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Saraca asoca – morphology and diversity across its natural distribution in India

Abstract

Aim: This review aims in consolidating the microscopic, propagative, and diversity studies on *Saraca asoca* (Roxb.) de Wilde. Studies on seed germination, biochemical and Physico-chemical parameters, and population status are discussed. Microscopic and diversity studies highlight how the Ashoka tree is different from its adulterants and throws light on the genetic variation and polymorphism existing within the populations and the evolutionary relationship of the tree with other members of the same family. Propagation studies by tissue culture can help in replenishing the wild population through mass propagation. Thus, this review has tried to compile some of the research studies conducted on *Saraca asoca* and also looks at the areas yet to explore.

Background: *Saraca asoca* (Roxb.) de Wilde is an evergreen tree of the Fabaceae family, and the bark is used to treat gynecological problems. Unethical harvesting and deforestation have reduced the wild population, listing the Ashoka tree in the 'Globally vulnerable' category by IUCN. Huge market demand has led to adulteration with substitutes. The wide use of micropropagation techniques can help in replenishing the wild population. This article has listed the major research works done on this medicinal tree.

Review results: The studies reveal the importance of the micropropagation technique and throw light on the seriousness of replenishment of this red-listed tree. Marker studies can help in understanding the diversity within the populations and give ideas about preserving the germplasm. A brief botanical description of the tree and its parts also is done.

Conclusion: Studies indicate the importance of protecting the wild population of *Saraca asoca*. The Government and local bodies should work together to create awareness among the people about the species for protecting and replenishing their depleting population.

Keywords: *saraca asoca*, review, micropropagation, markers, genetic variation, germination

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Introduction

Trees play a chief role in the existence of human civilization. We get a glimpse of the trees that have to be planted at a site in Varahamihira's Brihat Samhita. In Brihat Samhita tank bunds are discussed to be shaded with *Mangifera indica*, *Terminalia arjuna*, *Ficus religiosa*, *Ficus benghalensis*, *Syzygium cuminii*, *Mitragyna parviflora*, *Borassus flabellifer*, *Madhuca longifolia*, *Mimusops elengi*, and *Saraca asoca*[1].

Saraca asoca or *Jonesia asoca* Roxb., commonly known as Ashoka, is a medicinal tree used to treat gynecological problems, as found in records of Charaka Samhita, Bhavprakasha Nighantu, and the treatise of Susruta[2-4]. Belonging to the genus Saraca, it comes under the Fabaceae family.

The genus Saraca includes *Saraca asoca* (Roxb.) De Wilde, *Saraca indica* Linné, *Saraca thaipingensis* Cantley ex Prain, *Saraca celebica* De Wilde, *Saraca griffithiana* Prain, *Saraca hullettii* Prain, *Saraca declinata* (Jack) Miq., and *Saraca dives* Pierre, to name a few[5]. Of these, *S.asoca* (Roxb.) de Wilde, *S.indica* L., *S.declinata* Miq. (Red Saraca) and *S.thaipingensis* Prain (Yellow Saraca) has been reported in India. While *S.asoca* grows in the wild, the other three are grown in botanical gardens.[6-7] Though mainly used for ornamental purposes, they possess immense medicinal values.[7]

Ashoka is a perennial evergreen tree, growing to a height of 10m.[9] Though a native of the Indian sub-continent, it is also seen in Sri Lanka and the Indo-Malaysian region. In India, Ashoka trees are found in Peninsular India, comprising the Western and Eastern Ghats and the sub-Himalayan tracts, to an altitude of 750m. It also prefers to grow in semi-evergreen and moist deciduous forests and is found along river streams.[10,11] Red lateral alluvial soil is suitable for growth and requires an annual rainfall of 2000-4000mm.[12]

Saraca asoca (Roxb.) de Wilde, though known as Ashoka, has many common names in different languages - Ashoka in Hindi, Kankeli in Sanskrit, Ashokadamara in Kannada, Ashokapatta in Telugu, Asokam in Malayalam, and Asogam in Tamil.[13] It is considered a 'sacred tree' by both Hindus and Buddhists and holds high significance and importance in mythology, literature, and history.[2] The first variety of Ashoka – Aswani-I - with high bark yield and more tannin content has been released through a single plant selection method.[14]

