9.2	4.4	4	2.1	0.67	1006.3
PBR 1	26	2	7.9	2.3	4	2.3	0.78	1485.0
PBR 2	27.9	2	9.2	2.8	4	2.5	0.8	1709.6
PTB 1	25.6	3	9.5	4.7	4	2.0	0.72	1092.0
PTB 2	21.9	3	7.1	3.8	4	1.8	0.68	1006.3
CTA 1	27.8	3	10.1	5.8	4	2.4	0.82	1567.5
CTA 2	29.4	3	10.9	6.5	4	2.7	0.86	1872.0
CTA 3	25.3	2	8.2	5.1	4	2.4	0.8	1243.1
MNT 1	28.2	4	9.4	2.2	6	2.1	0.8	1545.6
MNT 2	26.2	3	7.4	1.5	6	1.8	0.75	1454.0
MNT 3	25.5	5	8.1	1.7	4	2.0	0.72	1085.0
ALV 1	19.6	3	8.3	1.9	4	2.4	0.61	759.4
ALV 2	23.2	3	8.9	2.1	6	2.6	0.7	1172.1
ALV 3	20.7	3	7.8	1.7	4	2.5	0.64	906.3
CBG 1	10.2	1	4.2	0.98	4	1.6	0.42	665.0
CBG 2	15.2	2	5.8	1.4	4	1.8	0.54	1168
CBG 3	12	1	4.9	1.2	4	1.8	0.47	828.8
CBG 4	13.4	2	4.5	0.99	4	1.9	0.49	960.0
TVM 1	34.6	3	10	5.0	4	2.3	0.87	2346.5
TVM 2	36.4	3	9.5	4.4	6	2.6	0.88	2307.9
TVM 3	33.4	3	8.1	3.8	4	2.4	0.85	2190.0

Contd.

Table 40 Continued

Seedling accessions	Height of plant (cm)	No. of leaves	Leaflet length (cm)	Leaflet breadth (cm)	No. of leaflets /leaf	Internodal length (cm)	Stem girth (cm)	Seedling vigour
KRA 1	33.6	4	9	3.3	4	2.5	0.85	2307.9
KRA 2	29.3	3	7.4	2.0	4	2.4	0.86	1693.9
PMA 1	27.8	3	7.2	3.0	4	2.4	0.80	1306.3
PMA 2	31.8	3	9.2	3.6	4	2.5	0.82	1571.7
PMA 3	23.7	3	7.7	3.3	4	2.2	0.78	1033.5
TTY 1	34.2	3	7.1	2.4	4	2.2	0.86	1862.5
TTY 2	32.7	3	6.4	1.9	4	1.8	0.86	1631.7
TTY 3	36.2	4	8.6	2.1	4	2.4	0.85	2190.0
KPM 1	33.5	3	8.3	3.2	4	2.5	0.87	2197.5
KPM 2	36.1	3	9.4	3.4	6	2.7	0.87	2300.1
KPM 3	31.7	3	7.2	3.1	4	2.4	0.87	1645.0
KLA 1	25.4	3	7.4	3.0	4	1.8	0.75	1290.0
KLA 2	31.6	4	8.5	3.3	4	2.1	0.78	1996.8
CKM 1	29.5	3	8.1	3.2	4	2.4	0.82	1708.5
CKM 2	35.2	3	9.5	4.0	4	2.6	0.85	2205.8
CKM 3	28.4	2	7.5	3.4	4	2.4	0.80	1351.9
CKM 4	33.4	3	8.8	4.2	4	2.7	0.86	2230.1
VCY 1	32.5	3	9.4	4.0	4	2.2	0.80	1518.7
VCY 2	35.4	3	10	4.6	6	2.5	0.82	1818.2
VCY 3	29.2	3	8.1	3.3	4	2.1	0.78	1324.7
KPA 1	25.5	3	7.2	3.9	4	2.5	0.70	865.0
KPA 2	29.3	3	8.4	5.4	4	2.7	0.75	1462.5
KPA 3	27.9	3	7.8	4.9	4	2.4	0.72	1079.1
Mean	27.23	2.97	8.29	3.14	4.3	2.27	0.76	1537.04
SE	±6.30	±0.77	±1.54	±1.46	±0.71	±0.33	±0.11	±548.6

Among the seeds collected, maximum seed size was identified for OKL2 and OKL4 collected from Odakkali. Big sized seeds were also produced by OKL1, OKL3, KKL1, KKL2, KKL3, KKL4 (Kottakkal) and TVM1, TVM2, TVM3 (Thiruvananthapuram). Seed length varied from 2.5 cm to 4.5 cm. Lowest seed breadth (1.4 cm) was observed for the accession CKD1 while OKL4 indicated maximum seed breadth. The accessions OKL2, OKL4, KKL2 and TVM2 recorded a maximum seed volume of 13 ml by water displacement method. Seed germination was completed within a minimum of 16 days for KMK2 (Kumarakom), whereas maximum of 45 days was pointed out for the accession PTB2 from Pattambi. Big seed sized accessions OKL2 and OKL4 took 25 and 30 days for germination respectively. Seeds collected from Odakkali accessions OKL1 (84.6%), OKL2 (85.7%), OKL3 (84.6%) and OKL4 (85.7%) showed maximum germination percentage.

Height of seedling when assessed in different accessions, maximum mean height was recorded for OKL4 (37.6 cm), OKL2 (37.4 cm), TVM2 (36.4 cm) and KKL2 (36.3 cm), while CBG1 (10.2 cm) from Kozhikode had the minimum seedling height. KKL3, VKA5 (Vellanikkara) and MNT3 (Mannuthy) had the highest number of leaves (5) and TTR3 (Tripunithura), CBG1, CBG3 showed the minimum number (1). The accessions KMK2, KKL2, TTR3, VKA6, VKA7, VKA8, MNT1, MNT2, ALV2, TVM2, KPM2 and VCY2 reported a maximum of 6 leaflets per leaf. Among the accessions, KKL2 had the longest leaflets followed by TTR3. Maximum width for the leaflets were observed in the accessions CTA2 (Chettupuzha) and TTR3. Internodes were long (2.9 cm) in the case of the accessions KKL2, KMK2 and KMK3. The accession CKD2 had the shortest internode (1.7 cm). Stem girth observed indicated that the accession OKL4 followed by OKL2, KKL2 and TVM2 had the maximum value. The minimum stem girth was reported for CBG1, CBG3 and CBG4. Most vigorous seedlings assessed from their seedling vigour based on germination percentage and seedling height were OKL2 (2790.5) and OKL4 (2690.9). Among the other accessions

KKL1, KKL2, KKL3, TVM1, TVM2, OKL1 and KRA1 (Kottarakara) also indicated high seedling vigour.

4.3.2 Genetic variability

The genetic variability was estimated with respect to different seedling traits in eighty accessions of *S. asoca.* Abstract of analysis of variance are presented in Table 41. Results from analysis of variance revealed highly significant difference among the eighty accessions for the seedling characters studied. The characters included height of seedling, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length and stem girth.

Variability parameters such as phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) computed for seedling characters are presented in Table 42. Among the characters of seedling accessions, high magnitude of PCV and GCV were observed for leaflet breadth (45.18, 45.00) number of leaves (28.83, 24.95) and height of seedling (22.79, 22.02). Number of leaflets per leaf exhibited a higher PCV (22.51) compared to moderate GCV (19.28). The level of PCV and GCV were moderate for leaflet length, stem girth and internodal length.

4.3.3 Correlation

The correlation coefficients among seed traits and with seedling vigour are presented in Table 43. Traits that indicted maximum association with seedling vigour were seed volume (0.826) and seed breadth (0.820). Other traits germination percentage and seed length also exhibited significant positive correlation with seedling vigour, whereas number of days for germination was negatively correlated with seedling vigour. Association among the seedling vigour component traits showed that seed length, seed breadth, seed volume and germination percentage were intercorrelated. Number of days for germination did

Table 41. ANOVA for seedling traits studied in asoka seedlings from May 2008 to April 2009

Source of variation	Degrees of freedom	Mean sum of squares						
		Height of seedling	Number of leaves	Leaflet length	Leaflet breadth	Number of leaflets	Internodal length	Stem girth
Replication	5	2430.12**	33.88**	23.12**	15.37**	49.85**	12.99**	1.09**
Treatment	79	2743.51**	3.80**	14.25**	13.25**	3.49**	0.65**	1.48**
Error	395	2.85	0.20	0.01	0.01	0.09	0.01	0.01

** Significant at 1% level

Table 42. Estimation of variability parameters for seedling traits studied from May 2008 to April 2009

Sl. No.	Characters	PCV (%)	GCV (%)
1.	Height of seedling	22.79	22.02
2.	Number of leaves	28.83	24.95
3.	Leaflet length	18.30	18.25
4.	Leaflet breadth	45.18	45.00
5.	Number of leaflets/leaf	22.51	19.28
6.	Internodal length	14.11	13.78
7.	Stem girth	15.76	14.57

Table 43. Correlation among seed traits and with seedling vigour

	Seed length	Seed breadth	Seed volume	No. of days for germination	Germination %	Seedling vigour
Seed length	1	0.456**	0.855**	-0.107	0.673**	0.636**
Seed breadth	0.456**	1	0.808**	-0.091	0.648**	0.820**
Seed volume	0.855**	0.808**	1	-0.109	0.730**	0.826**
No. of days for germination	-0.107	-0.091	-0.109	1	-0.378**	-0.219
Germination %	0.673**	0.648**	0.730**	-0.378**	1	0.716**
Seedling vigour	0.636**	0.820**	0.826**	-0.219	0.716**	1

** Significant at 1% level

not indicate association with these traits except for negative correlation with germination percentage.

Association among seedling traits and with seedling vigour are presented in Table 44. The seedling traits height of seedling (0.902) and stem girth (0.811) had maximum correlation with seedling vigour. Other characters such as leaflet length, internodal length, leaflet breadth, number of leaves and number of leaflets per leaf also reported significant positive association with seedling vigour. Among the seedling vigour component traits, height of seedling and leaflet length presented significant correlation with all the traits. Stem girth was associated with most of the seedling traits except number of leaflets per leaf. Internodal length showed correlation with all characters except number of leaves and number of leaflets per leaf.

Among seed and seedling traits, the characters which are highly associated with seedling vigour are seed breadth, seed volume, height of seedling and stem girth. The ranking of eighty accessions based on these traits identified OKL4, OKL2, KKL2, TVM2 from Odakkali, Kottakkal and Thiruvananthapuram as better accessions.

4.4 Evaluation of therapeutical components of *S. asoca*

The assessment of biochemical contents such as tannin and phenol were undertaken in different age groups of *S. asoca* which included KAU campus trees (9-31 years), germplasm accessions (3-4 years) and the seedlings (1 year) collected.

4.4.1 Tannin content

Tannin content was estimated in bark, leaves and flowers of KAU campus trees, bark and leaves of germplasm accessions and in leaves of seedlings.

In the *S. asoca* trees, tannin content in bark, flowers and leaves were estimated in March 2009. The results are presented in Table 45. The tannin

Table 44. Correlation among seedling traits and with seedling vigour

	Height of seedling	No. of leaves	Leaflet length	Leaflet breadth	No. of leaflets/leaf	Internodal length	Stem girth	Seedling vigour
Height of seedling	1	0.377**	0.700**	0.534*	0.225*	0.667**	0.912**	0.902**
No. of leaves	0.377**	1	0.286*	-0.126	-0.032	0.141	0.289**	0.308**
Leaflet length	0.700**	0.286*	1	0.562**	0.352**	0.671**	0.736**	0.652**
Leaflet breadth	0.534**	-0.126	0.562**	1	-0.076	0.576**	0.560**	0.445**
No. of leaflets/leaf	0.225*	-0.032	0.352**	-0.076	1	0.172	0.210	0.231*
Internodal length	0.667**	0.141	0.671**	0.576**	0.172	1	0.700**	0.644**
Stem girth	0.912**	0.289**	0.736**	0.560**	0.210	0.700**	1	0.811**
Seedling vigour	0.902**	0.308**	0.652**	0.445**	0.231*	0.644**	0.811**	1

* Significant at 5% level
** Significant at 1% level

Table 45. Tannin content estimated in *S. asoca* trees

Sl.No.	Accession No. of trees	Tannin content (mg/g)		
		Bark	Flowers	Leaves
1	KAU 1	54.72	26.39	13.61
2	KAU 2	51.67	24.45	11.67
3	KAU 3	54.17	26.94	14.17
4	KAU 4	47.78	21.66	10.83
5	KAU 5	56.67	28.06	15.27
6	KAU 6	60.00	30.55	17.78
7	KAU 7	60.83	30.83	18.61
8	KAU 8	62.50	32.50	20.00
Mean		56.04	27.67	15.24

content in bark ranged from 47.78 mg/g to 62.50 mg/g. Bark of KAU 4 had the lowest tannin content. KAU 8 expressed the highest tannin content of 62.50 mg/g followed by KAU 7 (60.83 mg/g) and KAU 6 (60 mg/g). In flowers, tannin content recorded the lowest value of 21.66 mg/g for KAU 4. Maximum flower tannin content was also identified for the tree KAU 8 (32.50 mg/g). The accessions KAU 6 and KAU 7 also exhibited high tannin content in flowers. The tree studied showed a range of 10.83 mg/g (KAU 4) to 20 mg/g (KAU 8) for tannin content in leaves. High tannin content was identified for trees KAU 8, KAU 7 and KAU 6. Generally, *S. asoca* leaves exhibited low tannin content than bark and flowers. Mean tannin content in bark, flower and leaves in tree accessions was found to be 56.04 mg/g, 27.67 mg/g and 15.24 mg/g respectively.

Tannin content was worked out in bark and leaves of *S. asoca* germplasm accessions and values are given in Table 46. The estimation of tannin content in bark was carried out twice, in March 2008 and March 2009. The accessions IC 566474, IC 566467 and IC 566476 exhibited high tannin values in both the cases. The bark tannin content of accessions ranged from 14.56 mg/g to 37.69 mg/g in first year analysis and 21.11 mg/g to 43.34 mg/g in second year analysis. Low bark tannin content was observed for the accessions IC 566460, IC 566478 and IC 566488. Generally, *S. asoca* accessions indicated high tannin estimates in bark than in leaves. The tannin content in leaves varied from 2.22 mg/g to 12.77 mg/g. The lowest leaf tannin content was exhibited by IC 566478, IC 566495, IC 566488, IC 566477, IC 566473, IC 566472 and IC 566481. The highest leaf tannin content was recorded for accession IC 566476. The accessions IC 566474, IC 566461 and IC 566467 expressed higher leaf tannin content than rest of the accessions. Mean tannin content in bark and leaves of *S. asoca* germplasm accessions were identified as 32.73 mg/g and 6.33 mg/g.

Table 46. Tannin content in *S. asoca* germplasm accessions

Sl.No.	Accession No.	Tannin content in bark (mg/g)		Tannin content in leaves (mg/g)
		March 2008	March 2009	March 2009
1	IC 566463	29.63	36.11	7.50
2	IC 566465	27.59	34.45	6.95
3	IC 566466	28.52	35.28	7.22
4	IC 566467	36.11	43.33	11.67
5	IC 566468	28.48	34.72	6.38
6	IC 566469	28.34	34.16	6.11
7	IC 566464	24.82	31.11	5.00
8	IC 566456	22.41	29.16	4.44
9	IC 566457	15.37	22.77	2.77
10	IC 566458	25.28	31.39	5.83
11	IC 566459	18.61	25.55	3.05
12	IC 566460	14.56	21.66	2.77
13	IC 566461	34.57	41.39	11.11
14	IC 566462	33.34	40.00	10.55
15	IC 566485	29.07	36.11	7.22
16	IC 566484	34.91	39.44	9.72
17	IC 566479	29.98	35.28	6.66
18	IC 566478	15.18	21.11	2.22
19	IC 566471	15.47	22.22	2.50
20	IC 566470	16.19	23.33	2.77
21	IC 566475	34.08	40.55	10.83
22	IC 566498	31.26	37.50	7.50
23	IC 566497	26.82	32.50	5.28
24	IC 566496	33.53	39.16	9.17
25	IC 566495	15.47	22.22	2.22
26	IC 566494	30.35	36.66	7.22
27	IC 566492	33.61	40.28	10.27
28	IC 566489	29.17	36.94	7.50
29	IC 566488	15.28	21.39	2.22

Sl.No.	Accession No.	Tannin content in bark (mg/g)		Tannin content in leaves (mg/g)
		March 2008	March 2009	March 2009
30	IC 566480	27.60	33.33	5.55
31	IC 566477	16.21	23.61	2.22
32	IC 566474	37.23	43.34	12.50
33	IC 566473	25.47	30.55	2.22
34	IC 566472	16.30	22.22	2.22
35	IC 566483	30.00	37.23	8.33
36	IC 566493	33.98	40.28	10.00
37	IC 566491	29.96	36.39	7.22
38	IC 566490	29.57	35.55	6.11
39	IC 566487	31.78	38.61	8.61
40	IC 566486	16.08	23.33	2.50
41	IC 566482	23.43	31.11	5.28
42	IC 566476	37.69	43.06	12.77
43	IC 566481	16.72	23.06	2.22
Mean		26.27	32.73	6.33

The seedlings collected were evaluated for tannin content in leaves during the month March 2009 and presented in Table 47. The mean tannin content for seedlings was 1.31 mg/g, that ranged, between 1.11 mg/g to 1.94 mg/g. The highest leaf tannin content was exhibited by VKA 6, VKA 7,VKA 8 and KMK 3. Out of 80 accessions collected, thirty eight accessions expressed low tannin content of 1.11 mg/g. The accessions collected from Kumarakam (KMK 1, KMK 2, KMK 3), Odakkali (OKL 1, OKL 3), Vellanikkara (VKA 5, VKA 6, VKA 7, VKA 8), Kodakara (KDR 3), Mannuthy (MNT 3), Aluva (ALV 1, ALV 2), Thiruvananthapuram (TVM 2) and Kuttipuram (KPM 2) proved to have high tannin content in their leaves.

4.4.2 Phenol content

In the bark, leaves and flowers of KAU Campus trees, in bark and leaves of indigenous collections and in leaves of seedlings, the phenol content was worked out.

Phenol content in bark, flowers and leaves of *S. asoca* trees were estimated in March 2009 and the results are presented in Table 48. In bark, phenol content ranged from 30.11 mg/g to 43.54 mg/g. Bark of KAU 4 had the lowest phenol content. The accession KAU 8 expressed the maximum phenol content of 43.54 mg/g followed by KAU 7 (41.68 mg/g) and KAU 6 (41.31 mg/g). In flowers, minimum phenol content was observed for KAU 4 (15.61 mg/g). The highest flower phenol content was identified for the tree KAU 8 (25.33 mg/g). The accessions KAU 6 and KAU 7 also exhibited high phenol content in flowers. In leaves, the tree accessions showed a range of 9.34 mg/g (KAU 4) to 15.82 mg/g (KAU 8) for phenol content. High leaf phenol content was identified for trees KAU 8, KAU 7 and KAU 6. In general, *S. asoca* leaves exhibited low phenol content than bark and flowers. Mean phenol content in bark, flowers and leaves in tree accessions was found to be 36.74 mg/g, 20.0 mg/g and 12.60 mg/g.

