SEED SOURCE VARIATION IN THE SEED AND SEEDLING CHARACTERS OF ASHOKA [*Saraca asoca* (Roxb.) de Wilde]

by

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Forestry

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF TREE PHYSIOLOGY AND BREEDING
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2010

DECLARATION

I hereby declare that this thesis entitled **"Seed source variation in the seed and seedling characters of ashoka [*Saraca asoca* (Roxb.) de Wilde]"**, is a bonafide record of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society to me.

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ACKNOWLEDGEMENT

With utmost respect and great devotion, I wish to acknowledge the expert guidance of my advisor, ***Dr. A. V. Santhoshkumar****, Associate Professor and Head, Department of Tree Physiology and Breeding, College of Forestry, whose pragmatic suggestions sustained and valuable guidance, timely help and moral support throughout the study period made my thesis work an easy task. I express my heartfelt and sincere thanks to him.*

Iam deeply indebted to my advisory committee member, ***Dr. N. K, Vijayakumar****, Emeritus scientist, Dept. of Tree Physiology and Breeding, for his valuable suggestions and help rendered to me for the smooth conduct of my study.*

I place on record my sincere gratitude to, ***Dr. P. K, Ashokan,*** *Professor, Dept. of Tree Physiology and Breeding and member of advisory committee, for his uninhibited support and encouragement.*

I extend my unreserved thanks to my advisory committee member, ***Dr. E. V. Anoop****, Associate Professor and Head, Dept. of Wood Sciences, for the whole hearted cooperation and valuable advice extended to me during the study.*

With great reverence and commitment I would like to express my heartfelt thanks to ***Mrs. Rekha. R.*** *Scientist, Rubber Board, Kottayam, for her keen interest and valuable suggestions she has provided throughout the entire period of my study.*

My sincere thanks are due to ***Dr. B. Mohankumar****, Associate Dean College of Forestry,* ***Dr. K, Vidyasagaran****,* ***Dr. K, Sudhakara****,* ***Mr. S. Gopakumar****,* ***Dr. T. K, Kunhamu*** *and* ***Dr. P.O. Nameer*** *of College of Forestry, for kindly providing me valuable advice and various facilities for the conduct of my study. The help rendered by* ***Dr. K, C. Chacko****,* ***Dr. Giji Joseph****,* ***Dr. Rose Mary*** *and* ***Dr. Jim Thomas*** *is also remembered with deep sense of gratitude.*

I have no words to express my heartfelt thanks to my dearest friend, ***Miss. V. Vaani*** *for the valuable help rendered to me at the time of great need.*

It was a great pleasure working in the ***Department of Tree Physiology and Breeding.*** *The staff and students here always provided constant support and friendly cooperation throughout my study period. Special mention goes to* ***Mr. Srinivasan****, Teaching Assistant,* ***Mr. krishnadas****,* ***Mrs. Rejani****,* ***Miss. Shoba****,* ***Mr. John Kutty****,* ***Mr. Prashanth****,* ***Miss. Divya*** *and* ***Mr. Arun****. The help rendered to me by* ***Mrs. Sharada*** *and* ***Mrs. Padmavathy*** *is also remembered with gratitude.*

My heartiest thanks goes to ***Miss. Natalya krishnambika****, Teaching Assistant, College of Forestry for her support and help. I owe my sincere thanks to* ***Miss. Neenu Somaraj****,* ***Miss. Neethu Lakshmi****,* ***Mr. Malik Fasil****,* ***Mr. Ajayghosh****,* ***Mr. Sijo Samuel*** *and* ***Mr. Aneesh****, for their constant encouragement and suggestions. I am also indebted to my friends of junior batch for the effort taken by them to help me at the time of need. Special thanks goes to* ***Miss. Delphy Rocha, Miss. Sneha., Miss. Keerthi, Mr. Sreehari, Mr. Shine, Mr. Aneesh, Mr. Sreejith and Miss Sindhumathi.*** *The help rendered by* ***Miss. Jyothy, Miss. Deepa*** *and all the* ***laboures of college nursery****, is remembered with deep sense of gratitude.*

Let me take this opportunity to place on record my heartfelt gratitude to the whole crew of ***College of Forestry*** *for the timely cooperation, constant help and support throughout my study period. I express my sincere thanks to* ***Kerala Agricultural University****, for extending financial and technical support for my research programme.*

I would like to express my unfathomable love and gratitude to ***my parents*** *and* ***brothers*** *who are with me, with all their blessings and prayers and boundless affection for all my achievements in my life.*

Finally I bow my head before ***ALMIGHTY.***

DEEPA. K. S.

Dedicated to
*My **Parents** and **Brothers***

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Introduction

INTRODUCTION

Ashoka literally means 'remover of sorrow' attesting to its ability to cure pain and discomfort. Ashoka, scientifically known as *Saraca asoca* (Roxb.) de Wilde, is an indigenous small evergreen tree of fabaceae family, attaining a height of 6-9 m. Ashoka tree is found wild along streams or in the shade of evergreen forests. This tree is found almost throughout India up to an altitude of 750 m. It is also found in Srilanka and in the islands of Malayan archipelago. Ashoka possess a blackish bark and reddish-brown wood and is also an example for drooping young leaves (Blatter and Millard, 1997). Young leaflets hang downwards in bunches from the ends of the branches, initially with greyish white colour that turns to a reddish tint and then as the leaflets mature it becomes dark green. Flowers are orange to scarlet in colour, in dense corymbose panicles and without petals; pods tapering at both ends, seeds 4-8 ellipsoid-oblong. Flowering time is from December to May and fruiting time is in June-July (Subramanian, 1995).

Ashoka is historically important, with its traditional usage in ayurveda. In most of the ancient ayurvedic text, *Saraca asoca* has been mentioned as famous, for its use in treating gynecological disorders. It is especially relied upon as an astringent to treat excessive uterine bleeding from various causes (including hormone disorders and fibroids), but also for regulating the menstrual cycle and in various complex formulae as a tonic for women. The bark reportedly cures biliousness, dyspepsia, dysentery, colic and piles (Warrier et al., 1996). As medicine, bark, flower, leaves, roots and seeds of ashoka are used. According to ayurveda, bark is astringent, anodyne, analgesic, refrigerant, emollient and uterine tonic. Flowers are antidiabetic and complexion promoting. Roots are anticonvulsant and seeds antilithiasis. Leaves have antispasmodic properties and are local analgesic. Ashoka is also used in

treatment of thirst, burning sensation, blood disorders, biliousness, tumors, piles and ulcers (Gupta, 2004).

Several ancient texts has described about the relevance of ashoka. *Baishajya ratnavali*, a treatise on ayurvedic pharmacopia, describes pharmaceutical preparations out of ashoka – ashokarishtam, ashokaghrutham and ashokavati. Other formulation is ashokaksheerapakam, mentioned in *chakradratham*. Ayurvedic gynoecia products available in the market are prepared out of ashoka as main ingredient and are presented in the form of tablets, tinctures, extracts, decoction, powder, syrups and capsules.

The plant is one of the most sacred trees of Hindus and Buddhists, the flowers being much used for religious ceremonies and temple decorations (Troup, 1921). According to "Brihat Samhithas", ashoka should be planted in each and every home premises. The leaves are used in the celebration of Durga pooja. Folk songs in praise of ashoka tree are sung with the belief that these plants are abode of several gods and goddesses. In Ramayana, Sita Devi, the wife of Lord Rama, when abducted by Ravana escapes from the caresses of the demon and seeks refuge in a grove of ashoka. In addition, Buddhists believe that Buddha was born under this tree.

Moreover, ashoka is reported to be one among the 36 threatened and endangered medicinal plants of South India. The international union for conservation of nature and natural resources (IUCN) has featured it in the red list of threatened species with vulnerable status (Kurian, 2002). The tree is fast vanishing from the wild. The estimated annual requirement of the bark of the ashoka by the Indian pharmaceutical industry was about 2300 tonnes in 2002-2003. The demand for the bark is increasing with an annual growth rate of 15%. It has been reported that around 2250 tonnes of bark is used in the country for preparing varieties of ayurveda

medicines. It is estimated that approximately 70 tonnes of ashoka bark and one tonne of ashoka flowers are used by various ayurveda industry in Kerala (Prabhu, 2007).

The indiscriminate use and unscientific extraction of ashoka bark has lead to acute scarcity of the genuine raw drug and this in turn has lead to cost escalation and wide spread adulteration or substitution of the drug. Taxonomic identification becomes difficult in the case of crude drugs where only plant parts like root, stem, bark etc. are being used. Normally the adulteration or substitution of the drug is being done with materials which look very similar to the raw drug and that are available in plenty. In the case of ashoka the bark is being adulterated with the bark of an ornamental tree polyalthia having similar external appearance (Iyer and Kolammal, 1978). Apart from *Polyalthia longifolia* other species like *Shorea robusta*, *Brownea ariza, Bauhinia variegata* and *Trema orientalis* are sold under the name ashoka and used as adulterants (Srivastava et al., 1988). Such adulteration leads to a wide variation in quality and medical efficacy of the drug.

Requirements of ashoka bark is very high compared to other medicinal plants and species is in short supply (Kala et al. 2006). The tree is therefore vulnerable to extinction. Thus there is a demand for conserving the existing wild populations. An extensive systematic explorations and testing of the available stands are required for providing a sound choice of ashoka for domestication. It is under such circumstances that the present study has been formulated with the following objectives:

1. To evaluate the seed source variation in *Saraca asoca* with respect to its seed characters and germination aspects.
2. To study the variation among different seed sources with respect to its seedling attributes.
3. To understand the karyomorphological aspects of the species *Saraca asoca*.

It is expected that the results obtained from this study will be helpful to know the best available seed source of *Saraca asoca*, among the tested seed sources. In addition, the present study also investigates the karyomorphology of *Saraca asoca*, to confirm its chromosome number. Cytogenetic studies addressing the genus saraca is rather few. The aim of this perspective is to provide more insight into understanding the cytogenetics of the species and to provide additional information on the species for future cytogenetic research. Thus the study to evaluate the seed source variation of ashoka, is expected to screen the naturally available genetic variation within the species and to choose the best available type for reforestation or for future breeding programme.

Review of literature

REVIEW OF LITERATURE

Saraca asoca (ashoka) is a species that has been known for its ethnomedicinal values since ancient times. Various medicinal uses of ashoka have been mentioned in the classical textbooks of ayurveda like *Charaka samhitha*, *Susrutha samhitha* and *Ashtangahridaya*. *Abhidhanamanjari*, *Bhavaprakasha*, *Chakradhutta*, *Bhaishajya retnavali*, *Danavantharinighantu*, *Rajanighantu, Oushadinighantu*, *Amarakosham*, *Sahasra yogam*, *Chikitsa manjari* etc are some of the other texts in which medicinal properties of the tree is highlighted. Apart from the ancient knowledge, studies have also been carried out in modern times to further investigate its medicinal, pharmacological and botanical aspects. Reviewing the literature, it is evident that studies on medicinal properties of ashoka are innumerable while reports on morphological traits of seed, seedling and karyomorphological studies are scanty. This section reviews various studies that form the foundation for the present investigation.

2.1 Medicinal properties and pharmacology of *Saraca asoca*

Saraca asoca or ashoka holds a place of pride among the various medicinal plants because of the wide spectrum medicinal properties. The bark, leaves and flowers of the tree are of medicinal relevance. Prajapati et al. (2003) and Dastur (1970) added the information on medicinal qualities of ashoka. Cosmetic importance of the plant is available in *planta cosmetica* (Kunda and Bole, 1997). The wood qualities were thoroughly investigated by Chauhan and Rao (2003).

Phytochemical investigation revealed the presence of various bio-active components like steroids, sterols, tannins, catechol, catechins, haemetoxylin, ketosterol, crystalline glycoside, saponins and several flavanoids (Sharma et al., 2001). Later on the presence of epicatechin, procyanidin, deoxyprocyanidin, catechin,

leucopelargonidin and leucocyanidin was also detected in the bark. In another study lignan glycosides, lyoniside, nudiposide, 5-methoxy-9-β-xylopyranosyl-isolariciresinol, icariside, and schizandriside, and three flavonoids, epicatechin, and procyanidin B2, together with β-sitosterol glucoside, were isolated from dried bark (Dhawan et al., 1977). The floral part of plant contains oleic, linoleic, linolenic palmitic, and stearic acids, P-sitosterol, quercetin, kaempferol, quercetin- 3-0-P-D-glucoside, apigenin- 7-0-p-D-glucoside, pelargonidin- 3, 5- diglucoside, cyanidin-3, 5-diglucoside, palmitic, stearic, linolenic, p and y sitosterols, leucocyanidin and gallic acid. Seed and Pod contains oleic, linoleic, palmitic and stearic acids, catechol, epicatechol and leucocyanidin (Pradhan et al., 2009; Rastogi, 2003; Jain, 1968; Sadhu et al., 2007).

2.2.1 *Utility of bark*

The bark is the most important part in medicine and is used in the preparation of *Ashoka arishta, Ashoka gritha, Madhukadya awaleha, Devadarvya arishta, Mahamarichyadi taila, Pradarari rasa* and *Kasisadi taila*. Several pharmacological properties of the bark were reported by different workers which include spasmogenic, antibacterial, oxytocic, uterotonic, antioestrogenic, antiprogestational, anti-implantation, antimenorrhagic, antitumour and anticancer (Warrier et al., 1996; Annapurna et al., 1999; Katiyar et al., 2007)

The decoction of the stem bark is an excellent uterine tonic used to treat leucorrhea, dysmenorrhea and excessive uterine bleeding and it regulates female fertility, haemorrhagic dysentery (Sharma and Boissya, 2003). It is also known to regularize the menstrual flow (Sedani, 1990). In addition, bark extract can also control cervitis, leucorrhoea and cervical erosion (Upadyaya et al., 1990). It is suggested that the drug may prove useful in all cases of uterine bleeding. It is reported that the drug acts directly on the uterine muscles thereby reducing pain,

inflammation, excessive secretions and menstrual cramps. Moreover the drug is reported to stimulate the uterus, making the contractions more frequent and prolonged. It is therefore 'woman's best friend' or 'woman friendly tree'.

Saraca asoca in combination with *Emblica ribes* and *Areca catechu* exhibited contraceptive activity (Gowri et al., 1982). No toxic symptoms were observed when this drug was administered. The herbal formulations, containing *Saraca asoca, Tinospora cordifolia* and *Symplocos racemosa* when administered to immature female rats produced uterine weight increase, uterine glycogen increase like ethinyl oestradiol, indicating oestrogen activity (Joglekar et al., 1984). Utility of the bark extract is also extended in treating ulcers, diseases of the blood and tumours (Warrier et al., 1996). In addition, bark of ashoka is good for people with weak heart, nervousness or those who have rapid heart beat (George et al., 2007).

Earlier studies on oxytocic activities of the bark of ashoka was explained by Satyavati et al. (1969, 1970) as well as Middlecoop and Labadie (1986). Pure phenolic glycosides extracted from the stem bark of *Saraca asoca* showed oxytocic activity on the uteri of different species of animals. Mukherjee et al. (1996) were successful in preparing a herbal tonic using an aqueous extract of *Ficus glomerata* fruit, *Saraca asoca* bark and *Woodfordia floribunda* flowers. This tonic was evaluated by studying its effects on normal, estrogen- and tonic-pretreated rat uterus. It induced contractions of the estrogen-pretreated uterus similar to oxytocin. Vasaka (vasicine) and cinchona (quinine) are other plants with oxytocic effects.

Apart from its utility as female friendly tonic the herb is also found to possess antibacterial activity. In a study conducted by Annapurna et al. (1999), *Saraca asoca* bark extracts were screened against the enteric pathogen isolates, namely, *Escherichia coli, Shigella sonnei* and *Salmonella enteritis*. All the extracts used for

the study, other than aqueous extract showed antimicrobial activity with the methanol extract having the highest percentage of activity. In a different study, the phytochemical and antibacterial properties of the bark extract of *Saraca asoca* have been reported by Katiyar et al. (2007). They found that 10 mg/ml acetone and benzene extracts of bark were found to possess high to moderate antibacterial activity against a wide range of bacteria. The study also assured high inhibitory activities of extracts, against *Staphylococcus aureus, Lactobacillus sp., Serratia rubidaea, Proteus vulgaris, Bacillus pumilus and Salmonella typhi*.

The methanolic extracts of *Saraca asoca* was assayed against *Alternaria cajani, Helminthosporium* sp., *Bipolaris* sp., *Curvularia lunata* and *Fusarium* sp. at different concentrations (1000, 2000, 3000, 4000 and 5000 μg/ml). The extracts exhibited good inhibitory activity against *A. cajani*. It was also found to be also effective at lower concentrations against other fungi (Dabur et al., 2007). Along with antibacterial activity, the crude extracts of leaves, flowers, and bark of *Saraca asoca* also exhibited larvicidal activity when kept for 24-48 h at an initial concentration of 1,000 ppm against mosquitoes viz., *Culex quinquefasciatus, Aedes aegypti* and *A. stephensi*. The petroleum ether extract of *S. asoca* leaves and chloroform extract of the bark exhibited more than 50% larval mortality against *C. quinquefasciatus* larvae at an exposure period of 48 hour (Varghese et al., 1992a).

Various researches have shown the anticancerous property of ashoka. Studies are still in progress to explore the anti cancerous property of the tree. According to Panikkar (1994), epicatechin from the bark, inhibited antitumour, anticarcinogenic and antiproliferative activities and produced two to four fold elevations of intracellular reduced glutathione and related enzymes of sarcoma-180 tumour cells. These studies confirmed the anti-tumour chemopreventive and chemoprotective properties of *S. asoca. Saraca asoca* inhibits nuclear factors Kappa B (NF-KB), DNA

interaction. Nuclear factors Kappa B are host cell transcriptional factors which play a central role in regulating transcriptional oncogenes such as that of human papilloma virus indicating its antiviral properties. Study also revealed that the anticancer principles isolated and characterized from *Saraca asoca* helped in reducing toxicity of cisplatin and cyclophosphamide.

It was shown by Hattori et al., (1995) that on repeated oral administration of extracts of *Rhus acuminata* (galls), *Saraca asoca* (bark) *or Strychnos potatorum* (seeds) appreciably suppressed the development of skin lesions induced by herpes simplex virus-1 (HSV-1). The aqueous extract of *Saraca asoca* (bark), inhibited enzyme activity of human immunodeficiency virus type 1 by more than 70% (Kusumoto et al., 1995).

2.1.2 ***Utility of flower***

Like the bark the floral region of the tree is of immense utility. Its flowers ground into paste with water are used to treat haemorrhagic dysentery and the dried flowers are known to reduce diabetes. The flower are considered as a uterine tonic and is used in vitiated conditions of pitta, syphilis, cervical adenitis, hyperdypsia, burning sensation, haemorrhoides, dysentery, scabies in children and inflammation (Warrier et al., 1996). Dried and powdered flowers in the butter milk are recommended twice daily for bleeding piles. To cure head ache, a paste of flowers in the juice of coconut husk is smeared over the forehead. Apart from this, the flowers are useful in promoting complexion. A decoction of the flowers is used as a face wash to remove the black spots (George et al., 2007).

An earlier study to test the antibacterial activity of dried flower buds of *Saraca asoca* in methanolic extract on agar plate showed good results against *Salmonella viballerup, Shigella boydii, Escherichia coli, Vibreo cholera, Shigella*

flexneri and *Shigella dyserteriae* (Jain and Sharma, 1967). Report from the study of Annapurna et al. (1999), has confirmed that the methanolic and aqueous extracts of the leaves showed good activity against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhimurium, Alternaria alternata, Colletotrichum gloeosporioides* and *Drechslera specifera*, and further it exhibited moderate activity against *Saccharomyces cerevisiae* and *Candida albicans* in the disc diffusion assay. Later on another study found that the methanolic extracts from petals of *Saraca asoca* could show broad spectrum inhibitory activity against five gram-positive and five gram-negative bacteria. *Staphylococcus aureus* and *Klebsiella pneumoniae* were found to be most susceptible (Mohan et al., 2006).

Anti cancerous activity of flowers were also explored by various investigations. Cytotoxic studies confirmed the potential anti cancer activities of *Saraca asoca* flowers in vitrousing various tumour cells lines like DLA, S-180, Hela, Vero etc. The extract inhibited the growth of ascites and solid tumours experimentally induced in mice (Varghese et al., 1992b). Oral administration of extract reduced the size of 20-methyl cholanthrene induced fibrosarcomas in mice while the same extract prevented the growth of papillomas induced by 7, 12 di-methyl benz (a) anthracene in mice, beside delaying the onset of papilloma formation significantly (Varghese et al., 1993).

2.1.3 *Utility of leaves*

Scientific studies combined with traditional knowledge disclosed several medicinal properties of leaves of ashoka. Methanol and water extracts of *Saraca asoca* leaves exhibited good activity against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhymurium*. Both extracts showed marked activity against *Alternania alternate, colletotrichum gloesporioides* and *Drechlera specifera* (Seetharam et al., 2003). *Saraca asoca* leaves in ethanol (95 per cent) and water

extract on agar plate were found to possess antibacterial activity when tested against *Escherichia coli,* and *Staphylococcus aureus*. *Escherichia coli* was susceptible whereas *Staphylococcus aureus* gave negative result (Singh et al., 2009).

Another work discovered the blood purifying ability of the leaves. Study revealed that dried leaf powder along with lemon juice is often recommended to restore and purify blood and their juice mixed with cumin seeds is used for stomachache. Studies have shown that leaves can be used to reduce the serum cholesterol level. Ayurvedic practitioners use leaves for skin disease and leucorrhea. It is also recommended to bath children infected with impetigo and scabies, in water boiled with the leaves of *Syzigium cumini* and *S. asoca.* The tender leaves are used against intestinal worms, hoarseness of voice and stomachalgia (George et al., 2007).

2.1.4 *Utility of seed and fruit*

Studies on seeds of ashoka showed its usefulness in treating bone fractures, strangury and vesical calculi. A paste of seed powder in water is applied on the skin to soften the rough or dry skin. Seeds are also useful in problems related to urinary discharges. A herbal preparations of powdered seeds in tender coconut water is used as a remedy for urinary obstruction. Powdered seeds in warm water are given to cure mucous stool. Powdered seeds when mixed with wild turmeric powder and green gram powder are used as a natural soap to prevent skin diseases (Warrier et al., 1996). In Assam the fruits are chewed to control urinary discharge. The bark, flowers and fruits are prescribed in combination with other drugs for the treatment of snake bite and scorpion stings (Rajashekharan and Latha, 2006). Flow cytometric studies and agarose gel electrophoresis of lymphocyte DNA revealed that saracin, a lectin obtained from *S. asoca* seed integument, could induce apoptosis in activated human T-lymphocytes (Ghosh et al., 1999).

2.1.5 *Utility of root*

Dried root of ashoka is used in paralysis, hemiplegia and visceral numbness. It acts as a vulnerary and hastens healing time of skin trauma and broken bones. Paste of roots is useful in freckles and external inflammations, ulcers and skin diseases. It is also used to clean, cool and clear the blood. Other functions are its curable property in itching in eczema, psoriasis and dermatitis. The drug is favorite herb to help relieve pruritis. Also used in scabies and Tinea pedis. It is also known to rejuvenate the complexion and skin tone by curing discoloration or loss of pigmentation. Root is also used in obstruction of urinary passage and ammenorhea. It is drunk after delivery to procure copious lochial discharge. It is capable enough to dissolve oxalic stones present in kidney. There are studies supporting its usefulness in signs of congested uterus and pain, painful periods, clots, ammenorhea and endometriosis. Decoction is also said to be useful in rickets, delayed bone consolidation and calcium deficiency (Nadkarni, 1957).

2.1.6 *Other utilities*

The study conducted by Goyal et al. (2008) explores the effectiveness of *Saraca asoca* leaf powder, a surplus low value agricultural waste, in removing lead ions from aqueous solution. Morphological changes observed in scanning electron micrographs of untreated and metal treated biomass confirmed the phenomenon of biosorption. The findings showed that *Saraca asoca* leaf powder can easily be envisaged as a new, vibrant, low cost biosorbent for metal clean up operations.

As per the tribals of Chattisgarh, persons suffering from mental disorder are recommended to take bath under the shade of ashoka tree. For mental piece, the natives prepare special herbal mala using root pieces of sita ashok and give it to the patients. The patients are advised to put the powdered seeds inside the Pan (Betel vine) and eat it empty stomach (Pradhan et al., 2009).

2.2 Germination studies

Different studies have revealed that seed setting in natural populations in the Western Ghats is very poor and the seed viability lasts only for a short period. *S. asoca* produces large recalcitrant seeds typical to wet evergreen forest tree species with critical moisture content of around 35 per cent. Further insects and mammals heavily predate on young developing fruits as well as mature seeds. The attack of lepidopterous insect is also common in the early stages of fruit formation (Kumar et al., 2004).

Germination studies on ashoka are relatively meager. It is noted that germination of *S. asoca* continues up to 80 days after sowing. Hundred per cent germination was achieved within a span of around 80 days (Kumar et al., 2007). In another study conducted in Kerala, to investigate the seed germination and establishment of *Saraca asoca*, germination started one month after sowing with an average germination percentage of 83 per cent (Singh et al., 2005). After 150 days, the overall shoot length varied from 14.5 to 18.7 cm and root length varied from 12.5 to 28.9 cm. On an average 13 leaves were produced. The seedlings were observed to be healthy and free of diseases.

Reports on poor seed set in natural populations of the species in the Western Ghats and difficulties in large scale propagation of the species have been recorded (Warrier et al., 2007). In this respect, findings of Sasidharan and Pathrose (2007) were of immense help. The Study proved that stem cuttings excised at the nodal region can be taken as the best method for maximum rooting and only a small concentration of rooting hormone was required. Further the study revealed that clonal plantlets by air layering were also possible. It is a recognized fact that seeds of ashoka can germinate without any pre-germination treatment.

A number of reports on germination pattern of various species have been cited in the literature. In an investigation carried out by Manga and Sen (1996) genetic parameters for 6 seed traits (weight, length, width, thickness, volume and density) and percentage germination were studied in 51 accessions of *Prosopis cineraria*, mostly from the state of Rajasthan, but with four each from Gujarat and Haryana. Seed weight and volume exhibited high genetic variability, heritability and genetic gain. A correlation study showed that seed weight, seed volume and seed thickness had significant and positive associations with germination percentage. Therefore, these traits should be given priority for improving germination in *Prosopis cineraria*. Variation in seed germination and juvenile growth of 12 provenances of Albizzia *lebbeck* in arid India was studied by Kumar and Toky (1996). Significant differences among most of the provenances in seed germination, with variations of 5-94 per cent in the incubator and 8-50 per cent in the nursery was observed. The variations did not show a significant relation with the latitude or longitude of the seed source, but are likely to be important in the selection of vigorous provenances based on seed germination and juvenile characters.

In a different study, variation in germination was investigated (Siril et al., 1998) in seeds of the multipurpose tree *Sapium sebiferum* obtained from 3 populations in Uttar Pradesh, India. The influence of seed weight and treatment on germination was also studied. Within each population, seed germination was much higher (84.3-89 per cent) for larger seeds than medium (24.6-44.3 per cent) and small (9.6-11.6 per cent) seeds. A similar experiment was conducted to study the germination variation of different *Acacia mangium* provenances at a nursery in Coimbatore, Tamil Nadu. Results showed that the overall germination was 71.9 per cent. Germination variation due to provenances was statistically significant. The highest germination rate was found to be of 84.9 per cent while the lowest was 60.9 per cent.

Differences in seed morphological traits (length, width, thickness, volume, 100-seed weight) and germination were compared for seven provenances of *Grewia optiva* from different parts of the Tehri-Garhwal District of Uttar Pradesh and the Solan District of Himachal Pradesh (Tyagi et al., 1999). The analysis demonstrated genetic variation between the provenances, and highly significant correlations between different pairs of characters. A multiple regression analysis showed that seed length and 100-seed weight might be used as the predictors of germination in *G. optiva*.

2.3 Studies on seed source variation

It can be said that the determination of the best geographic source for a species should be used as the first step in any tree improvement programme. Forest tree improvement programme starts with the study of available variations in the entire range of species (Suri, 1984). Knowledge of variability within a species is a pre requisite for developing effective tree-improvement/ breeding strategies (Vakshaya, et al. 1992). The significance of the genetic variation studies and provenance testing in forest tree improvement is very well realized by different authors. Use of proper seed source helps to attain maximum gains in most of the tree improvement programmes (Zobel and Talbert, 1984). Literature citing the variation existing between different seed sources of ashoka is lacking.

Results of provenance trials in *Dalbergia sissoo* showed that seedlings from Riva, Katni, Bhopal, Guna, Shivpuri and Karera districts were excellent (Solanki et al., 1999). Likewise, ten seed sources of *Dalbergia sissoo* from over a wide range of its natural occurrence in India were studied for germination, and nursery and early field performance at the Forest Research Institute at Jabalpur, Madhya Pradesh (Gera et al., 1999). Significant variations among the provenances were observed in

germination percentage, seedling height and collar diameter, and field height and survival percentage.

A study was conducted to elucidate the pattern of variation in pods, seed characteristics and germination behaviour among *Dalbergia sissoo* seed sources to develop selection criteria for future tree improvement programmes (Singh and Pokhriyal, 2001). Six seed sources were selected to represent widely divergent areas. All pod traits (except number of seeds per pod), seed characters, i.e. length, width, weight (except seed thickness) and germination parameters, that is germination percentage, germination value, germination period, etc., varied significantly among different seed sources. Seed weight and thickness showed highly positive and seed length negative correlation with germination percentage; hence, these can be considered as important traits for the selection of superior seed source. In the same year, Srivastava et al. (2001) collected *Dalbergia sissoo* seeds from 58 marked candidate plus trees from 7 seed sources in Uttar Pradesh, India to evaluate their morphological characteristics and viability. Almost all the morphological characteristics studied revealed significant variations among different seed sources. Mathura seed source obtained the highest seed length, seed thickness, shape index and seed weight of 100 seeds. The maximum viability values of 90 per cent and above were obtained by all seed sources except Ramnagar.

Observations on genotype x environment interactions and stability in neem (*Azadirachta indica*) were carried out by Kundu et al. (1998). Variation in plant height, collar diameter and survival rate of six neem provenances was examined at three test sites in Bangladesh and India after a growing period of about seven months in the field. Three out of the six provenances showed significant genotype x environment interactions. Positive correlations between collar diameter and survival rate at two sites were detected among provenances. Clinal variations were observed

for collar diameter, survival rate and production percentage. In case of studying provenance variation of *Azadirachta indica* in Bangladesh and India (Jodhpur, Rajasthan) 20 sites were taken. Significant differences were observed between provenances in height and collar diameter. Eco-climatic attributes played an important role in the differentiation of neem populations and thereby affected their growth and survival during the early stages of establishment (Kundu, 2000).

A study was conducted by Jalil et al. (2000) to investigate the performance of 10 provenances of *Azadirachta indica* at Seoni in Madhya Pradesh, India. Significant variation was observed among the ten provenances with respect to germination percentage, height, collar diameter and survival. The provenance Betul showed best results with respect to height and survival percentage after four years of growth. Amarkantak provenance was found superior in terms of collar diameter while germination percentage was recorded highest in Indore provenance. Based on the results obtained after four years, provenances of Betul and Bilaspur can be ranked as overall best. Study furnished that, except provenance of Ujjain (the lowest growth performer), all the provenance are suitable for the plantation with fastest growth rate.