Other than 'Ashokarishta' and 'Ashokaghrita' many tablets, tonics, and syrups made from *S.asoca* are available in the market like 'Ashotone', and 'Ashonil'.[10] The Ayurvedic formulation of Ashokarishta comprises of *Saraca asoca* bark, *Woodfordia fruticosa* flower, *Cuminum cyaminum* fruit, *Cyperus rotundus* rhizome, *Zingiber officinale* rhizome, *Berberis aristata* stem, *Nymphea stellata* flower, *Terminalia bellirica* fruit pericarp, *Terminalia chebula* fruit pericarp, *Phyllanthus emblica* fruit pericarp, *Mangifera indica* endosperm, *Adhatoda vasica* root, *Santalum album* heartwood, and jiggery.[15] The bark helps in stopping excessive menstrual bleeding and hemorrhagic dysentery due to the presence of tannins.[16] Chemical investigations have reported the presence of epicatechin, catechin, procyanidin, leucocyanidin, linoleic acid, β-sitosterol, Quercetin, catechol, epicatechol, and ceryl alcohol.[17]

Saraca asoca seeds germinate quickly during the rains. They have two cotyledons and an embryonic axis covered with a thin seed coat and are non-endosperm.[18] Recalcitrant seeds are the source of reproduction and cannot be stored long. This could be a reason behind the inhibition of germination on exposure to gamma irradiation (Figures 1& 2).[19]

Figure 1 Occurrence of Saraca asoca across the globe.

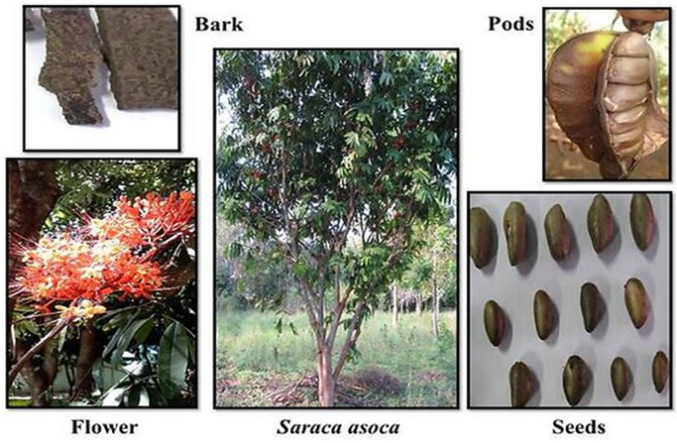

Figure 2 *Saraca asoca* and plant parts.

Overharvesting and exploitation of bark, poor seed viability, narrow ecological niche, increasing market demands, and deforestation have resulted in depletion of the wild population of these trees, thus listing them under 'Endangered' and 'Vulnerable' categories, by CAMP (2001) and IUCN (2013), respectively.[10] It is one of the 32 medicinal plant species prioritized under the National Medicinal Plant Board (NMPB), Government of India.[20] The unavailability of *Saraca asoca* bark to meet rising market demands has led to the adulteration of the drug with barks of *Polyalthia longifolia* Benth, *Trema orientalis, Shorea robusta*, *Bauhinia variegata, Mallotus nudiflorus, Sicalpinea pulchirena,* and *Afanamexis polystakis.*[21-24] Shubhashree et al. had listed out the uses and identification of the two common adulterants – *Polyalthia longifolia* (Sonn.) Thwaites and *Cananga odorata* (Lam.) Hook. f. and Thomson –, along with that of *Saraca asoca* (Roxb.) de Wilde, which can help in easy differentiation.[4] Sometimes these adulterants can cause adverse effects in the patients, which are not usually developed upon intake of the actual drug. Adulteration happens when the harvesting of the specific plant part is not able to meet the rising market demands when both the original and its substitute have the same vernacular name leading to identification difficulties and sometimes just for profit. In the case of *S.asoca* and *P.longifolia* - both commonly called Ashoka – they are chemically equivalent and biologically different.[25]

In vitro propagation helps in producing a large number of true-to-type plants in a short time, which can help in the afforestation of this species.[26] Many reviews have explored the various aspects and uses of *S. asoca.*[12,23] Through this review, the authors have attempted to compile the different research works that have been conducted on *Saraca asoca*. An attempt has also been made to list out the areas, yet to be explored.

Review results

Botanical description of *Saraca asoca*

Bark

The bark is dark brown, grey, or almost black with a reddish wood[27] and has a warty surface, sometimes cracked. Rough and uneven bark is channeled and smooth with circular lenticels.[14] Bark has a bitter, sweet, and astringent taste.[28]

Leaves

Glabrous leaves are long, corky at the base, and bitter.[29] Petioles are short and the leaves are united such that 6-12 pairs form a leaflet. Leaflets are glabrous and oblong-lanceolate in shape.[14] The young leaves are copper red when they germinate and start turning light green and then dark green as they mature.[30]