Table 47. Tannin content in *S. asoca* seedlings collected

Sl.No.	Accession No. of seedlings	Tannin content in leaves (mg/g)
1	KMK 1	1.67
2	KMK 2	1.67
3	KMK 3	1.94
4	KMK 4	1.39
5	OKL 1	1.67
6	OKL 2	1.39
7	OKL 3	1.67
8	OKL 4	1.39
9	KKL 1	1.11
10	KKL 2	1.11
11	KKL 3	1.11
12	KKL 4	1.11
13	CKD 1	1.11
14	CKD 2	1.11
15	TTR 1	1.39
16	TTR 2	1.11
17	TTR 3	1.39
18	TTR 4	1.11
19	VTR 1	1.11
20	VTR 2	1.11
21	VTR 3	1.11
22	VTR 4	1.39
23	VTR 5	1.39

Sl.No.	Accession No. of seedlings	Tannin content in leaves (mg/g)
24	VTR 6	1.39
25	VKA 1	1.39
26	VKA 2	1.39
27	VKA 3	1.39
28	VKA 4	1.11
29	VKA 5	1.66
30	VKA 6	1.94
31	VKA 7	1.94
32	VKA 8	1.94
33	KDR 1	1.39
34	KDR 2	1.39
35	KDR 3	1.66
36	KRT 1	1.11
37	KRT 2	1.39
38	PBR 1	1.11
39	PBR 2	1.11
40	PTB 1	1.39
41	PTB 2	1.11
42	CTA 1	1.11
43	CTA 2	1.11
44	CTA 3	1.39
45	MNT 1	1.11
46	MNT 2	1.11
47	MNT 3	1.66

Sl.No.	Accession No. of seedlings	Tannin content in leaves (mg/g)
48	ALV 1	1.66
49	ALV 2	1.66
50	ALV 3	1.39
51	CBG 1	1.11
52	CBG 2	1.11
53	CBG 3	1.11
54	CBG 4	1.11
55	TVM 1	1.39
56	TVM 2	1.67
57	TVM 3	1.39
58	KRA 1	1.39
59	KRA 2	1.11
60	PMA 1	1.11
61	PMA 2	1.11
62	PMA 3	1.11
63	TTY 1	1.11
64	TTY 2	1.11
65	TTY 3	1.39
66	KPM 1	1.39
67	KPM 2	1.67
68	KPM 3	1.39
69	KLA 1	1.11
70	KLA 2	1.11
71	CKM 1	1.11

Sl.No.	Accession No. of seedlings	Tannin content in leaves (mg/g)
72	CKM 2	1.39
73	CKM 3	1.11
74	CKM 4	1.39
75	VCY 1	1.11
76	VCY 2	1.39
77	VCY 3	1.11
78	KPA 1	1.11
79	KPA 2	1.11
80	KPA 3	1.11
	Mean	1.31

Table 48. Phenol content in *S. asoca* trees

Sl.No.	Accession No. of trees	Phenol content (mg/g)		
		Bark	Flowers	Leaves
1	KAU 1	34.78	18.00	11.36
2	KAU 2	32.18	17.36	10.51
3	KAU 3	34.14	18.32	11.84
4	KAU 4	30.11	15.61	9.34
5	KAU 5	36.21	20.07	12.64
6	KAU 6	41.31	22.56	14.12
7	KAU 7	41.68	22.77	15.24
8	KAU 8	43.54	25.33	15.82
	Mean	36.74	20.00	12.60

The accessions in germplasm accessions of *S. asoca* were evaluated for phenol content in bark and leaves. The values of phenol content are presented in Table 49. The estimation of phenol content in bark was carried out twice in March 2008 and March 2009. High phenol values were exhibited by the accessions IC566474 (25.54 mg/g), IC566467 (25.22 mg/g), IC566476 (24.43 mg/g) and IC566461 (24.11 mg/g). Bark phenol content of accessions ranged from 8.62 mg/g to 19.22 mg/g in first year analysis and from 15.03mg/g to 25.54mg/g in second year analysis. Low bark phenol content was observed for the accessions IC566470, IC566460, IC566478, IC566495, IC566488, IC566477, IC566486 and IC566471. Generally, the accessions indicated high phenol estimates in bark than in leaves. The phenol content in leaves varied from 1.06 mg/g to 9.56 mg/g. The lowest phenol content was exhibited by IC566472 and IC566477. The accessions IC566476, IC566474, IC566473, IC566461 and IC566467 expressed higher leaf phenol content than rest of the accessions. Mean phenol content in bark and leaves of germplasm accessions was noted as 18.79 mg/g and 5.17 mg/g.

In seedlings, phenol content was estimated in leaves during the month of March 2009 and presented in Table 50. The highest phenol content was exhibited by VKA 8, VKA 6, VKA7 and KMK 3. The mean phenol content for seedlings was 0.90 mg/g, that ranged between 0.53 mg/g to 1.96 mg/g. Among the seedlings, 11 accessions expressed low phenol content of 0.53 mg/g. The accessions collected from Vellanikkara (VKA 8, VKA 6, VKA7), Kumarakom (KMK3, KMK 2, KMK 1), Odakkali (OKL 1, OKL 3) and Thiruvananthapuram (TVM 2) indicated high leaf phenol value.

Table 49. Phenol content in *S. asoca* germplasm acession

Sl.No.	Accession No.	Phenol content in bark (mg/g)		Phenol content in leaves (mg/g)
		March 2008	March 2009	March 2009
1	IC 566463	15.03	20.07	6.69
2	IC 566465	15.47	20.82	5.78
3	IC 566466	14.83	20.34	6.21
4	IC 566467	19.22	25.22	9.03
5	IC 566468	15.35	20.44	5.42
6	IC 566469	15.47	20.49	5.31
7	IC 566464	14.52	20.12	4.08
8	IC 566456	13.48	19.22	3.35
9	IC 566457	10.12	16.73	1.22
10	IC 566458	13.07	19.65	4.67
11	IC 566459	9.65	16.41	2.81
12	IC 566460	9.2	15.13	1.75
13	IC 566461	18.17	24.11	9.13
14	IC 566462	16.83	22.08	8.49
15	IC 566485	13.76	20.49	6.21
16	IC 566484	17.64	22.25	7.27
17	IC 566479	13.52	20.23	5.42
18	IC 566478	9.84	15.66	1.16
19	IC 566471	9.49	15.13	1.22
20	IC 566470	8.62	15.45	1.48
21	IC 566475	14.88	21.45	8.34
22	IC 566498	15.12	21.24	6.26
23	IC 566497	13.95	19.59	4.67
24	IC 566496	16.23	22.41	7.27
25	IC 566495	9.39	15.13	1.48
26	IC 566494	14.28	20.6	6.32
27	IC 566492	17.43	23.26	8.44
28	IC 566489	14.45	20.97	6.32
29	IC 566488	10.1	15.66	1.75

Sl.No.	Accession No.	Phenol content in bark (mg/g)		Phenol content in leaves (mg/g)
		March 2008	March 2009	March 2009
30	IC 566480	14.14	20.65	4.14
31	IC 566477	9.71	15.13	1.06
32	IC 566474	19.22	25.54	9.34
33	IC 566473	12.71	19.43	9.18
34	IC 566472	10.12	15.39	1.06
35	IC 566483	15.12	21.24	6.90
36	IC 566493	18.15	23.57	8.71
37	IC 566491	16.72	21.45	6.26
38	IC 566490	15.24	20.44	5.20
39	IC 566487	16.53	21.24	6.43
40	IC 566486	9.71	15.13	1.69
41	IC 566482	15.42	20.76	4.14
42	IC 566476	18.34	24.43	9.56
43	IC 566481	10.03	15.03	1.33
	Mean	13.59	18.79	5.17

Table 50. Phenol content in *S. asoca* seedlings collected

Sl.No.	Accession No. of seedlings	Phenol content in leaves (mg/g)
1	KMK 1	1.64
2	KMK 2	1.69
3	KMK 3	1.85
4	KMK 4	1.22
5	OKL 1	1.69
6	OKL 2	1.54
7	OKL 3	1.65
8	OKL 4	1.32
9	KKL 1	0.69
10	KKL 2	0.53
11	KKL 3	0.63
12	KKL 4	0.85
13	CKD 1	0.53
14	CKD 2	0.58
15	TTR 1	0.79
16	TTR 2	0.58
17	TTR 3	0.85
18	TTR 4	0.69
19	VTR 1	0.63
20	VTR 2	0.53
21	VTR 3	0.58
22	VTR 4	0.69
23	VTR 5	0.79

Sl.No.	Accession No. of seedlings	Phenol content in leaves (mg/g)
24	VTR 6	0.63
25	VKA 1	0.96
26	VKA 2	0.85
27	VKA 3	0.85
28	VKA 4	0.79
29	VKA 5	1.32
30	VKA 6	1.86
31	VKA 7	1.85
32	VKA 8	1.96
33	KDR 1	1.32
34	KDR 2	1.06
35	KDR 3	1.49
36	KRT 1	0.79
37	KRT 2	0.96
38	PBR 1	0.69
39	PBR 2	0.53
40	PTB 1	0.85
41	PTB 2	0.69
42	CTA 1	0.79
43	CTA 2	0.85
44	CTA 3	0.96
45	MNT 1	0.69
46	MNT 2	0.96
47	MNT 3	1.01

Sl.No.	Accession No. of seedlings	Phenol content in leaves (mg/g)
48	ALV 1	1.12
49	ALV 2	1.01
50	ALV 3	0.85
51	CBG 1	0.58
52	CBG2	0.63
53	CBG 3	0.53
54	CBG 4	0.53
55	TVM 1	1.54
56	TVM 2	1.65
57	TVM 3	1.32
58	KRA 1	0.63
59	KRA 2	0.53
60	PMA 1	0.63
61	PMA 2	0.85
62	PMA 3	0.53
63	TTY 1	0.58
64	TTY 2	0.53
65	TTY 3	0.63
66	KPM 1	0.69
67	KPM 2	0.96
68	KPM 3	0.79
69	KLA 1	0.53
70	KLA 2	0.58
71	CKM 1	0.63

Sl.No.	Accession No. of seedlings	Phenol content in leaves (mg/g)
72	CKM 2	0.69
73	CKM 3	0.58
74	CKM 4	0.79
75	VCY 1	0.63
76	VCY 2	0.79
77	VCY 3	0.69
78	KPA 1	0.53
79	KPA 2	0.58
80	KPA 3	0.63
	Mean	0.90

4.5 Molecular characterization

4.5.1 RAPD Marker analysis

The results of the experiments conducted for the extraction of genomic DNA of ten *S. asoca* accessions and the evaluation under RAPD assay are presented below.

4.5.1.1Genomic DNA isolation

The genomic DNA was isolated from leaflets of the third node leaf of ten *S. asoca* accessions using modified CTAB method. The DNA extracted after RNase treatment was tested on 0.7 per cent agarose gel electrophoresis revealed the presence of good quantity DNA in sufficient quantity. The leaflets of first and second node leaf which were very coppery red coloured did not yield good DNA.

The quantity and quality of isolated DNA are presented in the Table 51. The ratio of optical density (O.D.) at 260 nm/280 nm was between 1.92 and 2.14. The quantity of the DNA extracted varied between 63.7 to 803.9 ng/µl. High recovery was obtained for IC 566495 (803.9 ng/µl of DNA extract) whereas the quality was good for the accessions IC 566461, IC 566463 and IC 566488. The electrophoretic profile showed good clear band. The gel picture showing isolated genomic DNA and DNA after RNase treatment are presented in plate 3.

Table 51. Quantity and quality of genomic DNA isolated from indigenous collections of *S. asoca*

Sl. No.	IC No.	OD 260/ OD 280	Quantity (ng/µl)
1.	IC 566463	1.96	63.7
2.	IC 566461	1.92	80.7
3.	IC 566488	1.98	108.1
4.	IC 566493	2.07	163.7
5.	IC 566456	2.03	169.9
6.	IC 566497	2.06	186.1
7.	IC 566498	2.14	203.8
8.	IC 566496	2.09	247.4
9.	IC 566474	2.06	307.3
10.	IC 566495	2.12	803.9

4.5.1.2 Screening of random primers

DNA sample from one accession was amplified with ten random decamer primers (3 from OPA series, 3 from OPRN series, two from OPAH series, one each from OPF series and OPRY series) for screening the primers.

The results of screening are given in Table 52 and Plate 3. The number of bands ranged between zero and eight. Out of the ten primers screened, four viz., OPRN8, OPA8, OPAH9 and OPA21 gave good amplification and the number of amplification products were also more for these primers with OPRN8 and OPA8 producing eight bands whereas OPA21 producing six bands. Five bands were produced by OPAH 9. The other primers OPRY3, OPA24, OPAH12, OPF3, OPRN9 and OPRN5 gave only one or two bands or no amplification at all. Repeated tests gave similar results. OPRN8, OPA8, OPAH9 and OPA21 were selected (Table 53) for further studies based on distinct banding pattern with good amplification and reproducibility.

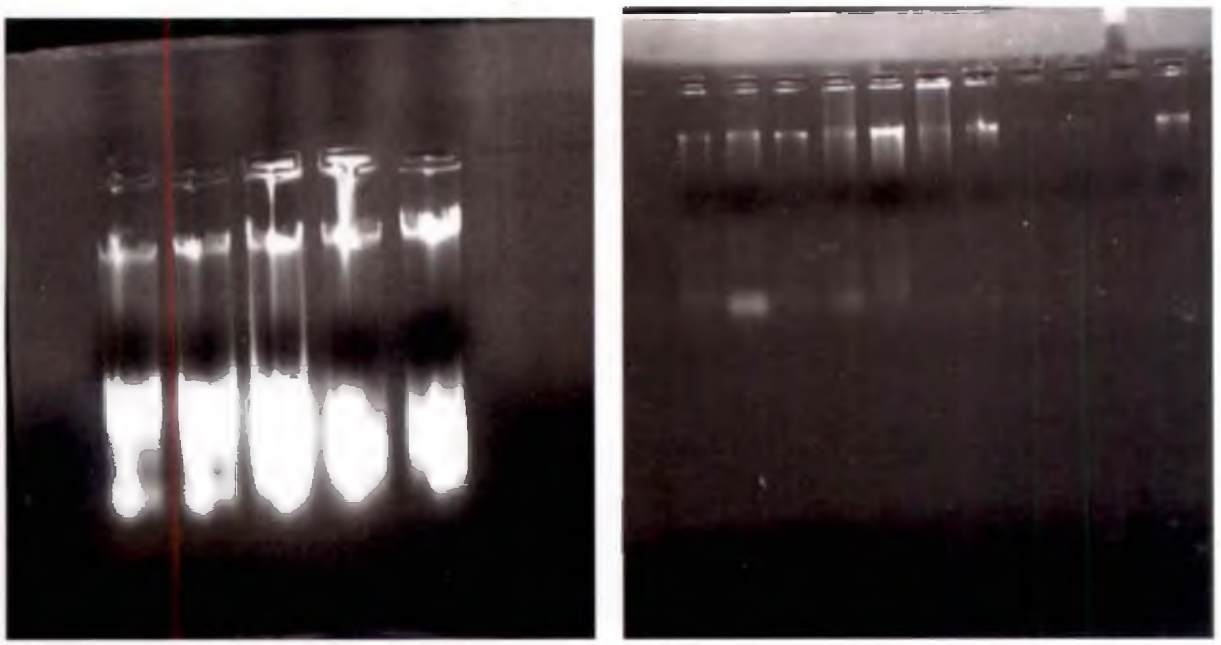
Table 52. Amplification pattern of *S. asoca* genomic DNA with different decamer primers screened

Sl. No.	Primer Code	No. of Bands	Quality of amplification
1.	OPF3	1	Poor
2.	OPRN5	0	Poor
3.	OPRN9	1	Poor
4.	OPRN8	8	Good
5.	OPA8	8	Good
6.	OPAH9	5	Good
7.	OPA21	6	Good
8.	OPRY3	2	Poor
9.	OPA24	1	Poor
10.	OPAH12	1	Poor

Table 53. List of selected decamer primers used for RAPD analysis

Sl. No.	Primer Code
1.	OPRN8
2.	OPA8
3.	OPAH9
4.	OPA21

Plate 3. Isolated Genomic DNA before and after RNase treatment



Screening with primers

MM-	Molecular marker
1	- OPF3
2	- OPRN5
3	- OPRN9
4	- OPRN8
5	- OPA8
6	- OPAH9
7	- OPA21
8	- OPRY3
9	- OPA24
10	- OPAH12

4.5.1.3 Screening of *S. asoca* accession with selected primers

The genomic DNA isolated from ten *S. asoca* accessions were subjected to RAPD analysis using four selected primers in order to assess the accession heterogeneity. The four random primers used for amplification gave 227 scorable amplified products of which 210 (92.5 per cent) were monomorphic and 17 bands were polymorphic.

Amplification with primer OPRN8 resulted in amplification in all ten *S. asoca* accessions. Molecular weight of bands ranged between 1 kb and 0.1 kb (Table 54, Plate 4). The high molecular weight bands in the other accessions were absent in IC566488, indicating that it is different from other accessions. Polymorphism was observed with respect to some bands in accessions IC566463, IC566461, IC566493 and IC566456.

RAPD banding pattern with primer OPA8 gave amplification in all the accessions with molecular weight of bands ranging between 0.7 kb and 0.1 kb (Table 55, Plate 5). The accessions IC566488 and IC566456 did not follow the banding pattern as other accessions. Bands indicating polymorphism were identified in IC566474 and IC566495.

Primer OPAH9 also gave a RAPD profile with amplification in 9 accessions (Table 56, Plate 6). The bands ranged between 1.9 kb and 0.1 kb. The accessions IC566461 and IC566456 indicated polymorphic bands.

Banding pattern for primer OPA21 gave amplification for most of the accessions except IC566493 and IC566498 (Table 57, Plate 7). IC566488 did not follow the same banding pattern as other accessions. The bands ranged between 1.3 kb and 0.3 kb. Polymorphism was observed in IC566456, IC 566497, IC566495, IC566496 and IC566474.

Plate 4. RAPD profile for *S.asoca* accessions with primer OPRN8

Table 54. RAPD profile for ten *S. asoca* accessions using primer OPRN8

	IC566463	IC566461	IC566488	IC566493	IC566456	IC566497	IC566498	IC566496	IC566474	IC566495
Band1	+	+	-	+	+	+	+	+	+	+
Band2	+	+	-	+	+	+	+	+	+	+
Band3	+	+	-	+	+	+	+	+	+	+
Band4	+	+	-	+	+	+	+	+	+	+
Band5	+	+	-	+	+	+	+	+	+	+
Band6	-	+	-	+	+	-	-	-	-	-
Band7	+	-	-	-	-	-	-	-	-	-
Band8	+	+	+	+	+	-	-	+	+	+
Band9	+	+	+	+	+	-	-	+	+	+
Band10	+	-	-	-	-	-	-	+	+	+

+ Band present
- Band absent

Plate 5. RAPD profile for *S.asoca* accessions with primer OPA8

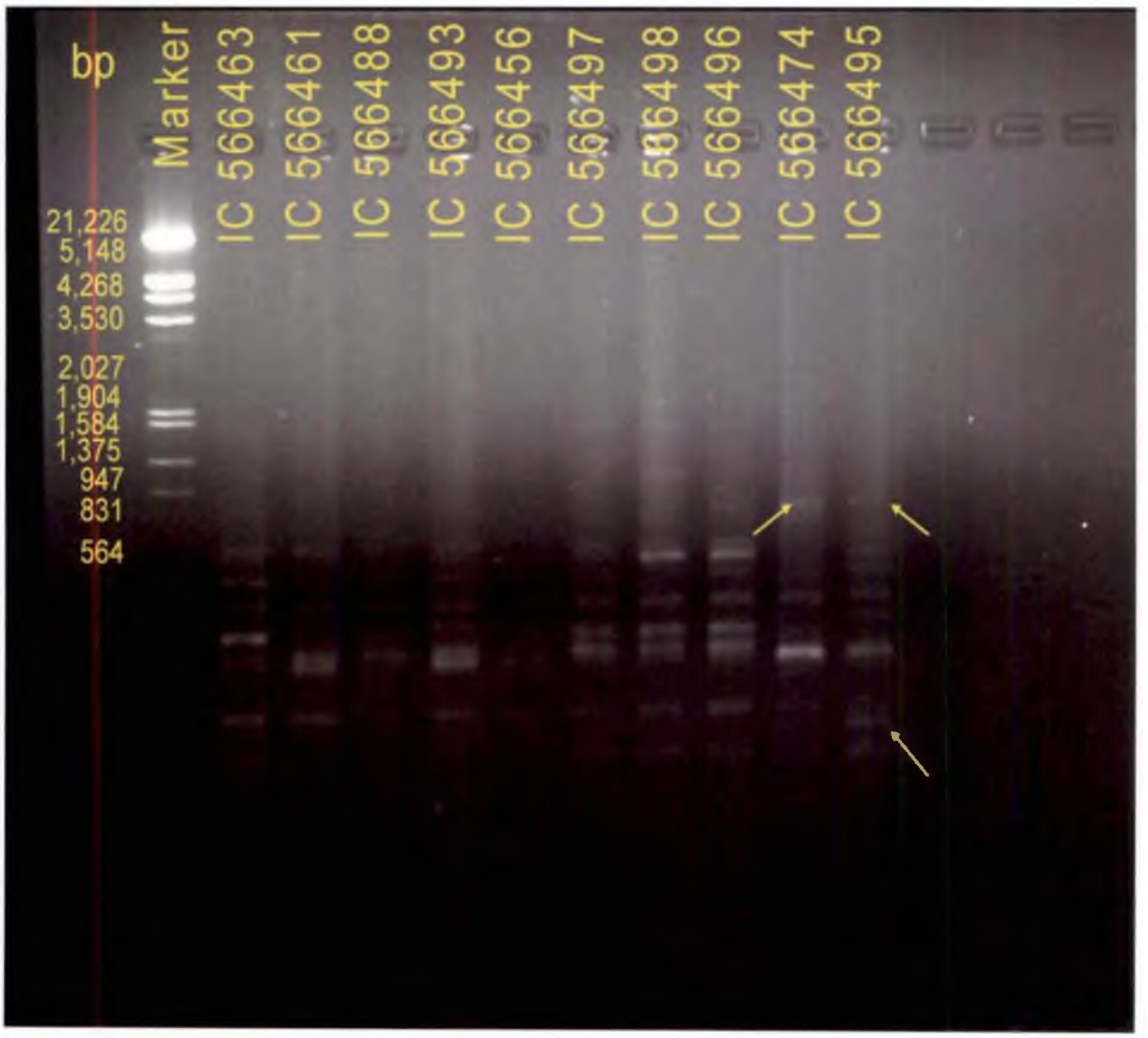


Table 55. RAPD profile for ten *S. asoca* accessions using primer OPA8

	IC566463	IC566461	IC566488	IC566493	IC566456	IC566497	IC566498	IC566496	IC566474	IC566495
Band1	-	-	-	-	-	-	-	-	+	+
Band2	+	+	-	+	-	+	+	+	-	+
Band3	+	-	-	+	-	+	-	+	-	+
Band4	+	+	-	+	-	+	+	+	+	+
Band5	+	-	-	+	-	+	+	+	-	-
Band6	-	+	+	+	+	+	+	+	+	+
Band7	+	+	-	+	-	+	+	+	+	-
Band8	-	-	-	-	-	-	-	-	-	+
Band9	+	-	-	-	-	+	+	+	+	+