In a study by Ginwal et al. (1996), twenty provenances of *Acacia nilotica* covering five countries (India, Sudan, Senegal, Yemen and Pakistan) were compared for variation in certain seed and seedling characteristics under nursery and field conditions of Jabalpur, India. A significant relationship was found between seed weight and growth of seedlings in nurseries and plantations, indicating it to be an important trait for the early selection of provenances. In a similar study to investigate the effect of seed source on physical and physiological qualities of *Acacia nilotica* seeds (Vanangamudi et al., 1999) showed significant differences between seed sources for all characteristics (seed length, width, thickness, 100-seed weight, percentage germination, vigour index and root and shoot length of seedlings) except

percentage germination. Studies were undertaken at the Forest research Institute, Dehra Dun (Uttar Pradesh) to assess the magnitude of seed-source variations among 27 seed-sources of *Acacia nilotica* (Bagchi, 1999). Ten quantitative characters (plant height, clear-bole length, collar diameter, number of branches, first, second and third inter-branch distances and angles of first, second and third branches with reference to the main stem) were recorded in 8-month-old seedlings.

In another case, twenty six seed sources of *Acacia nilotica* scattered over nine states of India were studied to determine the pattern and extent of variation in seed morphology and germination. Statistically significant differences were observed among the seed sources for all the characters studied. The variation in germination percentage was large (34-91.33 per cent) as compared to the other characters studied. Germination percentage was positively and significantly correlated with 100 seed weight and viability, and showed a negative association with purity and number of seeds in 100 g. The seeds from Daltonganj, Jaipur and Raipur had higher germination and were better suited for plantation and tree improvement programme. The study indicated the importance of germination percentage and 100 seed weight as the criterion for selection of seed sources for bulk commercial plantation (Shekar et al., 2002).

Further, Ginwal and Gera (2000) studied seed germination behaviour and growth characteristics of 12 different *Acacia nilotica* populations in a provenance trial at Jabalpur in Madhya Pradesh. The study showed significant difference between the provenances with respect to seed germination and growth (height, diameter, survival percentage). In another case, *Acacia catechu* collected from different seed source of Garhwal Himalayas (India) to understand the variations with respect to different pod and seed morphological characters, significant variations was observed with respect to various characters. Significant inter-genotypic differences were

recorded for all the pod and seed characteristics, that is total number of seeds/pod, healthy and damaged seeds/pod, seed length, width, thickness and seed weight. A number of traits were found to have interrelationship at varying levels of significance.

An experiment was carried out to investigate the effect of four seed zones of Karnataka, India, on the seed quality and seed germination parameters of *Acacia nilotica* (Shivanna, 2002). Significant effects of seed zones were observed in the germination value and seedling fresh weight. Results clearly indicated that sulfuric acid is a suitable treatment for hard coated seeds and in promoting seed germination in *A. nilotica*. Seeds from plantations on clay loam and sandy loam soils were largest, gave the best germination and the best quality seedlings, while seeds from plantations on calcareous, red and black cotton (vertisol) soils and on tank beds which are under prolonged waterlogging gave poor results.

Variation in the chlorophyll content of leaves under four levels of water stress levels was studied under controlled conditions in seedlings from 23 seed sources of *Acacia catechu* from its natural distributional range in India. Considerable differences were observed among seed sources with respect to contents (mg/g fresh weight) of chlorophylls a and b, and total chlorophyll (a+b). Chlorophyll content decreased with increasing water stress in all the sources, but the decrease was higher in seedlings from humid sources than dry sources. An overall high chlorophyll content (a, b and total) was observed in seed sources from drier regions, whereas low contents were observed in sources from humid regions. The chlorophyll contents were negatively correlated with rainfall (Ramachandra et al., 1997).

A study on provenance identification based on leaf morphology of teak was done in FRI, Dehradun (Rawat et al., 1998). The study revealed certain diagnostic characters in plants greater than two year old, on the basis of which different

provenances/sources could easily be identified. A provenance trial in teak (*Tectona grandis*) involving seven provenances from Kerala (India) was conducted (Jayasankar et al., 1999). Germination characteristics did not vary significantly among the provenances in the nursery. But profound variation in seedling growth rates among the provenances was observed. Parambikulam, Nilambur and Malayattur provenances consistently recorded better shoot and root growth, biomass allocation and relative growth rate, while the local provenance (Trichur) was poorest. Study recognized the existing fact that, performance of the provenances in the field followed nursery growth patterns, confirming that growth was under strong genetic control. Therefore, grading of seedlings based on their height and collar diameter at an early age is recommended for selection of best performing seedlings in the field. Results showed that Parambikulam, Nilambur and Malayattur were the best provenances suited for planting in the Trichur District of Kerala, India.

In another study on geographic variation in seed and seedling characteristics in *Pinus roxburghii* from Himachal Pradesh, significant differences among seed sources in seed weight, germination percentage, germination value (GV), root and shoot growth and seedling height after three months were observed. Seed weight significantly correlated with cotyledon numbers and germination percentage, whereas GV significantly correlated with seedling height. Seed weight and cotyledon numbers correlated with longitude of the seed source (Thapliyal and Dhiman, 1997).

Similarly, sandal seeds from various seed sources exhibited significant variation morphologically and physiologically. It is reported that seed parameters (seed length, seed width and seed weight) of nine provenances from Karnataka and Kerala (India), demonstrated a significant amount of variation among the different sources (Sindhuveerendra et al., 1999). Results of study conducted by Manonmani and Vanangamudi (2002) showed that, it is best to collect sandal seeds from

Coimbatore and select only big-sized seeds in order to obtain maximum germination percentage and increased seedling vigour. Mature sandal (*Santalum album*) seeds for this study was collected from Harur, Siruvani, Coimbatore, and Mettupalayam areas of Tamil Nadu, India.

The influence of seed source on the seed and seedling characteristics of *Grewia oppositifolia* was evaluated (Uniyal et al., 2003). There was considerable morphological and physiological variation between provenances for percent of sound seed, seed weight, speed of germination, time to complete germination and percent germination in the nursery. Further important differences in average plant height, growth and various other seedling attributes were found among the seed sources.

2.4 Cytological Studies

In the last few decades of the last century emphasis on classical plant cytogenetics largely declined due to the emergence of molecular biology tools. The first step in cytogenetic research is the investigation on karyotypes of the species. Chromosomal data can be regarded as a valuable tool for cytogeneticists and breeders. They are often providing more insight into taxonomic and phylogenetic relationship (Raven, 1975 and Stuessy, 1990). In India there are about 15,475 flowering plant species, of which chromosome number of only 6,973 (45.06 per cent) species are known. In world flora chromosome number for only 15 to 20 per cent of the 2,50,000 flowering plant is known (Stace, 1980). It appears from the published data that the tree legume species of the subfamily Caesalpinioideae have been poorly analysed cytogenetically. The investigations in this regard have been mainly confined to groups from temperate region. It seems from the literature that cytological research in the genus saraca has been scanty. Hence the report available on cytogenetics of different species has been cited in this section.

Cytological (chromosomal) studies are reported for 47 woody leguminous species from the forests of northern, central and southern India. Of these, *Acacia canescens* (n=13), *Dichrostachys cinerea* (n=26), *Erythrina caffra* (n=21), *Millettia brandisiana* (n=11), *Muscuna hirsuta* (n=11), *Pahudia martabanica* (n=12) and *Phanera* [Bauhinia] *glauca* (n=14) were recorded for the first time. Additional and/or variable cytotypes were recorded in *Bauhinia acuminata* (n=13) and *Prosopis glandulosa* (n=28). The existence of B-chromosome was recorded in *Erythrina caffra* (n=21+0-3B), *Millettia brandisiana* (n=11+0-2B), *Pongamia pinnata* (n=11+0-7B) and *Tamarindus indica* (n=13+0-4B) (Singhal et al., 1990). Many Australian acacias possess a somatic chromosome number of 2n=26. Some of them are *Acacia mangium*, *A auriculiformis* and *A. albida* (Ahmad, 1989; Shukor et al., 1994; Aloni, 1973). In a study conducted at Sudan by Elamin (1976) on *A. leata*, *A. mellifera*, *A. Senegal* it has been found that *A. mellifera* and *A. Senegal* are diploid (2n = 26). Wheras, *A. leata* is triploid (2n=39), but fertile. Many acacias including *A. nilotica*, *A. tortilis* and *A. arabica* evolved in the Indian subcontinent are reported to be polyploides (Ghimpu, 1929; Atchinson 1948; Bir and Kumari, 1985).

Beltran and Kam (1984) studied the cytotaxonomy of zingiberacea and found that, Asiatic kaempferias had a basic chromosome number of x = 11 while African ones have x = 14. In a study, regarding cytogenetic analysis in *Kaempferia galanga* carried out by Rekha (1993), the species was reported to be a polyploid (2n = 5x = 55). Results of studies conducted by Dhamayanthi (2005) in karyomorphological analysis of seven Gossypium species (*Gossypium arboreum*, *G. herbaceum, G. aridum, G. armourianum, G. davidsonii, G. hirsutum, G. barbadense*) genome was carried out and found that all the species invariably shows the basic chromosome number as x=13.

In a recent study investigated by Candan et al. (2009), it was found that the chromosome numbers of *Crocus fleischeri*, *C. pallasii* subsp. pallasii, *C. cancellatus* subsp. lycius and *C. pulchellus* were 2n = 20, 2n = 14, 2n = 16 and 2n = 12,

respectively. Chromosome numbers and karyotype of *Butia eriospatha* and *B. odorata* are being reported for the first time by Correa et al. (2009). Study revealed that that the karyotype of *B. capitata* and *B. yatay* had been never reported before although its chromosome number has previously recorded. Nine specimens of *B. capitata*, three of *B. eriospatha*, three of *B. odorata,* two of *B. paraguayensis* and two of *B. yatay* were analyzed. All species showed the same chromosome number, 2n = 2x = 32, and the same chromosome morphology. In another study, four species of Stigeoclonium from Argentina were investigated by means of transmission electron microscopy and light microscopy. The chromosome number for *Stigeoclonium aestivale* was reported to be 8, *Stigeoclonium tenue* exhibited 5, *Stigeoclonium variabile* gave 3 and *Stigeoclonium farctum* showed 8 chromosome number (Michetti et al., 2010)

Another research work revealed that *Dracocephalum molarica* has five pairs of chromosomes, *Salvia officinalis* L. has seven pairs of chromosomes and *Stachys byzantine* has four pairs of chromosomes. The study followed a completely randomized design with four replications (Tarinejad and Mirshekari, 2010). In an investigation carried out by Stanko et al. (2010), the basic chromosome number for species belonging to Ocimum was found to be x = 12. A karyotype analysis of 147 populations of 25 Brazilian species of *Eleocharis* (Cyperaceae) was carried out, including representatives of the three subgenera that occur in the country: *Limnochloa*, *Scirpidium* and *Eleocharis*. The analyses showed chromosomes without centromeres, but with terminal nucleolar constrictions (satellites) in some chromosomes. The chromosome numbers varied from $2n = 6$ in *E. subarticulata* and *E. maculosa* to $2n = 60$ in *E. laeviglumis*, but the chromosome basic number $x = 5$ was confirmed (Da silva et al., 2010).

Reviewing the literature, it was noted that studies regarding the seed source variation in *Saraca asoca* was scanty. The literature cites only few works with respect to studies related to the germination aspects in *Saraca asoca*. Also, investigations regarding karyomorphological works in ashoka were not cited. Hence the present study is oriented to explore on the above mentioned topics and thus would be useful in cultivation and establishment of better quality ashoka plantations.

Materials and Methods

MATERIALS AND METHODS

The present investigation on "Seed source variation in seed and seedling characters of *Saraca asoca* [*Saraca asoca* (Roxb.) de wilde]" was carried out in the nursey of College of Forestry nursery, Thrissur, Kerala, India, at a latitude of 10^0 32′ N and 76^0 10′ E longitude with an altitude of 22.25 m from mean sea level. The climate is warm and humid with an average rainfall of 3000 mm. The mean maximum temperature is 28.6^0 C (July) to 36.2^0 C (March) and the minimum temperature is 22.2^0 C (December) to 24.7^0 C (May).

3.1 Seed source variation studies

3.1.1 Collection of seed

The seeds of ashoka, for this study were collected from six seed sources of Kerala, India. The whole of Kerala was divided into six equal parts latitude wise (Table 1 and Fig. 1). The seeds were collected in the month of May-June from different locations of each seed source (Table 2). Some of the important localities of seed collection is shown in the Plates from one 1-12. The seeds collected were bulked and used for the study.

3.1.2 Observations on seed traits

Data on various quantitative traits of seeds were recorded for all six seed sources. The seed parameter studies were carried out with a random sample of 40 seeds from each source with four replications. The characters studied and techniques adopted to record the observations are given below.

Table 1. Different seed sources taken for the seed source variation study in *Saraca asoca* based on the latitude

Seed source (SS)	Latitude (from)	Latitude (up to)
SS 1	8° 17′ 30″ N	9° 2′ 31.67″ N
SS 2	9° 02′ 31.67″ N	9° 47′ 33.3″ N
SS 3	9° 47′ 33.3″ N	10° 32′ 35.01″ N
SS 4	10° 32′ 35.01″ N	11° 17′ 36.68″ N
SS 5	11° 17′ 36.68″ N	12° 02′ 38.35″ N
SS 6	12° 02′ 38.35″ N	12° 47′ 40.02″ N

1. Trivandrum
2. Haripad
3. Cherthala
4. Kallara
5. Mala
6. Chalakkudy
7. Ollur
8. Vellanikkara
9. Kottakkal
10. Thenjipalam
11. Kozhikode
12. Kannur
13. Puthiyatheruvu
14. Payyannur
15. Vellur
16. Pilicode

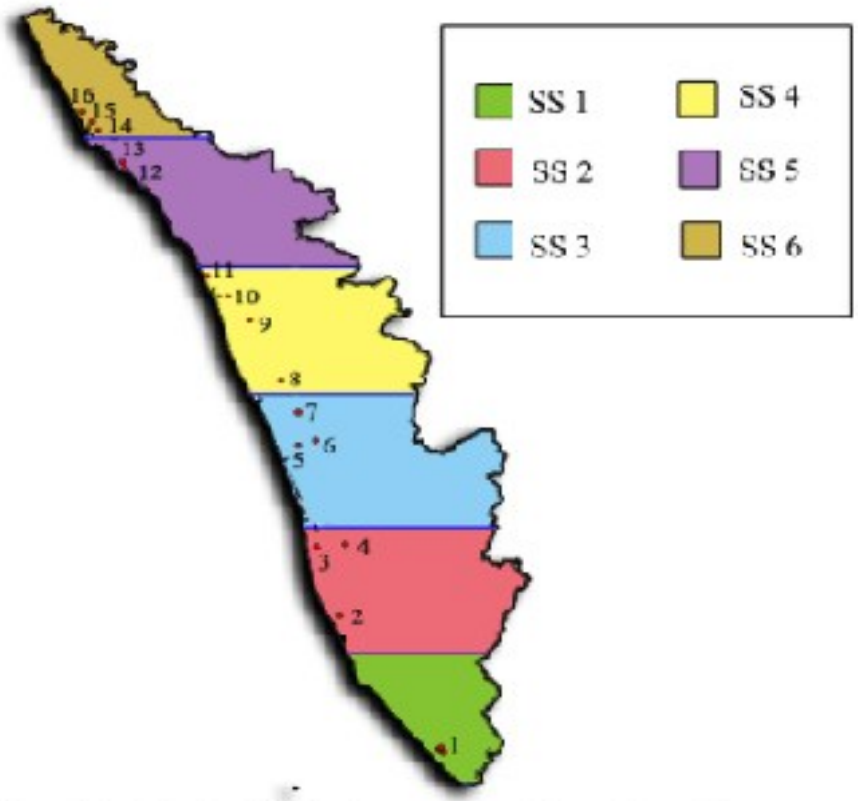


Fig 1. Important locations of seed collection in *Saraca asoca* from six seed sources

Table. 2 Location of seed collection of *Saraca asoca* from different seed sources

Sl. No.	SS	No. of trees	Location	Latitude (N)	Longitude (E)	Altitude (m)
1	SS 1	Tree no.1	Poojapura, Ayurveda hospital	8º29·786	76º58.713	39
		Tree no.2	Poojapura, Panchakarma	8º29.445	76º58.463	60
		Tree no.3	Poojapura, Panchakarma	8º29.443	76º58.466	60
		Tree no.4	Poojapura, Panchakarma	8º29.447	76º58.470	60
		Tree no.5	Poojapura, Panchakarma	8º29.444	76º58.469	60
		Tree no.6	Trivandrum museum	8º30.527	76º57.269	55
		Tree no.7	Trivandrum museum	8º30.514	76º57.328	48
		Tree no.8	Kowdiar, homestead	8º31.052	76º57.907	52
		Tree no.9	Kowdiar, homestead	8º31.003	76º58.058	51
2	SS 2	Tree no.1	Haripad, homestead	9º16.881	76º27.226	6
		Tree no.2	Haripad, homestead	9º16.892	76º27.005	14
		Tree no.3	Haripad, homestead	9º16.801	76º27.283	8
		Tree no.4	Haripad, Subramanya temple	9º16.990	76º27.095	15
		Tree no.5	Cherthala, homestead	9º41.166	76º20.359	3
		Tree no.6	Kallara, Kottayam, homestead	9º41.827	76º28. 971	4
		Tree no.7	Kallara, Kottayam, homestead	9º41.824	76º28.968	4
3	SS 3	Tree no.1	Ollur, Ayurveda college	10º27.851	76º14.532	17
		Tree no.2	Ollur, Thaikkattusery temple	10º27.425	76º14.507	27
		Tree no.3	Chalakudy, Forest office	10º18.457	76º19.981	34
		Tree no.4	Chalakudy,Sacred heart college	10º18.139	76º19.981	18
		Tree no.5	Mala, Ambalaparambu road	10º14.384	76º14.732	8
		Tree no.6	Mala, Puthenchira,homestead	10º16.353	76º14.732	22
		Tree no.7	Mala, Puthenchira,homestead	10º16.077	76º14.696	18
		Tree no.8	Mala, Meladoor roadside	10º13.903	76º18.006	10
4	SS 4	Tree no.1	Vellanikkara, KAU campus	10º32.456	76º10.341	22
		Tree no.2	Vellanikkara, KAU campus	10º32.231	76º10.227	20
		Tree no.3	Vellanikkara, KAU campus	10º32.244	76º10.112	15
		Tree no.4	Kottakal, medicinal garden	10º59.952	75º59.956	89
		Tree no.5	Kottakal, medicinal garden	10º59.958	75º59.925	87
		Tree no.6	Kottakal, medicinal garden	10º59.988	75º59.932	71
		Tree no.7	Kottakal, medicinal garden	11º00.016	75º59.136	71
		Tree no.8	Kozhikode town, Thali temple	11º14.977	75º47.243	6
		Tree no.9	Thenjipalam,Calicut university	11º08.005	75º53.378	69
		Tree no.10	Thenjipalam,Calicut university	11º07.983	75º53.409	65
		Tree no.11	Thenjipalam,Calicut university	11º08.008	75º53.442	86
5	SS 5	Tree no.1	Kannur, Puthiyatheruvu, homestead	11º54.979	75º21.655	6
		Tree no.2	Kannur, Puthiyatheruvu, homestead	11º54.975	75º21.654	6
		Tree no.3	Kannur, Talap, homestead	11º52.941	75º22.427	6
		Tree no.4	Kannur, opp. AKG hospital	11º53.053	75º22.354	21
		Tree no.5	Kannur, Mahathma Bhavan	11º52.637	75º22.341	-2
6	SS 6	Tree no.1	Payannur, Edat, homestead	12º 05.681	75º14.389	12
		Tree no.2	Payannur, Kokkanassery homestead	12º 07.026	75º12.800	2
		Tree no.3	Vellur, homestead	12º08.686	75º12.859	12
		Tree no.4	Pilicode, near RARS, homestead	12º12.197	75º09.982	5
		Tree no.5	Pilicode, Melmattalayi temple	12º12.224	75º09.757	15
		Tree no.6	Pilicode, Melmattalayi temple	12º12.221	75º09.755	15
		Tree no.7	Pilicode, Melmattalayi temple	12º12.222	75º09.756	15

Plates 1-12: Representative tree of *Saraca asoca* from some selected locations

Plate 1: *Saraca asoca* in Trivandrum museum (SS1)

Plate 2: *Saraca asoca* in Poojapura Ayurveda hospital (SS 1)

Plate 3: *Saraca asoca* in Haripad Subramanya temple (SS 2)

Plate 4: *Saraca asoca* in homestead of Kallara (SS 2)

Plate 5: *Saraca asoca* in Ollur Ayurveda College (SS 3)

Plate 6: *Saraca asoca* in Ollur Thaikattusery temple (SS 3)

Plate 7: *Saraca asoca* in Vellanikkara KAU campus (SS 4)

Plate 8: *Saraca asoca* in Calicut university botanical garden (SS 4)

Plate 9: *Saraca asoca* in Thali temple, Kozhikode town (SS 4)

Plate 10: *Saraca asoca* in homestead of Puthiyatheruvu (SS 5)

Plate 11: *Saraca asoca* in homestead, Talap (SS 5)

Plate 12: *Saraca asoca* in Melmattalayi temple, Pilicode (SS 6)

3.1.2.1 Seed length

The length of the seed from base to the tip was measured using digital vernier caliper, expressed in mm.

3.1.2.2 Seed breadth

The breadth at the middle of the seed was measured using digital vernier caliper, expressed in mm.

3.1.2.3 Seed length to breadth ratio

The ratio between seed length and breadth was assessed.

3.1.2.4 Seed weight

Weight of each seed was taken in the electronic weighing balance and expressed in grams.

3.1.2.5 Thickness of pericarp

Pericarp thickness was measured using digital vernier caliper and expressed in millimeters (mm).

3.1.3 Sowing and layout of the experiment

Seeds were sown in polybags of 5 x 6 inches filled with potting mixture of sand, soil and cow dung in the ratio 1:1:1. The polybags were arranged in CRD with four replications for each source. There were 100 polybags in each replication and altogether 400 polybags were arranged in a single seed source. A total of 2400 seeds of ashoka were sown for the nursery study.

3.1.4 Seed germination attributes

Number of seeds germinated was monitored everyday from 8.30 am to 9.30 am, till no further germination was noticed. Based on the germination account the following parameters were recorded.

3.1.4.1 Days required for germination to initiate

Also known as imbibition period (number of days from sowing to commencement of germination) was recorded for each replication of the six seed sources (Hossain et al., 2005).

3.1.4.2 Days required for germination to cease

The day on which the germination came to an end was recorded for each replication.

3.1.4.3 Total germination days

Total germination days = Days required for germination to end –
Days required for germination to start

3.1.4.4 Days required for 50 per cent of germination

Total days required for 50 per cent of germination to occur was estimated. It was assessed from the day after sowing onwards.

3.1.4.5 Germination percentage (GP)

Germination percentage was calculated using the formula,

$$\text{Germination percentage} = \frac{\text{Total seeds germinated}}{\text{Total seeds sown for germination}} \times 100$$

3.1.4.6 Germination energy (GE)

Germination energy was calculated as the percentage summation of daily seed germinated up to the day of peak germination to the total seeds sown for germination (Paul, 1972).

3.1.4.7 Mean daily germination (MDG)

Mean daily germination was assessed using the formula,

$$\text{Mean daily germination} = \frac{\text{Germination per cent}}{\text{Total number of days}}$$

3.1.4.8. Peak value of germination (PV)

Peak value is the maximum mean daily germination reached at any time during the period of germination test.

3.1.4.9 Germination value (GV)

Germination value was estimated according to the method prescribed by Czabator (1962).

$$GV = PV \times MDG$$

3.1.5 Seedling attributes

Seedling attributes were recorded for ten uniform seedlings, which were randomly selected from each replication. Following parameters were recorded for the seedlings.

3.1.5.1 Height

The plant height was measured from the ground level to the tip of the stem by using a meter scale. Mean was calculated for each seed source and expressed in centimeters.

3.1.5.2 Collar diameter

Collar diameter of the seedlings was measured with a digital vernier caliper and it was expressed in millimeters (mm).

3.1.5.3 Leaf number

Numbers of leaves produced by seedlings were recorded. Leaves of ashoka are pinnately compound.

3.1.5.4 Leaf area

Leaf area of the seedlings was measured using CI-202 leaf area meter.

3.1.5.5 Number of branches

Number of branches other than the main stem was noted and its average was calculated.

3.1.5.6 Leaf thickness

Thickness of the leaf was calculated by measuring 50 leaves using digital vernier caliper. The thickness of individual leaves was calculated from the observation and expressed in mm.

3.1.5.7 Biomass

Two seedlings were taken randomly from each replication by destructive sampling method. The following parameters were recorded at monthly intervals:-

a) Fresh weight of shoot

Shoot weight was measured monthly by electronic weighing balance and expressed in grams.

b) Fresh weight of root

Roots were removed carefully from the soil, washed thoroughly, weighed in an electronic weighing balance and expressed in grams.

c) Dry weight of shoot

The dry weight of the shoot was observed after oven drying the shoots at 70°C ± 5°C for 24 hours and expressed in grams.

d) Dry weight of root

The dry weight of the root was observed after oven drying the roots at 70°C ± 5° C for 24 hours and expressed in grams.

e) Shoot-root dry weight ratio

Shoot-root dry weight ratio was worked out for each month using the formula,

$$\text{Shoot-root dry weight ratio} = \frac{\text{Shoot weight (g)}}{\text{Root weight (g)}}$$

3.1.5.8 Relative growth rate (RGR)

Relative growth rate was estimated by the formula,

$$RGR = \frac{\ln W2 - \ln W1}{T2 - T1}$$

where,

ln = natural logarithm

W1 = initial dry weight

W2 = final dry weight

T1 = initial time period

T2 = final time period

3.1.5.9 Chlorophyll content

Chlorophyll content was measured using SPAD 502 leaf chlorophyll meter. The output value can be converted into the amount of leaf chlorophyll content ($\mu mol/m^2$) by the exponential equation $10 \times M^{0.261}$, where M represents the meter output (Markwell et al., 1995).

3.1.5.10 Photosynthetic rate

Rate of photosynthesis of the plant was measured using LI-6400 portable photosynthesis system. Results were expressed in μ mol/m^2/s.

3.1.6 Incidence of pest and disease

The occurrence of pest and disease was observed throughout the study period.

3.1.7 Statistical analysis and cluster analysis

The data obtained from the field were tabulated and subjected to statistical analysis using SPSS software. Cluster analysis was carried out to find the similarity in relationship among different seed sources. It was based on, seed characters, germination attributes and seedling characters of ashoka. The Euclidean distance was selected as distance measure in cluster analysis.

3.2 Karyotype analysis

3.2.1 Collection and germination of seeds

The seeds were collected from the trees growing in Kerala Agricultural University main campus, Vellanikkara, Thrissur. Seeds were then sown in a bed of absorbant cotton and moistened daily.

3.2.2 Cytological studies

In order to do the karyotype analysis of *Saraca asoca,* mitotic studies were carried out using root tip squash method. The roots of newly germinated seeds were used for this purpose.

3.2.2.1 Pre-treatment of roots

Two pre-treatment chemicals namely 8-hydroxyquinoline and colchicine were used for the present study. The pre-treatment chemicals along with its preparation and application technique is shown in the Table 3. Roots of about 2 cm, collected from freshly germinated seeds and thoroughly washed were used for treatment.

3.2.2.2 Fixing of the roots

Two fixatives, namely Carnoys I and Carnoys II were tried for the study. The compositions of the fixatives are given in Table 4. The pretreated roots were washed and put into the fixatives for 24 hours.

3.2.2.3 Squashing and staining of the roots

After 24 hours, fixed roots were taken out and treated with stain. The two different stains used for the study were acetocarmine and fuelgen stain. Details regarding the preparation of the stain squashing techniques are given in Table 5.

Table 3. Pre-treatment chemicals used for standardization procedure in the cytogenetic analysis of *Saraca asoca*

Pretreatment Chemicals	Preparation	Application technique
8-hydroxyquinoline	Saturated solution is prepared in distilled water	Root tips were immersed in the solution for 4 hours at refrigeration.
Colchicine	0.5 % solution is prepared in distilled water	Root tips were immersed in the solution for 1- 2 hours at room temperature.

Table 4. Fixatives used for standardization procedure in the cytogenetic analysis of *Saraca asoca*

Fixatives	Preparation	Application technique
Carnoys I	1 part glacial acetic acid + 3 parts ethyl alcohol	Root tips were kept in the solution for 24 hours at room temperature.
Carnoys II	1 part acetic acid + 3 parts chloroform + 6 parts ethyl alcohol	

Table 5. Stains used for standardization procedure in cytogenetic analysis of *Saraca asoca*

Stain	Preparation	Application technique
Acetocarmine 1%	Hundred ml of 45 per cent acetic acid was boiled. Then 1 g of carmine powder was added with constant srirring. Boiling was continued for 2-3 minutes until the dye got dissolved and the colour changed to grape red. The stain was cooled to room temperature, filtered and stored in a glass stoppered bottle (Sharma and Sharma, 1980)	A few drops of stain was added over the root tip placed on a slide. The slide was then heated for nearly 10-15 minutes simultaneously adding to it. The treated root tip was placed on the slide and covered with a cover slip. Root tip was squashed using the thumb and also gently tapped over the cover slip. The preparation was viewed through the microscope.
Fuelgen stain	In 100 ml boiling distilled water, 1 g of basic fuchsin was dissolved. It was cooled to 58^{o}C and filtered. Allow to cool up to 26^{o}C. Then 30 ml 1 N HCl and 3 g potassium meta-bisulphite were added followed by vigorous shaking for three minutes, the bottle was sealed well, wrapped with black paper and kept overnight in a cool dark chamber. Next day, a straw yellow colour was observed. If it does not occur, 2 g charcoal can be added, shake vigorously and filter it. The stain was kept in dark coloured bottle in a refrigerator (Sharma and Sharma, 1980)	After washing the root tip thoroughly it was hydrolysed in 1N HCl at 60^{o}C for 20 minutes. After that root tips were rinsed in double distilled water and transfer it to fuelgen stain. Adequate staining was achieved after 1 to 2 hours. The stained portion of the root was cut and placed over a clean glass slide. A drop of 1% acetic acid was added to the preparation a cover glass was placed over it. Squashed preparation was viewed through the microscope.

3.2.2.4 Standardization procedures

Standardization of the cytological procedure was done by varying combination of pre-treating chemicals, fixatives and stains as detailed in Table 6.

3.2.3 Preparation of karyotype

Photographs of well spread metaphase stages were used for the preparation of karyotype. Individual chromosome figures were cut and arranged in an order according to their lengths. Chromosomes having equal length were pasted together and considered as homologous pairs.