Flowers

Morphological characters: Flowers are seen as dense axillary corymb of orange and yellow color seen from January to March, though they are seen throughout the year. They are aromatic, hermaphrodite, and staminate, and astringent in taste.[31] Each cluster contains many small, long tube flowers opening into four oval lobes and has half-white, half crimson stamens protruding from the ring at top of each tube. The flowers are yellow when young, gradually turning to orange and crimson as they mature. Eventually, they turn into vermillion due to the sun's ray's effect. The tree bears flowers from December to May, with peak flowering from February to March. The flowers were visited by white ants, butterflies, Arctiidae moth, Syrphid fly, black ant, and bees (especially Giant Asian honey bee), which helps in pollination.[14, 32]

Anatomic and diagnostical characters: - Petals are partial gamopetalous and the flowers are arranged in compact sessile and open in acropetal succession.[14,30] Colorful bracteoles are shorter than hypanthium. The tetramerous petaloid overlaps calyx and corolla is absent. Out of the seven stamens, two of the outer whorls and one of the inner whorls are slightly larger than the rest. Sigmoidal shape gynoecium is long stipitate and grows closely attached to one side of the hypanthium. The style is coiled threadlike, stigma is obtuse and many ovules are present indicating the pubescent stage of the ovary. The anther is versatile.[31-32]

Seeds and pods

The seeds are flat, ovoid-ellipsoid in shape, and are covered with a brown seed coat. Two to eight seeds are found within each pod.[14] The pods are dehiscent and tapering at both ends.[33] The green pods are leathery and turn brownish-purple and black, as they ripen and get ready to disperse the matured seeds. The green seeds turn black as they mature and are recalcitrant.[30]

Roots

Roots are long, slightly hard, and grey-brown. They have a taproot system with profused side roots.[34]

Germination studies

The seeds are sown in a germination bed or polybags and the potting mixture consists of soil, sand, and farmyard manure (FYM) in an equal ratio. Intercropping system is also followed.[33]

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Seeds from Sri Lanka were studied to understand the kind of dormancy and storage behavior patterns. The recalcitrant seeds were found to be physiologically dormant, with a non-orthodox storage behaviour.[35] Maturation drying, leachate conductivity, storability, germination, and presence or absence of oligosaccharides can help in differentiating the very similar desiccation-tolerant *Caesalpinia pulcherrima* and desiccation-sensitive *Saraca asoca*, of the same family. While *S.asoca* seeds are recalcitrant, *C.pulcherrima* seeds are orthodox.[36] Different germination behavior was noticed when the seeds were stored at different temperatures. The Ashoka seeds were found to not withstand drying to a low moisture content of 20-23%.[37] Storage of seeds at different temperatures reduced the critical moisture level of seeds, which resulted in decreased viability and vigor of the seedlings.[38]

Seeds were harvested at different developmental stages to determine the best collection time, which was found to be when the brownish-white seeds were premature. The optimum temperature for seed germination was found to be 30-50°C and storing seeds at 15°C increased longevity to 4 years.[39] Multiple shoots - root induction and traits like number of leaflets, number of leaves, seedling height, shoot height, maximum root length, and maximum root diameter were analyzed for seedlings that germinated from seeds stored at different temperatures.[18]

A higher rate of Polyembryony was observed in Ashoka and these multiple seedlings performed similarly in comparison to the normal seedlings.[40-41] Albino seedlings were reported for the first time in *Saraca asoca* and these seedlings could only survive for 45-60 days as they were not able to produce their food due to the absence of chlorophyll.[42] Twin seedlings were also observed during a seed germination study.[43] Abscisic acid (ABA) level was found to decrease as the seed develops indicating their recalcitrant nature, while an increase in the ABA levels in the embryonic axis was found to decrease the seed viability when the seeds were artificially dehydrated.[44] Seedling growth, biomass, and germination were higher in large-sized seeds with medium-sized seeds being at par with it, moisture content was observed to be more in small-sized seeds. So, for large-scale planting, large and medium-sized seeds are a better choice.[45]

Seeds treated with 200ppm of Gibberellic acid (GA_3) were found to take fewer days to initiate germination. Also, germination percentage, seedling vigor, seedling height, and other growth parameters were found to have increased in these treated seedlings.[20] Just the conventional method of soaking the seeds in distilled water for 12 hours before sowing, had a better germination percentage, followed by those of treatment with 0.1N Sulphuric acid and Vrikshayurveda methods.[33,46] Healthy and disease-free seedlings were found to germinate in semi-arid conditions, even when the seeds were collected from semi-moist conditions and no pre-treatment methods were required for seed germination.[47]

The germinated plants initially require shade and high amounts of water for survival and if these conditions are not met, their survival percentage declines.[48] A seed production system was established by raising seedlings of *Saraca asoca*, *Aegle marmelos*, *Terminalia bellirica*, *Oroxylum indicum*, *Asparagus racemosus*, *Acacia concina,* and *Caesalpinia sappan* and was reintroduced into the land at Attapady, Kerala.[49]