+ Band present
- Band absent

Plate 6. RAPD profile for *S.asoca* accessions with primer OPAH9

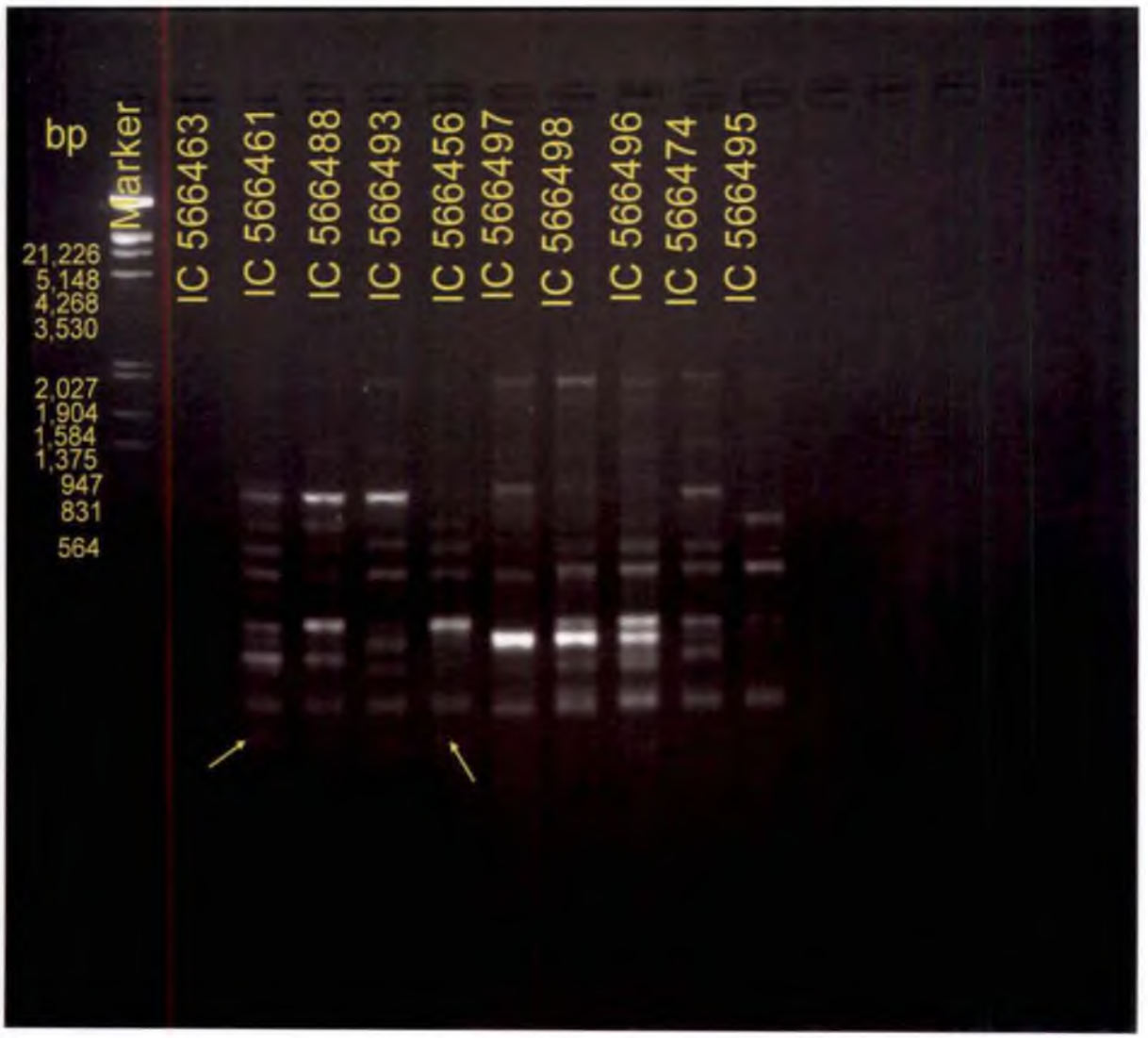


Table 56. RAPD profile for ten *S. asoca* accessions using primer OPAH9

	IC566463	IC566461	IC566488	IC566493	IC566456	IC566497	IC566498	IC566496	IC566474	IC566495
Band1	-	+	-	+	-	+	+	+	+	-
Band2	-	+	+	+	-	+	+	-	+	-
Band3	-	+	+	-	+	-	-	+	-	+
Band4	-	+	+	+	+	-	+	+	+	-
Band5	-	+	+	+	+	+	+	+	+	+
Band6	-	+	+	-	+	-	+	+	+	+
Band7	-	-	-	+	-	+	+	+	-	-
Band8	-	+	+	+	-	-	+	+	+	-
Band9	-	+	+	+	+	+	+	+	+	+
Band10	+	+	-	-	+	-	-	-	-	-

+ Band present
- Band absent

Plate 7. RAPD profile for *S.asoca* accessions with primer OPA21

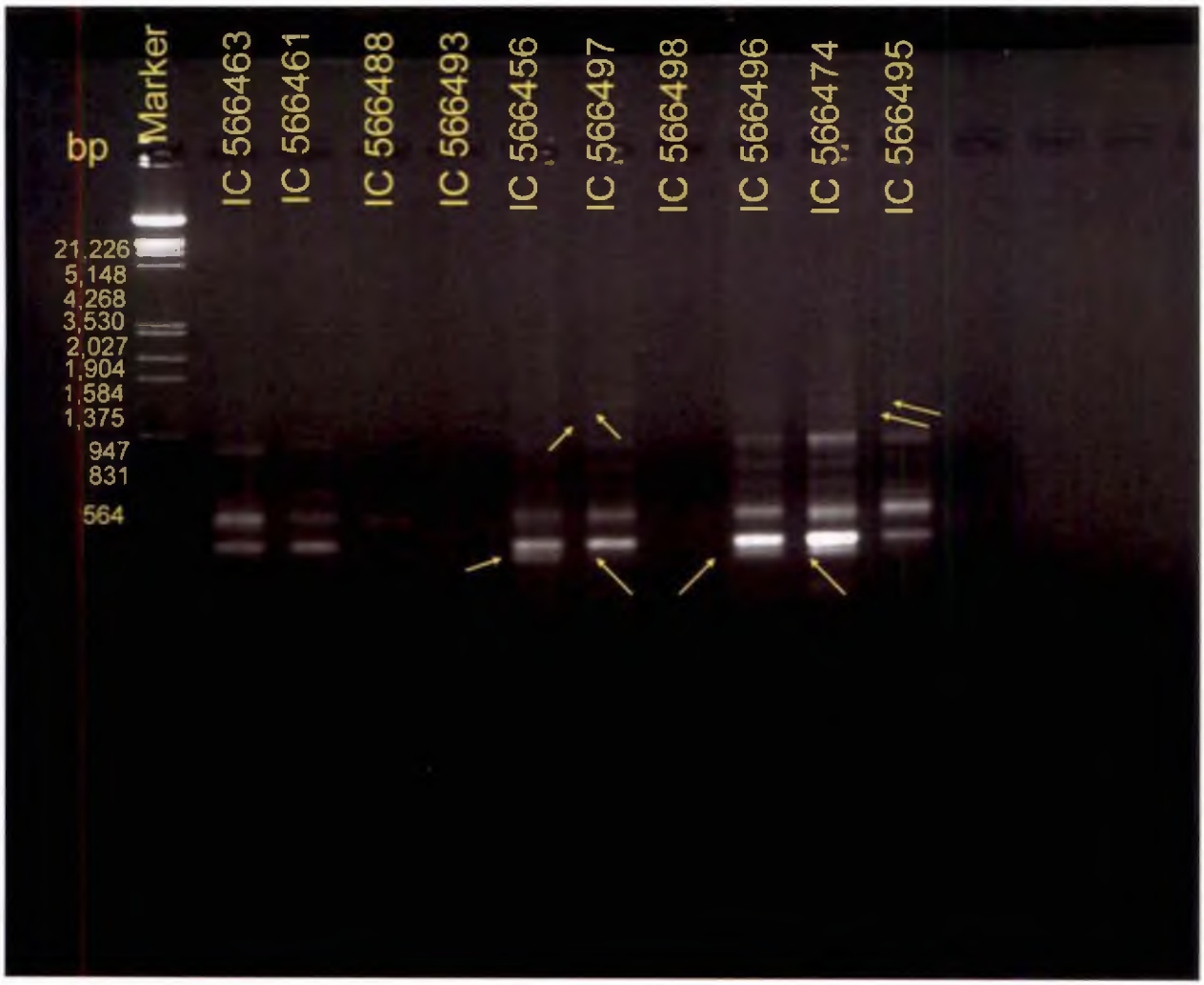


Table 57. RAPD profile for ten *S. asoca* accessions using primer OPA21

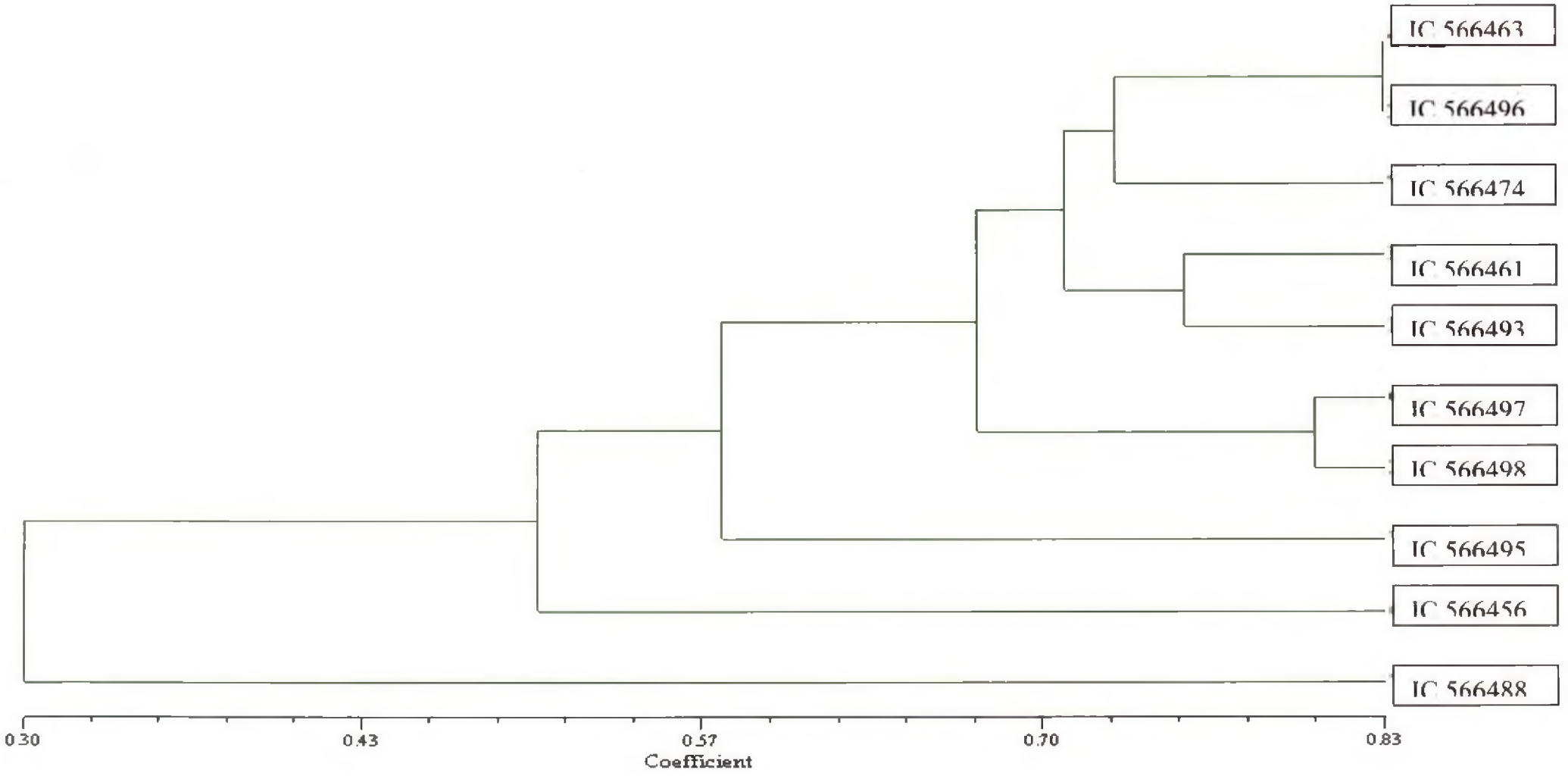
	IC566463	IC566461	IC566488	IC566493	IC566456	IC566497	IC566498	IC566496	IC566474	IC566495
Band1	-	-	-	-	-	+	-	-	-	+
Band2	-	-	-	-	-	+	-	-	-	+
Band3	+	+	-	-	+	+	-	+	+	+
Band4	+	+	-	-	-	+	-	+	+	+
Band5	+	+	-	-	-	+	-	+	+	+
Band6	+	+	+	-	+	+	-	+	+	+
Band7	+	+	-	-	+	+	-	+	+	-
Band8	-	-	-	-	+	+	-	+	+	-
Band9	-	+	-	-	+	+	-	+	+	-

+ Band present
- Band absent

4.5.1.4 Molecular genetic relationship

Dendrogram was constructed for asoka accessions from pooled RAPD data using UPGMA clustering. At similarity coefficient 0.30 the dendrogram got divided into two clusters, one large cluster with nine asoka accessions and other small cluster with IC566488 (Fig. 2). This indicated that the accession IC566488 from Thiruvananthapuram was genetically distinct from the other accessions. The nine accessions were subdivided into two clusters at 0.49 similarity coefficients with IC566456 in one cluster and other eight accessions in another distinct cluster. At similarity coefficient of 0.58, eight accessions were divided into two clusters, one large cluster with seven accessions and other small cluster with IC566495. The seven accessions were subdivided into two clusters at 0.67 similarity coefficients with IC566463, IC566496, IC566474, IC566461 and IC566493 in one cluster and IC566497, IC566498 in another distinct cluster. IC566497 and IC566498 came under one cluster at a similarity coefficient of 0.80. IC566463 and IC566496 clustered at 0.83 similarity coefficient along with IC566474 at similarity coefficient of 0.73. At similarity coefficient of 0.75, separate cluster was obtained for IC566461 and IC566493. The accessions IC566463, IC566496, IC566474 and IC566461, IC566493 formed a cluster at similarity coefficient of 0.71. The clustering pattern did not follow the geographical distribution of accessions.

Fig.2. Dendrogram of S.asoca accessions from pooled RAPD data using UPGMA clustering



Discussion

5. DISCUSSION

Asoka (*S. asoca*) is a sacred tree among the Buddhists. This medicinal plant tree commonly seen in South India in Hindu temples, *sarpakavu,* national gardens and sides of main roads. The word 'asoka' means an agent removing sorrow. It is mainly used for correcting uterine problems and thus healing their sorrows. It has a stimulating effect on endometrium and ovarian tissue and was found to be useful in menorrhagia due to uterine fibroids, in leucorrhoea and internal bleeding hemorrhoids and hemorrhagic dysentry (Ambasta, 1992). Due to over exploitation of this plant or its bark, this has now become extinct in many of the areas where it is commonly seen. The international Union for conservation of Nature (IUCN) has listed this species under 'globally vulnerable' category (IUCN, 2009). It is also enlisted among the 36 threatened and endangered medicinal plants of India. Due to its acute short supply compared to its demand, various development and research activities are being prioritized by National Medicinal Plant Board. Due to its short supply, it is being adulterated with other species like *Polyalthia longifolia.* Now it is the present need to identify better asoka genotypes having higher biochemical constituents and also to popularise its cultivation, to free from its adulteration and minimize the gap between demand and supply.

In Kerala, the existing variability in *S. asoca* germplasm has not been exploited so far, as a result not a single known variety is available for commercial cultivation. In order to select superior accessions for future breeding programme of *S. asoca*, the extent of variability with respect to morphological traits is indispensable. Radhakrishnan *et al.* (2007) reported that morphological architecture of asoka plants varied among accessions. From the view point of conservation, utilization and improvement of this species, there is a felt need to assess the morphological variations in *S. asoca* accessions in different ecogeographical regions of Kerala.

Basically, breeding system for improvement of a particular species is devised from the knowledge of its reproductive behaviour. In *S. asoca*,

reproductive phenology, pollination systems, inflorescence, flower, pod and seed traits were studied by Kumar *et al.* (2007a), Dhiman (2006), Singh and Somadey (2005), Vardhana (2007) and Chatterjee and Prakashi (1992). But several aspects of reproductive biology such as anthesis, anther dehiscence, stigma receptivity, pollen studies, pollinating agents, influence of weather parameters were not identified. Therefore an investigation of all the reproductive traits of *S. asoca* is essential.

The study of seed biology of any species reveals the fact about germination of seed as well as storability of seeds and loss of viability of seeds, which helps in increasing the storability of the seeds for a long period without compromising for the loss of seed viability. Pattern of seed germination and seedling growth in *S. asoca* was studied by Kumar *et al.* (2007b) and by Nayagam and Varghese (2007).But the relationship between seed characters and seedling traits is not studied. The variation among *S. asoca* seedlings in different ecogeographical regions of Kerala is not determined yet. Therefore there is a need to assess the variation among *S. asoca* seedlings, so that better accessions can be selected at seedling stage itself, so as to save time and resources used in germplasm evaluation.

S. asoca is an overexploited medicinal species, which is of high commercial value in herbal industry. The bioactivities of this plant are due to the large number of secondary metabolites present in the various parts of the plant. The biochemical study by George and John (2007) revealed that these secondary metabolites show a vast diversity in their structure and bioactivities. Qualitative and quantitative estimation and thin layer chromatography of the major phytochemical classes of *S. asoca*, namely tannins, flavanoids and ketosterol were determined by Srikant *et al.* (2007). Quality studies in *S. asoca* were also conducted by Mathew *et al.* (2007). But no investigation was undertaken in *S. asoca* to identify a better accession with higher therapeutically active constituents for commercial cultivation.

Information on the genetic relationships and diversity of available germplasm is essential for the identification of potential germplasm and conservation biology. DNA markers represent polymorphism in the actual base sequence of DNA (Singh, 2006). The optimization of DNA isolation and PCR conditions for RAPD analysis of selected medicinal plants containing high levels of polyphenols and secondary metabolites including *S. asoca* had been presented by Padmalatha and Prasad (2006). To confirm the relationship of morphological variations in asoka with its DNA level, molecular characterization of accessions representing different ecogeographical regions is to be studied.

Based on the above aspects, the present investigation in *S. asoca* envisages studies on morphological variations of existing germplasm, reproductive biology, collection of seeds and evaluation of seeds and seedlings for variation in its traits. The evaluation of therapeutical components and the molecular characterization of various accessions are also included.

5.1 Morphological variations of existing germplasm

Variability in morphological traits were assessed in the forty three indigenous collections of *S. asoca* germplasm. The study was undertaken in the first year (April 2007 to March 2008) and second year (April 2008 to March 2009). The pooled morphological data from April 2007 to March 2009 was derived from the mean values of morphological traits of two years. In pooled data, tannin and phenol content was also considered along with morphological traits. The estimates of this analysis included genetic variability, heritability, genetic advance, genetic gain and correlation. Path analysis, cluster analysis and discriminant function analysis were also carried out in morphological data of 43 accessions. The results of the above statistical analysis are discussed below.

5.1.1 Genetic Variability, heritability and genetic gain

In the process of crop improvement, desirable plants are continuously being selected from genetically variable population. Genetic improvement thus depends on the existence of genetic variability. Therefore an insight into the magnitude of variability present in a crop species is of utmost importance as it is a key factor, which determines the amount of progress expected from selection. The extent to which the variability of a quantitative character is transferable to the progeny is referred to as heritability for that particular character. Genetic advance refers to the improvement in genotypic value of new population as compared with the base population and when it is expressed in percentage of mean, the new parameter is termed as genetic gain. So to have an effective selection, along with genetic variability, heritability and genetic gain measurement are also important.