3.2.4 Preparation of idiogram

Chromosome count of ashoka was made from a well spread mitotic metaphase stage. Length of each chromosome was measured using the image analyser (LABOMED iVu 3000 model). Two parameters, namely total chromosome length (TCL) and relative chromosome length (RCL) was also calculated from the measurements. While TCL was estimated as the sum total of the haploid complement, RCL was estimated as the percent of TCL. RCL was calculated for each of the chromosome as below.

$$RCL = \frac{\text{Length of individual chromosome}}{\text{Total haploid chromosome length}} \times 100$$

Table 6. Different combination of chemicals used for standardizing the cytological procedure in *Saraca asoca*

Pre-treatment chemical	Fixative	Stain
8-hydroxyquinoline	Carnoys I	a) Acetocarmine b) Fuelgen
	Carnoys II	a) Acetocarmine b) Fuelgen
Colchicine	Carnoys I	c) Acetocarmine d) Fuelgen
	Carnoys II	c) Acetocarmine d) Fuelgen

Results

RESULTS

The study on "Seed source variation in the seed and seedling characters of ashoka [*Saraca asoca* (Roxb.) de Wilde]" was carried out during the period 2008-2010 in College of Forestry, Vellanikkara, Kerala, India. The results obtained from this study are presented below:-

4.1 Seed source variation studies

Seed source variation in *Saraca asoca* was evaluated from six seed sources of Kerala. The seeds collected from different localities of a seed source were bulked and used for the study. In order to understand the variation, seed traits, germination aspects and seedling parameters were studied. The results obtained are presented here under.

4.1.2 Observations on seed traits

Data pertaining to the morphological variations of seed from the six seed sources have been furnished in the Table 7. Different seed traits, like seed length, seed breadth, seed length to breadth ratio, thickness of pericarp and seed weight was assessed in the present experiment with respect to different seed sources. The photographs of seeds collected from different seed sources are displayed from Plate 13 to Plate 18. The result for different seed source is described in detail ahead.

4.1.2.1 Seed length

The data on seed length revealed that seeds collected from seed source SS 3, was the shortest with a recorded value of 33.1 mm (Plate 16 and Fig. 2). The remaining seed sources, SS 1, SS 2, SS 4, SS 5 and SS 6, exhibited values which were on par with each other. Therefore, marked variation was not displayed among these seed sources, in terms of seed length.

Table 7. Seed characters of *Saraca asoca* collected from six seed sources

Seed source	Length (mm)	Breadth (mm)	Length/breadth	Weight (g)	Pericarp thickness (mm)
SS 1	40.3[a]	25.3[ab]	1.6[a]	14.0[ab]	2.3[a]
SS 2	40.3[a]	27.4[a]	1.5[b]	14.4[a]	2.7[a]
SS 3	33.1[b]	21.3[c]	1.6[ab]	8.8[d]	2.3[a]
SS 4	39.9[a]	26.9[a]	1.5[b]	13.2[ab]	2.5[a]
SS 5	37.6[a]	24.0[b]	1.6[ab]	10.9[c]	1.7[b]
SS 6	38.6[a]	25.9[ab]	1.5[b]	12.7[b]	2.3[a]
SEm±	1.0	0.8	0.0	0.4	0.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Plates 13-18: *Saraca asoca* seeds collected from the six seed sources

Plate 13: Seeds from seed source SS 1

Plate 14: Seeds from seed source SS 2

Plate 15: Seeds from seed source SS 3

Plate 16: Seeds from seed sourcs SS 4

Plate 17: Seeds from seed source SS 5

Plate 18: Seeds from seed source SS 6

4.1.2.2 Seed breadth

Observation on seed breadth revealed that significant differences were existing between various seed sources (Table 7 and Fig. 3). Seed breadth was maximum and on par with each other in case of seed sources SS 2 (27.4 mm) and SS 4 (26.9 mm). Likewise seed source SS 1 gave near to the same value with seed source SS 6 in terms of seed breadth, with a recorded value of 25.3 and 25.9, respectively (Plate 13 and Plate 18). Seed breadth for the seed source SS 3, exhibited the least value (21.3 mm) among different seed sources (Plate 15).

4.1.2.3 Seed length to breadth ratio

The data regarding seed length to breadth ratio is given in Table 7 and illustrated in the Fig. 4. The difference with respect to seed length to breadth ratio is also depicted in the plates 13 to 18. Seed length to breadth ratio was maximum for SS 1 (1.6). Seed sources SS 3 and SS 5 were also on par with each other. From the data given in the Table 7, it is clear that SS 2, SS 4 and SS 6 showed similarity in seed length to breadth ratio, the values exhibited by them were on par with each other. Seed source SS 1, SS 3 and SS 5 were more elongated when compared to seed source SS 2, SS 4 and SS 6, which appeared to be more roundish.

4.1.2.4 Seed weight

Statistically significant difference was exhibited by different seed source with respect to seed weight. The data pertaining to seed weight is tabulated and illustrated in the Fig. 5. Variation in seed weight was observed ranging from a maximum of 14.4 g for SS 2 to a minimum of 8.8 g for SS 3. Seed sources SS 1 with 14.0 g and SS 4 with 13.2 g was on par with SS 2 in terms of seed weight. Seed weight for SS 6 was comparatively lesser than SS 4 but was statistically par with each other. Seed source SS 5 was found to be better in weight when compared to SS 3. Seeds from seed source SS 3 being smaller in size also displayed the lowest weight.

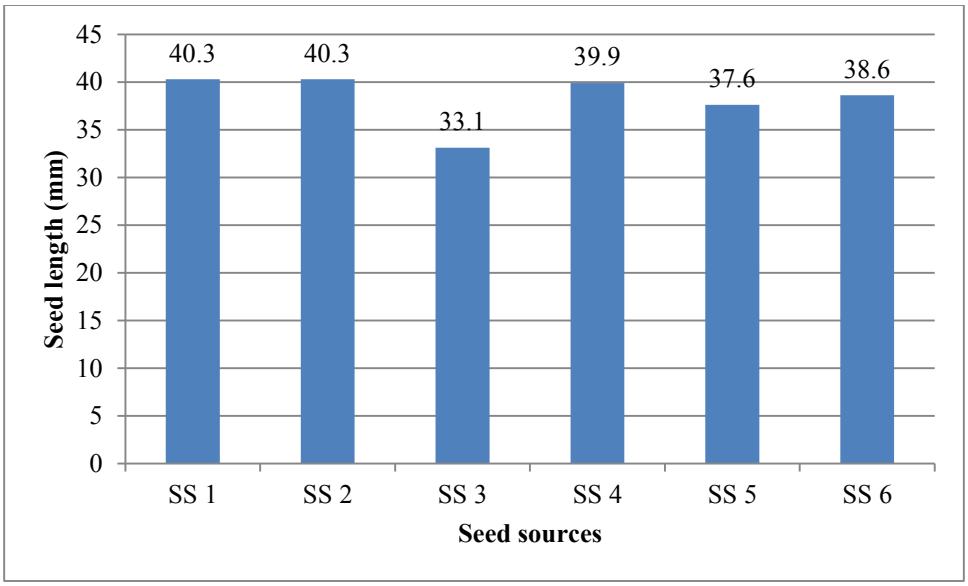


Fig. 2: Length of *Saraca asoca* seeds collected from six seed sources

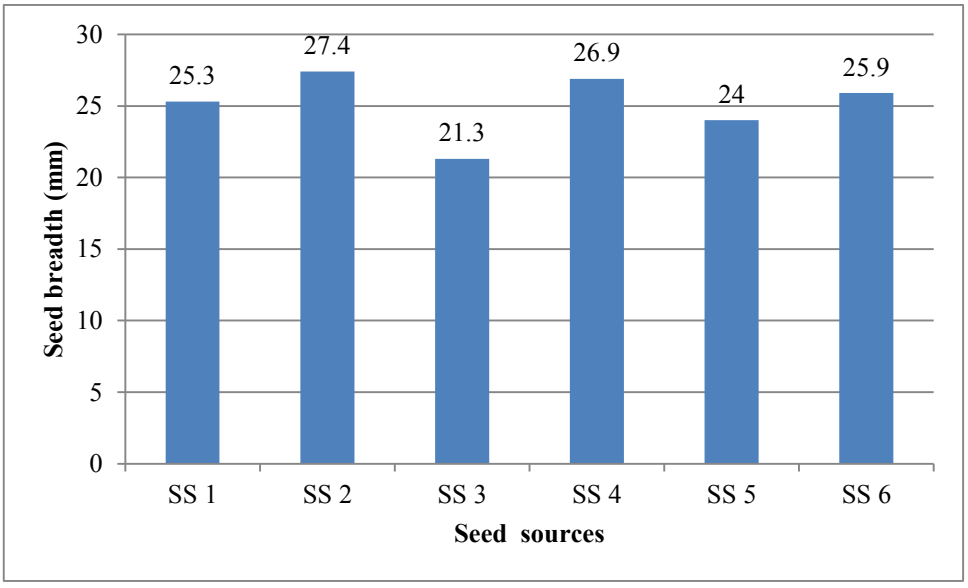


Fig. 3: Breadth of *Saraca asoca* seeds collected from six seed sources

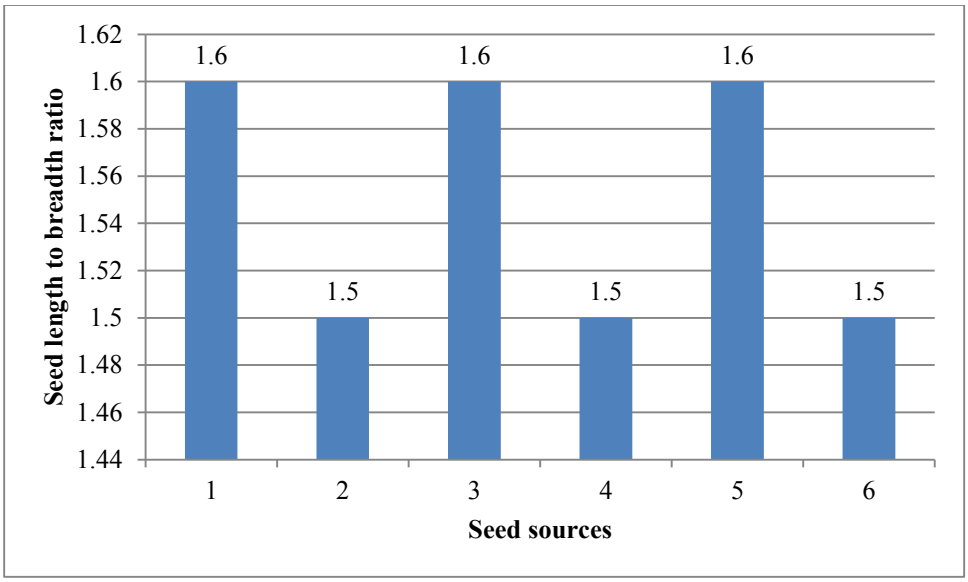


Fig. 4: Length to breadth ratio in *Saraca asoca* seeds collected from six seed sources

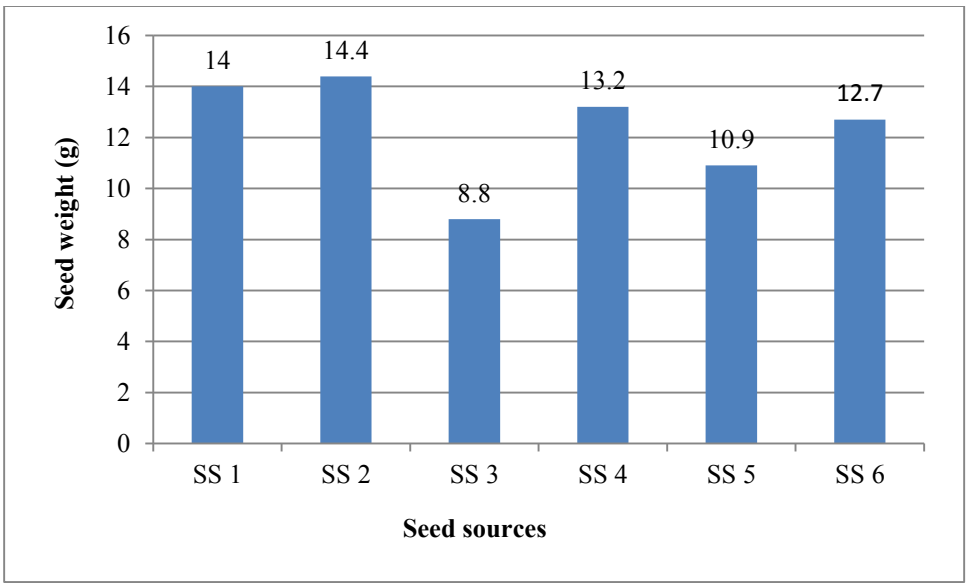


Fig. 5: Weight of *Saraca asoca* seeds collected from six seed sources

4.1.2.5 Thickness of pericarp

Pericarp thickness, with the exception of SS 5, did not vary significantly across different seed sources (Table 7, Fig. 6). They exhibited statistically similar values, with seed source SS 2 showing the maximum value (2.7mm) in the present study. Seed source SS 5 with 1.73 mm as pericarp thickness recorded the least value among the studied sources. Seed source SS 4 recorded a value of 2.5 mm. Rest of the seed sources SS 1, SS 3 and SS 6 recorded a value of 2.3 mm for each sources. The photograph of pod along with seeds is shown in the Plate 19.

4.1.3 Seed germination attributes

Seed germination attributes of ashoka was studied for the six seed sources and the results are given in Table 8. Germination in ashoka is also depicted in the Plate 20. Difference in germination pattern can be observed among various seed sources from the data furnished below.

4.1.3.1 Days required for germination to initiate

Number of days required for germination to initiate varied significantly across different seed sources (Table 8). Fastest germination was triggered in the seed source SS 4 (14 days). The performance of seed source SS 2 (15 days) was on par with seed source SS 4. Seed source SS 6 and SS 3 displayed relatively better performance among the remaining seed sources with a recorded data of 18.5 days and 19 days, respectively. Seed source SS 5 (33.5 days) exhibited significantly the lowest performance across the seed sources. Seed source SS 1 showed better performance when compared to the seed source SS 5, in terms of number of days for germination. The illustration on initiation of germination is given in Fig. 7.

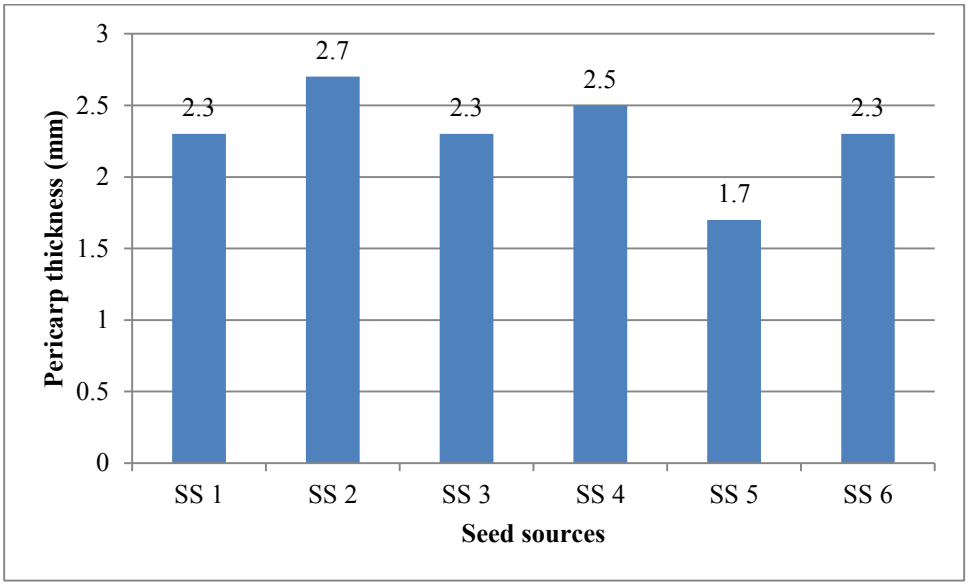


Fig. 6: Thickness of pericarp in *Saraca asoca* fruit collected from six seed sources

Plate 19: *Saraca asoca* seeds inside the pod

Table 8. Seed germination attributes of *Saraca asoca* collected from six seed sources.

Seed source	Days to germinate	Days to cease	Germination days	Days for50% germination	GP*	GE*	MDG	PV	GV
SS 1	23.8^{c}	69.5^{ab}	45.8^{b}	43.5^{b}	1.4^{a} (98.0)	0.54^{ab} (51.0)	1.3^{a}	1.6^{a}	2.1^{a}
SS 2	15.0^{ab}	71.0^{ab}	56.0^{a}	39.8^{ab}	1.2^{ab} (93.8)	0.30^{cd} (29.5)	1.3^{ab}	1.5^{a}	1.8^{ab}
SS 3	19.0^{b}	64.8^{b}	45.8^{b}	36.8^{a}	1.0^{c} (84.3)	0.61^{a} (57.0)	1.2^{b}	1.6^{a}	1.9^{ab}
SS 4	14.0^{a}	74.8^{a}	60.8^{a}	37.8^{a}	1.2^{bc} (89.0)	0.42^{bc} (40.5)	1.1^{c}	1.5^{a}	1.7^{b}
SS 5	33.5^{d}	67.0^{ab}	33.5^{c}	52.25^{c}	0.4^{d} (37.8)	0.23^{d} (22.8)	0.5^{d}	0.6^{b}	0.3^{c}
SS 6	18.5^{b}	65.3^{b}	46.8^{b}	36.5^{a}	1.0^{bc} (86.0)	0.42^{bc} (40.5)	1.2^{b}	1.6^{a}	2.0^{ab}
SEm±	1.4	2.4	2.7	1.8	0.06	0.05	0.03	0.07	0.1

Values having the same alphabets in a column do not differ significantly (significant level at 5 %).

*GP and GE before angular transformation are given in parenthesis.

Plate 20: Germination of seeds in *Saraca asoca*

4.1.3.2 Days required for germination to cease

Seed source SS 4 (74.8 days) took significantly more days for the viable seeds to germinate. Seed source SS 2, SS 1 and SS 5 ceased its germination on 71st day, 69.5th day and 67th day, respectively and their performance were on par with seed source SS 4. Seed source SS 3 and SS 6 noted the lowest days for germination to cease with recorded values of 64.8 days and 65.3 days, respectively (Table 8). The performance of different seed source, with respect to days required for germination to cease, is also displayed in the Fig. 8.

4.1.3.3 Total germination days

Days accounting to germination period of ashoka exhibited considerable variation among different seed sources (Table 8 and Fig. 10). Maximum germination days were taken for the seed source SS 4 (60.8 days) and SS 2 (56 days). The performance of seed source SS 1 (45.8 days), SS 3 (45.8 days) and SS 6 (46.8 days), where on par with each other with regard to total germination days. Total days for germination were the least in the case of seed source SS 5, recording 33.5 days.

4.1.3.4 Days required to reach 50 per cent of germination

Days required to reach 50 per cent germination was the quickest among the seed sources SS 3, SS 4 and SS 6 with 36.8 days, 37.8 days and 36.5 days respectively. Table 8 and Fig. 9 provides with information with respect to this character in *Saraca asoca* seed germination. The Days to reach 50 per cent germination was assumed from the day after sowing itself. The value was on par for seed source SS 2 with the above mentioned sources, recording 39.8 days. It took 43.5 days for seed source SS 1 to attain 50 per cent of germination. Still more days were taken by seed source SS 5 (52.3 days) to reach the target. In totality significant difference were shown by different seed sources with data varying from 36.5 days to 52.3 days.

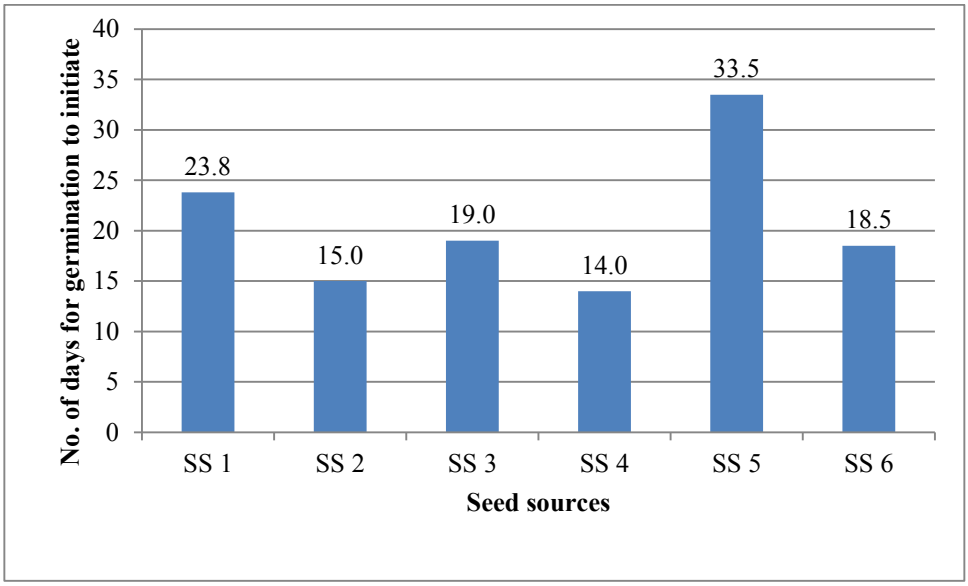


Fig. 7: Number of days required for germination to initiate in *Saraca asoca* seeds collected from six seed sources

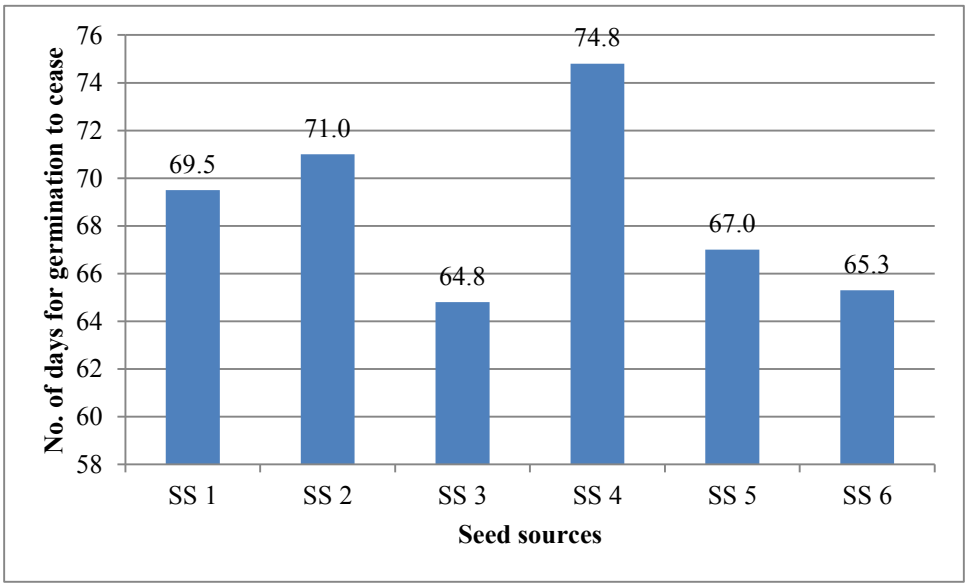


Fig. 8: Number of days required for germination to cease in *Saraca asoca* seeds collected from six seed sources

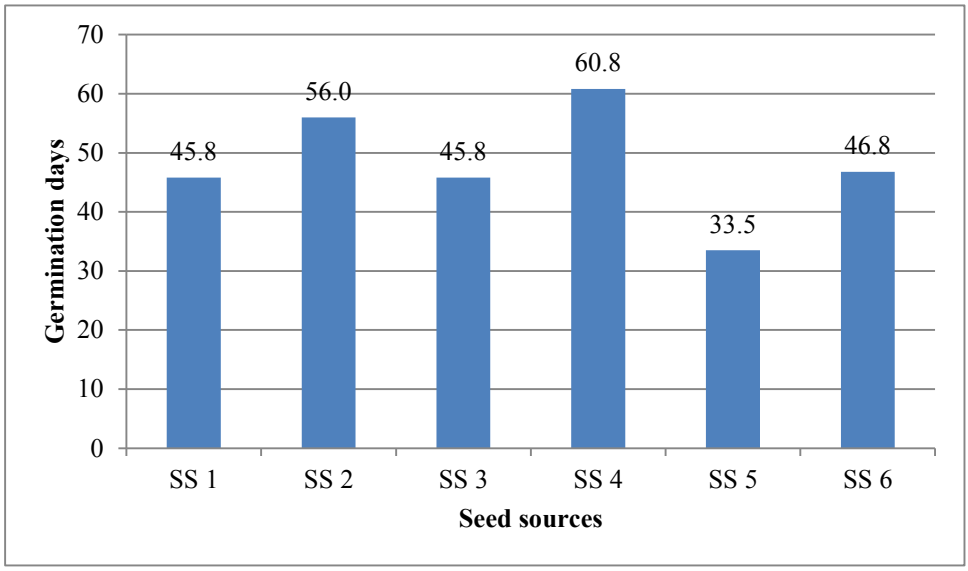


Fig. 9: Total germination days in *Saraca asoca* seeds collected from six seed sources

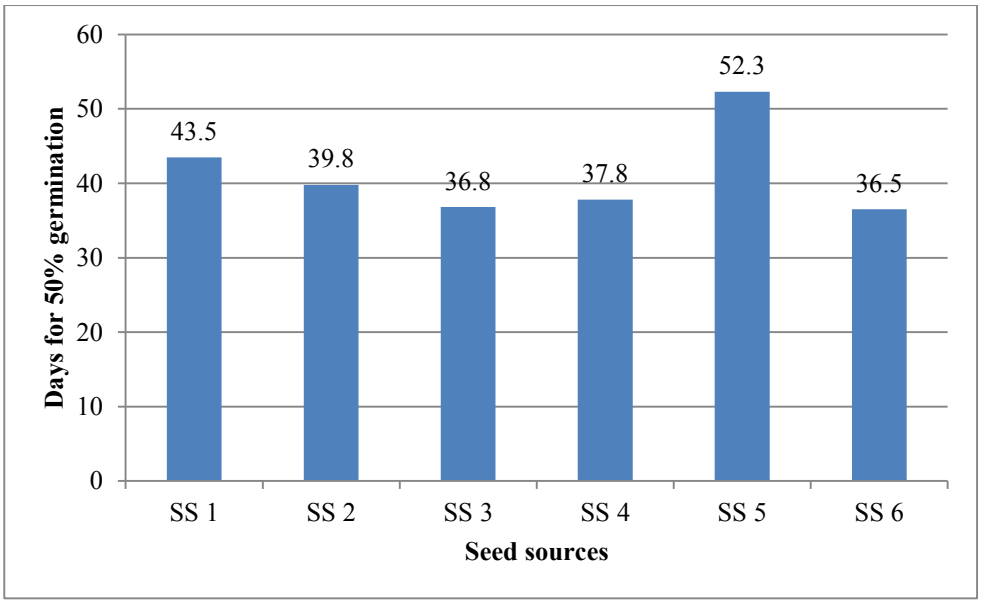


Fig. 10: Days required to achieve 50 per cent germination in *Saraca asoca* seeds collected from six seed sources

4.1.3.5 Germination percentage (GP)

Overall germination percentage gave good results for all the seed sources except for seed source SS 5 (Table 8 and Fig. 11). However, statistically significant variation was exhibited by different seed sources. Seed source SS 1 (98.0 per cent) came out to be the most superior among studied seed sources in terms of germination percentage. The next best sources after SS 1 were SS 2 and SS 4 with values 93.75 per cent and 89.0 per cent respectively. Seed sources SS 6 (86.0 per cent) and SS 3 (84.3 per cent) also showed moderate performance when compared to SS 1. In contrast to all the other seed sources, seeds collected from SS 5 exhibited significantly lower value. The performance was least with a recorded value of 37.8 per cent. On an average ashoka seeds gave 81.5 per cent as germination percentage.

4.1.3.6 Germination energy (GE)

Significantly high value was recorded for the seed source SS 3 (57). The value exhibited by SS 1 was on par with that of SS 3, with a recorded value of 51 (Table 8 and Fig. 12). Seed sources SS 4 showed similarity in performance with seed source SS 6 displaying a value of 40.5 each, for germination energy. Seed source SS 2 and SS 5 showed comparatively low germination energy. The data in the present study exhibited, SS 5 (22.8) with the lowest germination energy among different seed sources. Altogether all the seed sources showed significant differences with respect to germination energy.

4.1.3.7 Mean daily germination (MDG)

The variation was significant statistically for different seed sources in case of mean daily germination with a value of 1.3 for the seed source SS1 to 0.5 for SS 5 (Table 8). The performance of seed source of SS 2 was on par with the seed source SS 1. The performance of *Saraca asoca* in terms of MDG, is also illustrated in the Fig. 13. Seed source SS 3 and SS 6 followed SS 1 based on the performance in terms

of mean daily germination. It is evident from the tabulated data that seed source SS 4 exhibited significantly low value. When compared to other sources, mean daily germination was lowest for the seed source SS 5 with MDG value 0.5.

4.1.3.8 Peak value (PV)

Maximum MDG for a source is represented by peak value. Peak value differed significantly from one another as depicted in the Table 8 and Fig. 14. However, seed source SS 5 (0.5) showed exceptionally the lowest value among the given seed sources. In the present study, highest PV was exhibited by the seed source SS 3 (1.6). This was followed by the seed sources SS 6, SS 1, SS 4 and SS 2 in the decreasing order of performance with respect to PV.

4.1.3.9 Germination value (GV)

The tabulated data and Fig. 15, displayed significant variation among different seed sources with respect to GV. Germination value varied from a maximum of 2.11 for the seed source SS 1 to a minimum of 0.32 for the seed source SS 5. The performance of seed sources SS 6, SS 3 and SS 2 were on par with each other and the seed source SS 1. The performance of seed source SS 4 was better than the least germination value displayed by seed source SS 5.

4.1.4 Seedling attributes

Apart from assessing the seed traits and germination attributes of ashoka, from different seed sources, its seedling morphology and physiology in terms of rate of photosynthesis and chlorophyll content was studied. The study displayed significant differences among different seed sources in case of most of the seedling attributes. Plates from 21 to 26, shows the growth of two month old *Saraca asoca* seedlings. The results on seedling attributes explained here under, were used to examine the seed source variation in the present study.