Population studies

Studies were undertaken to study the distribution, regeneration, and population status of *S.asoca*. A team led by Ankur studied the status of Ashoka trees at eight localities in the Northern Western Ghats in Maharashtra, Goa, and Gujarat. The study area included one private forest, five sacred groves, and 2 protected areas inclusive of a wildlife sanctuary and a reserved forest, from where a total of 258 adult and 449 regenerating individual trees were recorded. The study indicated the sparse distribution of the trees and revealed that regeneration was favorable at canopy openings.[10] As an extension, another study was done on eighteen localities on the Sahyadri-Konkan ecological corridor of Maharashtra and concentrated on parameters like protection level, density, canopy cover, altitude, and girth at breast height of the trees. Studies on a private forest, six sacred groves, and 2 protected areas including a wildlife sanctuary and a reserved forest recorded 298 adults and 441 regenerating trees in the region. Girth at breast height was observed to be more for trees in the sacred groves. The studies suggested that it is the need of the hour for both the government and the local community to work together for the efficient protection of this tree.[11]

Microscopic studies

Transverse and longitudinal sections of *S.asoca, S.declinata,* and *P.longifolia* were taken for study and the results have been displayed in Table 1[21] (Khatoon et al., 2009) (Table 1).

Table 1 Macro and Microscopic characters of *S.asoca, S.declinata,* and *P.longifolia*

S.No	Macro-microscopic characters	*S.asoca*	*S.declinata*	*P.longifolia*
1	Stem bark	Channeled	Curved	Curved
2	Phelloderm and Stone cells	Phelloderm represented by 2-3 continuous bands of stone cells	Phelloderm differentiated in outer and inner zones- a cluster of rows of 6-7 stone cells	Stone cells throughout phelloderm
3	Distribution pattern of fibers in phloem region	In a group of 3-24	Solitary or in groups of 3-6	As broad concentric bands that alternate with parenchymatous bands
4	Mucilage canals and oil cells	Absent	Absent	Present
5	The broadness of Medullary rays	Uni-biseriate	Uni- to triseriate	Multiseriate

On comparing transverse sections of *S.asoca* and its common adulterant *P.longifolia*, smaller epidermal cells, as well as specialized cells, were seen in the cortical region of the latter, which can help in differentiating the two barks by microscopy.[50]

Microscopical study of flowers revealed uniseriate trichomes on the outer epidermis of calyx, oval- to spherical-shaped large pollen grains, presence of spiral xylem vessels, ovoid oil glands, etc.[30] Brachyparacytic stomata were found on the adaxial surface and were surrounded by irregular wavy cells.[51]

Microscopic examination of leaves revealed the polygonal shape of upper and lower epidermal cells. The vascular bundle was encircled in a sclerenchymatous ring in the midrib region, while multi-layered spongy tissue and single-layered palisade tissue were present in the mesophyll region.[29] Palynological studies indicated a radially-symmetric isopolar pollen with an oblate shape rugulate ornamentation.[52] Karyotyping in *Saraca asoca* revealed the presence of 34 chromosomes in the somatic cells arranged as 17 sets of homologous pairs. Pre-treating the root tips with 8-hydroxyquinoline, with acetocarmine as the stain and Carnoys II as the fixative, was standardized for the same.[53]

Physico-chemical studies

Haemagglutination can be used to differentiate *Saraca asoca* from its most common adulterant *Polyalthia longifolia,* as the former was found to contain haemagglutinin.[54] When *S.asoca, S.declinata,* and *P.longifolia* were compared, tannin content was found to be higher in *S.asoca* and *S.declinata*, while sugar and starch contents were higher in *P.longifolia.*[21] Parameters like total ash, acid insoluble ash, and water-soluble ash were studied up on dried flower powders.[55] Parameters like foreign matter, water-soluble extractive, alcohol-soluble extractive, water-soluble ash, acid-insoluble ash, total ash value, foaming index, moisture content, and crude fiber content evaluated on leaves, can be further developed for identification of the original drug material.[29]

Biochemical studies

Methanol and ethanol flower extract was found to contain carbohydrates, tannins, flavonoids, glycosides, saponin, proteins, and steroids.[55] Petroleum benzene, acetone, water, ethanol, and diethyl ether extracts of flower, leaves, and bark revealed the presence of saponins, steroids, phenols, carbohydrates, glycosides, phytosterols, and tannins.[56,57] The presence of terpenoids and phlobatannins has also been reported in Ashoka leaves.[58] Ethyl acetate extract of leaves revealed the presence of carbohydrates, protein, glycosides, tannins, flavonoids, and saponins.[29]