The analysis of variance revealed that the accessions differed significantly for all the characters studied indicating considerable variation among the accessions for all the characters. GCV, heritability and genetic gain values of various traits in first year, second year and pooled data are presented in Fig. 3,4 and 5. High genotypic coefficient of variation (GCV) was observed for bark yield, number of leaves, stem girth and height of plant in all data, indicating the scope of improvement of these traits. High GCV values indicate that there is little influence of environment on the expression of character. The first year, second year and pooled data indicated moderate GCV for the trait number of leaflets per leaf. Most of the characters showed low difference between PCV and GCV suggesting that these characters were least affected by environment and thus emphasized the importance of these traits during selection programme. Variability among different characters in *S. asoca* such as height of plant, number of leaflets per leaf and bark yield were previously observed by workers Dhiman (2003), Singh and Somadey (2005), Retnam and Martin (2006) and Kapoor (2001). Leaflet length indicated high GCV in first year and pooled data while moderate GCV in second year data. High GCV for leaflet breadth in April 2007 to March 2008 data was observed. In the second year and pooled data, moderate GCV was recorded for

Fig. 3 Genetic parameters of morphological traits in first year data

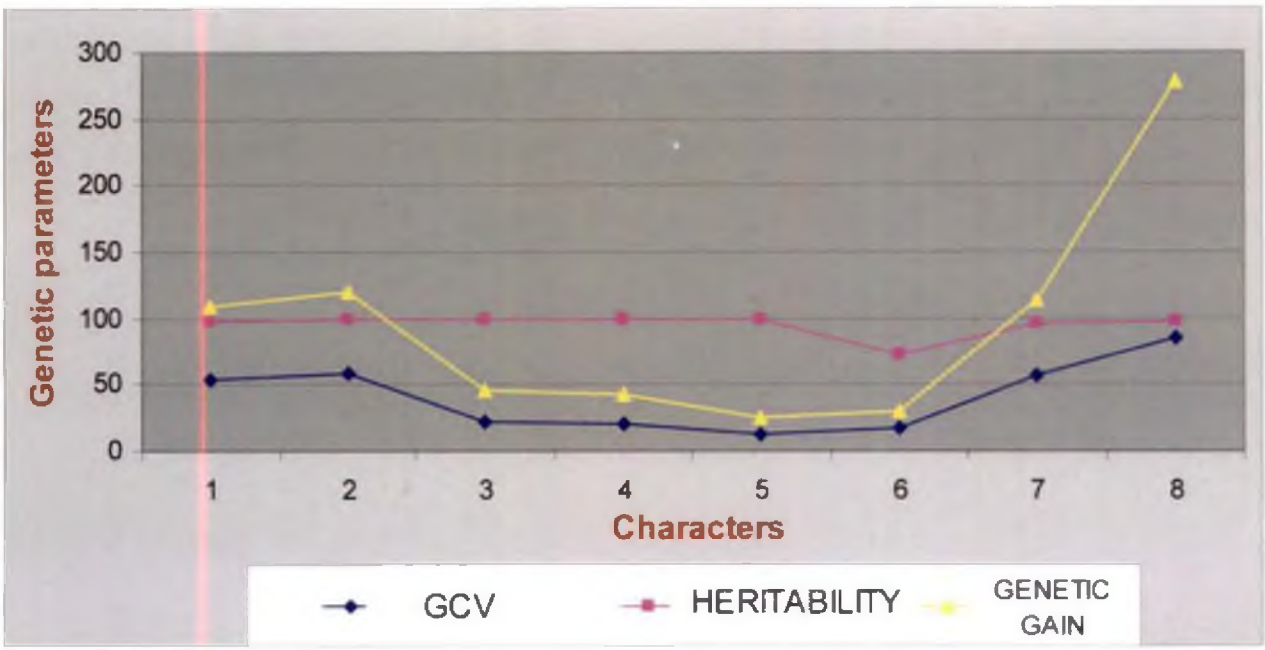


Fig. 4 Genetic parameters of morphological traits in second year data

Characters

1.Height of plant
2.Number of leaves
3.Leaflet length
4.Leaflet breadth
5.Number of leaflets per leaf
6.Internodal length
7.Stem girth
8.Bark yield

leaflet breadth. High variability in leaflet length and breadth in *S. asoca* was reported earlier by Subramanian (1995) and Sasidharan and Sivarajan (1996). Internodal length had high GCV in second year data but moderate GCV in first year and pooled data. In pooled data, biochemical traits like tannin content pointed out high GCV, while phenol content had moderate GCV. High GCV for biochemical trait catechin content in *A. catechu* was already reported by Singh *et al.* (2004). Among the morphological and biochemical traits studied, plant height, stem girth, number of leaves, tannin content and bark yield recorded high GCV. Therefore in asoka, tall plants with maximum stem girth, higher number of leaves and high tannin content may be selected as to obtain maximum amount of progress expected from selection.

In, general, high estimates of heritability was indicated by height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth and bark yield in both the years. In pooled analysis data, high heritability was exhibited by most of the characters except internodal length which indicated moderate heritability. The tannin and phenol content also exhibited high heritability values. High heritability estimates indicated that characters are genetically controlled and selection for these traits would be effective. Genetic advance as per cent of mean was high for height of plant, number of leaves, leaflet length, leaflet breadth, number of leaflets per leaf, internodal length, stem girth and bark yield. High genetic gain values for tannin and phenol content was observed in the pooled data. High genetic advance shows that character is governed by additive genes and selection will be rewarding for improvement of such trait. Singh and Narayanan (1993) suggested that estimates of heritability and genetic advance when considered together are more useful than heritability alone. High heritability accompanied with high genetic advance indicates that most likely the heritability is due to additive gene effects and selection may be effective. For a reliable conclusion for the present study, the characters which have high variability, high heritability and highest genetic gain were considered. This included height of plant, number of leaves, stem girth, bark

yield and tannin content. The results were in accordance with the findings of Singh *et al.* (2004) in *Acacia catechu*. Tree height, diameter at breast height, crown width and catechin content in *A. catechu* had high estimates of heritability, genetic gain and genetic advance. Based on variability, heritability and genetic gain values, it is identified that, in asoka, maximum improvement in genotypic value of new population over the base population is achieved only by selecting the accessions having high magnitudes of the traits namely height of plant, number of leaves, stem girth and tannin content.

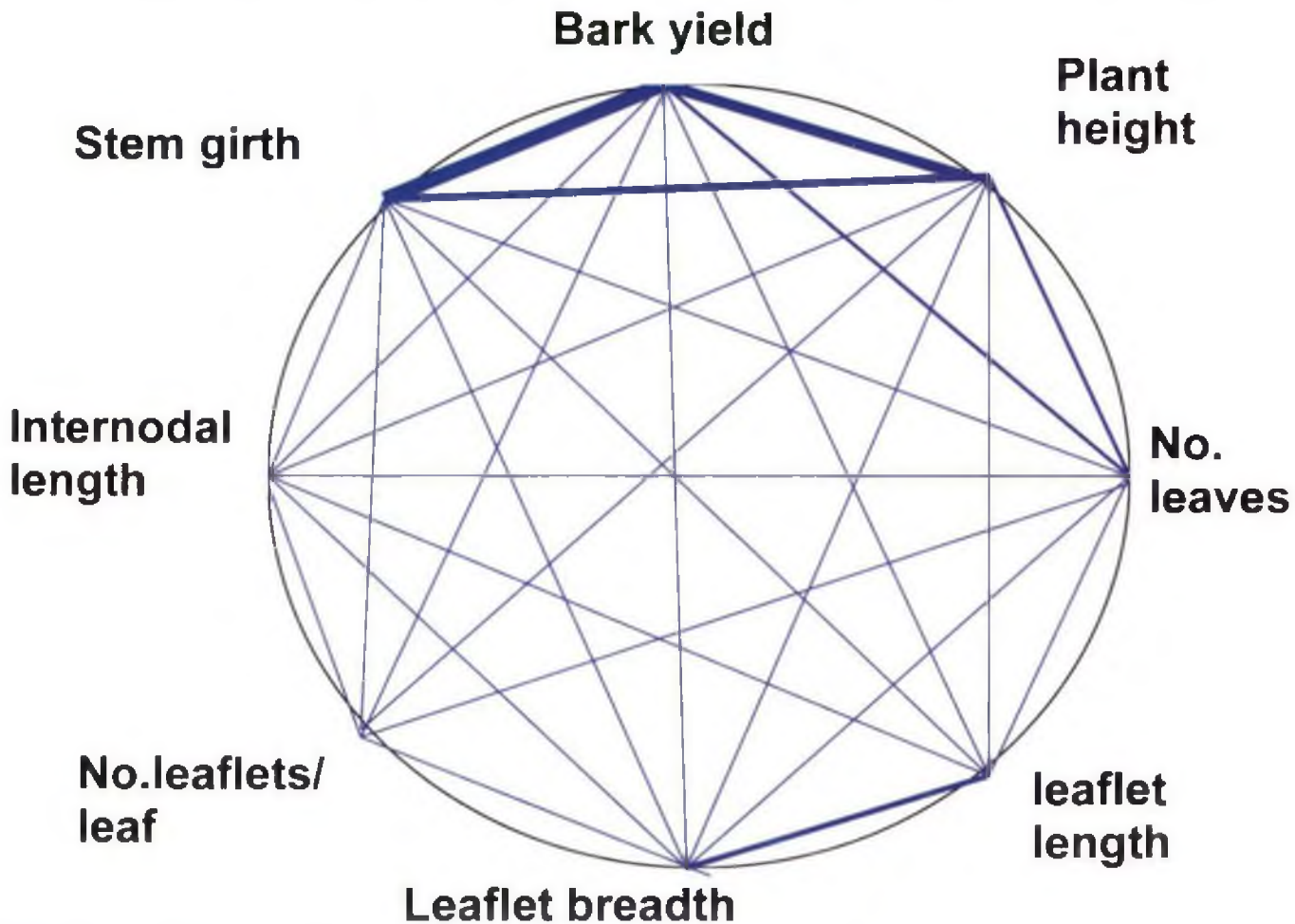
5.1.2 Association of characters

Association of characters is measured by assessing the inter and intra relationship among the various constituent characters on which selection can be relied for genetic improvement. Both phenotypic and genotypic level can be estimated and these assessment termed as phenotypic correlation coefficient and genotypic correlation coefficient. Estimation of these correlation coefficients among the constituent traits is very much important to know the type of association existing between the variables. Genotypic correlations are mainly considered for determining the genotypic association among the genotypes.

The genotypic association among different characters of asoka accessions identified in first year, second year and pooled data are diagrammatically presented (Fig. 6,7 and 8). Bark yield recorded significant genotypic correlation with height of plant, number of leaves, stem girth, internodal length, leaflet length and leaflet breadth and number of leaflets per leaf. These correlations were observed in first year, second year and pooled data. The biochemical traits tannin content and phenol content also exhibited significant association with bark yield as identified in pooled data.

All the component traits showed positive and significant intercorrelation among themselves, which was evident in first year data. The traits number of leaflets per leaf and leaflet length were an exception to this as they did not show inter correlation. The similar trend was also observed in the results of correlation

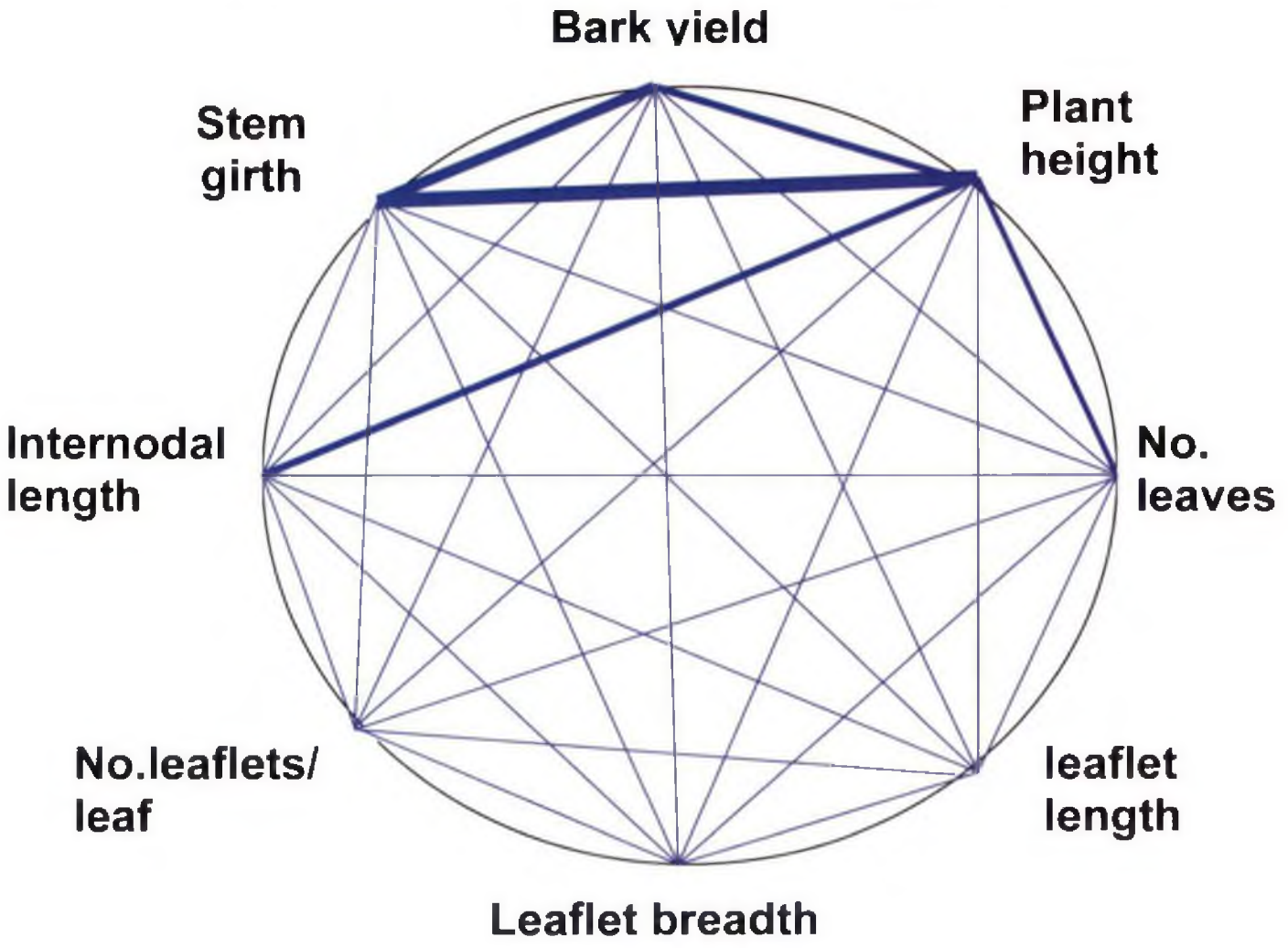
Fig.6 Genotypic correlation among bark yield and morphological traits in first year



——— Significant positive correlation

Width of line is apparently proportional to magnitude of correlation

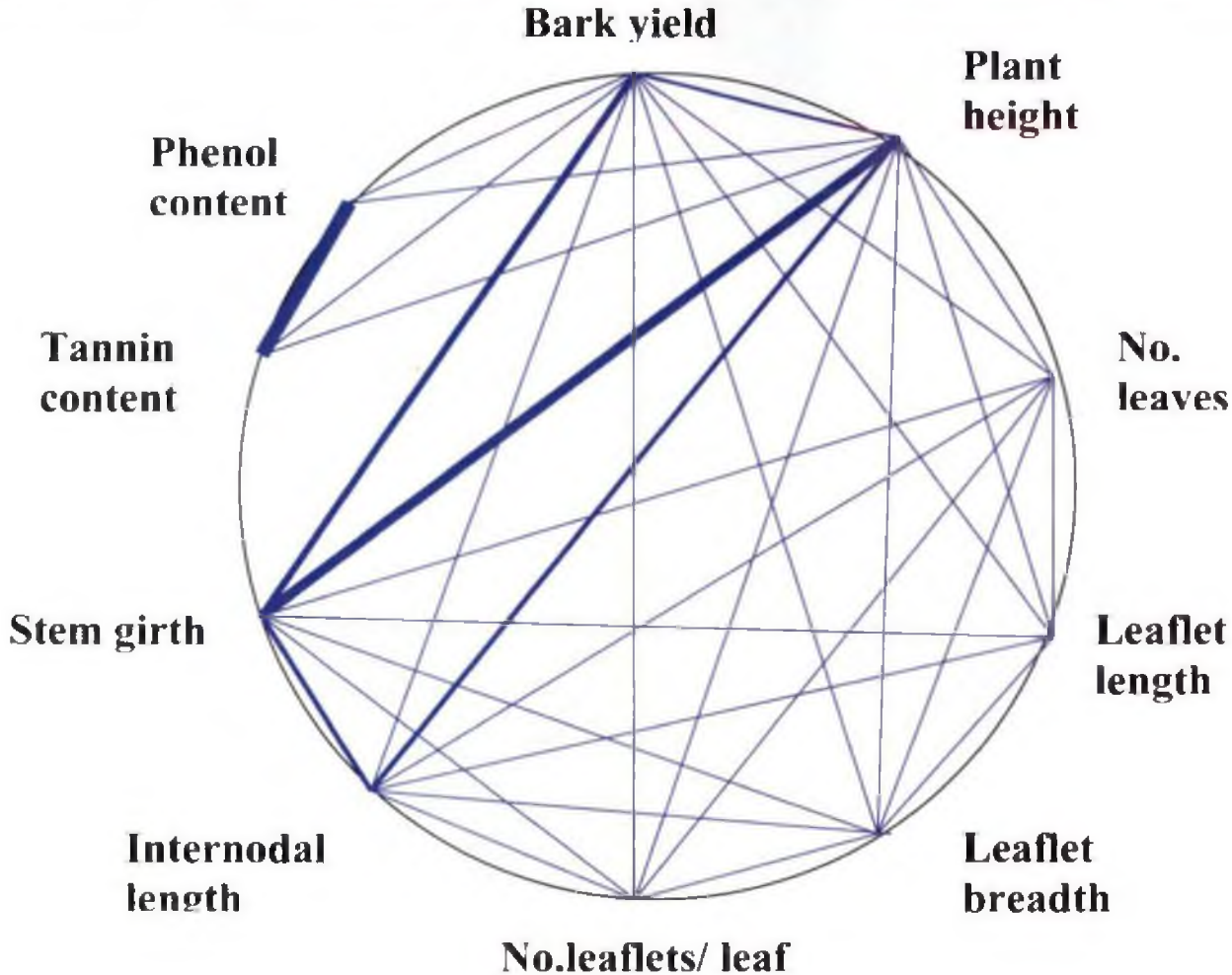
Fig.7 Genotypic correlation among bark yield and morphological traits in second year



—— Significant positive correlation

Width of line is apparently proportional to magnitude of correlation

Fig.8 Genotypic correlation among bark yield and morphological traits in pooled data



—— Significant positive correlation

Width of line is apparently proportional to magnitude of correlation

analysis of pooled data from April 2007 – March 2009. From the pooled data, it was noted that biochemical traits tannin content and phenol content did not exhibit association with the component traits except height of plant. In the second year data, the assessment of intercorrelation among the bark yield component traits indicated significant association between all the component traits. The association between plant height and stem girth had been pointed out earlier in ber by Saran *et al.* (2007). Correlation of leaflet length with leaflet breadth was indicated by Oboh *et al.* (2008) in *Terminalia catappa.*

The association analysis pointed out that the traits stem girth, height of plant and number of leaves exhibited highest correlation with bark yield. Therefore in asoka these traits are the selection parameters to obtain better accessions. Based on the association analysis, tall plants with maximum stem girth and maximum number of leaves may be selected to have better accessions with higher bark yield.

5.1.3 Path analysis

Path analysis provides an aid to sort out total correlation into direct and indirect effects of different characters on yield. Rapid improvement in yield is expected to result if selection is practiced for component characters. Rate of improvement is expected to be rapid if differential emphasis is laid on the component characters during selection.