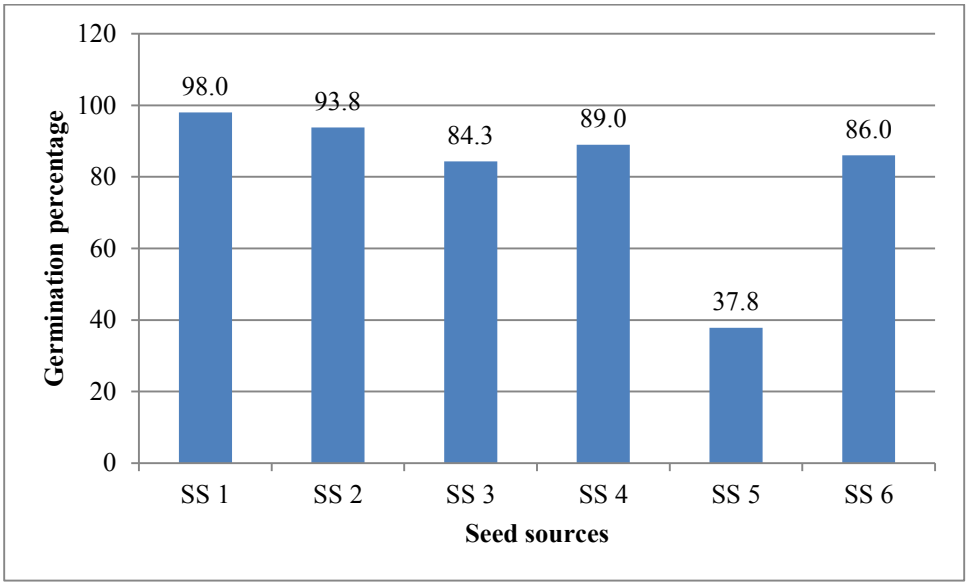


Fig. 11: Germination percentage in *Saraca asoca* seeds collected from six seed sources

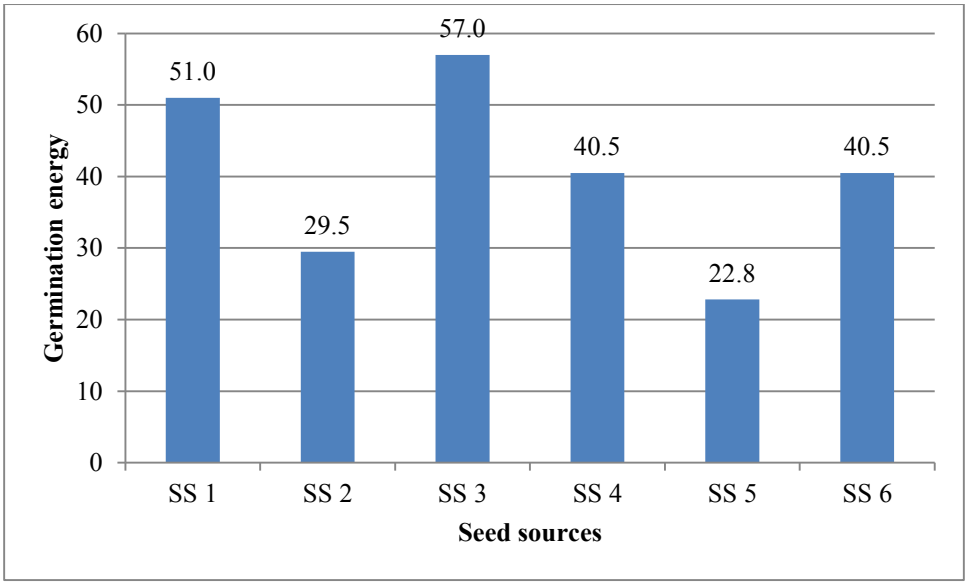


Fig. 12: Germination energy in *Saraca asoca* seeds collected from six seed sources

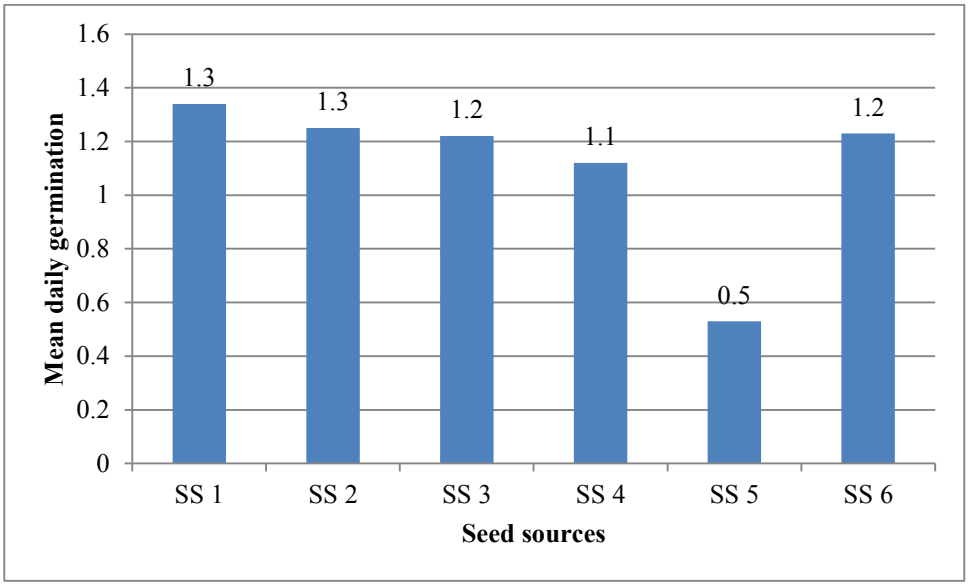


Fig. 13: Mean daily germination in *Saraca asoca* seeds collected from six seed sources

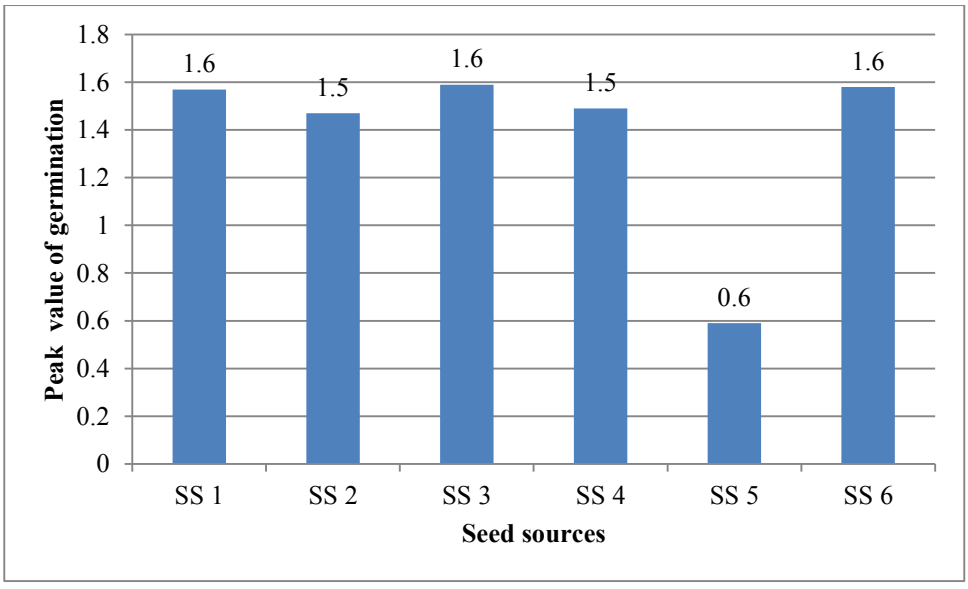


Fig. 14: Peak value of germination in *Saraca asoca* seeds collected from six seed sources

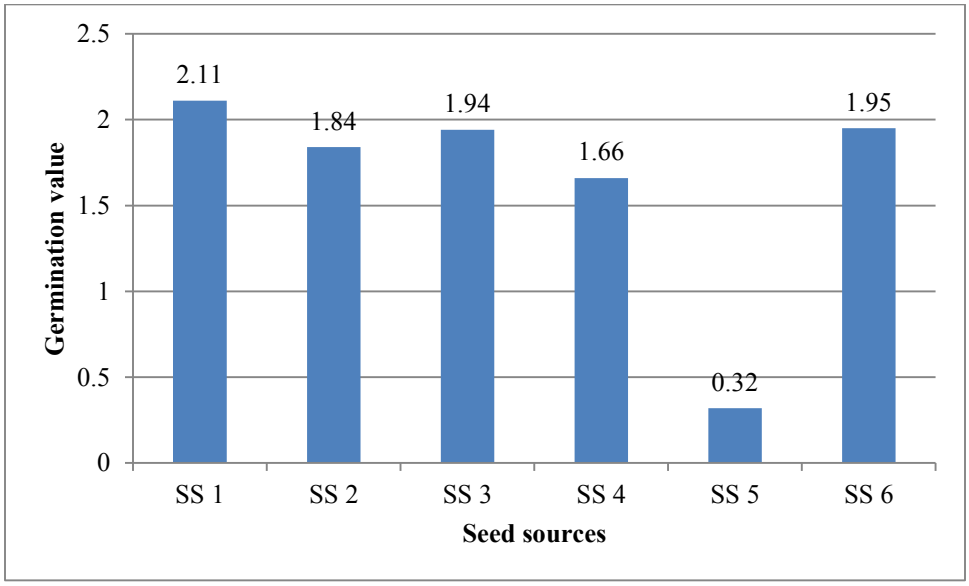


Fig. 15: Germination value in *Saraca asoca* seeds collected from six seed sources

Plates 21-26: Two month old seedlings of *Saraca asoca* of the six seed sources

Plate 21: Seedlings of seed source SS 1

Plate 22: Seedlings of seed source SS 2

Plate 23: Seedlings of seed source SS 3

Plate 24: Seedlings from seed source SS 4

Plate 25: Seedlings from seed source SS 5

Plate 26: Seedlings from seed source SS 6

4.1.4.1 Height

Significant variation was observed among different seed sources across the monthly intervals with respect to height (Table 9). However, the variation with respect to seed source SS 5 showed the lowest height throughout the observation period. Seed source SS 5 exhibited the least performance with a height of 32.2 cm at the end of observation. Maximum height towards the end of observation period was shown by the seed source SS 3 and SS 2. Height for the seed source SS 4, SS 1 were on par with SS 3 and SS 2, as seen in the final observation stages. Growth with respect to height was most rapid in case of seed source SS 2 recording 22.7 cm to 37.6 cm within a span of eight months. Whereas the growth was apparently slow with respect to seed source SS 5. It was also noted that, the height increment in seedlings of ashoka was slightly higher after 210 days of growth. In general the growth was almost uniform for all the seed sources throughout the study period. The performance of *Saraca asoca* seedlings in terms of height is also illustrated in the Fig. 16.

4.1.4.2 Collar diameter

Collar diameter of seedlings exhibited notable variation across different seed sources, especially after 210 days of germination (Table 10 and Fig. 17). Initial observation recorded highest collar diameter for seed source SS 3 (3.3 mm) among the studied seed sources. Initially, the performance of seed sources SS 4 and SS 6, followed seed source SS 3, in terms of collar diameter. The lowest value with respect to collar diameter was exhibited by the seed source SS 5 after 60 days of growth. It was observed that after shifting the seedlings from shade (after 210 days), the performance of all the seed sources started to show significant variation among each other when compared to earlier periods of observation. Even after shifting the seedlings, the performance of seed source SS1 was maintained on comparison with other sources. In the final observation seed source SS 1 (6.0 mm) exhibited superiority over other sources, in the present study. The performance of seed sources

Table 9. Height (cm) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	26.4^{a}	29.0^{a}	29.8^{a}	30.5^{a}	31.4^{a}	32.9^{a}	34.7^{a}	36.6^{a}
SS 2	22.7^{ab}	27.1^{a}	28.8^{a}	30.5^{a}	32.0^{a}	33.7^{a}	35.4^{a}	37.6^{a}
SS 3	26.1^{a}	27.7^{a}	28.7^{a}	30.1^{a}	30.6^{a}	32.3^{a}	34.6^{a}	37.7^{a}
SS 4	26.7^{a}	29.9^{a}	30.8^{a}	31.2^{a}	32.6^{a}	33.3^{a}	34.8^{a}	36.7^{a}
SS 5	20.4^{b}	23.5^{b}	24.3^{b}	25.4^{b}	26.5^{b}	27.7^{b}	29.5^{b}	32.2^{b}
SS 6	25.5^{a}	28.2^{a}	28.9^{a}	29.6^{a}	32.3^{a}	32.7^{a}	33.4^{a}	34.9^{ab}
SEm±	1.3	1.0	1.0	1.0	1.1	1.0	1.1	1.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Table 10. Collar diameter (mm) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	2.8^{bc}	3.5^{a}	3.6^{a}	3.9^{a}	4.2^{a}	5.1^{a}	5.5^{a}	6.0^{a}
SS 2	2.8^{bc}	3.5^{a}	3.6^{a}	4.0^{a}	4.5^{a}	4.9^{ab}	5.2^{ab}	5.7^{ab}
SS 3	3.3^{a}	3.7^{a}	3.7^{a}	3.9^{a}	4.3^{a}	4.5^{bc}	4.8^{ab}	5.3^{abc}
SS 4	2.9^{ab}	3.5^{a}	3.4^{ab}	3.8^{a}	4.3^{a}	4.7^{abc}	4.9^{ab}	5.3^{abc}
SS 5	2.4^{c}	2.8^{b}	3.1^{b}	3.3^{b}	3.7^{b}	4.0^{d}	4.2^{c}	4.6^{c}
SS 6	2.9^{ab}	3.3^{a}	3.4^{ab}	3.4^{b}	3.7^{b}	4.2^{cd}	4.7^{ab}	5.1^{bc}
SEm±	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

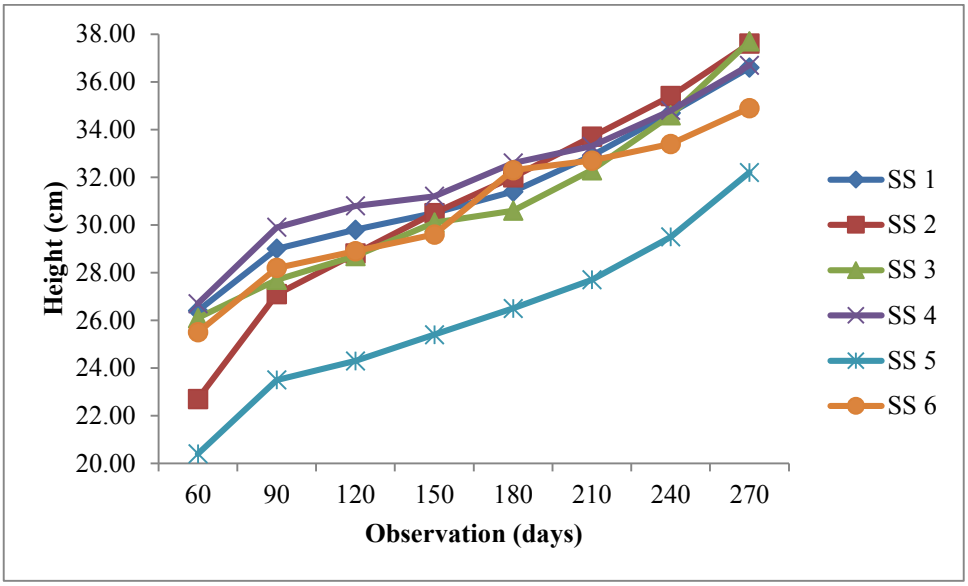


Fig. 16: Seedling height of *Saraca asoca* representing six seed sources

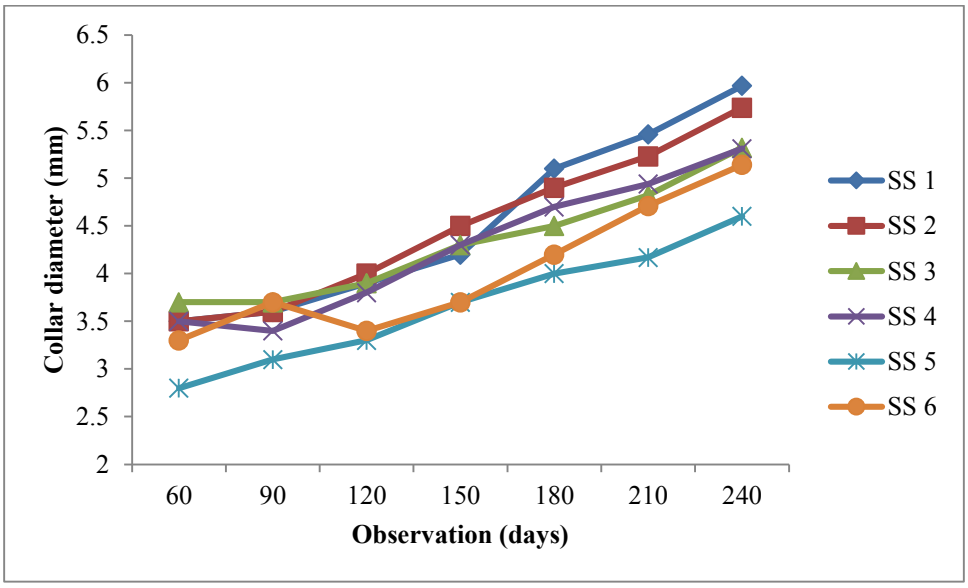


Fig. 17: Seedling collar diameter (mm) of *Saraca asoca* representing six seed sources

SS 2, SS 3 and SS 4 were on par with the performance of seed source SS 1. Least value for collar diameter was attained by seed source SS 5 (4.6 mm). It was noted that throughout the observation period, seed source SS 1displayed better performance. Whereas seed source SS 5, exhibited poor performance throughout the study period in terms of collar diameter.

4.1.4.3 Leaf number

Prominent variation among different seed sources was observed with respect to leaf number (Table 11). Significant difference in terms of leaf number was evident only after 60 days, 150 days and 180 days of growth. During the early growth stages, it was noted that the seed source SS 6 (2.3) displayed the best performance with respect to leaf production. The performance of seed source SS 6, continued till 150 days with respect to number of leaves. However, after 180 days, seed source SS 1, came out to be better performer when compared to other seed sources. Towards the final observation in the present study, all the seed sources showed similar performance in terms of leaf production, with a maximum value of 4.6 in case of seed source SS 3. The results are also depicted in the Fig. 18.

4.1.4.4 Leaf area

The leaf area of ashoka varied markedly across different seed sources, throughout the observation period (Table 12). The data showed significantly higher values for leaf area in case of seed sources SS 1, SS2 and SS 4, throughout the study period. The value recorded for seed sources SS 1, SS 2 and SS 4 after 270 days were 131.1 cm^2, 132.3 cm^2 and 125.3 cm^2. However seed source SS 5 gave significantly the least performance across different monthly intervals of the study. In the final observation seed source SS 5 recorded a value of 76.6 cm^2. The illustration regarding leaf area is given in the Fig. 19.

Table 11. Number of leaves per seedling of *Saraca asoca* representing six seed sources

Seed sources	Observation period							
	60 days	90 Days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	1.9^{abc}	2.2^{a}	2.2^{a}	2.6^{ab}	3.3^{a}	3.7^{a}	3.9^{a}	4.2^{a}
SS 2	1.7^{c}	2.3^{a}	2.3^{a}	2.6^{ab}	3.0^{ab}	3.4^{a}	4.0^{a}	4.5^{a}
SS 3	2.2^{ab}	2.3^{a}	2.3^{a}	2.7^{ab}	3.0^{ab}	3.7^{a}	4.2^{a}	4.6^{a}
SS 4	2.1^{abc}	2.2^{a}	2.2^{a}	2.5^{ab}	2.6^{b}	3.1^{a}	3.4^{a}	3.8^{a}
SS 5	1.8^{bc}	2.1^{a}	2.1^{a}	2.4^{b}	2.6^{b}	3.4^{a}	3.8^{a}	4.3^{a}
SS 6	2.3^{a}	2.4^{a}	2.4^{a}	2.9^{a}	3.1^{ab}	3.7^{a}	3.9^{a}	4.3^{a}
SEm±	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.3

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Table 12. Leaf area (cm^2) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 Days
SS 1	77.3^{a}	86.0^{a}	92.6^{a}	97.5^{a}	104.8^{a}	116.4^{a}	123.1^{ab}	131.1^{a}
SS 2	72.7^{ab}	78.3^{a}	84.6^{a}	95.0^{a}	110.5^{a}	118.2^{a}	127.0^{a}	132.3^{a}
SS 3	77.7^{a}	84.1^{a}	93.7^{a}	97.3^{a}	103.7^{a}	109.1^{a}	111.8^{b}	117.5^{ab}
SS 4	78.5^{a}	84.4^{a}	91.0^{a}	103.9^{a}	111.9^{a}	114.9^{a}	123.9^{ab}	125.3^{ab}
SS 5	54.7^{b}	59.8^{b}	63.1^{b}	68.4^{b}	70.5^{b}	72.0^{b}	74.4^{c}	76.6^{c}
SS 6	78.0^{a}	84.0^{a}	89.9^{a}	94.6^{a}	103.7^{a}	106.9^{a}	110.6^{b}	112.9^{b}
SEm±	6.7	5.5	4.7	5.3	4.7	4.8	4.2	4.8

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

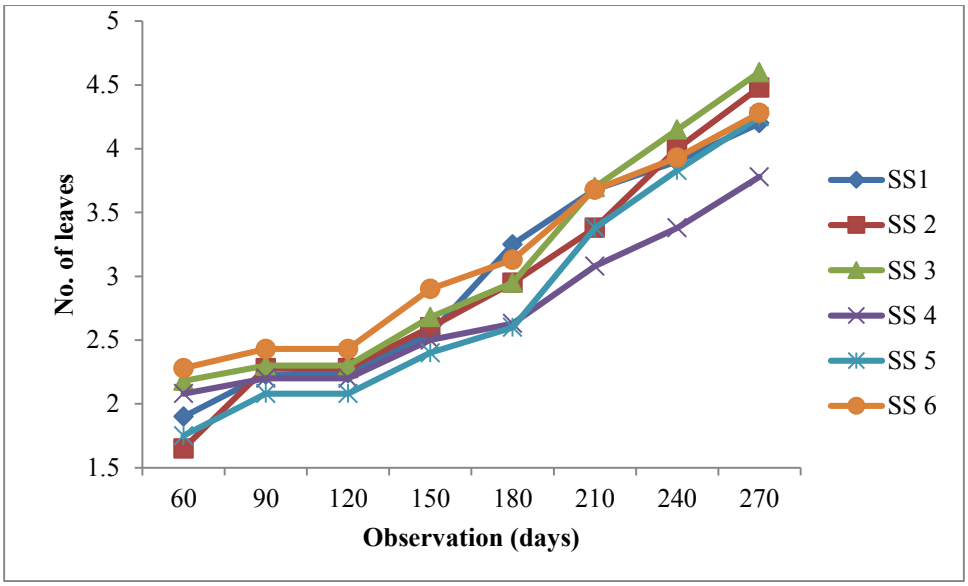


Fig. 18: Number of leaves per seedling of *Saraca asoca* representing six seed sources

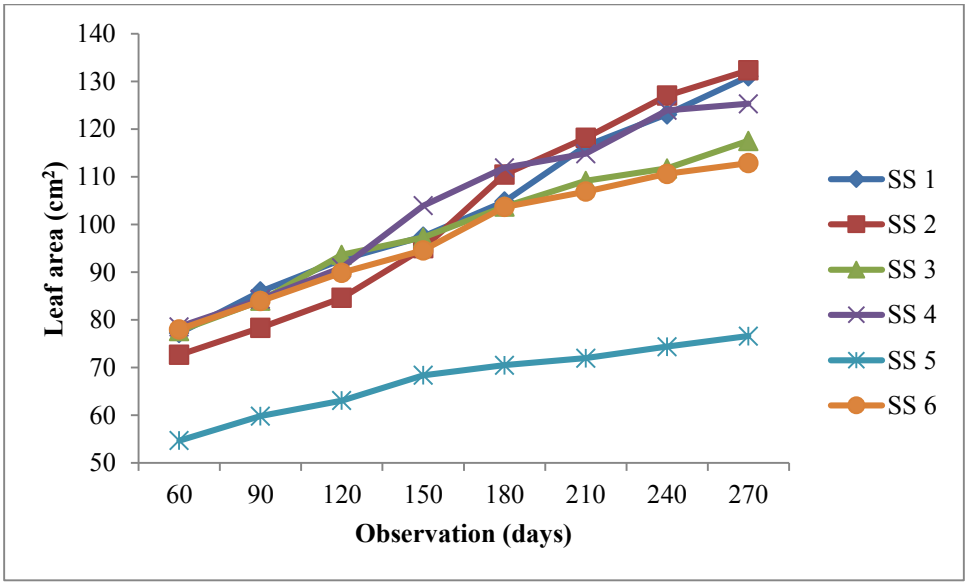


Fig. 19: Leaf area (cm^2) of *Saraca asoca* seedling representing six seed sources

The performance of seed sources SS 3 and SS 6 were also the best along with seed sources SS 1, SS 2 and SS 4, up to 210 days of growth in seedlings. It becomes evident from the tabulated data that the increment rate in leaf area was comparatively higher after shifting the seedlings from the shade (after 210 days).

4.1.4.5 Number of branches

Data on number of branches per plant recorded at different growth intervals are presented in Table 13. No significant differences in number of branches were observed during the study period. The results are illustrated in Fig. 20. At the end of observation, maximum branching was observed in the seed source SS 3. Initially branching was seen only in case of seed source SS 3 and SS 6 but after 210 days of growth, all the seed source exhibited branching. It took relatively more number of days for SS 4 seedlings to branch. In general profuse branching was absent in *Saraca asoca* seedlings as observed in the study.

4.1.4.6 Leaf thickness

Throughout the observation period the parameter leaf thickness did not show any significant results among different seed sources (Table 14 and Fig. 21). There were no wide differences with respect to this parameter among different seed sources as depicted in the table and Fig. 20. Leaf thickness in the present study varied from a value from 0.13 mm to 0.19 mm. Generally, varying trend was shown with respect to leaf thickness along the observation period among different seed sources.

4.1.4.7 Biomass

Biomass was assessed by taking the fresh weight and dry weight of the root and shoot portions. The study showed significant variation with respect to different seed sources in case of biomass production. The results with respect to this character have been tabulated and explained below.

Table 13. Number of branches per seedling of *Saraca asoca* representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	0.00	0.00	0.03	0.03	0.03	0.03	0.05	0.05
SS 2	0.00	0.03	0.03	0.08	0.08	0.08	0.10	0.10
SS 3	0.03	0.05	0.05	0.08	0.13	0.13	0.13	0.15
SS 4	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.05
SS 5	0.00	0.00	0.03	0.05	0.05	0.05	0.05	0.10
SS 6	0.03	0.03	0.05	0.05	0.08	0.08	0.10	0.10
SEm±	0.02	0.02	0.03	0.04	0.05	0.06	0.05	0.05

Table 14. Leaf thickness (mm) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	0.15	0.15	0.16	0.17	0.18	0.16	0.18	0.19
SS 2	0.15	0.16	0.17	0.17	0.18	0.18	0.18	0.19
SS 3	0.16	0.18	0.17	0.15	0.18	0.18	0.17	0.18
SS 4	0.13	0.16	0.17	0.16	0.16	0.17	0.18	0.19
SS 5	0.15	0.15	0.18	0.15	0.16	0.18	0.16	0.18
SS 6	0.16	0.15	0.15	0.15	0.16	0.19	0.17	0.18
SEm±	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00

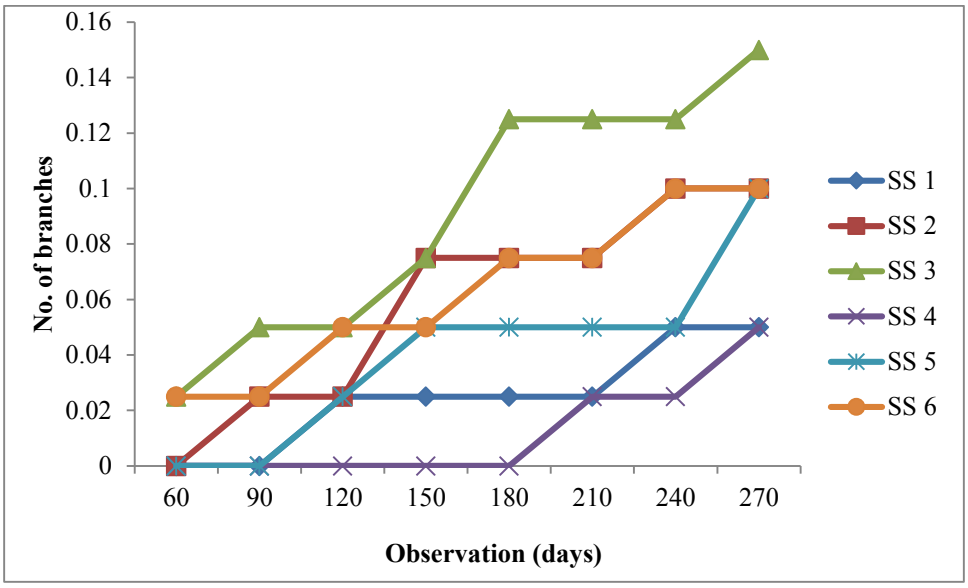


Fig. 20: Number of branches per seedling of *Saraca asoca* representing six seed sources

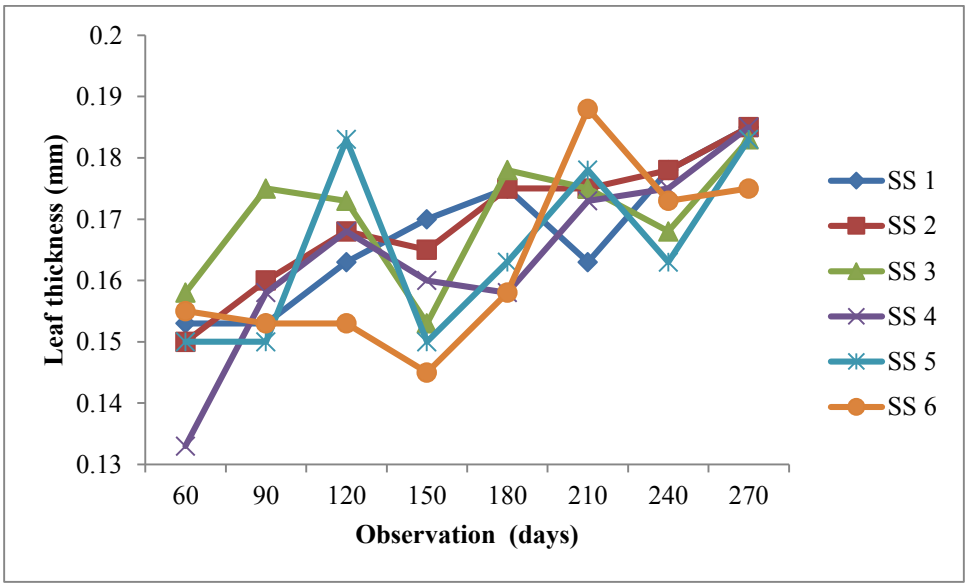


Fig. 21: Leaf thickness (mm) of *Saraca asoca* seedling representing six seed sources

4.1.4.7.1 Fresh weight of shoot

Observation regarding mean fresh weight of shoot showed significant variation throughout the study period. The data furnishing information in this respect is depicted in the Table 15 and displayed in Fig. 22. Significant difference was shown in the first observation, were seed source SS 4 (4.4 g) stood with maximum shoot fresh weight. Seed source SS 3 was on par with seed source SS 4. The performance of seed soure SS 1, SS 2 and SS 6 followed SS 4 and SS 3. Later on varying trend was shown with respect to this character among different seed sources. Throughout the observation, seed source SS 1 displayed the maximum performance. Whereas the seed source SS 5, produced the poorest result, along the observation period, with regard to shoot fresh weight. It was noted that after 210 days of growth, all the seedlings exhibited better performance. However, seed source SS 5 continued to display poor performance with respect to fresh weight of shoot.

4.1.4.7.2 Fresh weight of root

Fresh weight of root showed significant variation between different seed sources along the observation period. It is clear from Table 16, that the performance of seed source SS 5 was poor throughout the observation period. Whereas the seed source SS 6 also produced poor results from 150 days of observation. At the end, seed source SS 5 and SS 6 recorded a value of 3.5 g and 3.4 g respectively. Root fresh weight of seed source SS 2, gave the best result after 150 days of growth onwards. In the initial stages seed source SS 4 displayed better performance when compared to other sources. Gradually seed source SS 2 (5.1 g) replaced SS 4 and it gave the maximum fresh root weight at the end of the observation. Seed sources SS 1 and SS 3 could also display their maximum in terms of fresh weight of root, after 150 days, 180 days and 210 days of seedling growth. The performance of different seed sources have been shown in Fig. 23.