S.asoca barks had more tannin content when compared with that of *P.longifolia.*[50] In the three phases of rooting in IBA-treated cuttings, Polyphenol oxidase activity was seen till the initiation phase, IAA-oxidase activity only in the expression phase, and Peroxidase activity till the expression phase. Maximum phenolic content in the last two phases and activities of both Peroxidase and Polyphenol oxidase was found to help in the formation of adventitious roots.[59]

NMR studies: 1D and 2D NMR spectra of methanolic extracts revealed 3 market samples to match with *S.asoca* samples, indicating the extent of adulteration in most of the market samples. Phenolic metabolites were also found to be in decreased levels in the market samples.[22]

Diversity studies

Isolating DNA or RNA from the plant source is the most important step for genetic studies. The process of isolation varies according to plant source, species, age, etc. It is also very tedious to isolate the genetic material from woody species, as the presence of polyphenols and polysaccharides can interfere with the isolation process. So, the process is usually modified according to the plant source. Chloroplast DNA isolated from leaves was sequenced, with a total length of 137743 bp and GC content of 35.26%. The plastome consisting of 5,206,216,851 paired-end filtered reads and 126 coding genes, can be used to differentiate the genuine Ashoka sample from its adulterants.[60] The evolutionary relationship of *Saraca asoca* was compared with that of other species of the same tribe Detarieae, using the chloroplast *matK* gene. Results indicated that *S.asoca* may have had synonymous substitution and was found to be closely related to *Saraca palembanica*, *Saraca declinata*, *Endertia spectabilis*, and *Lysidice rhodostegia.*[61]

Markers like RAPD (Randomly Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Single sequence Repeat), DNA Amplification Fingerprinting (DAF), Inter-Simple Sequence Repeats (ISSR), Sequence Characterized Amplified Regions (SCAR), etc. can be used for characterization of the DNA.[12] Higher polymorphism was observed in populations at different habitats and so was the variation within a population.[27]

(a) Marker studies

Ten microsatellite markers of 2-22 alleles per locus identified can be used to study the genetic structure of a population.[62]

Randomly Amplified Polymorphic DNA (RAPD) profiling carried out in five natural populations in Orissa, found relatively high genetic variation as well as polymorphism between the populations, thus recommending *in situ* conservation of the species.[63] Fluorescent-labeled (6-carboxyfluorescein) RAPD primer based study on 6 accessions from Hyderabad, showed more polymorphism, indicating high genetic diversity within the species, even in a small population.[64]

Fingerprinting DNA by Amplified Fragment Length Polymorphism (AFLP) helps in differentiating *Saraca asoca* from its adulterants like *Polyalthia longifolia* bark samples, as *Saraca asoca* was less diverse when compared to the latter.[65]

The identity of *S.asoca* samples was confirmed by amplifying and sequencing the matK region.[66] DNA isolated from powdered bark samples of *S.asoca* and *P.longifolia* and market samples of *S.asoca* were amplified successfully with SCoT (Start Codon Targeted) and ISSR (Inter Simple Sequence Repeats) markers. The poor amplification, quality, and DNA concentration as well as differences in the DNA profile of market samples and *P. longifolia* from that of *S. asoca,* can be used as a marker for the identification of the true sample.[67] While Analysis of Molecular Variance (AMOVA) was used to find out the relationship between geographical and genetic distance, STRUCTURE software was used to determine the genetic structure of the population.[66]

ISSR primers were used to study and understand the level of genetic variation in 106 accessions representing 11 populations collected from the Western Ghats. Using AMOVA and Polymorphic Information Content (PIC), the molecular variance among and between populations as well as variability at specific loci was calculated, respectively.[68] ISSR markers found higher genetic variability among genotypes collected from different locations and UPGMA clustering was used to generate a dendrogram of the same.[69]

(b) DNA barcoding studies

DNA barcoding helps in the differentiation of adulterants from *S.asoca* in drug samples obtained from the market. Two chloroplastic regions, rbcL, and psbA-trnH were used to barcode the Biological Reference Material (BRM- sample with a specific identification number) and market samples. While amplified BRM sequences matched with rbcL sequences of *S.asoca,* only 3 samples out of 25 market samples matched with the rbcL region, thus confirming a wide-spread adulteration.[22]