In the present investigation, path analysis was performed in first year and second year using eight characters, which were significantly correlated with bark yield at genotypic levels. In the pooled data, biochemical traits like tannin content and phenol content which had correlation with bark yield were also included for path analysis. The direct and indirect effects of component traits on bark yield are given in Fig. 9,10 and 11. On bark yield, high positive direct effect was indicated by stem girth and height of plant in the first year. In second year data and pooled data, the trait stem girth had high positive direct effect whereas height of plant exhibited moderate positive direct effect on bark yield. These characters

Fig.9 Path diagram showing direct and indirect effects of component traits on bark yield in first year

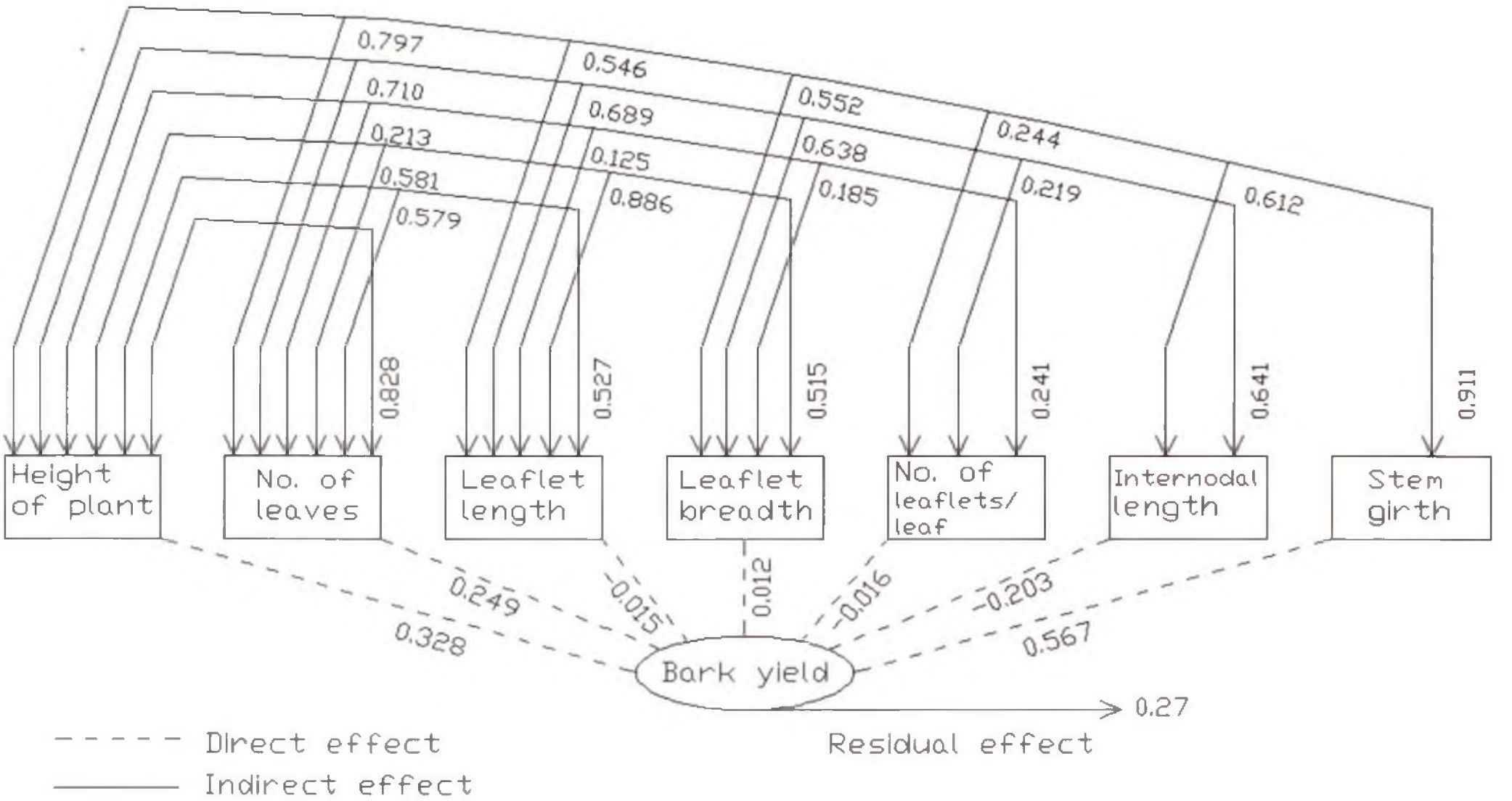


Fig. 10 Path diagram showing direct and indirect effects of component traits on bark yield in second year

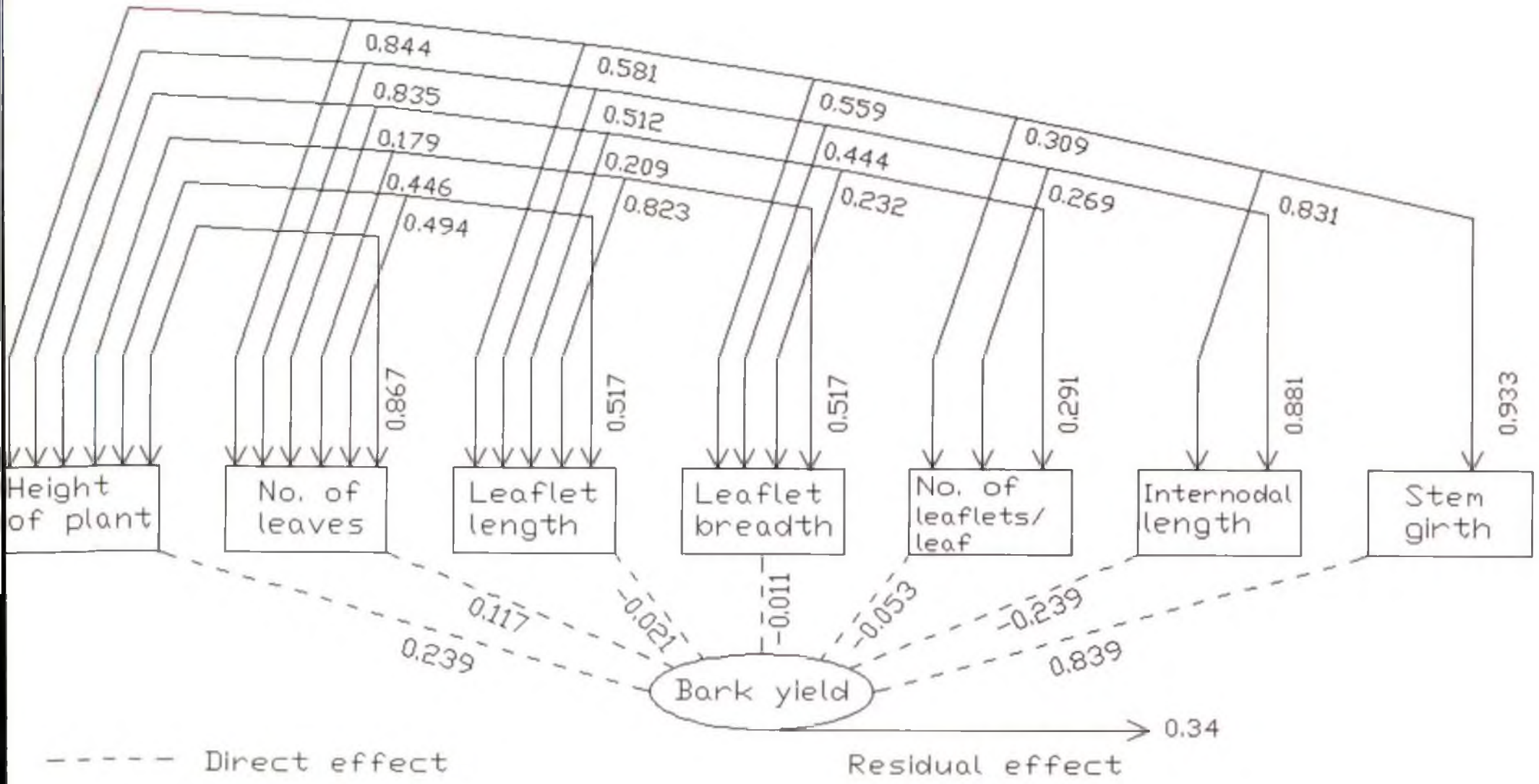
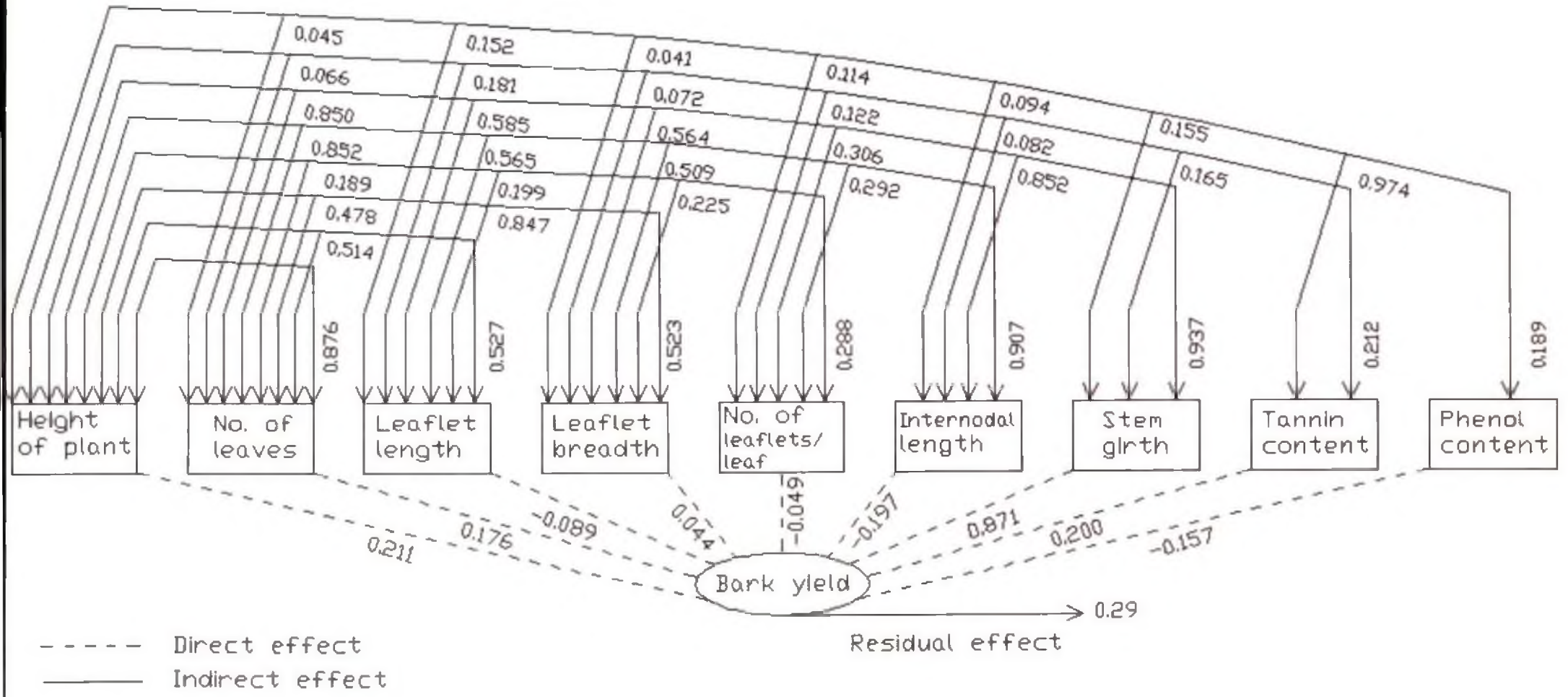


Fig.11 Path diagram showing direct and indirect effects of component traits on bark yield in pooled data



exhibited significant correlation with bark yield suggesting that selection of these traits could bring improvement in yield of bark. Saito *et al.* (2009) observed that stem girth and height of plant had direct effect on bark yield in *Broussonetia papyrifera.* Internodal length had moderate negative direct effect on bark yield as evident from first year and second year data. Number of leaves and tannin content was positively associated with bark yield as evidenced in the pooled data. The correlation of the characters leaflet length and leaflet breadth with bark yield was due to high indirect effect of these traits through stem girth.

Among the morphological and biochemical traits studied, general selection for bark yield could be efficient if it based on stem girth and height of plant as these characters satisfied both the requirements of association analysis and path coefficient analysis. Therefore accessions with maximum height and stem girth may be selected for better accessions with higher bark yield.

5.1.4 Cluster analysis

The variability present among different genotypes of a species is known as genetic diversity. Genetic diversity arises either due to geographical separation or due to genetic barriers to crossability. Variability differs from diversity in the sense that the former has observable phenotypic differences, whereas the latter may or may not have such an expression. One of the potent techniques of assessing genetic divergence is the cluster analysis. This technique helps in the selection of genetically divergent parents for exploitation in breeding programmes.

Hierarchical cluster analysis grouped the forty three accessions into two major clusters at an average linkage distance of 14. At an average linkage distance of 2, accessions formed 6 clusters. Cluster 3 was represented by all the six districts whereas cluster 1 by Thrissur, Malappuram, Thiruvananthapuram, Kollam, Kozhikode. Cluster 2 was indicated by Thrissur, Ernakulam while cluster 5 by Thrissur, Thiruvananthapuram. Cluster 4 and cluster 6 was pointed out by Thrissur and Thiruvananthapuram respectively. Accession IC566488 from

Thiruvananthapuram formed a separate cluster indicating that it is different from the other accessions. The accessions from Thrissur was observed in all the other clusters which lead to the conclusion that the Thrissur accessions indicated some relationship with the accessions from other districts. The clustering patterns of the 43 accessions did not follow the geographical distributions. Similar type of clustering in *Plumbage rosea* was indicated by Radhakrishnan *et al.* (2008b). Cluster analysis in Brahmi accessions pointed out that there was no parallelism between geographical distribution and clustering pattern of accessions (Radhakrishnan *et al.* 2008a). Similar results were also observed in *Artocarpus heterophyllus* by Maiti *et al.* (2002).

Cluster analysis showed that the clustering pattern did not follow the geographical distributions. The accession IC566488 from Thiruvananthapuram grouped alone in one cluster indicated that this accession was different from other accessions. In all the other 5 clusters, accessions from Thrissur was included. This indicated that some of the Thrissur accessions may have relationship with accessions from other districts. The accessions in each cluster are genetically divergent from the accessions in other clusters. These genetically divergent accessions may be selected for exploitation in breeding programs, as it exhibit high variability.

5.1.5 Discriminant function analysis

Selection index

The aim of most plant breeding programmes is simultaneous improvement of several characters. An objective method involving simultaneous selection for several attributes becomes necessary. It has been recognized that most rapid improvement in the economic value is expected from selection applied simultaneously to all the characters, which determine the economic value of a plant. Selection for economic value is thus a complex matter. If the component characters are combined together into a score or an index in such a way that when selection is applied to the index, as if index is the character to be improved, more

rapid improvement of economic value is expected, such an index is termed as selection index.

The traits which were significantly and highly associated with bark yield namely height of plant, number of leaves, leaflet length, leaflet breadth, internodal length and stem girth were used for discriminant function analysis. All possible combinations of these six characters were formulated and models with maximum expected genetic advance were selected from models with different character combinations. The selection index using single yield component, namely height of plant was found to have maximum expected genetic advance. Similar selection procedures were previously carried out by Francies (1998) in cocoa.

But a selection index with five yield components, namely height of plant, number of leaves, leaflet length, leaflet breadth and internodal length exhibited an expected genetic advance almost equal to earlier selection index. Hence in this study, the selection indices were formulated, one having single character and other with five yield components. Among these selection indices, selection index 1 with single trait namely height of plant was selected. This is because in this model, only single trait have to be considered to select better accessions when compared to selection index with five traits. By using the selection index 1, the forty three accessions were ranked. The top ten accessions selected were IC566463, IC566489, IC566488, IC566482, IC566493, IC566481, IC566462, IC566459, IC566475, IC566480. Among these accessions, eight accessions were ranked among the top ten accessions based on yield performance, but there was difference in ranking order. So we can conclude that for further breeding programme, these top ten promising accessions can be selected.

In discriminant function analysis, the selection index involving height of plant along with bark yield constituted the selection criteria among asoka accessions. Using this selection index, accessions IC566463, IC566489, IC566488, IC566482, IC566493, IC566481, IC566462, IC566459, IC566475,

IC566480 were selected as better accessions for higher bark yield. These promising accessions (Plate 8) may be utilized in further breeding programmes.

5.1.6 Bark thickness

A significant variation was not observed in bark thickness assessed during first year and second year of the study period April 2007 to March 2009. Bark thickness values of accessions ranged from 0.5 mm to 2.5 mm in the second year. The accessions IC566463, IC566489, IC566483, IC566465, IC566462, IC566475 recorded maximum bark thickness. Low bark thickness values were indicated by IC566477, IC566471, IC566474 and IC566473.

Kapoor (2001) reported that the thickness of asoka bark varied from 5 mm to 1 cm, depending upon the age of the tree.

5.2 Reproductive biology of *S. asoca*

A clear understanding of reproductive biology is necessary to develop orderly and efficient breeding methods for a given species (Tewari *et al.*, 2002). Reproductive biology studies were carried out in eight mature asoka trees located in KAU campus of age varying from 9 to 31 years. The results obtained form this study are discussed here under the subtitles namely flowering season, inflorescence and flower traits, pod and seed traits, reproductive period traits. The other aspects of reproductive biology include anthesis, pollen studies and pollination studies. The association among the reproductive biology traits and with weather parameters are also discussed below.

Plate 8. Better genotypes selected for higher bark yield

5.2.1 Season of flowering

Asoka trees showed blooming throughout the year. Profuse flowering was seen in the months January to May. Similar reports been made by Kumar *et al.* (2007a) in *S. asoca* trees. Asoka tree flowers in large compact orange-red clusters in February-March (Randhawa, 1983).

5.2.2 Inflorescence characters

Inflorescence is an axillary corymb (Plate 9) blooming in an irregular manner. Around 30-65 flowers are present in an inflorescence. On an average 15 to 185 inflorescence are present per tree (Fig.12). Among the various trees, KAU7, KAU6 had maximum inflorescence and flowers. Although KAU8 produced less number of flowers per inflorescence, the pods set per tree and seeds per pod were maximum in this tree. This is because the maximum number of pods were set in an inflorescence in KAU8. Better trees identified based on flower, pod and seed traits are presented in Plate 10. Vardhana (2007), Dhiman (2006) have pointed out the inflorescence of asoka is a corymb.

5.2.3 Floral morphology and biology

The flowers are short stalked, fragrant, bisexual and 5.9 cm long, 3.2 cm wide. Of the accessions studied, larger flowers were observed in KAU3, KAU4, KAU6 and KAU8 (Fig.13). The orange-yellow flowers eventually turns to vermillion. Calyx is long tubular which open out into four lobes. Corolla is absent. Stamens are seven with slender filaments. Anthers are elliptic-oblong, dithecous, dorsifixed and dehisce by longitudinal slits. Superior, unilocular ovary with ten to eleven ovules in marginal placentation (Plate 11). Style long, slender, ending in a minute, capitate stigma. These findings are in concurrence with the results obtained by Vardhana (2007), Singh and Somadey (2005) and Kumar *et al.* (2005). Floral diagram and floral formula of asoka are presented in Plate 12.

Plate 9. Inflorescence of asoka

Plate 10. Better trees identified based on flower, pod and seed traits

Fig.12 Inflorescence, flower and pod traits of trees

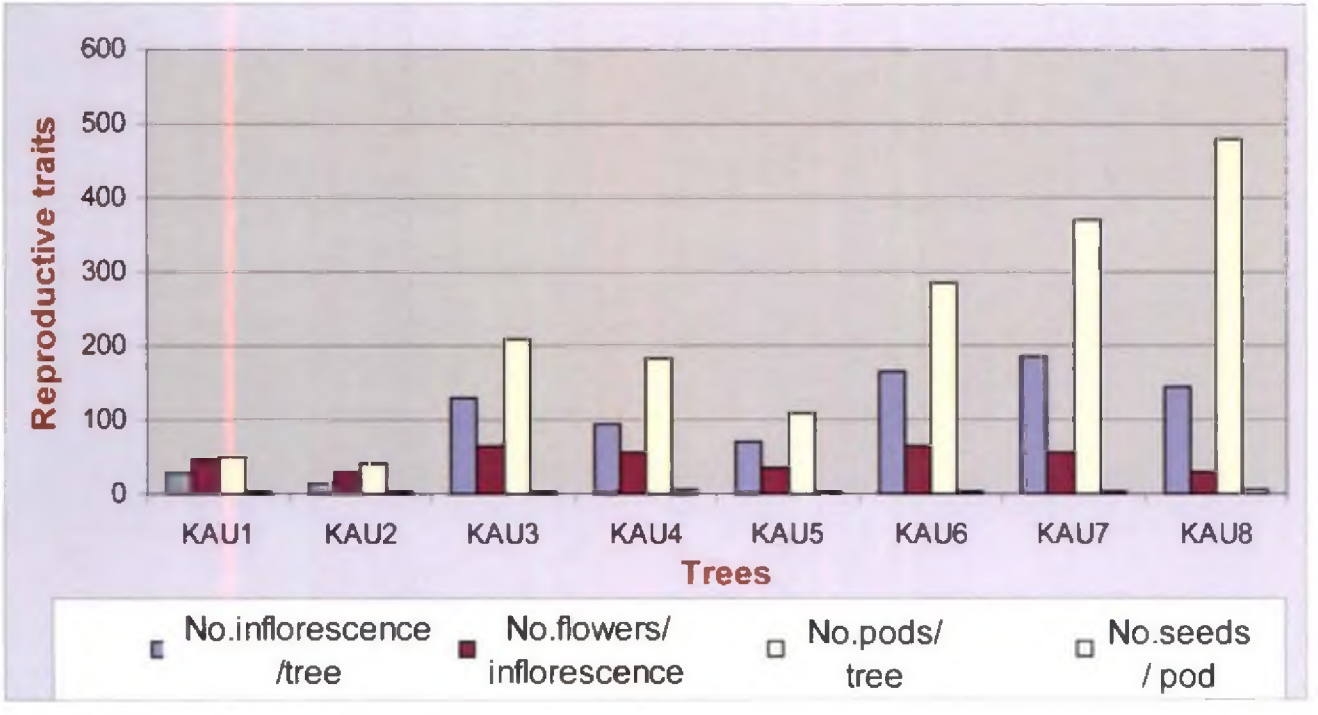
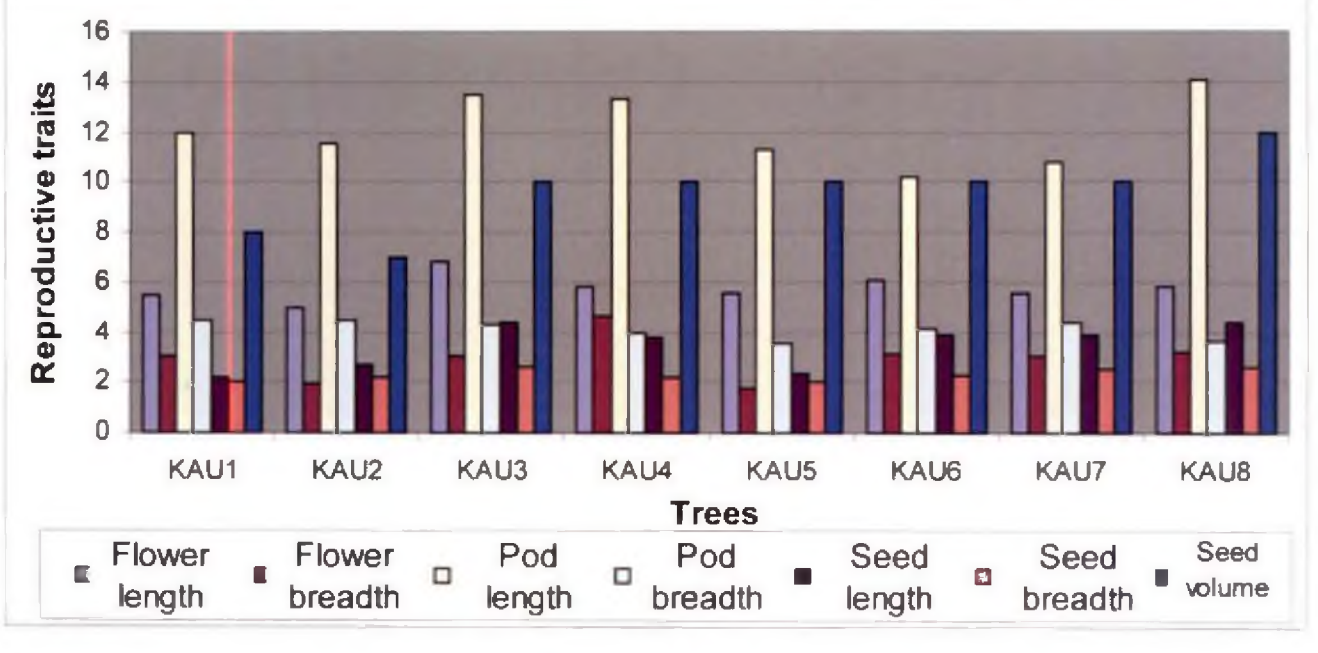
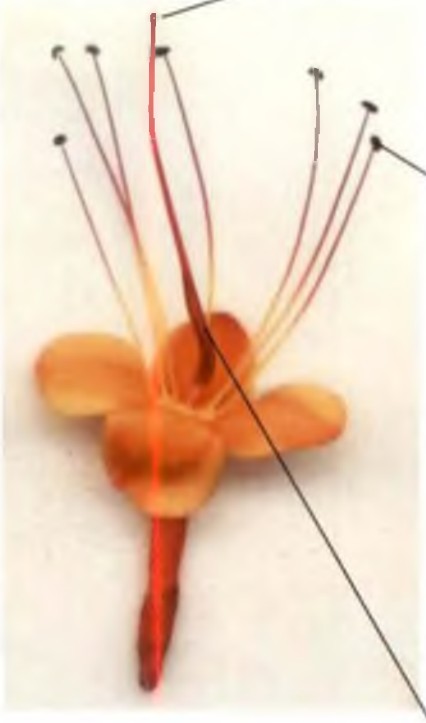