Table 15. Shoot fresh weight (g) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	3.6^{b}	4.4^{cd}	4.6^{bc}	4.7^{c}	5.1^{c}	5.4^{b}	6.2^{a}	6.6^{a}
SS 2	3.7^{b}	5.1^{b}	5.2^{b}	5.6^{b}	5.8^{ab}	6.1^{ab}	6.7^{a}	7.0^{a}
SS 3	3.9^{ab}	4.7^{bc}	4.9^{b}	5.0^{bc}	5.2^{bc}	5.5^{b}	6.3^{a}	6.7^{a}
SS 4	4.4^{a}	5.8^{a}	6.0^{a}	6.1^{a}	6.0^{a}	6.2^{a}	6.5^{a}	7.1^{a}
SS 5	2.5^{c}	3.0^{e}	3.1^{d}	3.2^{d}	3.3^{d}	3.6^{c}	4.2^{b}	4.9^{b}
SS 6	3.7^{b}	3.9^{d}	4.3^{c}	4.5^{c}	4.9^{c}	5.4^{b}	6.0^{a}	6.5^{a}
SEm±	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Table 16. Root fresh weight (g) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	2.7^{ab}	3.0^{b}	3.1^{ab}	3.3^{a}	3.4^{a}	3.7^{a}	4.1^{ab}	4.4^{b}
SS 2	2.4^{bc}	2.6^{cd}	3.0^{ab}	3.4^{a}	3.5^{a}	3.9^{a}	4.3^{a}	5.1^{a}
SS 3	2.5^{abc}	2.9^{bc}	3.0^{b}	3.2^{a}	3.4^{a}	3.7^{a}	3.8^{b}	4.4^{b}
SS 4	2.8^{a}	3.4^{a}	3.5^{a}	3.6^{a}	3.7^{a}	3.9^{a}	3.9^{ab}	4.5^{ab}
SS 5	2.0^{d}	2.1^{e}	2.2^{c}	2.4^{b}	2.5^{b}	2.7^{b}	3.1^{c}	3.5^{c}
SS 6	2.3^{c}	2.3^{de}	2.6^{bc}	2.6^{b}	2.9^{b}	3.0^{b}	3.0^{c}	3.4^{c}
SEm±	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

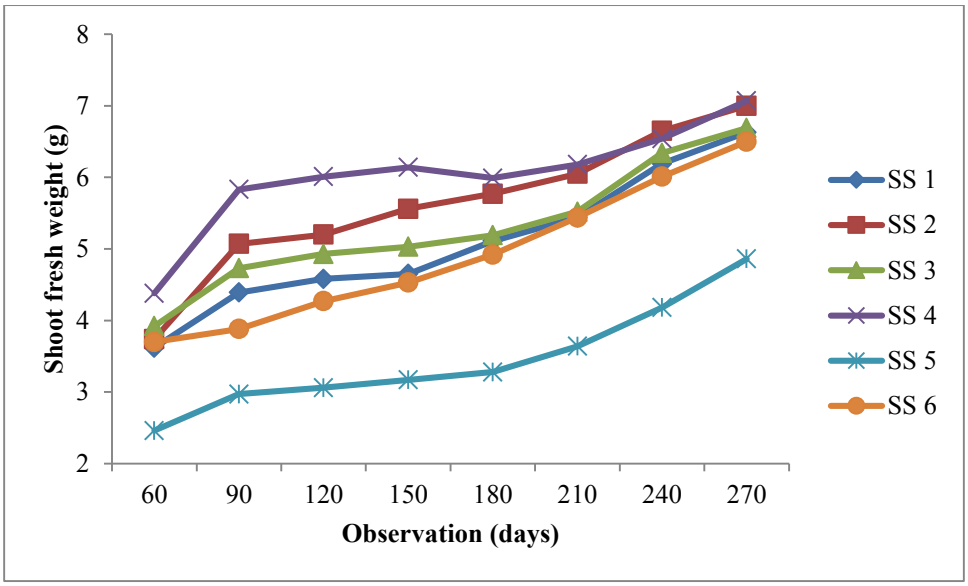


Fig. 22: Shoot Fresh weight of *Saraca asoca* seedling representing six seed sources

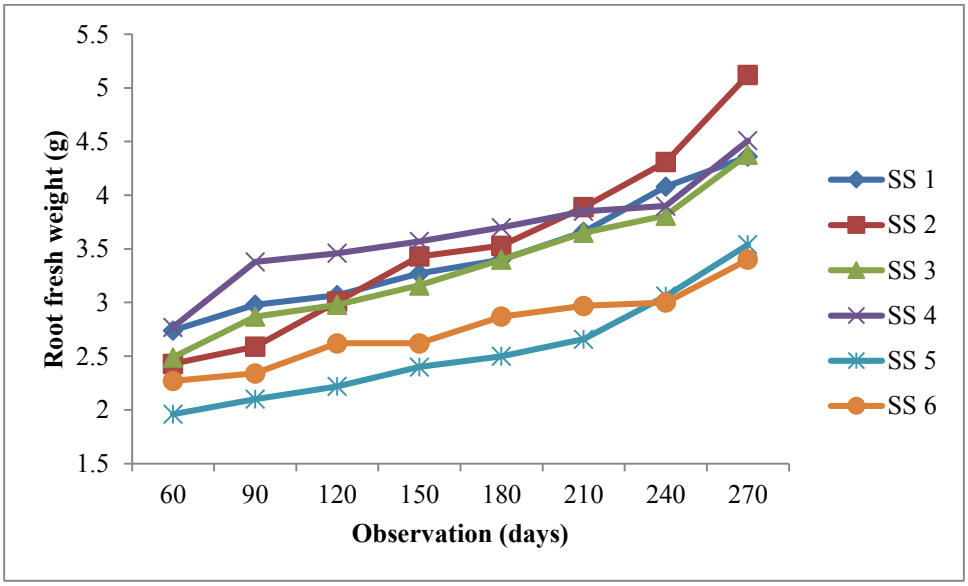


Fig. 23: Root fresh weight of *Saraca asoca* seedling representing six seed sources

4.1.4.7.3 Dry weight of shoot

Dry weight of shoot exhibited significant variation between different seed sources throughout the observation period (Table 17). Varying trend of performance was shown by different seed sources with respect to different observation period. However, it was noted that the seed source SS 2, showed the best performance throughout the entire period of observation and recording a final value of 3.3 g. Along with seed source SS 2, performance of seed source SS 4, was also notably superior. Seed source SS 4 displayed its superiority from 90 days to 210 days of seedling growth. The tabulated data indicated least performance along the study period in shoot dry weight, with respect to seed source SS 5. Similarly seedlings of seed source SS 6 displayed poor performance along with seed source SS 5, in terms of dry weight of shoot. The performance of seed source SS 1 and SS 3 was almost par with each other, in terms of dry weight of shoot, throughout the study period. The Fig. 24 shows the seedling performance of *Saraca asoca*, with respect to shoot dry weight.

4.1.4.7.4 Dry weight of root

Dry weight of root varied among different seed sources as given in the Table 18. The illustrated representation of the result is also given in the Fig. 25. In general, significant differences were exhibited throughout the observation period in a varying trend. It was noted that, seed source SS 2, could display almost better dry weight of root along the observation period. Seed source SS 6 with a recorded value of 2.59 g gave the maximum performance towards the end of the observation period. It was also evident from the table that the performance of seed sources SS 1, SS 2, SS 4 and SS 5, were on par with each other in the final observation period.

Table 17. Shoot dry weight (g) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	1.2^{b}	1.4^{b}	1.5^{b}	1.7^{b}	1.9^{bc}	2.2^{abc}	2.4^{bc}	2.6^{bc}
SS 2	1.5^{a}	1.8^{a}	2.1^{a}	2.3^{a}	2.4^{a}	2.6^{a}	2.9^{a}	3.3^{a}
SS 3	1.1^{b}	1.3^{b}	1.5^{b}	1.6^{b}	1.8^{bc}	2.0^{bc}	2.3^{bc}	2.5^{bc}
SS 4	1.2^{b}	1.9^{a}	2.1^{a}	2.1^{a}	2.4^{a}	2.5^{a}	2.6^{ab}	2.8^{b}
SS 5	1.0^{b}	1.1^{b}	1.3^{b}	1.5^{b}	1.6^{c}	1.8^{c}	2.1^{c}	2.3^{c}
SS 6	1.3^{b}	1.4^{b}	1.4^{b}	1.5^{b}	2.1^{ab}	2.4^{ab}	2.6^{ab}	2.7^{bc}
SEm±	0.08	0.1	0.1	0.2	0.1	0.1	0.1	0.1

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Table 18. Root dry weight (g) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	0.9^{a}	1.1^{ab}	1.4^{a}	1.5	1.8^{a}	2.0^{ab}	2.4^{bc}	2.4^{ab}
SS 2	1.0^{a}	1.4^{a}	1.4^{a}	1.6	1.8^{a}	2.0^{ab}	2.9^{a}	2.3^{ab}
SS 3	0.8^{a}	1.0^{b}	1.1^{b}	1.2	1.4^{b}	1.7^{b}	2.3^{bc}	2.1^{b}
SS 4	0.9^{a}	1.1^{ab}	1.2^{ab}	1.4	1.5^{ab}	1.6^{b}	2.6^{ab}	2.2^{ab}
SS 5	0.5^{b}	1.0^{b}	1.2^{ab}	1.4	1.6^{ab}	1.8^{ab}	2.1^{c}	2.4^{ab}
SS 6	0.8^{ab}	0.9^{b}	1.3^{ab}	1.5	1.8^{ab}	2.1^{a}	2.6^{ab}	2.6^{a}
SEm±	0.1	0.1	0.1	NS	0.1	0.1	0.1	0.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

4.1.4.7.5 Shoot-root dry weight ratio

The result on shoot-root dry weight ratio is presented in Table 19 and illustrated in Fig. 26. In the initial two months significant difference was not shown among different seed sources. Shoot-root ratio started to show considerable difference after 120 days onwards. The performance of seed source SS 4 displayed better values, from 120 days to 240 days of seedling growth. However, in the final observation seed source SS 2, replaced SS 4 with a value displaying 1.44 for SS 2. In the final observation seed source SS 5, gave the least ratio (0.95). In general the performance of seed source SS 5 and SS 6, showed poor performance with respect to shoot-root dry weight ratio.

4.1.4.8 Relative growth rate (RGR)

Relative growth rate altogether showed varying trend at each stage of growth of seedlings (Table 20 and Fig. 27). It was evident from the observation that relative growth rate in *Saraca asoca* gave generally lower values. Statistically significant differences were displayed among different seed sources after 90 days, 120 days, 180 days and 210 days. The tabulated data indicated the best performance in case of seed source SS 2 followed by seed source SS 4 in terms of relative growth rate. It was noted that maximum growth rate was observed in the initial months of observation. Towards the final two observations, relative growth rate values for seed source SS 4, were on par with seed source SS 2. In general relative growth rate showed declining trend across the entire period of observation.

4.1.4.9 Chlorophyll content

The data regarding the chlorophyll content of leaves have been tabulated below (Table 22 and Fig. 28). Significant variation was seen among different seed sources with respect to chlorophyll content in *Saraca asoca* leaves. Significant variation was observed after 120, 150, 240 and 260 days of seedling growth. In the

Table 19. Shoot-root dry weight ratio of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 Days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	1.3	1.3	1.1^{b}	1.1^{ab}	1.1^{b}	1.1^{b}	1.1^{c}	1.1^{bcd}
SS 2	1.6	1.4	1.5^{ab}	1.4^{ab}	1.4^{ab}	1.3^{ab}	1.3^{ab}	1.4^{a}
SS 3	1.6	1.4	1.5^{ab}	1.3^{ab}	1.3^{ab}	1.2^{b}	1.2^{bc}	1.2^{abc}
SS 4	1.4	1.8	1.8^{a}	1.6^{a}	1.6^{a}	1.6^{a}	1.5^{a}	1.3^{ab}
SS 5	2.0	1.4	1.2^{b}	1.2^{ab}	1.0^{b}	1.0^{b}	1.1^{c}	1.0^{d}
SS 6	1.7	1.6	1.1^{b}	1.0^{b}	1.2^{b}	1.1^{b}	1.1^{bc}	1.0^{cd}
SEm±	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Table 20. Relative growth rate (RGR) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period						
	90 days	120 Days	150 Days	180 days	210 Days	240 days	260 Days
SS 1	0.0066^{ab}	0.0047^{ab}	0.0032	0.0041^{b}	0.00383^{ab}	0.0031	0.0032
SS 2	0.0083^{ab}	0.0036^{ab}	0.0026	0.0030^{b}	0.0025^{ab}	0.0037	0.0034
SS 3	0.0052^{b}	0.0043^{ab}	0.0023	0.0047^{ab}	0.0048^{ab}	0.0038	0.0046
SS 4	0.0137^{a}	0.0024^{b}	0.0026	0.0031^{b}	0.0024^{b}	0.0096	0.0046
SS 5	0.0101^{ab}	0.0064^{a}	0.0038	0.0041^{b}	0.0041^{ab}	0.0031	0.0059
SS 6	0.0043^{b}	0.0067^{a}	0.0032	0.0074^{a}	0.0053^{a}	0.0025	0.0030
SEm±	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Plate 27: Two month old albino plant of *Saraca asoca* obtained from seed source SS 1

Plate 28: Appearance of albino *Saraca asoca* obtained from seed source SS 1 after six months

final observation the seed source SS 2 and SS 4 produced the maximum chlorophyll content. Whereas the lowest value was represented by the seed source SS 5 throughout the observation period. After 60 days of germination an albino plant of ashoka was noticed in the seed source SS 1 (Plate 27). The plant survived for about five months. The Plate 28 depicts the final appearance of the albino ashoka. Initially the plant had a height of 16.4 cm, collar girth of 2.2 mm and three leaves. Towards the final stages, the plant attained a height of 21.5 cm, collar girth of 3.5 mm and six leaves. The height and collar girth increment achieved in the albino plant was low when compared to other seedlings. Whereas the leaf production in ashoka, was satisfactory in the survival period of albino seedling. However, the size of the leaves appeared to be small when compared to other seedlings.

4.1.4.10 Photosynthetic rate

Significant variation was evident with respect to the rate of photosynthesis after 90 days and 180 days. After 90 days of seedling growth, seed source SS 4, gave notably better performance when compared to other seed sources. The data regarding photosynthetic rate is depicted in the Table 23 and illustrated in the Fig. 29. But after 180 days, seed sources SS 2, SS 3 and SS 5 showed values which were on par with the performance of seed source SS 4. Apart from these two variation observed during the study period, the performance of the seedlings were consistent throughout the observation period. No significant variation was observed during any stages of growth, with respect to photosynthetic rate among different seed sources other than 90 days and 280 days of observation period.

4.1.5 Incidence of pest and disease

The entire observation period was invigilated for the occurrence of pest and disease in the seedlings. However, all the seedlings were found to be healthy and no serious pest and disease was noticed during the study period.

Table 21. Chlorophyll content recorded in leaves (µ mol/m^2) of *Saraca asoca* seedlings representing six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	26.0	26.4	26.9^{ab}	27.2^{ab}	27.6	27.9	27.9^{ab}	28.2^{ab}
SS 2	26.2	26.9	27.5^{a}	27.7^{a}	27.8	28.1	28.2^{a}	28.3^{a}
SS 3	25.8	26.3	26.3^{b}	27.1^{ab}	27.0	27.2	27.4^{bc}	27.6^{bc}
SS 4	25.9	26.6	27.2^{ab}	27.4^{ab}	27.7	28.0	28.1^{a}	28.3^{a}
SS 5	25.4	26.1	26.4^{b}	26.7^{b}	27.1	26.9	27.2^{c}	27.4^{c}
SS 6	25.8	26.5	27.2^{ab}	27.4^{ab}	27.5	27.8	28.0^{ab}	28.1^{ab}
SEm±	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

Table 22. Rate of photosynthesis (µ mol/m^2/s) recorded for *Saraca asoca* seedlings collected from six seed sources

Seed sources	Observation period							
	60 days	90 days	120 days	150 days	180 days	210 days	240 days	260 days
SS 1	27.2	26.8^{ab}	27.2	29.8	28.3^{ab}	29.9	31.1	30.8
SS 2	25.7	25.4^{ab}	27.6	30.5	29.5^{a}	30.2	30.8	31.1
SS 3	27.1	25.0^{ab}	27.0	27.5	29.4^{a}	27.4	29.7	29.4
SS 4	26.8	28.1^{a}	28.5	29.4	29.3^{a}	29.8	29.5	30.9
SS 5	24.1	21.5^{b}	26.5	28.8	26.5^{b}	27.5	28.0	29.9
SS 6	25.0	24.1^{ab}	29.5	27.8	29.0^{a}	29.8	29.7	30.2
SEm±	1.2	1.9	1.2	1.0	0.8	1.0	1.0	1.0

Values having the same alphabets in a column do not differ significantly (significant level at 5 %)

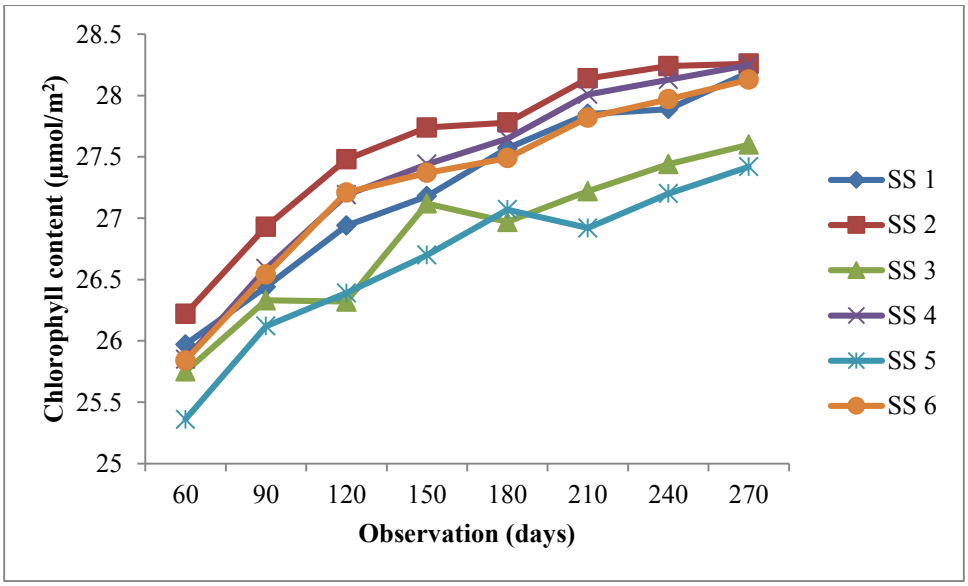


Fig. 28: Chlorophyll content recorded in leaves of *Saraca asoca* seedling representing six seed sources

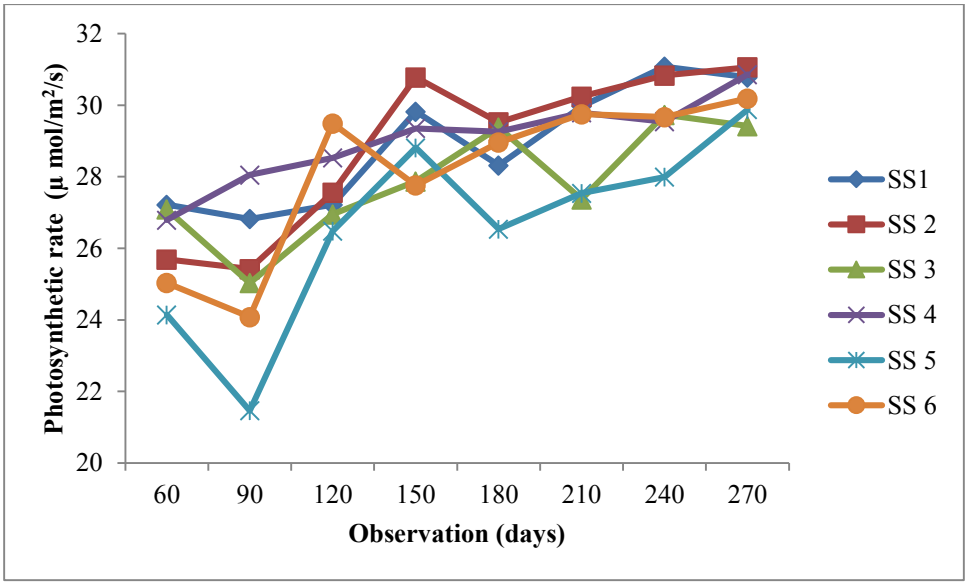


Fig. 29: Rate of photosynthesis recorded for *Saraca asoca* seedling collected from six seed sources

4.1.6 Cluster analysis

The dendrogram in the present study identified five cluster grouping with respect to the six seed sources (Fig. 30). Dendrogram revealed a very close relationship between the seed source SS 2 and SS 4. Similarly seed source SS 3 and SS 6 exhibited more similarity with respect to each other when various traits were considered. The seed source SS 1 showed similarity with the cluster SS 3 and SS 6. The cluster analysis revealed a distant relationship between the seed source SS 2 and the cluster grouping, consisting of seed sources SS 3, SS 6 and SS 1. A highly distant genetic relationship was shown by the seed source SS 5, with the rest of the seed sources.

4.2 Karyotype analysis

Standardization of different cytological steps were carried out, for better observation of somatic chromosomes. Chemicals were used in different combination for standardizing the technique so as to obtain good slide preparation.

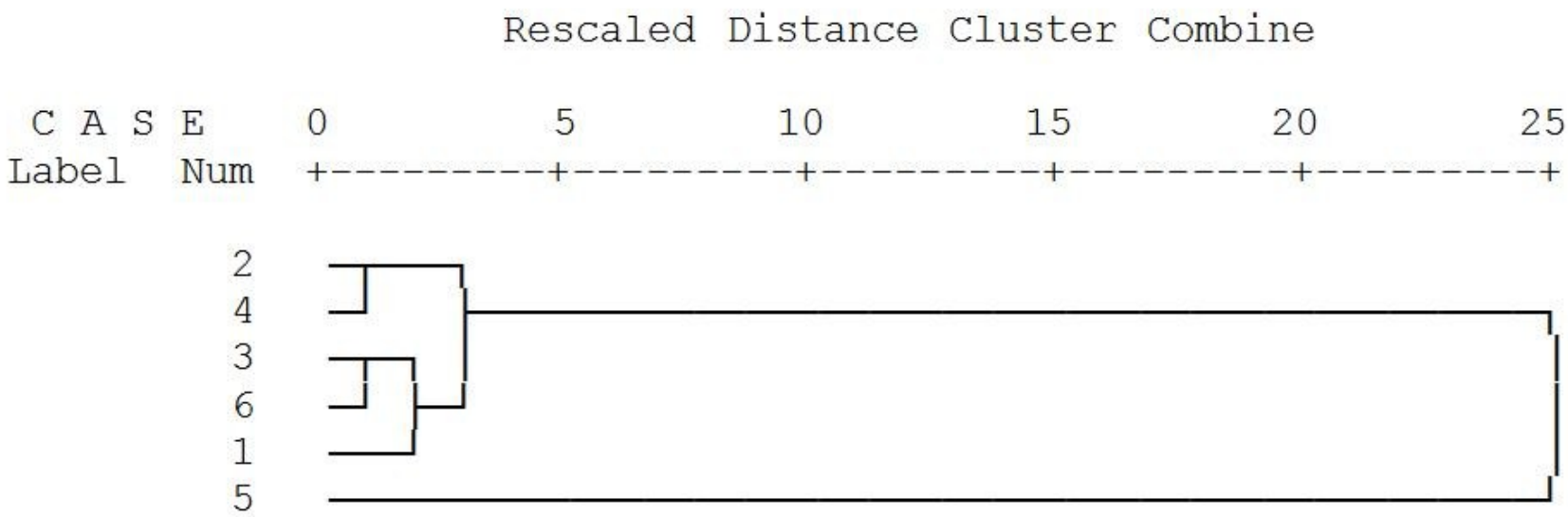
4.2.1 Pre-treatment of roots

As per the present investigation, 8-hydroxyquinoline gave better results when compared to colchicine. In most of the cases choromosomes were distinctly visible when pre-treated with 8-hydroxyquinoline (Plate 29, Plate 30, Plate 32, Plate 34, and Plate 35). Thus it can be said that condensation and chromosome separation was comparatively better in case of 8-hydroxyquinoline. Apart from this, root tips pre-treated at 10.30 am gave the maximum cell division in *Saraca asoca* (Plates 30-35).

4.2.2 Fixing of the roots

Among the two fixatives used Carnoys II was found to be the effective one (Plate 29, Plate 30, Plate 31 and Plate 34). In case of Carnoys I, cytoplasm staining was frequently observed in the slides when viewed through the microscope.

Fig. 30 Dendrogram using average linkage (between groups)



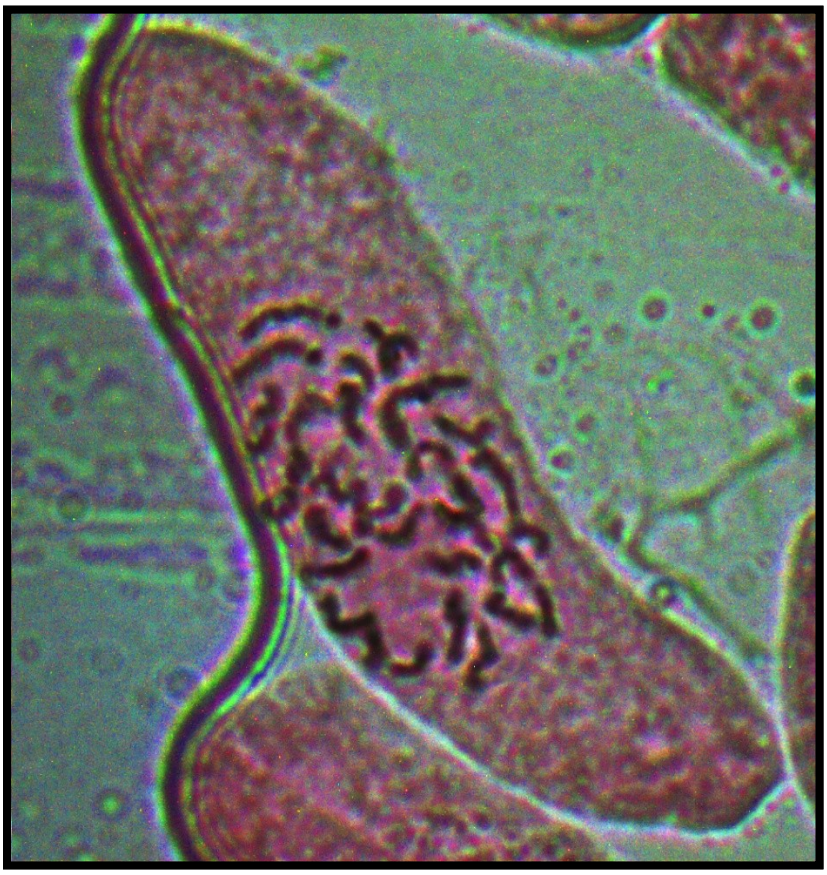


Plate 29: Photograph of *Saraca asoca* cell with chromosomes (X 10000)

Plates 30-35: Cells of *Saraca asoca* treated in different combination of acetocarmine and fuelgen stain fixed at 10.30 am.

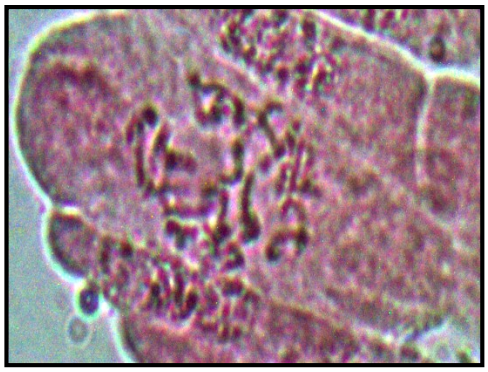


Plate 30: View of cell treated in 8-hydroxyquinoline, Carnoys II and acetocarmine (X 10000)

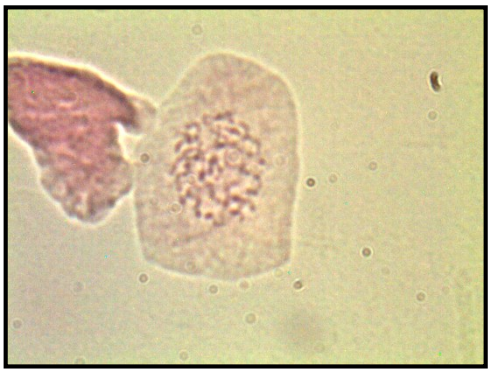


Plate 31: View of cell treated in colchicine, Carnoys II and acetocarmine (X 10000)

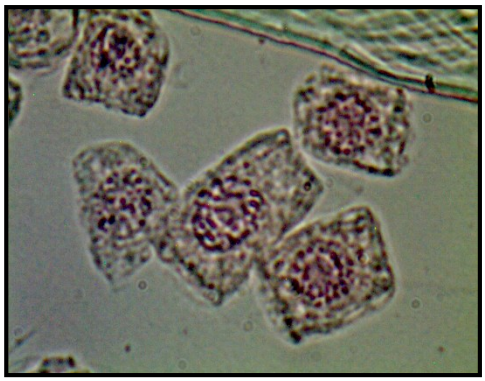


Plate 32: View of cell treated in 8-hydroxyquinoline, Carnoys I and acetocarmine (X 10000)

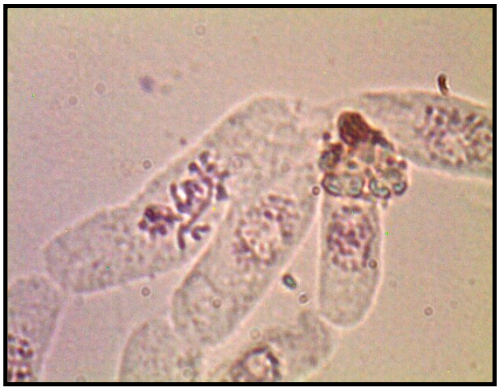


Plate 33: View of cell treated in colchicine, Carnoys I and fuelgen stain (X10000)

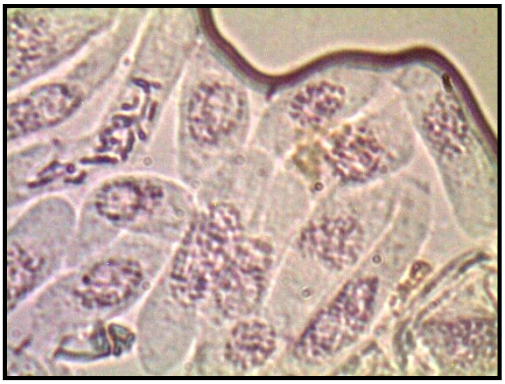


Plate 34: View of cell treated in 8-hydroxyquinoline, Carnoys II and fuelgen stain (X6000)

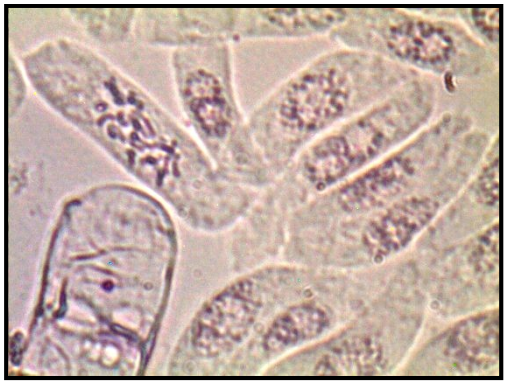


Plate 35: View of cell treated in 8-hydroxyquinoline, Carnoys I and Fuelgen stain (X6000)

Chromosomes usually appeared to be darker in Carnoys II reagent. Clusters of shrinked cells were commonly observed in root tips fixed in Carnoys I solution.