Vegetative Propagation works

Vegetative propagation helps in maintaining the superior traits of the mother plant, whereas plants propagated from seeds are mostly heterozygous. On comparing the effect of Indole-3-Acetic Acid (IAA). Indole-3-Butyric Acid (IBA) and α-Naphthalene Acetic Acid (NAA) on the rooting and sprouting of cuttings, cuttings dipped in 800ppm α-Naphthalene Acetic Acid (NAA) was found to develop better rooting and sprouting percentage, along with more biomass, leaf number, and root number.[70] Maximum rooting -sprouting percentage, minimum days for sprout initiation, and more number of sprouts per cutting were some of the characteristics found in cuttings treated with 2000ppm IBA. In air-layering, the number of primary and secondary roots, root length, root diameter, and rooting percentage were found to increase, when treated with 2500ppm IBA.[20] Maximum rooting was seen on cuttings pre-treated with 500ppm IBA, than those with IAA and NAA, indicating the effectiveness of IBA in adventitious rooting of cuttings under *in vivo* conditions.[59]

For rooting *in vitro* grown plants, a two-step treatment was adopted, where treatment with 200µM IBA for 5 days, was followed by transfer to lower IBA levels along with Phloroglucinol, as this yielded more number of roots with maximum length.[71] Sprouting Value Index (SVI) and rooting were found to be high in stem cuttings planted in coir pith compost for 300ppm, 500ppm, and 1000ppm of IBA.[72] Foliar application of 1000ppm BAP to one-year-old seedlings from Kerala, Tamil Nadu, and Karnataka for three months, helped to understand their sprouting ability. It was observed that SVI was maximum for seedlings from Kerala, followed by Tamil Nadu and Karnataka, thus concluding the source-dependency of the sprouting potential.[73]

Air-layering gives rise to true-to-type plants. In a case study in air-layering, layered branches were covered in black-colored and transparent polythene sheets. Though 100% layerages were found in both the cases, root biomass - that could give rise to clones with higher growth rate in a given period – was more in shoots wrapped in black-colored polythene sheets.[74]

Fungal strains as bio inoculants were tried out for increasing plant productivity under nursery conditions. Shoot height, leaf area, leaf number, plant biomass, and root length were found to increase in seedlings treated with Phosphate-solubilizing fungi- *Aspergillus kanagawaensis* and *Aspergillus niger*. The numbers of branches were highest in seedlings treated with *Aspergillus japanicus.* Good leaf area was also observed in those treated with *Aspergillus japanicus, Penicillium citrinum,* and *Fusarium oxysporum.* This experiment proved the use of fungal strains as bio inoculants for increasing plant productivity under nursery conditions.[75]

Micropropagation

Stems, leaves, and flower segments initiated callus in MS media with 5mg/L BAP, and these calli were also found to have antibacterial activity.[16] Fungal and bacterial contaminations are one of the major hindrances faced during *in vitro* propagation. A surface sterilization treatment by 0.1% Mercuric chloride for 15 minutes and 1% Sodium hypochlorite for 2minutes was standardized, as they initiated 93.33% callus from leaf segments, with minimal fungal contamination.[26] Maximum callus induction was found in MS media with 2mg/l 2,4-D (2,4-Dichlorophenoxy acetic acid), inoculated with leaf bits and ovary as explants.[76]

Multiple shoots were obtained from axillary nodal explants, when grown on MS media supplemented with 9.30 µM Kinetin and 2.47 µM Adenine sulfate. MS media supplemented with 9.84 µM IBA was found to be the most suitable root induction medium and 390 out of the 414 transplanted plants were successfully established in the field.[64] Shoot tips, nodal and internodal explants were found to produce shoots in MS medium containing 0.5mg/l BAP (Benzyl aminopurine), though nodal explants were found to be the best source for regeneration. MS media with BAP, Kn (Kinetin), and 2, 4-D produced shoots with an intervening callus phase and the shoots rooted on MS medium with 4mg/l IBA (Indole-3-Butyric Acid) produced more number of roots when compared to those in IAA (Indole-3-Acetic Acid). Also, these *in vitro* grown plants successfully acclimatized to field conditions.[76] Gamborg's B_5 medium with 2.2µM BA (Benzyl Adenine or BAP) was found to be the best medium for shoot proliferation and Sucrose, the most effective carbon source. Maximum shoots were obtained upon the addition of 0.25X strength KNO_3.[71]

Conclusion

Though various studies have been done in *S. asoca* by many researchers, there are some lacunae in some of the fields that are yet to be studied. Such fields of study can add on to be beneficial for the sustainability of this tree. For example, more detailed studies on the phytochemical aspects, especially on leaves or flowers could identify them to be a prospective substitute for bark and could also lead to the development of a phytomarker that can help in the identification of the true Ashoka, thus preventing adulteration.

Similarly, it has been found that *Saraca indica* and *Saraca asoca* are often used as synonyms. A thorough study on their morphology, phytochemistry, and genetic material can confirm them to be two different species of the same genus.