Fig.13 Flower, pod and seed traits of trees



Style with stigma



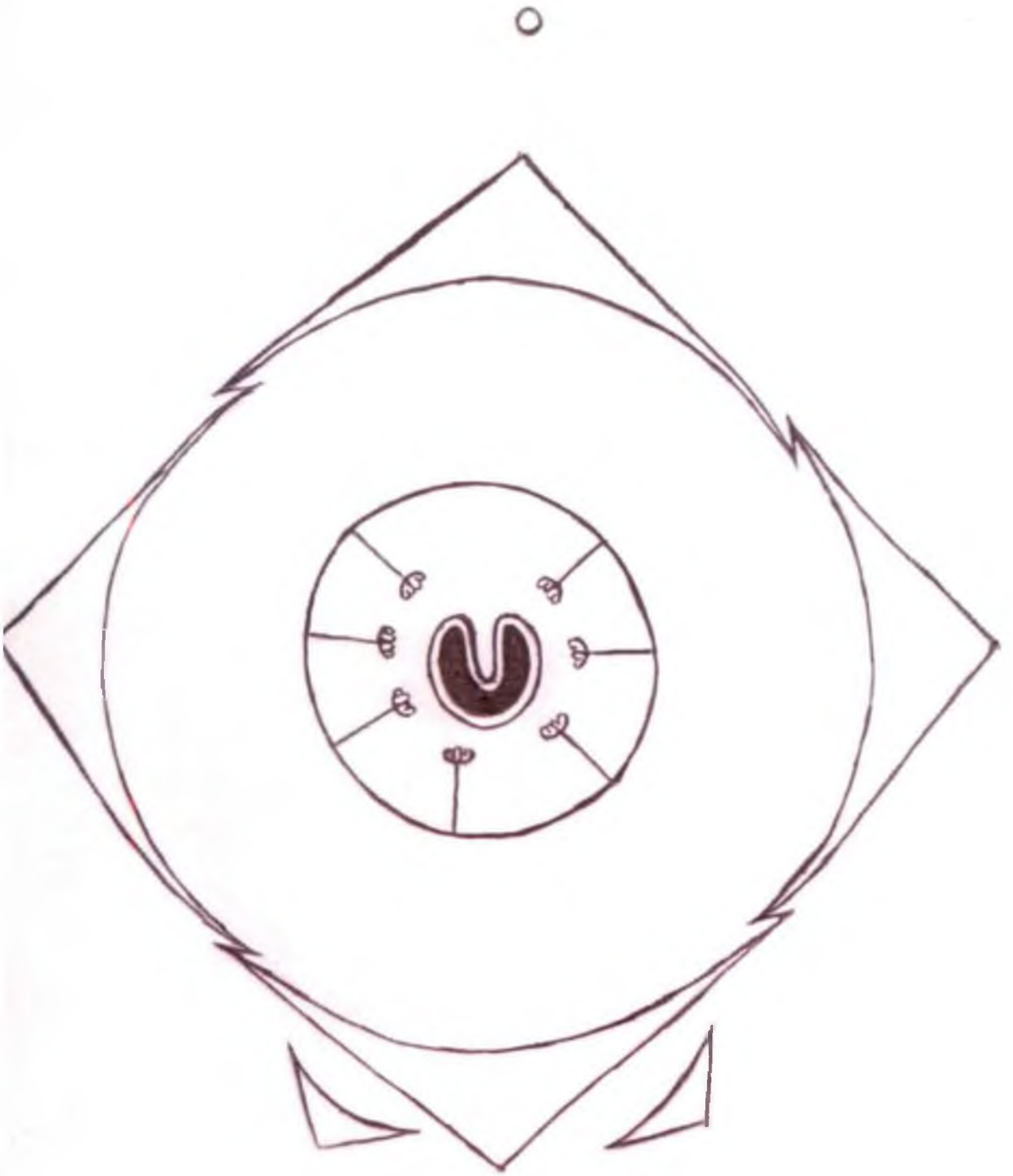
Flower

Anther

Gynoecium

Plate 11. Floral biology

Saraca asoca (Roxb.) De Wilde



Floral formula ♀ ⊕ $K_{(4)}\ C_0\ A_7\ \underline{G}_1$

5.2.4 Pod and seed characters

Pods are flat, oblong, woody with 2 to 5 seeds per pod. Average size of pods is 12.2 cm x 4 cm and around 50 to 480 pods are observed per tree (Fig.12 and 13). KAU3, KAU4 and KAU8 produced bigger pods and seeds (Plate 13). Dark brown immature pods gradually changes to full sized reddish brown pods with immature seeds. Matured pods are green in colour (Plate 14). Number of seeds per pod are presented in Plate 15. Similar pod features have been identified earlier by Vardhana (2007), Singh and Somadey (2005) and Dhiman (2006). Average seed size is 3.4 cm x 2.3 cm. Seed volume ranged from 7 ml to 12 ml. Seeds with maximum volume was identified in the tree accession KAU8. The trees KAU3, KAU4 and KAU8 may be selected to obtain big seeds with higher seed volume. Seeds are dark brown, ellipsoid-oblong, compressed (Chatterjee and Prakashi, 1992).

5.2.5 Reproductive period

The total reproductive period required from flower bud initiation to seed shedding is around 126-137 days. In visible buds, flowering starts in 20-26 days. Similar trend was noticed by Wani and Chauhan (2008) in Bauhinia variegata. Duration of blooming varied from 12 to 16 days. After anthesis, flowers took 30-34 days to form an immature pod. The immature pods dehisced in 62-69 days .Reproductive period traits in various trees studied are presented in Fig.14.

Based on reproductive studies in asoka, it was noted that mature pods are formed at 4 to 4 1/2 months from visual bud initiation. From the reproductive period observed, periods of profusion of flowers and pods could be identified. As the asoka flowers are used in medicinal preparations, the harvesting time is 1 to 1 ½ month after bud initiation.

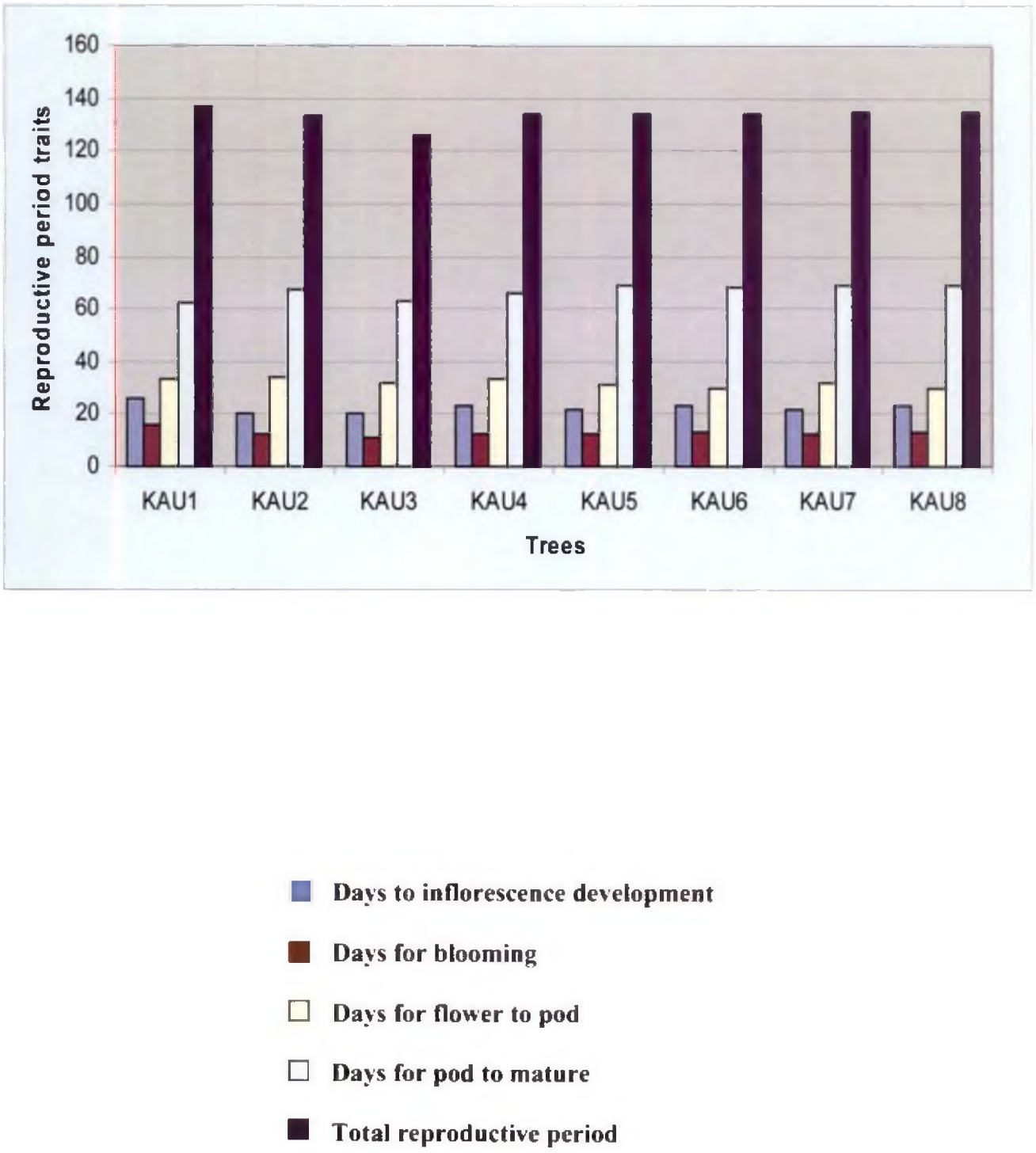
5.2.6 Anthesis

Plate 13. Seed and pods of trees studied

Plate 14. Stages of pod development

Plate 15. Variability in seed number per pods

Fig.14 Reproductive period traits of trees



Anthesis in the fully mature flower buds occurred between 5.00-6.00 am. The stages of anthesis are presented in Plate 16. Irrespective of the genotype and age of the tree, anthesis time in *S. asoca* is between 5-6 am.

5.2.7 Anther dehiscence and stigma receptivity

At the time of anthesis, anthers dehisced between 5-6 am. Anthers dehisced by longitudinal slitting of anther lobes. Similar dehiscence of anther lobes was reported by Raju and Rao (2006) in *Gmelina arborea*. Stigma was receptive one hour after anthesis and continued to be receptive for that same full day. In *Pterocarpus santalinus*, stigma became receptive when first two anthers dehisced and remained receptive until late evening of same day (Rao and Raju, 2002).

5.2.8 Pollen morphology and viability

Pollen grains are creamy white, tricolpate, round with smooth exine and 100 per cent fertile (Plate 17). The pollen of *Gmelina arborea* is tricolpate, oval with smooth exine (George, 2007). Pollen germination percentage was identified as 45%. Absolute pollen viability as assessed from the fertile pollen and germinated pollen indicated a value of 45. Pollen viability rate of *Balanites aegyptiaca* is 92 as reported by Ndoye *et al.* (2004).

Excess pollen grains does not guarantee a good pod set unless the pollen is viable with a higher germination percentage (Davarynejad *et al.*, 2008). Though the asoka pollen indicates hundred percentage fertility, germination capacity of pollen is less, thus causing less pollen to reach the ovules. This may be one of the reasons for lower pod set when compared to the huge number of flowers.

5.2.9 Pollination system and pollinating agents

S. asoca trees were identified to be cross-pollinated. Percentage pod set observed under open pollination was 42.5%. The occurrence of self pollination to a very small extent was observed. Percentage pod set under selfing was noted as

1

2

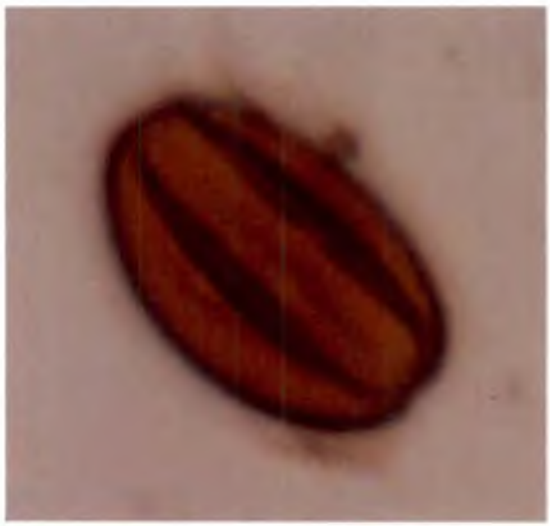
3

4

Plate 16. Stages of anthesis

Fertile pollen

Polar view



Equatorial view

Plate 17. Pollen morphology and fertility

4.37%. Kumar *et al.* (2007a) indicated that fruit set following geitnogamous selfing by hand pollination is very low (5.2%) compared to manual cross pollination (44.12%) in *S.asoca*. Ants were identified as major pollinators. Cross pollinating nature of asoka trees provide the basis for large amount of genetic variability in this species.

5.2.10 Evaluation of reproductive biology traits

The various reproductive biology traits were evaluated by statistical analysis and the results are discussed below.

5.2.10.1 Correlation among reproductive biology traits

Correlation coefficient is a statistical measure which is used to find out the degree and direction of relationship between two or more variables. Character association of a particular trait in relation to other traits is of greater importance in planning successful breeding programme.

In the present study, it was revealed that number of inflorescence per tree and number of flowers per inflorescence were highly and positively correlated with most of the reproductive biology traits except the reproductive period traits. The floral traits such as flower length and flower breadth also indicated significant correlation with all the reproductive biology traits.

The pod traits viz., number of pods per tree, pod length and breadth recorded significant genotypic correlation with all the reproductive biology traits.

Among the seed traits, number of seeds per pod had indicated a high positive genotypic correlation with number of flowers per inflorescence, flower length, pod breadth, seed length, seed breadth and seed volume. Seed length, seed breadth and seed volume had positive genotypic correlation with most of reproductive biology traits. Significant correlation were reported by Carpentar *et al.* (2003) in tropical rain forest tree between seed size and fruit size, between seed size and seed number.

The reproductive period traits such as duration of inflorescence development, duration of blooming, days for flower to pod, days for pod to mature expressed positive significant association with total reproductive period. At genotypic level of correlation, the reproductive period traits exhibited a high and significant correlation with most of the reproductive biology traits.

The association analysis indicated significant association among the reproductive traits. For higher production of big sized pods, there should be higher number of flowers per inflorescence along with higher number of inflorescence per tree.

5.2.11 Influence of weather parameters on reproductive biology traits

To assess the effect of weather parameters on reproductive biology traits studied, the statistical analysis was conducted and the results are discussed below.

5.2.11.1Genetic variability

Variation in weather parameters over months are presented in Fig.15. The reproductive biology traits of *S. asoca* were compared over various months from June 2007 to May 2008 and graphically represented in Fig.16. Graph indicates that best period for harvesting flowers is in February and March while for harvesting pods is from May to June. Asoka flowers through out the year, but during June to December, very few flowers are produced. Flowering behaviour of 15-20 years old *S. asoca* trees revealed that under Thrissur conditions, maximum flower production occurred during February – March, while maximum mature pods and number of seeds per pod was seen during March-April and February-April respectively (Maiti, 2008). Maximum number of seeds per pod was recorded in March and April.