4.2.3 Squashing and staining of the roots

Staining was preceded by an elaborate process of squashing technique. In case of acetocarmine staining the root tips were gently heated for 10-15 minutes until the tissue was softened. The softened root tips were easily squashed over a glass slide after covering it with a cover slip. It was observed that while squashing, the cell separation was very good in case of acetocarmine staining. Whereas, in case of fuelgen staining proper hydrolysis of the root tip was not attained hence, softening and squashing of the root tips were found to be difficult in the present study. Fuelgen stain is known for its ability to selectively stain the chromosomes (Plates 33-35). However, the main draw back with fuelgen staining was with respect to its preceding step of hydrolysis. Therefore in most of the cases proper squashing and spreading was not sufficient to get good slide preparations. Among the two stains used, acetocarmine stain gave better results (Plate 29 and Plate 30). The advantage with the acetocarmine staining was that a single layer of cells were able to view with good spread of chromosomes. This was achieved because of better softening and squashing of the root tips.

4.2.4. Karyotype preparation

A well spread cell in metaphase stage was used for the preparation of karyotype of *Saraca asoca* (Plate 29). The cell used for karyotype preparation has undergone a pre-treatment with 8-hydroxyquinoline, followed by fixation in Carnoys II and staining with Acetocarmine. Careful observation indicated the presence of 34 chromosomes in the cell. Karyotype of *Saraca asoca* is shown in the Plate 36. Chromosomes were classified into 17 sets of homologous pair and arranged according to its decreasing length. The pairs were numbered from one to seventeen in

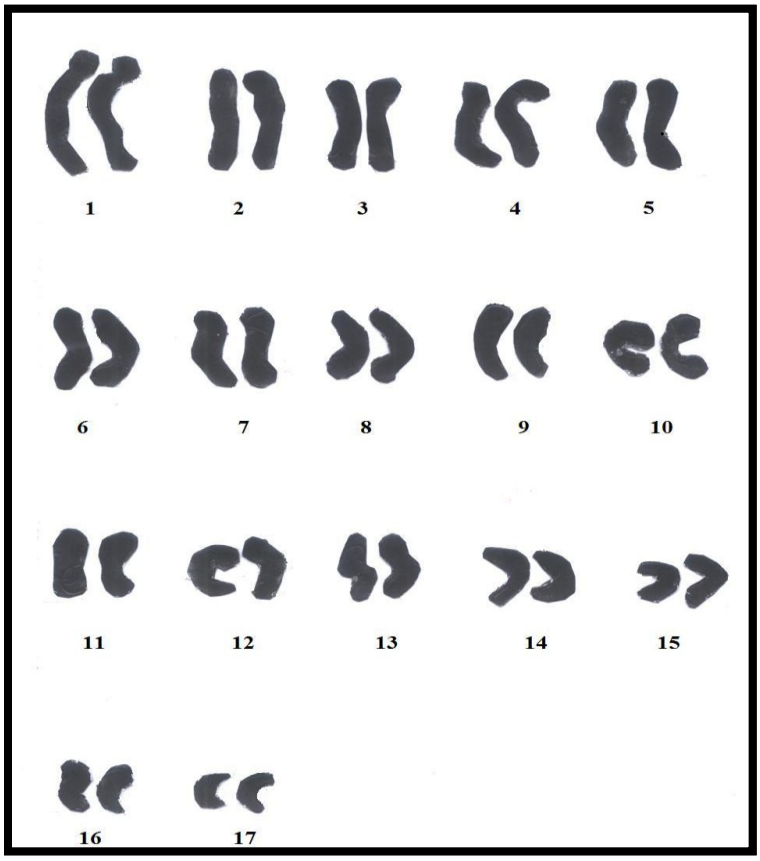


Plate 36: Karyotype of *Saraca asoca* showing chromosomes 2n=34

the prepared karyotype. Exact centromere location was unable to detect hence only the individual chromosome length was measured.

4.2.5. Idiogram preparation

In order to prepare the idiogram the length of each chromosome was measured with the help of image analyser (LABOMED iVu 3000 model) and the data is presented in Table 23. Idiogram based on relative chromosome length (RCL) was also constructed, apart from the idiogram obtained with absolute chromosome length (Fig. 31 and Fig. 32). Based on the measurements it was found that the length of chromosome in ashoka ranged from a maximum of 12.1 µm to 5.3 µm. Total chromosome length (TCL) was obtained as 127.2 µm in *Saraca asoca*. Likewise the relative chromosome length in ashoka ranged from 9.5 µm to 4.2 µm.

Table 23. Total chromosome length (TCL) and relative chromosome length (RCL) measured in *Saraca asoca*

Chromosome number	Total chromosome length (µm)	Relative chromosome length (µm)
1	12.1	9.5
2	9.9	7.8
3	8.8	6.9
4	8.3	6.5
5	8.0	6.3
6	7.8	6.1
7	7.7	6.1
8	7.5	5.9
9	7.2	5.7
10	6.8	5.3
11	6.8	5.3
12	6.6	5.2
13	6.5	5.1
14	6.4	5.0
15	6.2	4.9
16	5.3	4.2
17	5.3	4.2

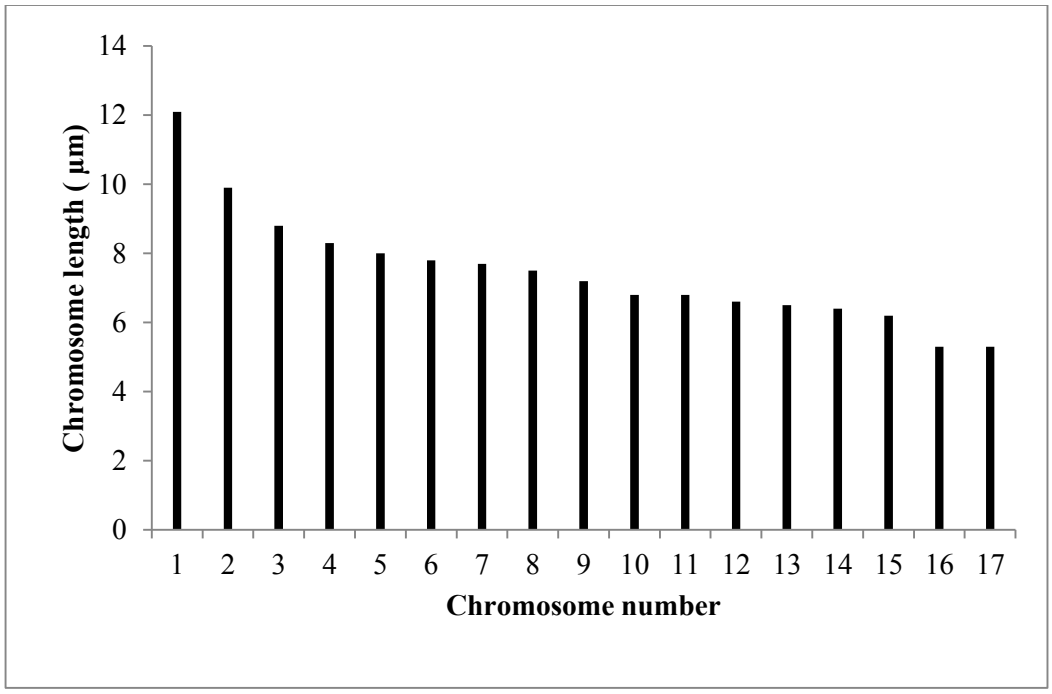


Fig.31: Idiogram based on absolute chromosome length

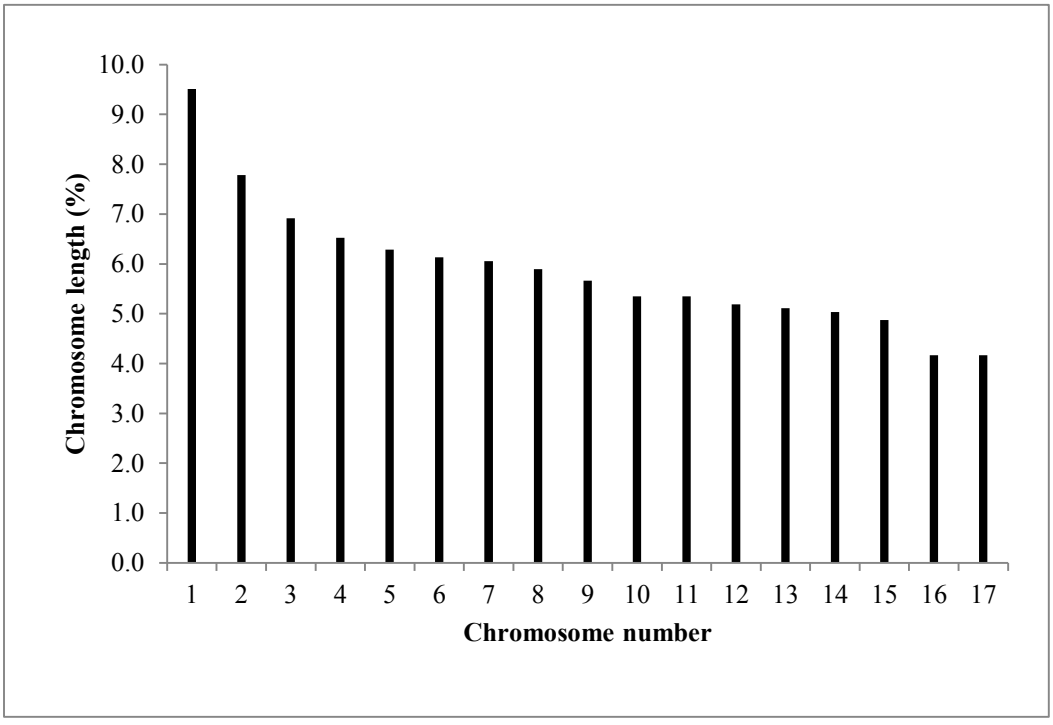


Fig 32. Idiogram based on relative chromosome length (RCL)

Discussion

DISCUSSION

Saraca asoca, an important medicinal tree is gaining popularity in the field of research. This 'divine' tree is known for its miraculous effect on gynaecological disorders and also have great effect on curing other diseases like ulcer, skin disorders, helminthes, ascites, heart ailments etc. Considering its potential in the field of pharmacology, due importance must be given in planting and cultivation of ashoka. Many studies on different ethno medicinal and pharmacological properties of ashoka had been already carried out and further investigations to explore in this respect, are still going on. However, studies to understand the provenance and seed source evaluation of ashoka have been meager.

The present study has made a successful attempt to understand the seed source variation in ashoka trees of Kerala. The study specifically tried to evaluate the variation among different seed sources on the basis of latitude. Moreover, the present investigation could also reveal the chromosome number in ashoka along with standardizing the procedure for cytological analysis. The information regarding the seed source variation and cytogenetics will therefore be useful in successfully establishing the best plantations of ashoka, which in turn could meet the rising demand for its different parts in the market. The results from the present study can be useful in further research works. This section, has dealt with discussing and interpreting the results obtained from the current study.

5.1 Seed source variation studies

5.1.1 Observations on seed traits

It is known that seed size is a key determinant of evolutionary fitness in plants and is a trait that often undergoes tremendous changes during domestication. Ashoka,

a tree that is under the verge of extinction from the wild, is mostly found in homesteads, institutions, parks, roadsides etc as domesticated tree. Thus the need to analyse the variation in seeds will help to understand any evolutionary changes in *Saraca asoca*. Thus evaluating the seed traits can be regarded as the first step in understanding the seed source variation.

Present study has attempted to depict the variation between seed sources of ashoka with respect to different seed traits. Seeds were collected from different locations across Kerala. Seed morphological aspects like seed length, seed breadth, seed length to breadth ratio, seed weight and pod thickness, have been assessed in the current study. These parameters will prove useful in determining the superiority of different seed sources from each other. The performance of seed morphological aspects can be indirectly co-related to its performance in future growth and development. The interpretation of the results obtained for different seed traits of ashoka have been discussed hereunder.

5.1.1.1 Seed length

The study showed a significantly low value for seed source SS 3 with respect to seed length, when compared to other seed sources. In spite of this, seed length did not vary markedly among other seed sources. The trait, seed length in general was found to influence the germination and growth ahead, in case of seed source SS 3. It was also noted that the performance of seedlings from the sources SS 1, SS 2 and SS 4 was found to be the most superior in most of the seedling growth attributes. Correspondingly, the seed length of SS1, SS 2 and SS 4 exhibited better performance as shown in the results. Thus a corresponding relation can be drawn for seed length and performance of seedlings. Supportingly, Gera et al. (1999) in the study on source variation in *Albizzia procera* have found that, seed length was positively and significantly correlated with germination percentage as well as seedling collar

diameter. The study strongly indicated the importance of seed length as a criterion for selection of seed sources and recommended seed collection from lower latitude populations for better seedling growth.

In another finding, Tyagi et al. (1999) suggested that seed length and 100-seed weight can be used as the predictors of germination in *Grewia optiva*. Contradictory to this, seed source SS 5 which displayed good seed length, could not perform well in terms of its seedling attributes. This might have occurred may be because of the presence of mostly non viable seeds obtained from the seed source SS 5, that appeared morphologically sound but may have possessed internal damage.

5.1.1.2 Seed breadth

Breadth of the seed was maximum for seed source SS 4 when compared to other sources. Similarly seed source SS 2 displayed seed breadth on par with SS 4. The observation recorded for seed source SS 6 and SS 1 followed the seed source SS 2. Significant differences among different seed sources was observed in case of seed breadth. Similarly, Vanangamudi et al. (1999) in their study on effect of seed source on physical and physiological qualities of *Acacia nilotica* seeds, could find significant difference among the sources with respect to seed breadth. Greater breadth of seeds points towards larger size of seeds. Studies in this regard, suggest that seedlings derived from larger seeds have a greater competitive ability than those emerged from smaller seeds, which results in slower growth (Cideciyan and Malloch, 1982; Howe and Richter 1982; Dolan 1984; Zhang and Hamill 1997), later flowering, and reduced seed production in adult plants derived from smaller seeds (Stanton, 1984; Wulff, 1986). As in the case of seed length, seed source SS 3 exhibited the least value for seed breadth of ashoka seeds. Correspondingly, their seedling attributes were not as good as of the performance of other seed sources like SS1, SS 2 and SS 4.

5.1.1.3 Seed length to breadth ratio

Seed length to breadth ratio was maximum for seed source SS 1, followed by seed sources SS 3 and SS 5. A good seed length to breadth ratio indicates nearness to round shape. It was observed that the seeds in all the seed source, either had a flattened or cylindrically bold appearance. The seeds from seed source SS 2 were bolder and distinctly dark when compared to other sources. This may be attributed towards the typical site quality existing in the 'Kuttanad' areas of Kerala that might have contributed towards variation with respect to the seed source SS 2. The Kuttanad area of Kerala is below sea level composed of riverine alluvial soil, which is generally clay loam in texture. The seeds from the source SS 3 were smaller and flattened in shape. Similarly seed sources SS 4 and SS 6 showed parallel performance in this respect and appeared to be more or less bolder in shape.

In general, seed source SS 5 gave significantly good seed length to breadth ratio. Inspite of this positive performance of seed source SS 5, it could not give better performance in germination and other growth aspects. It is assumed that good seed trait facilitates better germination and growth. But this does not seem to be true in case of seed source SS 5. Perhaps this may attributed towards constituting non viable seeds in the source SS 5. Unlike other studies a positive relation between seed size and seedling attributes could not be expressed in case of seed source SS 5. This may be because of the presence of sound seed dimension associated with internal damage, thus making the seeds non-viable.

5.1.1.4 Thickness of pericarp

Thickness of pericarp did not vary result across different seed sources. The variation however was notable with respect to the seed source SS 5 producing significantly the least value. In the findings of Ahlawat et al. (2007), significant variation among different studied seed sources was claimed with respect to seed

source variation study, in pod and seed characteristics of *Acacia nilotica*. In another study in *Dalbergia latifolia*, significant variation was observed with respect to pericarp thickness among the studied seed sources (Singh and Pokhriyal, 2001).

Generally the seed sources with greater volume produced larger seed coat thickness. Contrary to this, seed source SS 5 produced significantly the lowest seed coat thickness and not seed source SS 3. This may be because of the influence of degrading seed coat with respect to seed source SS 5. This in turn may be because of delayed seed collection from the seed source SS 3 and consequently degraded seed coats were available to assess from this source. The phenology of flowering and fruiting with respect to this region had already occurred before the normal collection period. Moreover, the seeds of *Saraca asoca* being recalcitrant, adequate storage techniques were not available. This might have resulted, mostly in the collection of non viable seeds with degraded pericarp. This aspect, regarding phenology was however not part of the study.

5.1.1.5 Seed weight

It is noted from different studies that seed weight exhibits highly positive co-relation with its germination behavior and vegetative growth. Aslan (1975) and Isik (1986) carried out detailed studies on the relations between seed dimensions and seedling growth of *Pinus brutia* and it has been observed that larger and heavier seeds produced better quality seedlings. The relevance of seed weight was also investigated in another study, in *Acacia nilotica* (Ahlawat et al. 2007). As per the study, an improvement in seed weight will improve other associated traits. In a similar study by Manga and Sen (1996) in *Prosopis cineraria*, seed weight, seed volume and seed thickness was found to have significant and positive correlations with germination percentage. Thus it can be recommended from the present study that seed weight should be given priority for improving germination in seedlings of ashoka.

In the current study, seed sources SS 2, SS 1 and SS 4 recorded significantly higher values with respect to seed weight. It was also noted that, the germination studies and the seedling attributes discussed further, recorded significantly higher values in case of these three seed sources. Similarly the seed sources SS 3 and seed source SS 6 were the next best performers in general terms. But seed weight of seed source SS 3 was reported to be lower than the seed source SS 5. This probably is because the seeds from the seed source SS 5 might have associated with lower viability. The seeds of ashoka being recalcitrant is supposed to be collected on time and sown as soon as possible. The early seeding and there by delayed collection, in seed source SS 5, effected severely in the growth and well being of the seeds and seedlings from this source.

5.1.2 Seed germination attributes

Evaluation of germination aspects of any species plays crucial role in anticipating its growth and further development. Hence, an early assessment of better quality seedlings for plantation can be done with respect to seed germination. Ginwal and Gera (2000) in their study in *Acacia nilotica* provenances have confirmed a strong positive correlation between seed germination with the growth of field planted seedlings. A number of works have established positive relationship between germination attributes and seedling growth (Gera et al. 1999; Tyagi et al. 1999; Neerja and Pokhriyal, 2001).

Different germination aspects gave varying results for different seed sources in the present study in ashoka. However, the seed source SS 5 showed the least performance with respect to all the germination attributes. This phenomenon can be attributed to the collection of mostly nonviable seeds from this seed source. Normally heavy seeding in Kerala is experienced in the month of May, June and July. Contradicting to this, the seed source SS 5 seeded earlier. This in turn produced

mostly non-viable seeds for the seed source SS 5, at the time of usual collection. As a result germination was severely hampered in most of the seeds of seed source SS 5. The discussion on the results obtained for germination attributes have been explained below.

5.1.2.1 Days required for germination to initiate

The commencement of germination in ashoka was observed after one month of sowing in a study conducted in Kerala (Singh et al. 2005). The germination was triggered the fastest in case of seed source SS 4. Imbibition period was the shortest with respect to seed source SS 5. The close resemblance of seed source SS 4 with the surrounding environment might have promoted faster germination in seed source SS 4. It can be noted that seeds from the experimental area had also contributed to the seed source SS 4. Hence it can be hypothesized that the environment was most conducive to trigger germination first in this particular seed source (Appendix I). It has already been observed that local seed source has a tendency to germinate faster (Hossain et al., 2005).

Seed source SS 2 followed the source SS 4 in terms of performance with regard to commencement of germination. Slightly delayed collection of seeds from this zone may be regarded as the reason for seeds to germinate fast in this particular source. Apart from this factor, all the seeds from the source SS 2 appeared to be healthy and heavy. This might have also contributed towards faster germination in seed source SS 2. Seed source SS 6 and SS 3 showed the next best performance in this regard. Even though seed source SS 3, possessed the lowest size, it was also found to be successful in faster germination. This, therefore suggest that seeds from this source were extremely healthy and viable seeds even though their size was comparatively small.

It took maximum number of days for seed source SS 5 to initiate germination. This suggests that the viability of some seeds were maintained for longer time in seed source SS 5. The germination of seed source SS 1 was also delayed, that is its imbibition period was longer. The reason can be attributed towards longer viability of seeds or may be because of the presence of impermeable seed coat in the SS 1 seeds. In support of this, Malavasi (1988) have explained that tropical tree seeds exhibits delayed or non-uniform germination because of impermeable seed coats to water or oxygen, mechanical restrictions, or a combination of these with presence of chemical inhibitors. This therefore might have triggered germination after longer time in seed source SS 1, which otherwise is regarded as one among the best seed source for ashoka.

5.1.2.2 Days required for germination to cease

Germination lasted for maximum days in case of seed source SS 4 (74.8). This was followed by seed sources SS 2, SS 1 and SS 5. Lowest days for germination were for SS 6 and SS 3. As seen already, initiation of germination was rapid in case of seed source SS 4. This indicates that the seed source may be comprised of a range of seeds, with different imbibition period. Seed source SS 3 took the least days for germination to cease. This suggests that the seeds were healthy and viable. In the succeeding discussion, it is evident that germination percentage of the seed source SS 3 was quiet better within this short span of time. The data therefore points towards the fact that better germination can be attained for all seed sources especially for SS 3, in minimum time if the seeds are collected and sown as per the present experimental procedure.

Seed source SS 2 and SS 1, which displayed maximum germination percentage, also took considerably long time for germination to cease. This fact suggests that most of the seeds can be in dormant state. In general, the variation

observed with respect to this trait among different seed sources may probably be influenced by different intensities of natural constraints acting upon these traits with respect to the prevailing geographic or climatic conditions. Nursery studies conducted on *Dalbergia sissoo* seedlings raised from six provenances indicated that environmental parameters can play an essential role in evaluating the seed sources in *D. sissoo* (Singh and Pokhriyal, 2000).

5.1.2.3 Total germination days

The period of germination or total germination days were assessed by subtracting the days required to initiate germination from the days required to cease the germination. Germination period gives an idea about the duration of germination. This can therefore be used to evaluate the vigour of whole seed source, after triggering germination process. Maximum germination period was attained by the seed sources SS 4 and SS 2. This suggests that the seeds did not display vigorous germination. It was also noted that germination per cent attained was highest in these two cases. Thus it can be because of some factors that lead to seed dormancy.

Seed source SS 4 and SS 2, utilized its full potential with respect to germination of seeds. According to the result seed source SS 5 showed the least germination period. However, this cannot be attributed to the vigorous nature of the seeds as germination percentage attained was too low. It was also observed that it took the longest days for SS 5 to initiate its germination. Thus it can be said that the performance of seed source SS 5, was comparatively the least, with respect to seed germination. The seed source SS 1, SS 3 and SS 6 took relatively small germination days when compared to SS4 and SS 2. Even though the seed source SS 1 took longer time to initiate the germination, the vigourosness with which the seeds germinated was commendable. On going through the germination percentage data it can be said that the seed source SS 1, with maximum germination percentage can be regarded as

the most vigorous seed source. Vigorousness was also high in case of seed source SS 3 and SS 6.

5.1.2.4 Days required for 50 per cent of germination

The days required for 50 per cent of germination was analysed from the day after sowing the seed. The data came out to be useful in interpreting the vigour of seed source based on its performance right from the first day of sowing. Days required for germination to initiate along with the total germination period was the deciding factors for making the days required for 50 per cent germination more effective. Seed source SS 6, SS 3 and SS 4 took the lowest days for attaining 50 per cent germination. This suggest that the coupled effect of days required for initiating germination as well as the total germination days, was most successful in case of seed source SS6, SS3 and SS 4. The maximum days required for attaining 50 per cent of germination was for seed source SS 5. This result can be attributed to the fact that the days required to initiate germination was very high in case of seed source SS 5 consequently the attainment of 50 per cent germination was also delayed.

5.1.2.5 Germination percentage

The performance of seedlings is considered as a measure of seed health and viability. Various studies with respect to germination percentage showed significant differences among the seed sources (Ginwal and Gera, 2000; Shekhar et al., 2002; Singh et al., 2005). Results from the present study indicated that, germination per cent achieved was maximum in case of seed source SS 1 (98 per cent). Seed source SS 1 took more number of days for attaining maximum germination per cent. This was followed by seed source SS 2, SS 4, SS 6 and SS 3. Altogether high germination per cent was attained for *Saraca asoca*. A germination percentage of 83 per cent was noted in ashoka by Singh et al. (2005) in the seed germination study of ashoka conducted in Kerala.

However the seed source SS 5, gave very low germination per cent when compared to other seed sources. The seed parameters like, better seed length breadth ratio, seed weight and pod thickness may have contributed towards better germination per cent in all the sources except for the source SS 5. However the seed source SS 3, showed relatively lower seed traits, still the germination per cent was on par with other seed source. It is therefore evident from the present study that size of the seeds is not a deciding factor for seed germination. The study showed that viability and healthiness of the seed determines good germination per cent and it differed between the seed sources.

5.1.2.6 Germination energy

Germination energy is regarded as a measure of speed of germination and hence is also assumed as a measure of vigor of seedlings. Considerable difference was observed among different seed sources. Maximum germination energy was observed for seed source SS 3 followed by seed source SS 1. It was observed that, seed source SS 3 experienced the least days for germination to attain 50 per cent. Apart from this, days required for germination to initiate and the total germination days were also comparatively less with respect to seed source SS 3. Along with these attributes germination percentage was also moderate in case of SS 3. All these factors points towards higher germination energy of SS 3. Seeds from this source appeared to be healthy even though it had small seed size.

The least germination energy was seen by the seed source SS 5. As mentioned in case of other measures of germination, it took comparatively more number of days for seed source SS 5 to initiate germination. Further seeds of this seed source probably had lower viability. Germination energy was also good in case of seed sources SS 4 and SS6, whereas seed source SS 2 had lower performance in terms of germination energy. Germination in seed source SS 2 continued for a longer period.

Hence more or less uniform germination of seeds were observed throughout this period. Thus it can be concluded that the seed source SS 3, SS 1, SS 4 and SS 6 were less uniform in germination, maximum germination being observed in the initial days.

5.1.2.7 Mean daily germination

Mean daily germination has a direct relation with germination per cent and simultaneously an indirect relation with total days required for germination. Mean daily germination (MDG) is a measure of number of seeds germinated per day. Maximum mean daily germination was observed in case of seed source SS 1. This had a corresponding result with germination per cent, where seed source SS 1 had the highest germination per cent. Seed source SS 2 followed SS 1with respect to MDG values. This again had a higher germination per cent. Seed source SS 6 and SS 3, exhibited the next best performance. This also can be attributed to corresponding values of germination per cent. Similarly as a consequence of lower germination percentage, the seed source SS 5, has produced the least MDG values. In case of seed source SS 4, the MDG values were comparatively less. Here, the reason point out towards more number of days taken for germination rather than higher germination per cent.

5.1.2.8 Peak value

The highest MDG value obtained for a source was regarded as the peak value. The peak value among the given sources did not vary significantly. However, seed source SS 5 displayed exceptionally lowest value. In comparison to one another, the seed source SS 3 gave the maximum peak value. Whereas the corresponding MDG value was not higher. Therefore it can be said that the seed source has a higher potential for daily germination contrary to the present MDG value. Seed source SS 1 immediately followed seed source SS 3. But the MDG value was the highest for this

particular source. These findings confirm that seed source SS 1 has more or less utilized its maximum potential for daily germination. Similar is the case with SS 2, SS 4 and SS 6. The lowest peak value was given by the source SS 5. It is interesting to note that the MDG value and peak value was almost similar in the case of seed source SS 5. This suggests that seed source SS 5 has utilized its full potential in germination of seeds. Even then the seed source SS 5 could not perform well. The findings therefore support that seed source SS 5 could have also been a better source in terms of germination aspects, if the recalcitrant seeds of ashoka were collected on time and consequently avoiding the old seeds from sowing.

5.1.2.9 Germination value

The product of mean daily germination and peak value is the germination value of a species. The maximum germination value was attained by the seed source SS 1. Germination value of seed source SS 2, SS 3 and SS 6 followed seed source SS 1. In a study on *Pinus roxburghii*, Thapliyal and Dhiman (1997) found out that GV was significantly correlated with seedling height. Germination value can therefore be regarded as an integrated measure of seed quality, which has been used by several tropical seed workers e.g. for *Terminalia ivorensis* (Okoro, 1976) and for *Pinus kesiya* (Costales and Veracion, 1978).

5.1.3 Seedling attributes

The performance of various seedlings attributes on morphology and physiological aspects can be considered as an important tool in locating the best seed source. Seed source variability in seedling attributes has been conducted in a number of species and has found out significant variation with respect to different seedling attributes (Ginwal et al., 1996; Thapliyal and Dhiman, 1997; Maideen et al., 1997; Kundu and Tigerstedt, 2007; Jalil et al., 2000). Studies have revealed that there exists a positive correlation between performance of a seed source with its seedling

attributes. It is known that the best performing seedlings correspondingly will also perform better in further growth stages. Based on these facts, the present study had tried to evaluate the seed source variation of ashoka. The results of different seedling attributes have been discussed further.

5.1.3.1 Height

Significant variation was observed with respect to height in case of seed source SS 5 and SS 6, almost throughout the observation period. Different studies have confirmed that germination and plant height is a factor strongly influenced by environment. The characters that showed greater genetic influence can be directly screened or selected for the improvement of this potential tree-crop. In a study conducted by Ginwal et al. (1996) in *Acacia nilotica*, the existence of significant variation among seed sources for some seed and seedling traits was demonstrated. Height variation was also observed significantly in *Azadirachta indica*, as per the findings of Kundu (1998) and Jalil et al. (2000). Variation obtained in these studies for seed morphology was thought to be due to geographical differences. A significant relationship was found between seed weight and growth of seedlings in nurseries and plantations.

Among the different seed sources, SS 5 exhibited significantly lowest height. This response of seed source SS 5 can be attributed towards its poor performance at seed trait and germination behavior. This in turn was the consequence of availability of poor quality seeds at the time of collection with respect to seed source SS 5. Apart from this, the presence of typical red lateritic soil in this zone, may have also contributed towards its poor performance in the new environment. The rate of height growth in all the seed source was quiet low up to the month of December. Afterwards the rate increment in height, was better. This may have occurred as the seedlings were transferred to shade net with seedlings receiving sufficient light. The result therefore

gives an impression that in general the variation in height for the entire seed source is not commendable.