The recalcitrant nature of seeds is another problem faced during the establishment of the plants. Transcriptome studies can identify the genes responsible for their viability and could reveal how their shelf life can be increased. Such studies can help in identifying genes behind the secondary metabolites pathway and help in the understanding of variations that are observed within the populations. Last but not the least, initiatives by the Government with local and expert support to identify and protect their habitats can prove beneficial to the already dwindling population. Creating awareness among the public and planting more trees will aid in this.

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None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

1. Umarani R, Aadhavan EK, Faisal MM. Understanding poor storage potential of recalcitrant seeds. *Current Science*. 2015:2023–2034.
2. Kolet M, Thosar S. *Saraca asoca* Roxb. De Wilde: Bridging mythology, taxonomy and modern research – A brief review. National Conference on New and Emerging Trends in Bioinformatics and Taxonomy 'NETBT 2015'.

3. Tewari R, Gupta M, Ahmad F, et al. Extraction, quantification and antioxidant activities of flavonoids, polyphenols and pinitol from wild and cultivated *Saraca asoca* bark using RP–HPLC–PDA–RI method. *Industrial Crops and Products*. 2017;103:73–80.

4. Shubhashree MN, Shanta TR, Thakur R, et al. Significance of identification of ayurvedic drugs with its different sources. *Research Journal of Pharmacology and Pharmacodynamics*. 2020;12(3):117–129.

5. Zuijderhoudt GF. A revision of the genus Saraca L.(Legum.—CAES.). *Blumea: Biodiversity, Evolution and Biogeography of Plants*. 196;15(2):413–425.

6. Begum SN, Ravikumar K, Ved DK. 'Asoka'–an important medicinal plant, its market scenario and conservation measures in India. *Current Science*. 2014;107(1):26–28.

7. Sil S, Mallick T, De KK, et al. Comparative morphological study of three species of Saraca L.*(Fabaceae)* by the statistical approach to find out the logic of potent morphological markers. *Beni-Suef University Journal of Basic and Applied Sciences*. 2018;7(4):612–619.

8. CAMP Workshops on Medicinal Plants, India (January 1997). *Saraca asoca*. *The IUCN Red List of Threatened Species*. 1998.

9. Kumar A, Kumar B, Bartariya G. Evaluation of α–amylase inhibition assay in various foliar extract of *Saraca asoca* (Roxb.). *Indo* Am *J Pharm*. 2018;5(1):119–123.

10. Patwardhan A, Mhaskar M, Pimputkar M, et al. Status of *Saraca asoca*: a species of conservation concern from Western Ghats biodiversity hot–spot. Biodivers. *Watch*. 2014;3:90–102.

11. Patwardhan A, Pimputkar M, Mhaskar M, et al. Distribution and population status of threatened medicinal tree *Saraca asoca* (Roxb.) De Wilde from Sahyadri–Konkan ecological corridor. *Current Science*. 2016:1500–1506.

12. Yadav, S. S., Woo, A. A., Choure, K. Review of medicinally important plant species *Saraca asoca* (Roxb.). Int. J. Adv. Res 2019*.;* 7(9): 154–166.

13. Pradhan P, Joseph L, Gupta V, et al. *Saraca asoca* (Ashoka): a review. *Journal of Chemical and Pharmaceutical Research*. 2009;1(1):62–71.

14. Smitha GR, Thondaiman V. Reproductive biology and breeding system of *Saraca asoca* (Roxb.) De Wilde: a vulnerable medicinal plant. *Springer Plus*. 2016;5(1):1–5.

15. Shikha M, Vidhu A. A comparative study of prepared and marketed asokarista with respect to physicochemical parameters and phytochemical markers. *International Journal of Pharmacognosy and Phytochemical Research*. 2015;7:144–149.

16. Shahid M, Shahzad A, Malik A, et al. Antibacterial activity of aerial parts as well as in vitro raised calli of the medicinal plant *Saraca asoca* (Roxb.) de Wilde. *Can J Microbiol*. 2007;53(1):75-81.

17. Baranwal, V. Asoka: Herbal boon to gynecological problems–an overview of current research. *Ayurveda J Health*. 2014;12:58–62.

18. Prajith TM, Chandran A. Multiple shoots and roots of germinating *Saraca asoca* seeds. *Int J Plant Animal Envir Sci*. 2015;5:273–277.

19. Chandrashekar KR. Gamma sensitivity of forest plants of Western Ghats. *J Environ Radioact*. 2014;132:100–107.

20. Madhushree SI, Shetty GR, Souravi K, et al. Standardization of Seed and Vegetative Propagation Techniques in *Saraca asoca* (Roxb.) De Wilde: An Endangered Medicinal Plant. *Int J Curr Microbiol App Sci*. 2018;7(4):1327–1335.