Significant differences were observed among the various months for all the characters studied except duration of inflorescence development, duration of blooming and days for pod to mature. Significant variation was also identified

Fig.15 Variation in weather parameters over months at College of Horticulture, Vellanikkara

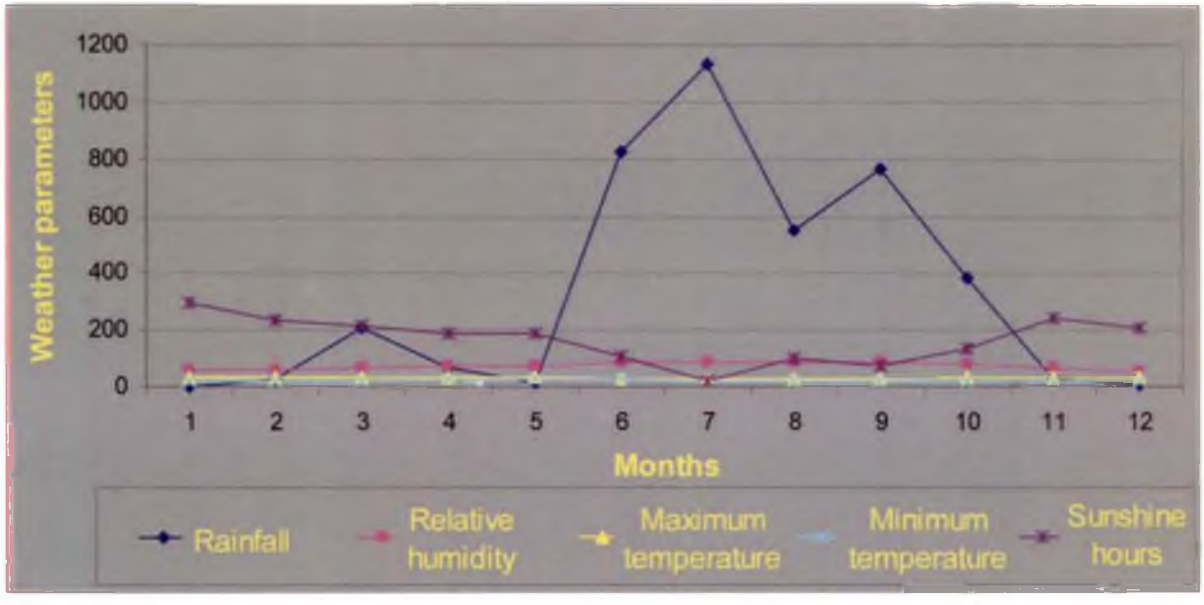
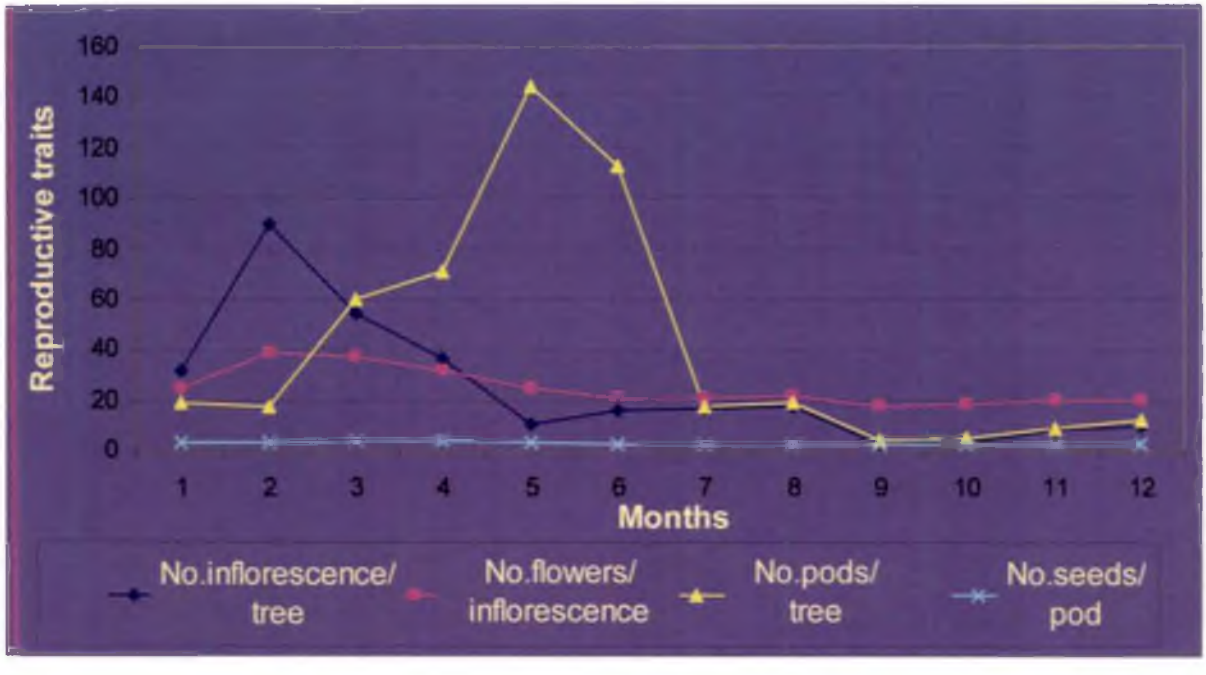


Fig.16 Variation in reproductive traits over months in asoka trees



1.January
2.February
3.March
4.April
5.May
6.June
7.July
8.August
9.September
10.October
11.November
12.December

between the trees for all the characters analysed. Among the reproductive biology traits, maximum variability were exhibited by number of inflorescence per tree, number of flowers per inflorescence and number of pods per tree indicated by their high GCV values. A moderate variability was expressed by the trait, number of seeds per pod. Vasudeva *et al.* (2004) observed that in teak, inter-clonal variation was significant for floral traits, while within the clone variation was negligible.

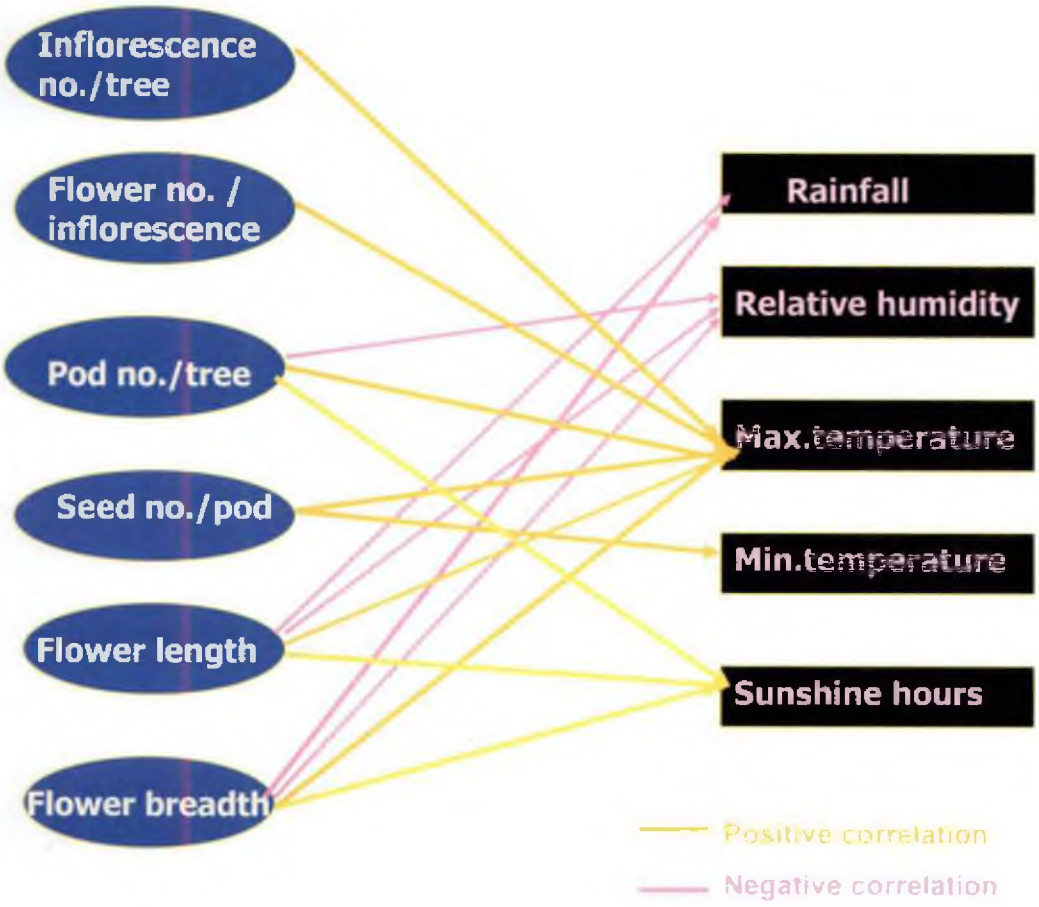
Moderate heritability was noted for the traits, number of inflorescence per tree, number of flowers per inflorescence, number of pods per tree, number of seeds per pod, flower length, flower breadth and days for flower to pod. Vasudeva *et al.* (2004) indicated that the broad sense heritability estimated based on clonal means of floral traits in teak were higher, suggesting a strong genetic control, hence selection could yield beneficial results.

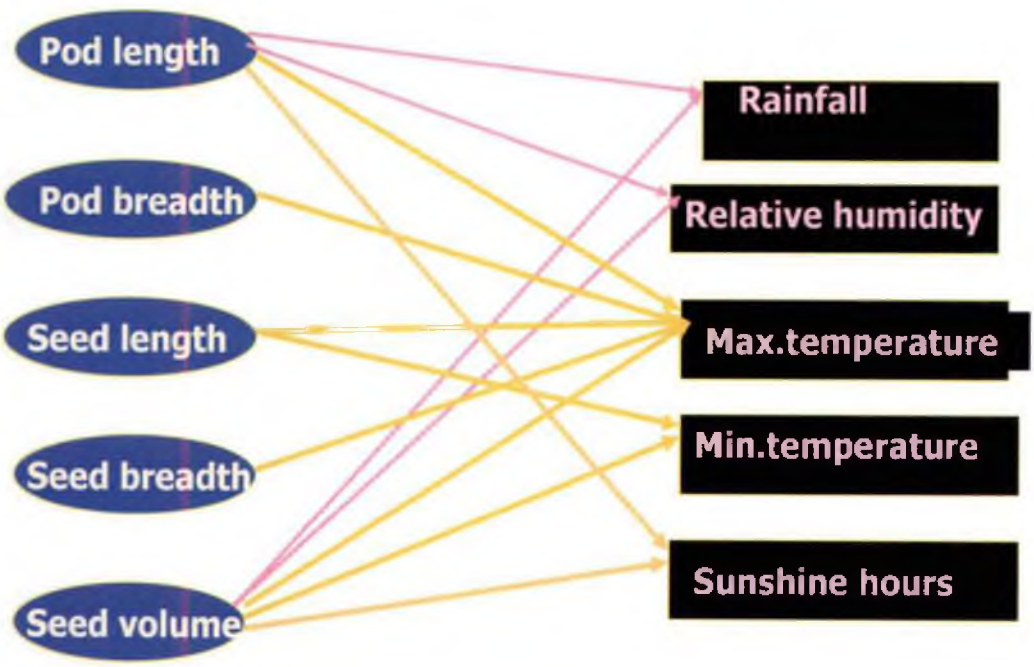
5.2.11.2 Correlation of weather parameters with reproductive biology traits

Correlation coefficient measures the intensity of linear relationship between variables. In genetic studies it is common to find the correlation between two or more characters. Association of reproductive biology traits with weather parameters rainfall, relative humidity, maximum temperature, minimum temperature and sunshine hours were determined and the results are discussed below.

Highest significant positive correlation was observed for maximum temperature with most of the traits except reproductive period traits (Fig.17). This indicated that increase in temperature during the various months had a profound significant influence in increment of these reproductive biology traits. Davarynejad *et al.* (2008) reported that floral traits changes with temperature in sour cherry cultivars. Effect of minimum temperature was observed only in seed traits like number of seeds per pod, seed length and seed volume. Hence a dip in temperature will have little influence on reproductive biology traits, provided the *S. asoca* trees experience a higher day temperature.

Fig.17 Association of weather parameters and reproductive traits in asoka





Other weather parameter viz., sunshine hours showed significant influence on the traits number of pods per tree, flower length, flower breadth, pod length and seed volume. As the sunshine hours increases, pod number, pod length, flower size and seed volume also tends to increase.

The association of weather parameters namely rainfall and relative humidity with reproductive biology traits were negligible except in the case of flower length, flower breadth, pod length, seed volume. Relative humidity also exhibited correlation with number of pods per tree. But rainfall and relative humidity had negative associations with these traits. This was earlier observed by Wolgast and Stout (1977) in Oak and by Krishnamani (2002) in woody plants. Therefore a reduction in rainfall and relative humidity produced in increase in flower size, pod length, seed volume and pod number per tree.

Based on these correlations, it was concluded that bright warm days cause the maximum productivity in asoka. Other weather parameters influenced the reproductive biology traits only when they can change the maximum temperature and the sunshine hours.

5.3 Collection and evaluation of seed and seedling traits

Asoka seeds were collected from 80 trees in different districts of Kerala. These seeds were evaluated for seed traits. Seedlings from these collected seed accessions were assessed for seedling traits. Evaluation of seedlings were carried out for one year from May 2008 to April 2009. The results of investigation of seed traits and seedling traits of the collected eighty accessions are discussed below.

5.3.1 Genetic variability

Variability with respect to the seed traits (Plate 18) were observed among the seeds collected from eighty accessions in different districts of Kerala. The seed traits included seed length, seed breadth, seed volume, number of days for

Plate 18. Variability in traits of seeds collected

germination and germination percentage. Hundred per cent germination in *S. asoca* was achieved within a span of around 80 days as per Kumar *et al.* (2007b). Kumaran *et al.* (1996) studied the variation of traits of seeds collected from 28-one-parent families of neem in seven agroclimatic zones of Tamil Nadu and results showed significant differences among families for all seed parameters studied.

Analysis of variance revealed highly significant difference among the eighty accessions for the seedling characters studied. High magnitudes of GCV were observed for leaflet breadth, number of leaves and height of seedling (Fig.18). Moderate GCV were observed for leaflet length, stem girth, internodal length and number of leaflets per leaf. The traits with high GCV leaflet breadth, number of leaves and height of seedling may be selected to obtain better seedlings. Seedling vigour of the seedling accessions also exhibited significant variation among the accessions.

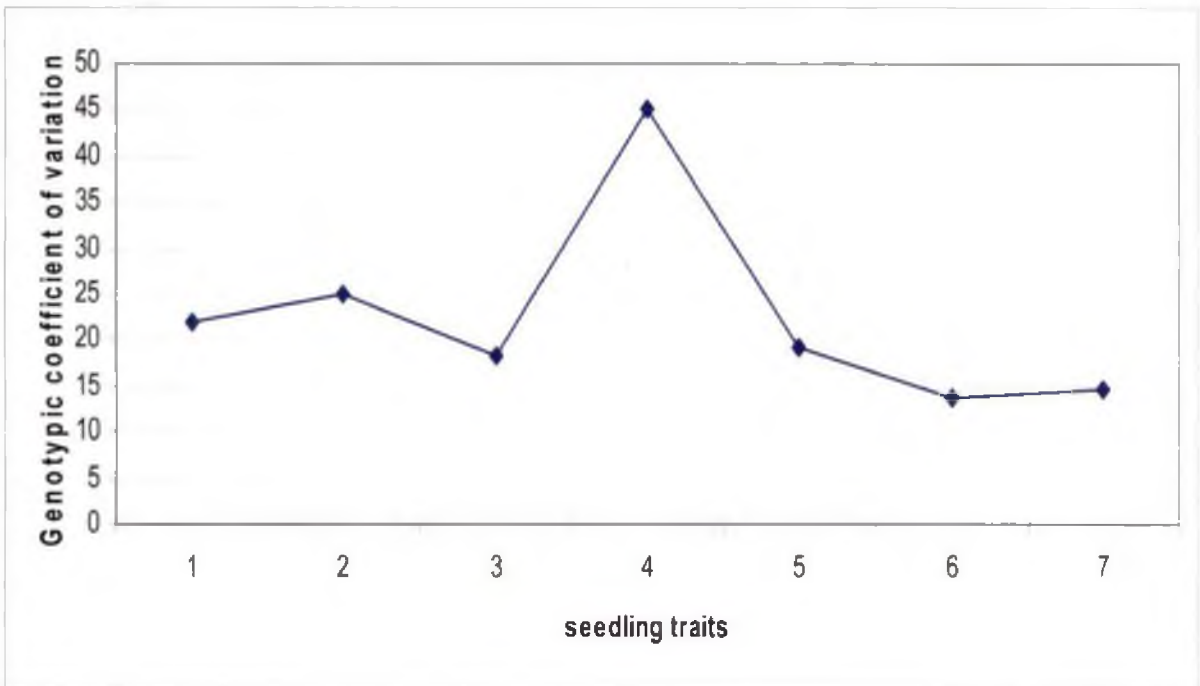
Variability among asoka seedlings had been reported earlier by Nayagam and Varghese (2007). All the seed and seedling traits in *Milletia ferruginea* exhibited highly significant differences among seed sources and the magnitude of genetic variation was substantially higher (77-99%) as per Loha *et al.* (2008). Shivanna *et al.* (2007) observed that the mean values of height of *Pongamia pinuata* seedlings indicated significant differences between seed sources.

In asoka, variability was observed among the seed and seedling traits of eighty accessions collected from different districts of Kerala.

5.3.2 Association of traits

The correlation of seed and seedling traits with seedling vigour were assessed. Among seed traits, seed breadth and seed volume indicated high positive significant association with seedling vigour (Fig.19). Of the seedling traits, height of seedling and stem girth pointed out maximum genetic association with seedling vigour (Fig.20). Therefore bigger sized seeds with tall seedlings

Fig.18 Variability in seedling traits



Seedling traits

1.Height of plant
2.Number of leaves
3.Leaflet length
4.Leaflet breadth
5.Number of leaflets per leaf
6.Internodal length
7.Stem girth

Fig.19 Association of seed traits with seedling vigour

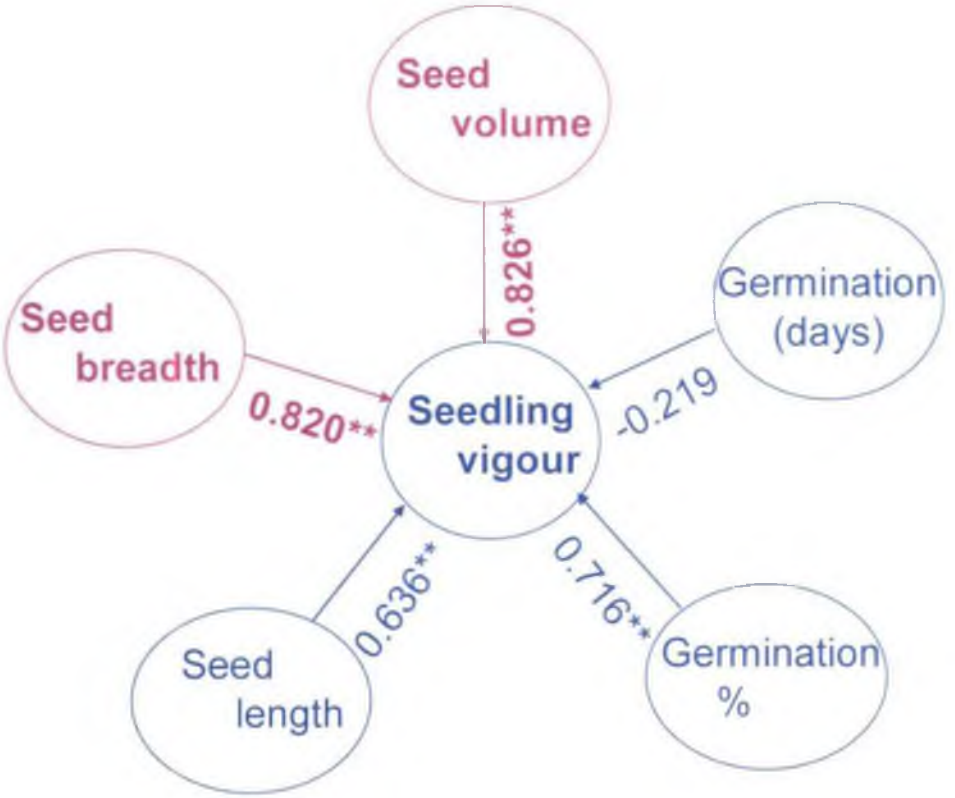
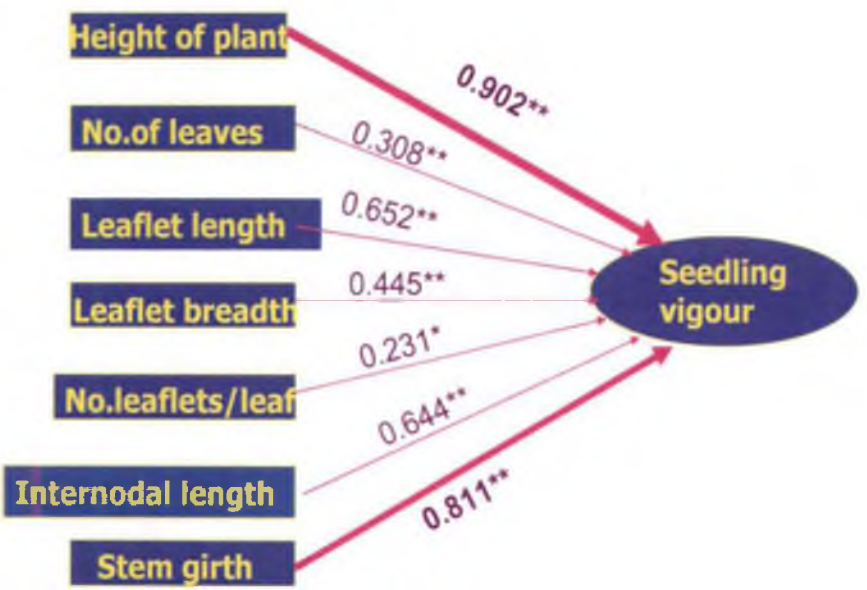


Fig.20 Association of seedling traits with seedling vigour



having higher stem girth can be selected as selection parameters for vigorous seedlings. Using this selection parameters, better asoka accessions can be selected at seed and seedling stage.

Seedling size and uniformity of growth were considered to be a function of germination patterns which were strongly influenced by seed size (Dunlap and Barnett, 1983). Seed sources with heavier seeds in *P. pinnata* possessed higher germination per cent than that of smaller and lighter seeds (Shivanna *et al.*, 2007). In *Gmelina arborea*, analysis of juvenile data showed that, of the two juvenile traits measured (basal diameter and total height), total height at 9 months of age was the best predictor of performance with genetic correlations ranging from 0.50 to 0.90. These results suggested that early selection at 9 months in gmelina clonal plantings is feasible and is a practical means of reducing the breeding and testing cycles (Padua, 2004).

Based on four selection parameters namely seed breadth, seed volume, height of seedling and stem girth, the 80 accessions collected were ranked. The four accessions (Plate 19) identified as better accessions as per selection parameters were OKL4 and OKL2 from Odakkali, KKL2 from Kottakkal and TVM 2 from Thiruvananthapuram.

5.4 Evaluation of therapeutical components of *S. asoca*

Asoka stem bark acts as a uterine sedative and flower is used as uterine tonic (Joy *et al.*, 1998). As per Dhiman (2006) asoka bark contain tannin and catechol and an active phenolic glycoside depending upon the availability place, time of collection and storage condition. Therefore the therapeutically active constituents namely tannin and phenol content were assessed in different age groups of asoka accessions. The materials used for the study included bark, flowers and leaves of KAU campus trees (9-31 years), bark and leaves of germplasm accessions (3-4 years) and the leaves of seedling (1 year) raised from seeds collected. The results of biochemical analysis in terms of tannin content and phenol content are discussed below.