5.1.3.2 Collar girth

Grading of seedlings based on their height and collar diameter at an early age is recommended for selection of best performing seedlings in the field (Jijeesh et al., 2007). Seedlings with high collar girth and large number of leaves are known to have good yield potential (Sathyabalan and Mathew, 1984). The seedlings from the source SS 1 generally presented good collar girth throughout the observation period. This therefore indicates towards the potentiality of the seed source SS 1 in producing better quality trees in future. Even though other seed sources showed statistically significant difference with each other in collar girth, their performance in this respect can be considered as good. Therefore it can be assumed that all the seed sources have the potentiality to develop into quality trees. However, as usual the performance of the seed source SS 5 was the least. The effect of shifting the seedlings into lower shade level have also evident in case of collar girth. A rapid increase in collar girth was experienced after 210 days, immediately after shifting from the shade.

5.1.3.3 Leaf number, leaf area and leaf thickness

Number of leaves of seedlings in different seed sources were at par towards the end of the observation. Leaf number is an important criteria in determining the best seedlings (Sathyabalan and Mathew, 1984). Good number of leaves indicate better photosynthesizing ability, hence ensures better growth of seedlings in future. The present investigation suggests that there was no significant difference among the seed sources with respect to leaf number. Contradicting to other results of seedling attributes, leaf number in case of seed source SS 5, could also display better number of leaves, despite with its smaller size.

Significant difference was observed in different seed source with respect to the leaf area. The trend of growth in leaf area was almost same throughout the observation period. Monitoring changes in leaf area is important for assessing the growth and vigour of the seedling. Different studies have confirmed direct relation of leaf area with photosynthetic activity (Kundu and Tigerstedt, 1999). The leaf area for the seed source SS 5 was the least among the different sources. As the number of leaves in SS 5 was comparable to other sources, this might because of the smaller leaves in the source. Previous result it was observed that the seedlings of SS 5 had good number of leaves with smaller size. This therefore reduces the potential of further growth and development of seed source SS 5, on comparison with other sources. The performance of seed sources SS 1 and SS 2 were significantly higher than the rest. The leaf number in these two sources was also good. The coupled effect of both good number of leaves and leaf area can therefore ensure better growth rate in seed sources SS 1 and SS 2.

Leaf thickness did not vary among different seed sources. No significant difference in leaf thickness among seed source was observed throughout the study period. Studies have shown a positive co-relation between photosynthesis and leaf thickness (Mc. Millan and Mc. Clendon, 1983). Correspondingly, in the present study the photosynthetic rate also did not vary significantly among the seed sources. Thereby supporting the correlation between photosynthesis and leaf thickness. As per the present study, leaf thickness does not become a criteria in classifying the seed source into different sources based on the latitude wise assumption.

5.1.3.4 Number of branches

The study on branching pattern in ashoka did not give any significant difference between the various seed sources. This shows that in general early branching is uncommon in ashoka seedlings. However, seedlings from the sources SS

3 and SS 6 had a tendency for branching in the early stages itself. Gradually branching appeared in all the sources at the end of the observation period.

5.1.3.5 Biomass

Biomass estimation enables to analyse the photosynthetic efficiency of the plant. Findings of Kundu and Tigerstedt (1999) in neem confirmed a positive correlation between net photosynthesis with whole-plant dry weight and leaf area. It was noted from the tabulated data, that the variation in shoot fresh weight, with respect to different seed sources was profound during the earlier periods of observation. In the final stages of observation, fresh weight of the shoot did not vary significantly across different seed sources with the exception of seed source SS 5. While in case of shoot dry weight, significant difference was observed among different seed sources.

The seed source SS 2 displayed maximum shoot dry weight attributing towards its greater photosynthesizing activity. Among the seed sources, SS 5 produced the least significant values in terms of dry weight of shoot. In general a proportionate relationship can be established for biomass with respect to height, collar girth, leaf number and leaf area. Significant variation was also obtained for Neerja and Pokhriyal (2000), in terms of biomass production in the nursery study of *Dalbergia sissoo* seedlings. In case of root fresh weight, considerable variation was observed with respect to all the seed sources. In studies of Harper (1977) and Stanton (1984), it was found that, seedlings originating from large seeds have the ability to develop deeper and larger roots which allow greater survival and growth. In general the seeds of ashoka are larger and supporting the above statement its root are found to be lengthy and deeply penetrating. In the present investigation, the seed sources differed significantly in terms of dry weight of root. Maximum dry weight was exhibited by seed source SS 6. This indicates towards greater biomass allocation in

the root of SS 6, when compared to other sources. Studies have also revealed that good root weight in correspondence to shoot weight will ensure better establishment of seedlings. The chances for survival of seedlings are therefore maximum with respect to seed source SS 6.

The shoot-root dry weight ratio was maximum with respect to seed source SS 2. This indicates towards better biomass allocation into the shoot portion in seed source SS 2. Conversely, the allocation of biomass was more towards the roots in case of seed source SS 5, indicating its strong capacity for survival and establishment. Significant difference was exhibited by shoot to root dry weight ratio among the different sources at varying trend across the observation.

5.1.3.6 Relative growth rate (RGR)

Altogether, the present study showed a very low relative growth rate value, ranging from a maximum of 0.01 to 0.0023. It has been reported that the growth in *Saraca asoca* is rather slow even though it germinate easily (Chithra, 2004). Many studies have found that larger seeded species tend to have slower seedling growth rate (Fenner, 1978; Fenner, 1983; Gross, 1984; Shipley and Peters, 1990; Reich et al., 1998). Ashoka seeds are large in size and their relative growth was found to be very less. Analyzing the mean relative growth rate of seedlings is one method used to compare growth differences that arise from experimental treatments. It could also be used to compare differences due to genotype (Sweet and Wells, 1974; Kolb and Steiner, 1990) and planting stock size (Britt et al., 1991; Van den Driessche, 1992; Haase and Rose, 1993).

In the present study, there was no marked difference with respect to relative growth rate. During the entire observation period showed varying trend with regard to RGR. This finding support the compound interest law, which says that the amount of

growth made in a unit of time is a constant percentage of the size of the plant at the beginning of the period and the constant percentage does not change with size (West et al., 1920; Snedecor and Cochran, 1967; Kramer and Kozlowski, 1979; Hunt, 1990). But on careful observation, it was found that in the initial stages, the rate of growth was higher than in the later stages. This indicates that the seedlings in its earlier stages were very active and had the potential to utilise the available resources. Later on this capacity slowly started to diminish. Decreasing of available resources can also be regarded as reason for the declining trend of RGR in ashoka seedlings. This fact therefore stand by the variable interest law, defined as the amount of growth made in a unit of time is a percentage of the size of the plant at the beginning of the period and this percentage changes as the plant increases in size (often the percentage declines as size increases). Generally this law is applicable in the initial stages of all seedling growth and this phenomenon could be applicable in the present study. But in the later stages of growth there was an inclination towards the compound interest law.

5.1.3.7 Chlorophyll content and photosynthetic rate

Chlorophyll content in leaves did not show any notable difference along the observation period. However, significant difference among the seed sources was exhibited in the final two observations. Generally, chlorophyll content of a leaf can be correlated with leaf thickness and leaf area. In the present study significant differences were not found in case of leaf thickness in any of the seed sources, while the difference was significant with respect to leaf area. Chlorophyll content was the maximum in case of seed source SS 2 and correspondingly its leaf area was also maximum. The chlorophyll content and leaf area was the least in case of seed source SS 5. It was interesting to note an albino ashoka seedling among the seedlings of seed source SS 1. Albinism in plants is caused by lack of chlorophyll pigment. This could be fatal in plants as the albino plant has no way to manufacture the food needed for survival and growth to maturity. Albino seedlings usually live to about a week to

few months depending upon available reserve in the seed. Albino seedlings probably result from the combination in the seed of genes that were recessive in the parent plants. Even though albino plants do not live long, their occurrence is useful to those who study forest genetics. The genes that create albinism can be used as markers to examine the rates and patterns of seed dispersal from those trees which carry those particular genes (Zasada, 1980).

The photosynthetic rate, in the study did not vary significantly across different seed source during the observation period. More or less insignificant results for leaf area, leaf thickness and chlorophyll content might have implicated seriously in producing the same trend for photosynthetic rate. Slightly increasing trend in Photosynthetic rate was observed towards the last couple of observation. This can be attributed to the availability of increased light intensity, after shifting the seedlings from shade.

5.1.4 Incidence of pest and disease

The seedlings in the experiment were found to be healthy throughout the observation period. In a study of ashoka in Kerala, Singh et al. (2005) have also found the seedlings to be healthy. No serious threats from pest and disease attack were experienced in the experimental plot of ashoka seedlings. Studies have shown that usually the seedlings suffer a lot from anthracnose disease caused by *Colletotrichum gleosporiodes*. The disease, result in the total loss of photosynthetic area of the leaf and brings about serious threat to the economy (Strange, 2005). Several antibacterial and pesticidal property of ashoka have been studied (Annapurna et al., 1999; Katiyar et al., 2007) in its different parts. The influence of the chemistry has to be explored in making the ashoka seedlings resistant to pest and disease.

5.1.5 Cluster analysis

The Information on seed traits, germination attributes and seedling attributes were used in cluster analysis to construct a dendrogram to assess the relationship among six seed sources (Bagchi, 1999; Kundu and Tigerstedt, 1999). It was clear from the dendrogram that seed source SS 2 and SS 4 also seed source SS 3 and SS 6 were more closely related. It was evident from the dendrogram that the degree with which the clusters related was same in both the cases. It was observed that seed source SS 2 and SS 4 showed similar results with respect to pod thickness, seed length, seed breadth, seed length to breadth ratio, total germination days, peak value, height, number of leaves, number of branches, leaf thickness, shoot fresh weight, root dry weight, RGR, photosynthetic rate and chlorophyll content. Hence the two seed source exhibited more similarity with each other in the dendrogram. Similarly a number of traits appeared to be same for seed source SS 3 and SS 6.

Seed source SS 3 and SS 1 were also related with each other, but was not as closely related as seed source SS 3 and SS 6. When compared to the similarity in traits for seed source SS 3 and SS 6, much lesser number of traits was commonly shared in case of seed source SS 3 and SS 1. The cluster containing seed sources SS 3, SS 6 and SS 1was related to the seed source SS 2 at a greater distance. This suggests that seed source SS 2 shared many common characters with seed sources SS 3, SS 6 and SS 1. The dendrogram exposed a highly distant relationship of seed source SS 5 from the rest of the seed sources. A number of traits in SS 5, expressed significantly least value, when compared to all the other seed sources. Very few traits like seed length, seed length to breadth ratio, days to cease germination, number of leaves, number of branches, leaf thickness, root fresh weight, root dry weight, RGR and photosynthetic rate exhibited similarity with one or two other seed sources.

5.2 Karyotype analysis

Due to the emergence of molecular biology tools, the emphasis on classical plant cytogenetics have largely declined. Cytogenetic analysis helps to understand the structural and numerical properties of chromosomes in a species. This inturn could find various abnormalities as well as polyploidy existing in various species. Present study has attempted to provide an insight into the classical plant cytogenetic analysis in the species *Saraca asoca.* Previous cytological studies conducted in plants, with a few exceptions have been confined to mainly groups from temperate regions. The present study was successful in assessing the chromosome number in ashoka as 2n=34.

5.2.1 Pre-treatment of roots

Pre-treatment is an important procedure carried out as the first step in cytological preparations. The most important function of pre-treatment is to arrest the mitotic cells at metaphase stage by acting on spindle fibres. Spindle formation is dependent on the viscosity balance between cytoplasmic and spindle constituents. A change in cytoplasmic viscosity because of the pre-treatment brings about destruction of spindle apparatus with chromosome remaining free in the cell (Sharma and Sharma, 1980).

Another objective of pre-treatment is to clear the cytoplasm as well as to facilitate separation of middle lamella, leading to softening of tissue. Pre-treatment also enables to achieve rapid penetration of the fixatives by removing undesirable deposits in tissues (La Cour, 1935). As per the present investigation, 8-hydroxyquinoline gave better results when compared to colchicine. The excised roots subjected to 8-hydroxyquinoline for four hours at refrigeration aws found to be most ideal pre-treatment for ashoka. Hommo and Sakilahn (1986) also recommended a

treatment of 8-hydroxyquinoline for four to five hours at 6^{o}C in case of *Betula papyrifera* and *Populous tremula*.

In most of the cases, choromosomes were distinctly visible when pre-treated with 8-hydroxyquinoline. Thus it can be said that condensation and chromosome separation was comparatively better in case of 8-hydroxyquinoline. Apart from this, it was observed that, root tips pre-treated at 10.30 am gave the maximum cell division in *Saraca asoca*. The temperature during this particular time of the day therefore appeared to be most appropriate for obtaining good number of mitotic division (Eifler, 1959). It was evident from the experiment that the optimum level of external conditions for initiating cell division process, including DNA replication was found to be triggered in the morning hours rather than afternoon conditions.

5.2.2 Fixing of the roots

After pre-treatment, the excised root tips are transferred to suitable fixatives for killing and fixing of the cells. While fixing, the cell wall and its contents are not supposed to be distorted (Sharma and Sharma 1980). Among the two fixatives used Carnoys II was found to be the effective one. In case of Carnoys I, cytoplasm staining was frequently observed in the slides when viewed through the microscope. Chromosomes usually appeared to be darker in Carnoys II reagent. Clusters of shrinked cells were generally observed in root tips fixed in Carnoys I solution. Chloroform in the Carnoys II, might have helped for rapid penetration and differential staining of chromosomes. Chloroform might have also helped in clearing the cytoplasm of ingredients, thereby preventing cytoplasmic staining in the cytological preparations.

5.2.3 Squashing and staining of the roots

Among the two stains used in the present study acetocarmine stain was found to be better than the fuelgen stain. In case of acetocarmine staining the root tips were gently heated for 10-15 minutes, by occasionally adding acetocarmine stain on the root tip kept over the slide. This was continued till the tissue was softened. The softened root tips were easily squashed over a glass slide after covering it with a cover slip. Tapping the coverslip using a rubber edged pencil and pressing over it using the thumb helped in proper spreading of the cells in a single layer.

The other stain used in the study was fuelgen stain. For softening of tissues prior to staining with fuelgen, the root tips have to be treated in 1N HCl at 60^{o}C for 30 minutes. The treatment was not sufficient in proper softening of the roots. Extending the time beyond 30 minutes and increasing the normality, did not prove useful, as poor results were attained because of denaturation of DNA components. The spreading of the cell in fuelgen stain was very poor because of insufficient softening. An attempt to separate the tissue clumps using a blunt end of needle and then pressing over it after covering with a cover slip, helped to achieve more spread in case of fuelgen staining.

In both the cases still more spreading can be achieved by slightly lifting the coverslip and adding a single drop of acetocarmine stain (or 45 per cent acetic acid when fuelgen is used). Pressing and tapping is then repeated. After clearing the excess stain from the slide using filter paper, view it through the microscope first under low power then in high power. Images were captured using image analyser connected to the microscope. Among the two stains used, acetocarmine stain was observed to be the better one. The advantage with the acetocarmine staining was that a single layer of cells were able to view with good spread of chromosomes. This was achieved because of better softening and squashing of the root tips. The

chromosomes exhibited distinctly from the cytoplasm. Fuelgen stain is known for its ability to selectively stain the chromosomes. However the draw back in fuelgen staining was its difficulty in softening the tissue. Therefore in most of the cases proper squashing and spreading was not sufficient to get good slide preparations. But once the proper spreading is ensured by separating the tissue clumps as mentioned above, good results can be achieved in fuelgen staining.

As per the findings of the present study standardization procedures can be recommended for good mitotic slide preparation of ashoka. Good results will be obtained when saturated solution of 8-hydroxyquinoline is used as pre-treatment agent and Carnoys II as fixative. A number of cells with metaphase stages can be viewed if the root tips are pretreated at about 10.30 am. Quality slides can be prepared if acetocarmine is used for staining.

5.2.4. Karyotype and idiogram preparation

Karyotype of ashoka was prepared out from a well spread cell in metaphase stage. The cell used for karyotype preparation has undergone a pre-treatment with 8-hydroxyquinoline, followed by fixation in Carnoys II and staining with Acetocarmine. Careful observation revealed the presence of 34 chromosomes in the cell. Various studies have reported karyotype in different species (Ghimpu, 1929; Atchinson, 1948; Aloni, 1973; Bir and Kumari, 1973; Shukor et al., 1994). Chromosomes were classified into 17 sets of homologous pair and arranged according to its decreasing length in *Saraca asoca*. The pairs were numbered from one to seventeen in the prepared karyotype. Exact centromere location was unable to detect hence only the total chromosome length was measured.

In order to prepare the idiogram the total chromosome length was assessed with the help of image analyser (LABOMED iVu 3000 model). Idiogram based on

relative chromosome length (RCL) was also constructed. Based on the measurements it was found that the length of the chromosome in ashoka ranged from 12.1 µm to 5.3 µm. Likewise the relative chromosome length (RCL) value ranged from a maximum 9.5 µm to 4.2 µm. The individual chromosome length of ashoka was found to be comparatively lengthier in appearance when a comparison is made with *Acacia auriculiformis* (1.61µm to 0.75 µm), *A. mangium* (2.6 µm to 0.67 µm), *A. nilotica* (1.25 µm to 0.51 µm) and *A. ferruginea* (1.44 µm to 0.56 µm), (Abideen, 1998), also coming under the family fabacea. This was confirmed from the idiogram constructed in *Saraca asoca*.

Summary

SUMMARY

An investigation was conducted on "Seed source variation in the seed and seedling characters of ashoka [*Saraca asoca* (Roxb.) de Wilde]" in the nursery of College of Forestry, Kerala Agricultural University. In this study six seed sources were taken, by dividing Kerala into six equal portions based on latitude. The study can be divided into five sections namely, collection of seeds of ashoka, study on seed traits, germination studies, seedling attributes and karyotype analysis. The salient features of the study under different headings are given below.

6.1 Collection of seeds

6.1.1 The tree *Saraca asoca*, is found almost throughout Kerala.

6.1.2 It was observed that the phenological process in seed source SS 5 took place earlier when compared to other seed sources. Hence healthy seeds were not available in plenty.

6.1.3 The seeds were mainly collected from domesticated trees especially from homesteads, temples, institutes, road sides, park and museum. *Saraca asoca* is almost vanishing from the forests of Kerala.

6.1.4 In the present investigation, seeds were collected in the month of May-June. Seeding in ashoka coincides with the arrival of monsoon.

6.2 Seed traits

6.2.1 Seed length was the least for seed source SS 3. Seed length was on par with each other for rest of the sources, showing similarity in performance.

6.2.2 Significant difference was shown by different seed sources with respect to seed breadth. Seed source SS 2 and SS 4, showed the best performance in seed breadth. The least performance in this respect was given by seed source SS 3.

6.2.3 The results of seed length to breadth ratio, indicated more elongated seeds for seed source SS 1. Seed sources SS 2, SS 4 and SS 6 appeared to be more roundish.

6.2.4 Significant difference was seen in case of seed source SS 5 with respect to pericarp thickness. All the remaining seed sources displayed similarity in performance with respect to pericarp thickness.

6.2.5 Seed weight was maximum for seed source SS 2 and the least for seed source SS 3. Moderate values were given by other seed sources with respect to seed weight.

6.3 Germination attributes

6.3.1 Germination behavior exhibited proportionate relationship with respect to its seed traits. Seeds with good size and weight showed better performance with respect to its germination attributes too.

6.3.2 Seed source SS 3 can be an exception to the above condition, unlike other seed sources. Seed size and weight were comparatively smaller in case of seed source SS 3, but it could perform relatively well in case of germination attributes.

6.3.3 Commencement of germination was the fastest in case of seed source SS 4 (14 days). This may be attributed towards the conducive circumstances of local seed source that came out to be suitable for triggering the germination fastest in seed source SS 4. Seed sources SS 2, SS 3 and SS 6, also showed better performance in this respect.

6.3.4 Germination was delayed in seed source SS 1, inspite of its better seed traits. This points towards its longer imbibition period. Similar was the case with seed source SS 5.

6.3.5 Germination ceased after 74.8 days in case of seed source SS 4. It took the least days for SS 3 and SS 6 to cease the germination process (about 64.8 days and 65.3 days).

6.3.6 Germination continued for maximum days in case of seed source SS 4. Germination days were least with respect to SS 1, SS 3 and SS 5. All the viable seeds germinated within this period.

6.3.7 Days required to attain 50 per cent germination was the fastest with respect to seed source SS3, SS 4 and SS 6. This indicates towards the vigorousness of seeds of these sources.

6.3.8 Germination percentage was maximum for seed source SS 1 with germination of 98 per cent. On an average 81.5 per cent germination was noted. The least germination was given by seed source SS 5 (37.8 per cent).

6.3.9 Almost all the seeds were viable in case of seed source SS 1. Most of the seeds in seed source SS 5 could not perform well. This can be because of delayed collection of seeds as result the viability might have lost for the seed source SS 5.

6.3.10 The speed with the germination occurred is given by germination energy and it was maximum for seed source SS 3 and the least with respect to seed source SS 5. A good germination percent in less number of days will give better performance for germination energy.

6.3.11 Mean daily germination (MDG) was maximum in case of seed source SS 1 (1.34) and the least in case of seed source SS 5 (0.53). Peak value of germination was the least for the seed source SS 5. The peak value was on par with each other in case of all the other seed sources.

6.3.12 Germination value is another parameter to assess the potentiality of seeds to germinate. Highest germination was exhibited in case of seed source SS 1. The least significant value was given by seed source SS 5.

6.4 Seedling attributes

6.4.1 Seedling attributes exhibited better performance correspondingly with respect to the seed traits and germination behavior of ashoka.

6.4.2 The increment in height followed almost similar trend in all the seed sources across the observation period. Significantly least performance was exhibited by the seed source SS 5, throughout the study period.

6.4.3 Mean collar girth of seed source SS 1, showed better performance throughout the study period. The collar girth was the least with respect to seed source SS 5 throughout the observation period.

6.4.4 The parameter, leaf thickness and number of leaves did not show any significant difference among different seed sources. Therefore it cannot be regarded as a criteria determining variation in seed sources.

6.4.5 Leaf area showed considerable variation among different seed sources, across the observation period. Throughout the study period seed source SS 5 displayed least leaf area.

6.4.6 Branching in ashoka seedlings were uncommon in the initial stages, even then the seed source SS 3 and SS 6, showed branching pattern. Towards the end of the observation period, all the seed sources showed branching.

6.4.7 Towards the final observation, mean fresh weight of shoot was almost par with respect to different seed sources, with the exception of seed source SS 5. Whereas root fresh weight, showed significant variation among different seed sources. The least root fresh weight displayed by seed source SS 5.

6.4.8 Dry weight of shoot and dry weight of root was assessed and both the parameters displayed significant values. The performance of seed source SS 5 was poor with respect to dry weight.

6.4.9 Significant difference among shoot-root dry weight ratio was evident from the observation. This implicates better shoot growth in case of seed sources SS 2. Shoot-root ratio was the least in case of seed source SS 5, indicating towards better root growth in comparison to its shoot.

6.4.10 Relative growth rate (RGR) was very low in *Saraca asoca* seedlings. RGR in the initial stages were higher, towards the end it showed a declining trend.

6.4.11 Chlorophyll content varied significantly among each other especially towards the final stages of observation. Maximum chlorophyll content was given by seed source SS 2 and the chlorophyll content was least with respect to seed source SS 5.

6.4.12 In general the values for photosynthetic rate did not show any significant difference among each seed sources. Accordingly the leaf thickness, leaf area, chlorophyll content did not show any marked difference in their respective value.

6.4.13 Throughout the study period, seedlings were invigilated for the occurrence of any pest and disease. No incidence of disease and pest was observed.

6.5 Karyotype analysis

6.5.1 Standardization of the cytogenetic procedure for the species *Saraca asoca* was carried out. Different chemicals were used in different combination inorder to obtain the best slide.

6.5.2 It was observed that the best time for obtaining the mitotic division in *Saraca asoca* is at 10.30 am.

6.5.3 As a pretreatment chemical, 8-hydroxyquinoline showed better performance in comparison to colchicine. Condensation and chromosome separation in the cells on using 8-hydroxyquinoline was better when compared to colchicines.

6.5.4 In case of fixation, Carnoys II appeared to be better than Carnoys I. Cytoplasmic staining was less in case of Carnoys II.

6.5.5 The stain used for the study was fuelgen and acetocarmine. It was found that softening of the root tips was not properly achieved after hydrolysis. This inturn made squashing difficult and hence application of fuelgen stain could not provide good results. As a result of good softening and squashing, acetocarmine stain was successful in providing better cytological preparation

6.5.6 It was found that a combination of 8-hydroxyquinoline as pretreatment, Carnoys II as fixative, and acetocarmine as stain produced good slide preparation.

6.5.7 The chromosome number in *Saraca asoca*, was confirmed as 2n=34.

6.5.8 Karyotype was prepared out of the photograph taken in the image analyser. The homologous pairs of chromosomes were arranged in the decreasing order of length from one to seventeen.

6.5.9 The length of chromosome was measured in the image analyser and idiogram was constructed. The length varied from a maximum of 12.1 µm to 5.3 µm.

6.5.10 Relative chromosome length was also calculated from the absolute length of chromosome. The value ranged from 9.5 µm to 4.2 µm.

CONCLUSION

The present study on "Seed source variation in the seed and seedling characters of ashoka [*Saraca asoca* (Roxb.) de Wilde]", was successful in identifying the best seed sources. The study showed significant differences among the seed sources with respect to seed traits, germination studies and seedling attributes. As per the study, seed size, seed weight and germination trends had a direct and positive influence on the seedling attributes of *Saraca asoca*. Altogether the performance of seedlings in seed sources SS 1, SS 2 and SS 4, can be regarded as the best, followed by the seed sources SS 3 and SS 6. The performance of seed source SS 5 was significantly the poorest in terms of most of the characters studied. Cluster analysis also revealed a highly distant relation of seed source SS 5 with respect to all the other seed sources. Apart from the seed source variation study, karyomorphological study was successful in finding out the chromosome number of *Saraca asoca* as 2n=34, after standardizing the procedure. The karyotype and idiogram of ashoka was also prepared.

FUTURE LINE OF STUDY

1. The wide range experienced in germination behaviour can be subjected to further study on seed physiological parameters. Different storage techniques have to be developed for the recalcitrant seeds of ashoka.
2. The seedlings were observed to be healthy throughout the study. Investigations can be carried out with respect to the insecticidal and pesticidal properties of ashoka.
3. Studies based on albino plants can be further taken ahead. This could prove useful in forest genetics.
4. Karyomorphological studies to understand the exact centromere location is required. Understanding the existence of any polyploidy or inducing polyploidy in ashoka can help to exploit the medicinal properties to its maximum extent.

References

REFERENCES

Abideen, K.A.M.Z. 1998. Karyomorphology and isoenzyme variation in certain Acacia species. MSc. (For.) thesis, Kerala Agricultural University, Thrissur, 61p.

Ahlawat, S.P., Kumar, R.V. and Gupta, V.K. 2007. Seed source variation in pod and seed characteristics of *Acacia nilotica*. *Ann. of Arid Zone*. 46(2): 189-196.

Ahmad, H.D. 1989. Growth performance and chromosome count of *Acacia mangium* plantlets derived from cultured nodal explants. *J. Trop. For. Sci*. 1 (3): 263-267.

Aloni, R. 1973. Studies of *Acacia albida* in Israel. *Israel Journal of Botany*. 22(3): 202.

Annapurna, J., Bhalerao, U.T. and Iyengar, D.S. 1999. Antimicrobial activity of *Saraca asoca* leaves. *Fitoterapia.* 70(1): 80-82.

Aslan, A. 1975. Relationship between seed dimensions and seedling percentage and seedling quality in *Pinus brutia*. *Orm. Ara . Enst. Tek. Bult*. 64: 39.

Atchinson, E. 1948. Studies in Leguminosae II. Cytogeography of *Acacia* (Tourn) L. *Am. J. of Bot*. 35: 651-65.

Bagchi, S.K. 1999. Seed source variation in *Acacia nilotica*: genetic divergence in 8-month old seedlings. *Ann. of For*. 7(1): 45-55.

Beltran, I.C. and Kam, V.K. 1984. Cytotaxonomic studies in the Zinigiberaceae. Notes from Royal Botanical Gardens. 41: 3.

Bir, S.S. and Kumari, S. 1973. In IOPB chromosome number reports LXIX. *Taxon*. 29: 459-464.

Bir, S.S. and Kumari, S. 1985**.** Chromosomal studies on the Indian legumes. In: Reddi, V.R. and Rao, M.K. (eds.), *Cytogenetics of Higher Plants: Chromosome Structure and Behaviour*. Andhra University: Waltair (India), pp. 39-41.

Blatter, E. and Millard, W.S. 1997. *Some Beautiful Indian Trees* (2nd ed.). Bombay Natural History Society, Calcutta, pp. 130-133.

Bonner, F.T. 1987. Importance of seed size in germination and seedling growth. In: Kamara, S.K. and Ayling, R.D. (eds.), *Proceedings of the International Symposium on Forest Seed Problems in Africa*. Harare, Zimbabwe, 23 August – 2 September. International union for forest reaearch organisation, pp. 53-61.

Britt, J.R., Mitchell, R.J., Zutter, B.R., South, D.B., Gjerstad, D.H. and Dickson, J.F. 1991. The influence of herbaceous weed control and seedling diameter on six years of loblolly pine growth - a classical growth analysis approach. *For. Sci.* 37: 655-668.

Candan, F., Sik., L. and Kesercioglu, T. 2009. Cytotaxonomical studies on some Crocus L. taxa in Turkey. *Afr. J. of Biotech*. 8: 4374-4377.

Chacko, K.C., Pandalai, R.C., Seethelakshmi K.K., Mohanan, C., Mathew, G. and Sasidharan, N. 2002. *Manual of Seeds of Forest Trees, Bamboos and Rattans in Kerala*. KFRI Publication, KFRI, Peechi. 331p.

Chauhan, L. and Rao, R.V. 2003. *Wood Anatomy of Legumes of India, Their Identification, Properties and Uses.* Bishen Singh Mahendra Pal Singh, New Connaught Place, Dehradun. 220p.

Chithra, R. 2004, Mar. 15. Evergreen elegance. *Metro Plus, The Hindu*. p2.

Cideciyan, M.A. and Malloch, A.J.C. 1982. Effects of seed size on the germination growth and competitive ability of *Rumex crispus* and *Rumex obtusifolius*. *J. Ecol*. 70: 227–232.

*Correa, L.B., Barbieri, R.L., Rossato, M., Buttow, M., Gustavo, H. 2009. Karyological Characterization of Butia (Arecaceae) Palm Trees. *Revista Brasileira de Fruticultura*. 31: 1111-1116.

Costales, A.B. and Veracion, V.P. 1978. Germination of benguet pine seeds at various intervals of watering. *Sylvatrop.* 3(4): 243-245.

Czabator, F.J. 1962. Germination value: An index combining speed and completeness of pine seed germination. *Forest Sci.* 8: 386–396.

Dabur, R., Gupta, A., Mandal, T.K., Singh, D.D., Bajpai, V., Gurav, A.M. and Lavekar, G.S. 2007. Antimicrobial activity of some Indian Medicinal Plants. *Afr. J. Trad. Cam.* 4(3): 313-318.

Da Silva, C.R.M., Trevisan, R., González, E.M.S., Ferreira, J.M. and Vanzela, A.L.L. 2010. Karyotypic diversification and its contribution to the taxonomy of *Eleocharis* (Cyperaceae) from Brazil. *Aust. J. of Bot.* 58(1): 49–60.

Das, D.K., 1990. Forest Types of Bangladesh. Bulletin 6, Plant Taxonomy Series. Bangladesh Forest Research Institute, Chittagong. p 9

Dastur, J.F. 1970. *Medicinal Plants of India and Pakistan*. D.B. Taraporevalasons and Co. Private Ltd., Dr. Dada Bai Naoroji Road, Bombay. 210p.

Dhamayanthi, K.P.M. 2005. Karyomorphological analysis and phylogenetic relationship of Gossypium species. *Cytologia*. 70(4): 421-425.

Dhawan, B.N., Patnaik, G.K., Rastogi, R.P., Singh, K.K. and Tandon, J.S. 1977. Screening of Indian plants for biological activity. *Indian J. Exp. Boil*. 15: 208-219.

Dolan, R.W. 1984. The effect of seed size and maternal source on individual size in a population of *Ludwigia leptocarpa* (Onagraceae). *Am. J. Bot* .71: 1302–1307.

*Eifler, I. 1959. Beschreibung ciner Fixier methods, die das Aus zahten von Birkenchromosomen creirchert. *Zeucter*. 29: 57-59.

Elamin, H.M. 1976. Geographical distribution of the Sudan acacias. *Sudan Silva* 3(21): 14–23.

Fenner, M. 1978. A comparison of the abilities of colonizers and closed-turf species to establish from seed in artificial swards. *J. of Ecol*. 66: 953-963.

Fenner, M. 1983. Relationships between seed weight, ash content and seedling growth in twenty-four species of Compositae. *New Phytologist* 95: 9-19.

George, K.V., Thomas, R.P., and Paul, J. 2007. Traditional botanical knowledge on *Saraca asoca* (roxb.) de wilde; an enquiry among the ayurvedic practitioners of Kerala. In: Sasidharan, N., Kumar, M.S.M., Ramachandran, K.K. and Jayson, E. A. (eds.), *Conservation, Cultivation and Sustainable Utilization of Saraca asoca*. Proceedings of the national

seminar, Peechi, Kerala. Kerala Forest Research Institute, Kerala, pp. 64-67.

Gera, M., Gera, N. and Ginwal, H.S. 1999. Seed source variation in germination and early growth among ten indigenous populations of *Dalbergia sissoo* Roxb. *Indian Forester*. 125(12): 1190-1197.

*Ghimpu, V. 1929. Sur I′ existence simultanee des mitoses diplodes didiploides, et teteraploides chezles *Acacia*. *Sec. Biol*. (Paris). 101: 1122-1123.

Ghosh, S., Majumder, M., Majumder, S., Ganguly, N.K. and Chatterjee, B.P. 1999. Saracin: a lectin from *Saraca indica* seed integument induces apoptosis in human T-lymphocytes. *Arch. of Biochem. and Biophys*. Academic Press. Orlando, USA. 371(2): 163-168.

Ginwal, H.S. and Gera, M. 2000. Genetic variation in seed germination and growth performance of 12 *Acacia nilotica* provenances in India. *J. Trop. For. Sci*. 12(2): 286-297.

Ginwal, H.S., Gera, M. and Srivastava, R.L. 1996. Seed source variability in some seed and seedling characteristics of twenty provenances of *Acacia nilotica* Willd. ex. Del. *Range Manage. and Agroforest*. 17(1): 49-59.

Gowri, N., Srinivasan, K. and Venkataraghavan, S. 1982. Clinical study of AC4-an ayurvedic preparation as oral contraceptive. *Ass. of Ancient Sci. of Life.* 2: 79-83.

Goyal, P., Sharma, P., Srivastava, S., and Srivastava, M.M. 2008. *Saraca indica* leaf powder for decontamination of Pb: removal, recovery, adsorbent characterization and equilibrium modeling. *Int. J. of Environ. Sci. and*

Technol. Center for Environment and Energy Research and Studies, Tehran, Iran. 5(1): 27-34.

Gross, K.L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *J. Ecol*. 72: 369-387.

Gupta, V. 2004. *Saraca indica*- The sorrow less tree of India. Ayurveda news (news letter), vol.5, issue V

Haase, D.L. and Rose, R. 1993. Soil moisture stress induces transplant shock in stored and unstored 2+0 Douglas-Fir seedlings of varying root volumes. *For. Sci.* 39: 275-294.

Harper, J.L. 1977. *Population Biology of Plants*. Academic press London. p.892.

Hattori, M., Nakabayashi, T., Lim, Y.A., Miyashiro, H., Kurokawa, M., Shiraki, K., Gupta, M.P., Correa, M. and Pilapitiya, U. 1995. Inhibitory effects of various Ayurvedic and Panamanian medicinal plants on the infection of herpes simplex virus-1 *in vitro* and *in vivo*. *Phytotherapy Res*. 9(4): 270-276.

Hommo, L.M. and Sakilahn, E.M. 1986. A method of counting chromosomes of hardwood trees using root tip and young leaves. *Can. J. of Forest Res*. 16: 401-403.

Hossain, M.A., Arefin, M.K., Khan, B.M and Rahman, M.A. 2005. Effects of seed treatments on germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. *Res. J. of Agric. and Biological Sci*. 1(2): 135–141.

Howe, H.F. and Richter, W.M. 1982. Effects of seed size on seedling size in *Virola surinamensis*: a within and between tree analysis. *Oecologia.* 53: 347-351.

Hunt, R. 1990. *Plant Growth Curves: The Functional Approach to Plant Growth Analysis.* Edward Arnold Ltd., London. p.248

Isik, K. 1986. Altitudinal variation in *Pinus brutia*, Ten: seed and seedling characteristics. *Silvae Genetica.* 35: 58–67.

Iyer, N.K. and Kolammal, M. 1978. *Pharmacognosy of ayurvedic drugs*. Series 1, No. 4. The department of pharmacognosy, Govt. Ayurveda College, Trivandrum, pp. 1-7.

Jain, S.K. 1968. *Medicinal Plants*. National book trust of India publications, New Delhi. 124p.

Jain, S.R. and Sharma, S.N. 1967. Hypoglyceamia drugs of Indian indigenous drugs. *Planta Med*. 15(4): 439-442.

Jalil, P., Kashyap, M.K., Chadhar, S. K. and Tiwari, K.P. 2000. Provenance trial of neem (*Azadirachta indica*) from different agroclimatic zones of Madhya Pradesh. *Vaniki Sandesh*. 24(3): 1-8.

Jayasankar, S., Babu, L.C., Sudhakara, K., and Kumar, P.D. 1999. Evaluation of provenances for seedling attributes in teak (*Tectona grandis* LINN.F.). *Silvae Genetica*. 48(3/4): 115-122.

Jijeesh, C.M. and Sudhakara, K. 2007. Variations in the number of seeds and physical characteristics of teak fruits of Nilambur Forest Division, Kerala. *Seed Res*. 35: 25-33.

Joglekar, S.N., Nabar, S.D. and Hedge, S.S. 1984. In vivo and in vitro effects of ayurvedic formulation M_2 on the uterus. *Indian practitioner*. 37(9): 847-854.

Kala, C.P., Dhyani, P.P., Singh, B. and Sajwan, B.S. 2006. Developing the medicinal plants sector in northern India: Challenges and opportunities. *J. of Ethnobiology and Ethnomedicine*, 2: 32.

Katiyar, K., Singh, L., Kishor, K. and Vats, P. 2007. Phytochemical and antibacterial studies on bark extracts of *Saraca asoca* (Roxb.) against Pathogenic Bacteria. *Biochemical and Cell. Arch*. 7 (2): 293-298.

Kolb, T.E., and Steiner, K.C. 1990. Growth and biomass partitioning response of northern red oak genotypes to shading and grass root competition. *For. Sci.* 36: 293-303.

Kramer, P.J., and Kozlowski, T.T. 1979. *Physiology of Woody Plants*. Academic Press, New York. 811p.

Kumar, A.B.N, Gowda, H.H.C. and Vasudeva, R. 2004. A note on air layering in *Saraca asoca* (Roxb.) de Wilde. *J. of Non Timber For. Prod*. 11(1): 34-35.

Kumar, A.B.N., Gunaga, R. and Vasudeva, R. 2007. Germination behavior of *Saraca asoca* seeds. In: Sasidharan, N., Kumar, M.S.M., Ramachandran, K. K. and Jayson, E. A. (eds.), *Conservation, Cultivation and Sustainable Utilization of Saraca asoca*. Proceedings of the national seminar, Peechi, Kerala. Kerala Forest Research Institute, Kerala. p.87.

Kumar, N. and Toky, O.P. 1996. Variation in seed germination and juvenile growth of 12 provenances of *Albizia lebbeck* (L.) Benth. in arid India. *Indian J. of For*. 19(2): 123-128.

Kunda, P.B. and Bole, P.V. 1997. *Herbal Cosmetics in Ancient India with a Treatise on Planta Cosmetica*. Bharathiya Vidya Bhavan, Kulapati Munshi Marg, Mumbai. p.45

Kundu, S.K. 2000. Evaluation of provenance variation on early growth and survival of neem (*Azadirachta indica*) in Bangladesh and India. *J. Trop. Forest Sci*. 12(3): 509-523.

Kundu, S.K., Islam, Q.N., Emmanuel, C.J.S.K. and Tigerstedt, P.M.A. 1998. Observations on genotype x environment interactions and stability in the international neem (*Azadirachta indica* A. Juss.) provenance trials in Bangladesh and India. *Forest Genetics*. 5(2): 85-96.

Kundu, S.K. and Tigerstedt, P.M.A. 1997. Geographical variation of seed and seedling traits of neem (*Azadirachta indica* A. Juss.) among ten population studies in growth chamber. *Silvae Genetica*. 46: 129-137.

Kundu, S.K. and Tigerstedt, P.M.A. 1999. Variation in net photosynthesis, stomatal characteristics, leaf area and whole-plant phytomass production among ten provenances of neem (*Azadirachta indica*). *Tree Physiol.* 19: 47-52.

Kusumoto, I.T., Nakabayashi, T., Kida., H., Miyashiro, H., Hattori., M., Namba, T. and Shimotohno, K. 1995. Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type I (HIV-I) protease. *Phytother. Res*. 93: 180-184.

Kurian, V. 2002. Nov. 4. Medicinal plants of Indian origin endangered. The Hindu Business Line. p 4

La Cour, L.F. 1935. *Stain tech*. Mc Millan and Co., London. pp. 10-57.

Maideen, S.K., Selvaraj, J.A. and Rai, R.S.V. 1997. Effect of geographic source and age of mother tree on seed viability and vigour in *Casuarina equisetifolia* F.R. and G. Forst. *Indian J. of For*. 20(3): 220-222.

*Malavasi, M.M. 1988. *Germinação de sementes*. In: Piña-Rodrigues, F.C. (Coord.). *Manual de Análise de Sementes Florestais.* Campinas : Fundação Cargill. pp. 25-40.

Manga,V.K and Sen, D.N. 1996. Genetic parameters for seed traits in *Prosopis cineraria* (L.). *Indian. J. For*. 19(2): 148-151.

Manonmani, V. and Vanangamudi, K. 2002. Effect of seed source and size on seed germination and seedling vigour of sandal (*Santalum album*). *J. Trop. For. Sci*. 14(1): 150-155.

Markwell, J., Osterman, J.C. and Mitchell, J.L. 1995. Calibration of the Minolta SPAD 502 leaf chlorophyll meter. *Photosynth Res.* 46: 467–472.

Mc. Millan, G.G. and Mc. Clendon, J.H. 1983. Dependence of photosynthetic rates on leaf density thickness in deciduous woody plants grown in sun and shade. *Plant Physiol*. 72: 674-678.

*Michetti, K.M., Leonardi, P.I. and Cáceres, E.J. 2010. Morphology, cytology and taxonomic remarks of four species of *Stigeoclonium* (Chaetophorales, Chlorophyceae) from Argentina. *Phycological Res*. 58(1): 35-43.

*Middlecoop, T.B. and Labadie, R.P. 1986. An *in vitro* examination of bark extracts from *Saraca asoca* (roxb.) de wilde. and *Rhododendron arboretum* for oxytocic activity. *Int. J. crude drug res*. Dept. pharmacog pharmaceut lab rijksuniversitiet Utrecht, Netherlands. 24(1): 41-44.

Mohan, J.S.S., Sharma, S., Mahida, Y. and Ishnava, K.B. 2006. Spectrum of antibacterial activity possessed by petal extracts of some Indian plants. *J. of Med. and Aromatic Plant Sci.* Central Institute of Medicinal and Aromatic Plants, Lucknow, India. 28(4): 557-561.

Mukherjee, P.K., Das, J., Balasubramaniam, R., Saha K., Pal, M. and Saha, B.P. 1996. A herbal uterine tonic with *Saraca asoca*. *Phytotherapy Res*. 10(7): 619-621.

Nadkarni, K.M. 1957. The Indian Materia Medica (3rd Ed.) Popular Book Depot. Bombay. p.1075

Neerja, S. and Pokhriyal, T.C. 2000. Biomass distribution pattern in relation to seed source variation in *Dalbergia sissoo* seedlings. *Ann. of Forestry.* 8(2): 238-249.

Neerja, S. and Pokhriyal, T.C. 2001. Variations in pod and seed traits in six different *Dalbergia sissoo* seed sources. *J. of Trop. For. Sci.* 13(1): 162-170.

Okora, O.O. 1976. Germination of *Terminalia ivorensis* seeds sown under various conditions of germination. In: (ed.), *Seed Problems*, Proceedings of the 2nd International Symposium on Physiology of Seed Germination. October, 1976. IUFRO, Fuji, Japan.

Panikkar, K.R. 1994. Reduction of toxicity of cisplatin and cyclophosphamide by plant products. Ethnobiology in human welfare: abstracts of the 4th International Congress of Ethnobiology; 17-21, Nov., 1994, National Botanical Research Institute, Lucknow, Uttar Pradesh, India.

*Paul, D.K. 1972. A handbook of nursery practice for *Pinus caribaeae* var. hondurensis and other conifers in West Malaysia. Wkg. Paper no. 19. FO: SF/MAL 12, UNDP/FAO Kuala Lumpur.

Prabhu, H.N. 2007. Vanishing asoka (*Saraca asoca*) – a challenge to ayurveda industry. In: Sasidharan, N., Kumar, M.S.M., Ramachandran, K. K. and Jayson, E. A. (eds.), *Conservation, Cultivation and Sustainable Utilization of Saraca asoca*. Proceedings of the national seminar, Peechi, Kerala. Kerala Forest Research Institute, Kerala, p.85

Pradhan, P., Joseph, L., Gupta, V., Chulet, R., Arya, H., Verma, R. and Bajpai, A. 2009. *Saraca asoca* (Ashoka): A Review. *J. of chemi. and pharmaceutical res*. 1(1): 62-71.

Prajapathi, N.D., Purohit, S.S., Sharma, A.K., and Kumar, T. 2003. *A Handbook of Medicinal Plants*. Agrobios (India), Agro house, Chopsani Road, Jodhpur. 928p.

Rajashekharan, S. and Latha, P.G. 2006. Asoka: the woman's best friend. *Sangamam Magazine on Ayurveda*. 2(4): 35-37.

Ramachandra, N.G., Nautiyal, S., Negi, D.S. and Thapliyal, R.C. 1997. Seed source variation in chlorophyll contents of leaves of *Acacia catechu* Willd. under different water stress conditions. *Ann. For*. 5(1): 88-96.

Rastogi, V.D. 2003. *Pharmacognosy and Phytochemistry*. Career Publication, Nashik. pp. 269-270.

*Raven, P.H. 1975. The basis of angiosperm phylogeny: Cytology. *Ann. Missouri Bot. Gard*. 62: 724-764.

Rawat, M.S., Uniyal, D.P. and Sharma, S.L. 1998. Identification of provenances based on leaf morphology in *Tectona grandis*. *Indian Forester*. 124(4): 248-251.

Reich, P.B., Tjoelker, M.G., Walters, M.B., Vanderklein, D.W. and Buschena, C. 1998. Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Funct. Ecol*. 12: 327–328.

Rekha, K. 1993. Cytogenetic Analysis in Kacholam, *Kaempferia galanga* L. MSc. (Ag.) Thesis, College of Horticulture, Kerala Agricultural University, Trichur. 85p.

Sadhu, S.K., Khatun, A., Phattanawasin, P., Ohtsuki, T and Ishibashi, M. 2007. Lignan glycosides and flavonoids from *Saraca asoca* with antioxidant activity. *J. Nat. Med.* 61(4): 480-482.

Sasidharan, N. and Pathros, P. 2007. Mass multiplicatipon of *Saraca asoca* (Roxb.) de wilde, through stem cuttings and air layering. In: Proceedings of National Seminar on Conservation, Cultivation and Sustainable Utilization of *Saraca asoca*. KFRI, Peechi. pp. 69-77.

Satyabalan, K. and Mathew, J. 1984. Identification of prepotent palms in West Coast Tall coconuts based on the early stages of growth of the progeny

in the nursery. In: (ed. Nayar, N.M.), Coconut Research and Development. Wiley Eastern Limited. pp. 15-22.

Satyavati, G.V., Prasad, D.N., Sen, S.P. and Das, P.K. 1969. Investigations into the uterine activity of *Saraca indica* L. (Ashoka), *J. Res. Indian Med*. 4(1): 37-45.

Satyavati, G.V., Prasad, D.N., Sen, S.P. and Das P.K. 1970. Oxytoxic activity of a pure phenolic glycoside (P_2) from *Saraca indica* Linn. (Ashoka): a short communication, *Indian J. Med. Res*. 58(5): 660-663.

Sedani, M.M. 1990. Role of ayurvedic injection of asoka in management of menorrhgia. *J. of Natl. integrated Med. Ass*. 32(9): 13p.

Seetharam N., Sujeeth, H., Jyothishwaran, G., Barad, A., Sharanabasappa, G. and Shabana, P. 2003. *Saraca asoca* (Ashoka); A Review. *Ind J. P. Sci* . 65(6): 658-659.

Sharma, A.K and Sharma, A. 1980. Chromosome techniques: theory and practice (3rd ed.). Butterworth-Heinemann Ltd. 724 p.

Sharma, P.C., Yelna, M.B. and Dennis, T.J. 2001. *Database on Medicinal Plants Used in Ayurveda*. Central Council for Research on Ayurveda and Siddha, New Delhi. 3: 76-87.

Sharma, U.K. and Boissya, C.L. 2003. Menstrual problems: ethnobotany practices among Mising tribes in Dhemaji District of Assam. *Advances in Plant Sciences*. 16(1): 17-21.

Shekhar, S., Shrigirishi, N., Shukla, N.K., Dubey, P. and Burfal, B.S. 2002. Effect of seed sources on germination and morphological traits in *Acacia nilotica*. *Indian J. of For.* 25(1/2): 1-6.

Shipley, B. and Peters, R.H. 1990. The allometry of seed weight and seedling relative growth rate. *Functional ecology*. 4: 523-529.

Shivanna, H., Nayak, B.G., Patel, J.S. and Sanjeev, D.K. 2002. Provenance variation for seed quality and seed germination in *Acacia nilotica*. *Karnataka. J. Agric. Sci*. 15(1): 88-93.

Shukor, N.A., Abd-Rashid, M. and Itam, K. 1994. Karyotypic comparison of *Acacia mangium* Willd*., A. auriculiformis A*. Cunn. Ex Benth and their F1 and F2 hybrids*. Silvae Genetica.* 43(2-3): 65-68.

Sindhuveerendra, H.C., Ramalakshmi, S. and Mallesha, B.B. 1999. Variation in seed characteristics in provenances of sandal (*Santalum album* L.). *Indian Forester*. 125(3): 308-312.

Singh, A., Singh, S., Sarma, B.K., Singh, U.P., Srivastava, R. and Singh, K.P. 2009. Phenolic acid analysis and biological activity of methanolic extracts of some medicinal plants against some phytopathogenic fungi. *Internet J. Alternative med*. 6(2): 1-2.

Singh, B.G., Warrier, R.R., Anandalakshmi, R., Sivakumar, V., Sivalingam, R., Mahadevan, N. P. and Chevanan, G. 2005. Seed germination studies in *Saraca asoca* (Roxb.) de Wilde. *Indian Forester*. 131(6): 841-843.

Singh, N. and Pokhriyal, T.C. 2000. Biomass distribution pattern in relation to seed source variation in *Dalbergia sissoo* seedlings. *Ann. of For*. 8(2): 238-249.

Singh, N. and Pokhriyal, T.C. 2001. Variations in pod and seed traits in six different *Dalbergia sissoo* seed sources. *J. of Trop*. *Forest Sci*. 13(1): 162-170.

Singhal, V.K., Gill, B.S. and Sidhu, M.S. 1990. Cytological explorations of Indian woody legumes. *Plant Sciences*. 100(5): 319-331.

Siril, E.A., Dhar, U. and Dhyani, P.P. 1998. Seed germination of Chinese tallow tree (*Sapium sebiferum*). *Forest, Farm, and Community Tree Research Reports*. 3: 55-58.

Snedecor, G. W. and Cochran, W. G. 1967. *Statistical Methods*. The Iowa State University Press, Ames, Iowa. 593p.

Solanki, K.R., Gupta, V.K. and Rai, P. 1999. Studies on genetic improvement of neem (*Azadirachta indica* A. Juss). In: Recent advances in management of arid ecosystem. Proceedings of a symposium, Jodhpur, India. Arid Zone Research Association of India, India. pp. 325-328.

Srivastava, G.N., Bagchi, G.D. and Srivastava, A.K. 1988. Pharmacognosy of ashoka stem bark and its adulterants. *Pharmaceutical Biology*, 26(2): 65-72.

Srivastava, S.K., Neeta, V., Mishra, C.M., Dubey, P. and Burfal, B.S. 2001. Evaluation of seed source variation on different seed parameters of *Dalbergia sissoo* Roxb. *Indian Forester*. 127(3): 358-362.

Stace, C.A. 1980. *Plant Taxonomy and Biosystematics*. Edward Arnold, Pitmann Press, Bath. 272 p.

Stanko, K.C., Liber, Z., Besendorfer, V., Javornik, B., Bohanec, B., Kolak, I. and Satovic, Z. 2010. Genetic relations among basil taxa (*Ocimum* L.) based on molecular markers, nuclear DNA content, and chromosome number. *Plant Systematics and Evolution*. 285(2): 13-22.

Stanton, M.L. 1984. Seed size variation in Wild Radish: Effect of Seed size on components of seedling and adult fitness. *Ecology*. 65: 1105-1112.

Strange, R.N. 2005. Plant Disease: A Threat to Global Food Security. *Phytopathology*. 43: 83-116.

Stuessy, T.F. 1990. *Plant Taxonomy- the systematic evaluation of comparative data*. Columbia University Press, New York. pp. 231-233.

Subramanian, K.N. 1995. *Flora of Thenmala.* International Book Distributors, Dehradun, pp. 119-120.

Suri, S.K. 1984. Analytical study of teak provenance tests in North Raipur Division of M.P. *Indian forester*. 110 (4): 345-363.

Sweet, G.B. and Wells, L.G. 1974. Comparison of the growth of vegetative propagules and seedlings of *Pinus radiata.* N. Z. *J. For. Sci*. 4: 399-409.

*Tarinejad, A.R. and Mirshekari, B. 2010. Study on variation of karyotypes between and within species of *Salvia officinalis* L., *Stachys bryzantine* L and *Dracocephalum molarica* L. *Acta Horticulturae* No.: 853.

Thapliyal, R.C. and Dhiman, R.C. 1997. Geographic variation in seed and seedling characteristics in *Pinus roxburghii* Sarg. from Himachal Pradesh. *Ann. of For.* 5(2): 140-145.

Troup, R.S. 1921. *The Silviculture of Indian Trees:* vol. 2. Oxford University Press, p.362

Tyagi, P.C., Agarwal, M.C. and Kumar, N. 1999. Provenance variation in seed parameters and germination of *Grewia optiva* (Drummond). *Indian Forester*. 125(5): 517-521.

Uniyal, A.K., Bhatt, B.P. and Todaria, N.P. 2003. Effect of provenance variation on seed and seedling characteristics of *Grewia oppositifolia* Roxb.: a

promising agroforestry tree-crop of Central Himalaya, India. *Plant Genetic Resources Newsletter*. 136: 47-53.

Upadyaya, B.I., Sastry, M.S. and Mistry, I.U. 1990. Effect of certain indigenous drugs in asrigdara. *Ayu.* 11 (4): 8-12.

Vakshaya, R.D., Rajora, O.P. and Rawat, M.S. 1992. Seed and seedling traits of *Dalbergia sissoo* roxb. seed source variation studies among ten sources in India. *Forest Ecology and Management*. 48: 265-275.

Vanangamudi, K., Umarani, R., Bharathi, A. and Venkatesh, A. 1999. Effect of seed source on physical and physiological qualities of *Acacia nilotica* seeds. *Seed Res*. 26 (2): 114-116.

Van den Driessche, R. 1992. Absolute and relative growth of Douglas-fir seedlings of different sizes. *Tree Physiol*. 10: 141-152.

Varghese, C.D, Nair, S.C, Beena, P. and Panikkar, K.R. 1993. Effect of asoka on the intracellular glutathione levels and skin tumour promotion in mice. *Cancer letters*. 69: 45-50.

Varghese, C.D, Nair, S.C and Panikkar, K.R. 1992a. Chemoprotection of *Saraca asoca* on cisplatin toxicity in mice. *Amala research bulletin*.12: 86-91.

Varghese, C.D, Nair, S.C and Panikkar, K.R 1992b. Potential anticancer activity of *Saraca asoca* towards transplantable tumours in mice. *Indian J. of pharmaceutical Sci*. 54: 37-39.

Warrier, P.K., Nambiar, V.P.K., and Ramankutty, C, 1996. *Indian medicinal plants – a compendium of 500 species. Vol. 5* Orient Longmans Ltd., Hyderabad, India. pp. 66-70.

Warrier, R.R., Anandalakshmi, R., Sivakumar, V., Sivalingam, S. and Gurudevsingh, B. Seed biology of *Saraca asoca*. In: Sasidharan, N., Kumar, M.S.M., Ramachandran, K. K. and Jayson, E. A. (eds.), *Conservation, Cultivation and Sustainable Utilization of Saraca asoca*. Proceedings of the national seminar, Peechi, Kerala. Kerala Forest Research Institute, Kerala, p.90

West, C., Briggs, G.E. and Didd, F. 1920. Methods and significant relations in the quantitative analysis of plant growth. *New Phytologist*. 19: 200-207.

Wulff, R.D. 1986. Seed size variation in *Desmodium paniculatum*. II. Effects on seedling growth and physiological performance. *J. of Ecol* .74: 99–114.

Zasada, J. 1980. Albino Plants. *Alaska Science Forum*. University of Alaska. Article No. 433.

Zhang, J. and Hamill, A.S. 1997. Seed weight, intraspecific competition and plant performance in Abutilon theophrasti. *Can. J. of Bot.* 75: 1614-1620.

Zobel, B. and Talbert, J. 1984. *Applied forest tree improvement*. John Wiley and Sons, New York, 505p.

* Originals of the literature not seen.

APPENDIx

Appendix- I

Weather data of Vellanikkara (2009 July to 2010 April)

Element	Year 2009						Year 2010			
	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Relative humidity (%)	86.5	85.5	81	80	72	62	57	63.5	63	70
Rain fall(mm)	686.9	454.2	245.6	302.1	104.4	17.8	1.9	3.9	6.6	68.8
Rainy days	24	21	12	13	5	1	0	0	0	4
Sunshine hours	3	3.8	5.9	5.9	7	8.5	9	9.3	9.2	8.5
Maximum temperature (^{0}C)	29.0	29.3	30.5	31.1	31.7	31.7	32.8	34.8	36.1	35.4
Minimum temperature (^{0}C)	22.9	23.1	23.2	23.0	22.8	22.5	22.2	22.6	23.9	25.1

SEED SOURCE VARIATION IN THE SEED AND SEEDLING CHARACTERS OF ASHOKA [*Saraca asoca* (Roxb.) de Wilde]

by

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Forestry

Faculty of Agriculture

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2010

ABSTRACT

An investigation was conducted on "Seed source variation in the seed and seedling characters of ashoka [*Saraca asoca* (Roxb.) de Wilde]" in the nursery of College of Forestry, Kerala Agricultural University, Thrissur, Kerala, India. In order to fix the seed sources, the state was divided into six equal portions based on the latitude. The seeds collected from each source were bulked and used for the study. Seed traits, germination attributes and seedling characters were used for investigating seed source variation in *Saraca asoca*. Different seed traits like seed length, seed breadth, seed length to breadth ratio, seed weight and pericarp thickness was evaluated in the present investigation. Study showed significantly lower size for the seeds collected from seed source SS 3. The performance of seed source SS 1, SS 2, SS 4 and SS 6, were on par with each other in case of most of the seed attributes.

In the present study altogether 2400 seeds were sown, with 400 seeds representing each seed source. Germination of seeds were noted daily. In general germination per cent in *Saraca asoca* was found to be good with an average of 81.5 per cent. Maximum germination per cent was exhibited by seed source SS 1 (98 per cent). Germination per cent of seed source SS 2 was also on par with SS 1, with a recorded value of 93.8 per cent. Significantly lower germination per cent was shown by seed source SS 5 (37.8 per cent). This may be attributed towards the presence of mostly non viable seeds in seed source SS 5. Apart from germination percentage, days required for germination to initiate, days required for germination to cease, germination days, days required for attaining 50 per cent of germination, germination energy, mean daily germination, peak value and germination value of *Saraca asoca* was assessed. Most of the germination attributes were the least in case of seed source SS 5.

A number of seedling parameters like height, collar diameter, number of leaves, leaf area, number of branches, leaf thickness, fresh weight of shoot and root dry weight of shoot and root, shoot to root dry weight ratio, relative growth rate, chlorophyll content and photosynthetic rate was measured. The study showed seed source SS 1, SS 2 and SS 4 to be the best followed by seed source SS 3 and SS 6. However the variation with respect to seed source SS 5 was significantly the lowest in most of the attributes studied. The cluster analysis revealed seed sources SS 2 and SS 4 along with seed sources SS 3 and SS 6 to be genetically closer. A highly distant relationship was exhibited by seed source SS 5 with rest of the seed sources.

Apart from the seed source variation study, karyomorphhological analysis was also carried out in ashoka. The study revealed 2n = 34 chromosomes, in *Saraca asoca*. In order to standardize the procedure for cytological preparation two pre-treatment chemicals namely 8-hydroxyquinoline and colchicine were used. Two fixatives Carnoys I and Carnoys II were used in the present study. For staining acetocarmine and fuelgen were used. It was found that pretreating the chemicals with 8-hydroxyquinoline and fixing it in Carnoys II and using the stain acetocarmine gave the best cytological preparation.