Plate 19. Promising accessions based on selection parameters

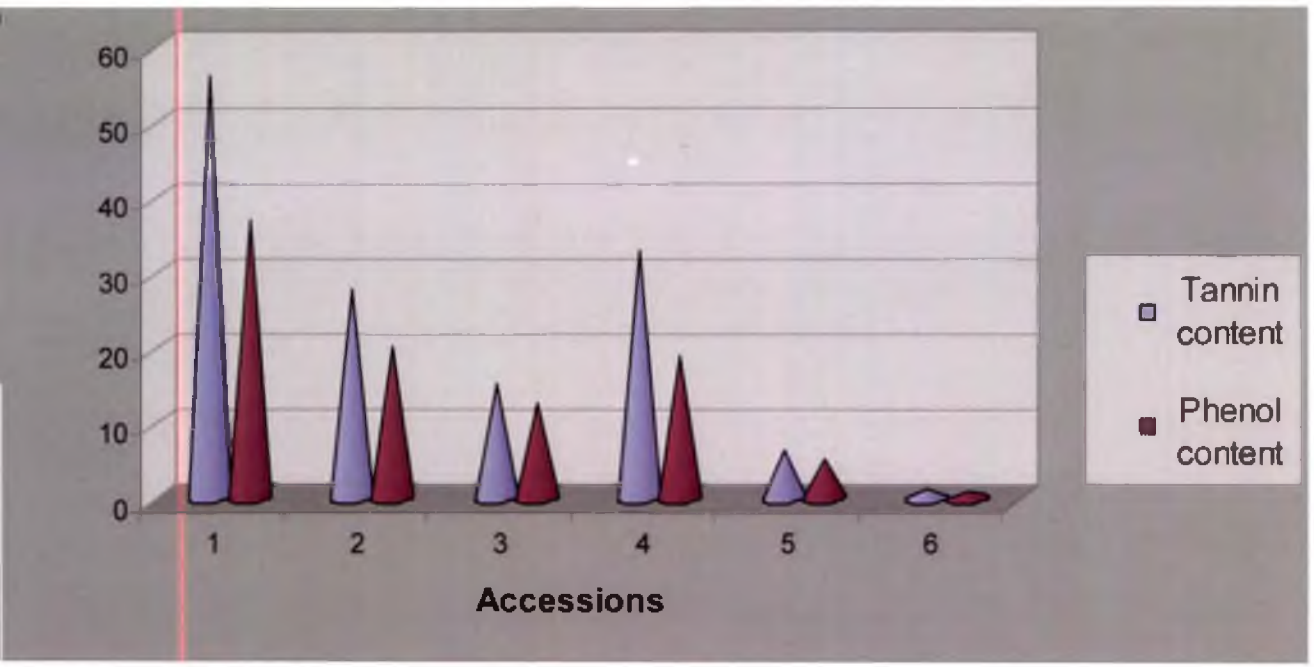
5.4.1 Tannin and phenol content

Tannins are one among the several secondary metabolites found in plants. Secondary metabolites are end products of primary metabolism and in general not involved in metabolic activity. These are synthesized in very small quantities in specialized cells at particular developmental stages, making their extraction and purification difficult. Phenols are widely distributed in plants, usually in combination with sugars as glycosides. Polyhydric phenols are powerful reducing agents. Simple phenols such as catechol are not widely distributed, the most common being hydroquinone. Phenols are said to offer resistance to diseases and pests in plants. The major therapeutical constituents in asoka are tannin content and phenol content. Therefore an insight into the magnitude of these biochemical contents in various parts as well as in different accessions of asoka was undertaken.

Results of biochemical analysis in terms of tannin content and phenol content in various parts in different age groups of asoka are graphically represented in Fig.21. Of the various materials used for analysis in asoka trees, bark had higher tannin and phenol content than flowers and leaves. In *S. asoca* bark, presence of fair amount of tannin (6%) had been reported earlier by Skaria *et al.* (2009), Singh and Panda (2005), Nesamony (1995), Madukakuzhi (1993). Phenol content in asoka bark was assessed earlier by Chatterjee and Prakashi (1992), Joshi (2000). In comparison to the bark, the flowers recorded 50% biochemical contents. Due to this, flowers are also used along with bark in Ayurvedic preparations. Asoka leaves exhibited low therapeutical constituents than bark and flowers. Asoka leaves indicated only 50% of tannin and phenol content in flowers, suggesting its absence in Ayurvedic preparations.

In 43 germplasm accessions, high tannin and phenol content was observed in bark than in leaves. Maiti (2008) reported that asoka bark contained tannins. More than 50% of therapeutical constituents in tree bark were identified in the bark of germplasm accessions. About 40% of tree leaf biochemical contents only

Fig.21 Biochemical contents in different age groups of asoka



1.Bark of mature trees
2.Flowers of mature trees
3.Leaves of mature trees
4.Bark of germplasm accessions
5.Leaves of germplasm accessions
6.Leaves of seedlings

were present in leaves of germplasm accessions. In germplasm accessions, tannin and phenol content were high compared to leaves. Therefore the bark can be used in Ayurvedic preparations. In contrast to this, leaves cannot be used in medicinal preparations as they recorded a low biochemical values.

Tannin and phenol content in leaves of seedlings were only 20% of that present in germplasm accession leaves. In comparison to the tree leaves, the biochemical contents were very low (7%) in seedling leaves. Therefore the estimates of therapeutical constituents in seedlings could only be used to distinguish the seedlings on the basis of tannin and phenol contents.

Based on estimation of biochemical contents in asoka, it was identified that higher tannin and phenol content are in bark than in flowers and leaves. About 50 per cent of therapeutical constituents in bark are available in flowers and hence at nondestructive level, flowers of asoka can be substituted for bark in Ayurvedic preparations. Among the asoka accessions, the trees such as KAU8, KAU7 and KAU6 (Plate 20) and germplasm accessions namely IC566474, IC566467 and IC566476 (Plate 21) were indicated as better accessions in terms of tannin and phenol content. Seedling accessions VKA6, VKA7, VKA8 and KMK3 (Plate 21) were better accessions with high biochemical constituents. Therefore these accessions may be selected for obtaining accessions with higher therapeutical constituents.

5.5 Molecular characterisation

5.5.1 RAPD analysis

Morphological and biochemical markers though widely used for characterization are influenced by environmental factors. An efficient method to fingerprint the genotypes without the effect of environment is the use of molecular markers.

Plate 20. Promising trees with high tannin and phenol contents

Plate 21. Promising accessions with high tannin and phenol contents

The RAPD technique is relatively simple and rapid approach. The use of RAPD for assessment of variability in different crops had been reported by several workers (Philip *et al.*, 2000; Darokar *et al.*, 2001; Das *et al.*, 2004).

Ten germplasm accessions of asoka representing six districts of Kerala were used for molecular characterization. Genomic DNA were isolated from these accessions using modified CTAB method and RAPD analysis was carried out.

5.5.1.1 Screening of random primers

The decamer primers supplied from Operon Technologies Inc. USA were used for the study. A total of ten decamer primers, three each from OPA series and OPRN series, two from OPAH series, one each from OPF series and OPRY series were screened initially. Four primers namely OPRN8, OPA8, OPAH9 and OPA21 were selected based on the number of bands, quality of amplification and cosistency, for further analysis. Padmalatha and Prasad (2006) had reported the use of OPA series of primers in asoka. Many workers have identified the utility of Operon primers in various medicinal species (Sharma *et al.*, 2009, Mandal *et al.*, 2007, Padmesh *et al.*, 1999, Joy and Maridass, 2008, Nair, 2005, Kalpesh and Mohan, 2008).

Among the ten primers screened, four gave good amplification in the present study. Maximum no. of bands were eight (for OPRN8 and OPA8) followed by six bands for OPA21 and five bands for OPAH9. These four primers were selected for RAPD analysis. Based on the amplification pattern OPRN8 and OPA8 were found to be good for amplification of DNA in asoka and this was further confirmed in screening studies with more asoka accessions. Jaleel (2006) carried out primer screening with twenty primers and among these, five were selected as suitable on the basis of good DNA amplification.

5.5.1.2 Screening of asoka accessions with selected primers

Four selected primers were used for screening ten *S. asoca* accessions in order to assess the variability. Out of total 227 scorable amplified products, 210 (92.5 per cent) were monomorphic and 17 bands were polymorphic (7.48 per cent). The molecular weight of amplified products ranged from 1.9 kb to 0.1 kb. The selected primers brought out only 7.48 per cent variability among the ten accessions.

Padmalatha *et al.* (2007) carried out molecular investigations by RAPD markers in accessions of *Pterocarpus santalinus*. Out of the 40 primers screened, 26 primers selected for the data analysis generated a total of 217 scorable markers, all of which were polymorphic. so use of more primers may be used for further analysis.

RAPD profile with primers OPRN8 and OPA8 indicated amplification in all the ten asoka accessions where as 9 accessions showed amplification with primer OPAH9. Banding pattern for primer OPA21 identified maximum polymorphism among the accessions. Among the primers used for RAPD analysis, amplification pattern of three primers pointed out that the accession IC566488 did not follow the same banding pattern as other accessions. Therefore this accession from Thiruvananthapuram was different from other accessions. The other accessions indicating polymorphism with respect to some bands were IC566456, IC566461, IC566495 and IC566474 (Plate 22).

5.5.1.3 Molecular genetic relationship

The dendrogram constructed from pooled RAPD data pointed out that the ten asoka accessions were grouped into two clusters one large cluster with nine accessions and other small cluster with IC566488 alone. The accession from Thiruvananthapuram IC566488 in separate cluster revealed that this accession was variable from other accessions at its genomic level. IC566497 and IC566498 from Malappuram were grouped in a single group. The accessions from the same

Plate 22. Accessions indicating polymorphic bands

district were observed in different clusters indicating that the clustering pattern of accessions did not follow the geographical distribution of the accessions.

The dendrogram drawn by Farooqui *et al.* (1998) in neem revealed that similarities in RAPD profile of 34 accessions leads to the fact that neem may be having a narrow genetic base. Darokar *et al.* (2001) carried out cluster analysis (UPGMA) for brahmi accessions to determine genetic diversity among accessions. The dendrogram indicated that the genetic similarity between accessions ranged from 0.8 to 1.0. In Gymnema accessions, the dendrogram of RAPD analysis indicated that accessions were divided into two distinct major clusters (Nair, 2005).

5.5.1.4 Comparison of clustering at morphological and molecular level

Morphological markers though widely used for characterization are influenced by environmental factors. An efficient method to fingerprint the genotypes without the effect of environment is the use of molecular markers. RAPD proved to be useful in molecular profiling of different accessions. In order to precisely confirm the morphological variations are due to the genetic variability at its genomic level, molecular characterization of accessions were undertaken.

Clustering at morphological and molecular level indicated a similar pattern. The different accessions were grouped as two major clusters at both levels of clustering. IC566488 from Thiruvananthapuram kept apart as a single cluster in morphological and molecular clustering showing that this accession was genetically variable from the other accessions. IC566497 and IC566498 were grouped in a single group in both the clustering techniques. These accessions collected from Malappuram were grouped in the same cluster. These accessions were divergent from the other accessions. The clustering pattern of accessions did not follow the geographical distribution of the accessions. This revealed that the accessions from the same district were genetically variable.

5.6 Conclusion

Based on the various experiments discussed, the breeding strategy for Asoka is proposed. S.asoca is a cross pollinated crop and hence the breeding strategy for a perennial crop tree can be followed. Based on morphological studies of germplasm, bark yield was identified as the most important selection parameter for better accessions. To select better accessions with high bark yield, traits height of plant and stem girth were the most prominent characters contributing to bark yield. Accessions identified based on these characters also correlated with the accessions identified from molecular characterization. Therefore the parameters in selection used should be bark yield, height of plant, stem girth at mature plant level and seed size, height of plant, stem girth at seedling level. But the biochemical characters does not indicate a side by side relationship with morphological traits. Accessions IC566489, IC566463, IC566482 and IC566488 performed better with respect to morphological traits whereas accessions IC566474, IC566467 and IC566476 indicated better performance with respect to biochemical traits. Medium biochemical and morphological traits may be incorporated in a single accession by adopting recombination breeding between divergent accessions. Elite accessions thus derived cannot be propagated through seeds as it induces variability in these accessions. Therefore prominent accessions resulted may be multiplied by *in vitro* clonal propagation techniques.

Summary

6. SUMMARY

Asoka, a sacred tree of India, literally means remover of sorrow. It is a very valued medicinal plant for its therapeutic constituents in the bark. Due to overexploitation, this became a vulnerable tree species and needs conservation and cultivation. For the development of this plant through breeding, the study was initiated at College of Horticulture, Vellanikkara during the period 2006-2009. The results obtained during the study are summarized as follows

1. High genetic variability was noticed among the 43 accessions maintained in the germplasm of asoka at AICRP on Medicinal and Aromatic Plants, KAU campus, Vellanikkara

2. Maximum genetic variability was for height of plant, number of leaves, stem girth and tannin content

3. Accessions having high magnitude of traits namely height of plant, number of leaves, stem girth and tannin content can be selected for genetic improvement of asoka

4. Association of characters indicated that stem girth, height of plant and number of leaves exhibited higher association with bark yield and hence these traits can be used for selecting better genotypes for higher bark yield

5. For higher bark yield, tall plants having higher stem girth may be selected based on path analysis

6. IC566489, IC566463, IC566482 and IC566488 were identified as better genotypes for higher bark yield

7. Cluster analysis grouped the 43 accessions into two major clusters

8. Clustering pattern did not follow the geographical distributions

9. Accession IC566488 from Thiruvananthapuram grouped alone in one cluster indicated that this accession was genetically different from other accessions.

10. IC566497 and IC566498 collected from Malappuram were grouped in the same cluster. These accessions were divergent from the other accessions.

11. Discriminant functions of different character combinations were formulated and models with maximum expected genetic advance were selected

12. Selection index using single yield component, namely height of plant was found to have maximum expected genetic advance and the selection criteria for asoka was formulated

13. IC566463, IC566489, IC566488, IC566482 were selected as better accessions based on the selection index

14. Inflorescence is an axillary corymb blooming in an irregular manner

15. Anthers are elliptic-oblong, dithecous, dorsifixed and dehisce by longitudinal slits

16. Superior, unilocular ovary with ten to eleven ovules in marginal placentation

17. Anthesis in the fully mature flower buds occurred between 5.00-6.00 am

18. Anthers dehisced between 5-6 am

19. Stigma was receptive one hour after anthesis and continued to be receptive for that same full day

20. Pollen grains are creamy white, tricolpate, round with smooth exine and 100 per cent fertile

21. Absolute pollen viability assessed indicated a value of 45.25

22. *S. asoca* is a cross-pollinated tree and ants were identified as major pollinators

23. Best period for harvesting flowers is in February and March while for harvesting pods it is from May to June

24. Bright warm days favour the maximum productivity in asoka

25. High genetic variability was observed among the seed and seedling traits of eighty accessions collected from different districts of Kerala

26. Bigger sized seeds and tall seedlings having higher stem girth are selection parameters for vigorous seedlings

27. OKL4 and OKL2 from Odakkali, KKL2 from Kottakkal and TVM 2 from Thiruvananthapuram were better accessions as per selection parameters

28. Tannin and phenol content are higher in bark followed by flowers and leaves

29. About 50 per cent of therapeutical constituents in bark are available in flowers

30. Trees KAU8, KAU7 and KAU6 and germplasm accessions IC566474, IC566467 and IC566476 were better accessions for tannin and phenol contents

31. Seedling accessions VKA6, VKA7, VKA8 and KMK3 were better accessions with high tannin and phenol contents

32. OPRN8, OPA8, OPA21 and OPAH9 were rated as the best primers for DNA studies in asoka

33. Dendrogram from pooled RAPD data grouped the asoka accessions into two clusters

34. IC566488 from Thiruvananthapuram in separate cluster indicated that this accession was distinct from other accessions at its genomic level.

35. Accessions IC566497 and IC566498 collected from Malappuram were grouped in the same cluster denoted that these accessions were divergent from the other accessions.

36. Clustering pattern of accessions did not follow the geographical distribution of the accessions

37. Clustering at morphological and molecular level indicated a similar clustering pattern formimg two clusters

38. IC566488 from Thiruvananthapuram kept apart as a single cluster in morphological and molecular clustering showing that this accession was genetically variable from the other accessions

39. IC566497 and IC566498 from Malappuram were grouped in a single cluster in both the clustering techniques. These accessions were divergent from the other accessions.

Future line of work suggested

Future research priorities in asoka are

1. Promote better accessions identified for commercial cultivation

As research programmes in asoka are meagre, there is no variety available that could be suggested for cultivation. The better accessions identified based on this study may be promoted for commercial cultivation.

2. *In vitro* clonal propagation of elite accessions

As asoka is a cross pollinated crop, the elite accessions identified cannot be propagated through seeds as it induces variability in the accessions. Therefore *in vitro* clonal propagation of elite accessions may be undertaken.

3. In depth studies in reproductive biology of asoka

The reproductive studies in asoka were carried out only for an year. So to thoroughly confirm the reproductive behaviour in asoka, further investigations in this area is proposed.

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VARIABILITY IN ASOKA (*Saraca asoca* (Roxb.) DE WILDE)

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ABSTRACT OF THE THESIS

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ABSTRACT

Asoka (*Saraca asoca*) is a sacred tree among the Buddhists and Hindus. It is called sorrow-less tree as it removes the grief. The tree has immense medicinal properties. Its bark is mainly used for correcting uterine problems. The well-known Ayurvedic preparations of asoka bark are Asokarishtam and Asokaghrutham. Due to over exploitation of this tree for its bark, this has now become almost extinct. The International Union for Conservation of Nature and Natural Resources (IUCN) has listed this species under 'globally vulnerable' category. It is also enlisted among the 36 threatened and endangered medicinal plants of India. Due to its acute short supply compared to its demand, various development and research activities are being prioritized to conserve, utilize and improve this species. Therefore the present study was undertaken to assess morphological variations in existing germplasm and to study the reproductive biology of *S. asoca*. Collection of seeds and evaluation of seed and seedling traits, evaluation of therapeutical components and molecular characterisation of asoka were the other objectives of the study.

Variability studies for morphological traits of asoka indicated that height of plant and stem girth have high correlation with bark yield as well as higher direct effect. These traits can be used for identifying better genotypes for higher bark yield. In discriminant function analysis, the selection index involving height of plant along with bark yield constituted for selection criteria among asoka genotypes. Using this selection index, accessions IC566463, IC566489, IC566488, IC566482 were selected as better accessions for higher bark yield. The 43 accessions maintained in germplasm of asoka at AICRP on M&AP were grouped into two major clusters based on morphological traits. These clusters further formed 6 clusters. Accession IC566488 grouped alone in one cluster indicated that this accession was different from all other accessions. In all the other 5 clusters, accessions from Thrissur was included. This indicated that accessions did not follow geographical distributions. Some of the accessions from Thrissur may have relationship with accessions from other districts. IC566497 and

IC566498 collected from Malappuram were grouped in the same cluster. These accessions were divergent from the other accessions.

Reproductive biology of asoka was studied. It indicated that the reproductive traits like floral biology, anthesis, pollen morphology, its viability have variability among eight trees studied in KAU campus. Among the eight trees studied, 'KAU8' have better reproductive traits compared to the rest. Preliminary studies were carried out in pollination system and agents for pollination in asoka. The studies indicated that ants may be one of the pollinating agents.

Seed and seedling behaviour of asoka were studied on seeds collected from 80 trees located in different districts of Kerala. Seed breadth, seed volume, height of plant and stem girth were identified as selection traits for better seedlings. Hence at seed stage, bigger sized seeds with higher seed volume will result into better seedlings. Vigorous seedlings is produced from tall seedlings with higher stem girth and in mature plants for higher bark yield; height of plant and stem girth can be selection traits. Thus the selection parameters were worked out in three stages in asoka. Based on seed and seedling selection parameters, OKL4, OKL2 from Odakkali, KKL2 from Kottakkal and TVM2 from Thiruvananthapuram were selected as better mother plants for higher bark yielding accessions.

The biochemical constituents (phenol and tannin content) imparting medicinal properties were estimated among the different age groups of asoka. Both phenol and tannin contents were higher in bark compared to flower and leaves. About 50 per cent of therapeutical constituents are available in flowers and hence at non destructive level, flowers of asoka can be substituted for bark. The trees KAU8, KAU7, KAU6 and among the accessions IC566474, IC566467 and among the seedlings VKA6, VKA7, VKA8, KMK3 indicated higher phenol and tannin content compared to rest.

Molecular characterisation among the selected asoka accessions representing the different districts of Kerala were studied. RAPD analysis was attempted among ten accessions selected. Dendrogram was constituted based on pooled RAPD data. The ten selected accessions were grouped into two major clusters as done in morphological grouping. In grouping at molecular level, the accession IC566488 kept apart as a single group indicating the same trend at its morphological level. Accessions IC566497 and IC566498 collected from Malappuram were grouped in the same cluster denoted that these accessions were divergent from the other accessions.

The clustering pattern based on molecular characterisation did not follow geographical distribution of accessions.