21. Khatoon S, Singh N, Kumar S, et al. Authentication and quality evaluation of an important Ayurvedic drug– Ashoka bark. *J Sci Ind Res*. 2009;68:393–400.

22. Khatoon S, Singh N, Kumar S, et al. Authentication and quality evaluation of an important Ayurvedic drug–Ashoka bark. *J Sci Ind Res*. 2009;68(5):393–400.

23. Urumarudappa SK, Gogna N, Newmaster SG, et al. DNA barcoding and NMR spectroscopy–based assessment of species adulteration in the raw herbal trade of *Saraca asoca* (Roxb.) Willd, an important medicinal plant. *Int J Legal Med*. 2016;130(6):1457–1470.

24. Borokar AA, Pansare TA. Plant Profile, Phytochemistry, and Pharmacology of Ashoka (*Saraca asoca* (Roxb.), De. Wilde)–A Comprehensive Review. *Int J Ayurvedic Herb Med*. 2017;7(2):2524–25241.

25. Mahajan S, Shivaram NG, Lalitha BR, et al. Ashoka [*Saraca asoca* (Roxb.) Willd.]: A Salubrious Plant. *Journal of Ayurveda and Integrated Medical Sciences*. 2019;4(5):184–190.

26. Rout S, Khare N. Effect of Various Surface Sterilant on Contamination and Callus Regeneration of Ashoka (*Saraca asoca* Roxb. De Wilde) from Leaf Segment Explant. *Int J Curr Microbiol App Sci*. 2018;7(7):2027–2033.

27. Warrier RR, Joshi G, Arunkumar AN. DNA fingerprinting in industrially important medicinal trees. *Ann Phytomed*. 2019;8(1):19–35.

28. Rathee P, Rathee S, Rathee D, Rathee D. Quantitative estimation of (+)–Catechin in stem bark of Saraca asoka Linn using HPTLC. *Der Pharma Chemica*. 2010;2(1):306–314.

29. Pradhan P, Joseph L, George M, et al. Pharmacognostic, phytochemical and quantitative investigation of *Saraca asoca* leaves. *J Pharm Res*. 2010;3(1):776–780.

30. Chauhan S. Floral and pollination biology of *Saraca asoca* (Roxb.) De Wilde (Caesalpinioideae). *The International Journal of Plant Reproductive Biology*. 2019:11(1):31–37.

31. Bisht A, Irshad S, Rawat AK, et al. Pharmacognostical studies on *Saraca asoca* (Roxb.) Willd. flower. *Tropical Plant Research*. 2017;4(1):153–160.

32. Mishra SB, Vijayakumar M. Floral morphology and HPTLC fingerprint profile of *Saraca asoca* (Roxb.) De Wilde (Caesalpiniaceae): A consecrated tree of India. *Int Lett Nat Sci*. 2015*;*43:54–62.

33. National Medicinal Plant Board, 2008. Agro–techniques of selected Medicinal Plants, Volume I. India: TERI Press; 2008.

34. Sanghamitra S, Paria ND. Seedling morphology as a tool for taxonomic study in some members of Leguminosae (Fabaceae). *International Journal of Plant, Animal and Environmental Sciences*. 2015;5(1):1–5.

35. Jayasuriya KG, Wijetunga AS, Baskin JM, et al. Seed dormancy and storage behavior in tropical Fabaceae: a study of 100 species from Sri Lanka*. Seed Science Research*. 2013;23(4):257.

36. Prajith TM, Anilkumar C, Rajani K, et al. Comparative analysis of seed development and desiccation aspects of *Saraca asoca* (Roxb.) WJ de Wilde and Caesalpinia pulcherrima (L.) Sw. *Asian Journal of Plant Sciences*. 2014;13(4/8):164–171.

37. Smitha GR, Das M. Effect of seed moisture content, temperature, and storage period on seed germination of *Saraca asoca*—an endangered medicinal plant. *Medicinal Plants*. 2016;8(1):60–64.

38. Prajith TM, Anilkumar C. Seed desiccation responses in *Saraca asoca* (Roxb.) WJ de Wilde. *Current Science*. 2017:2462–2466.

39. Kundu M, Tiwari S, Haldkar M. Collection, germination and storage of seeds of *Saraca asoca* (Roxb.) Willd. *Journal of Applied Research on Medicinal and Aromatic Plants*. 2020;16:100231.

40. Rane A, Narkhede S, Bhave S. A note on polyembryony in *Saraca asoca* (Roxb.) Wilde, a critically endangered medicinal tree species. *Karnataka J Agric Sci*. 2010*;*23(4):662.

41. Singh BG, Sivalingam R, Mahadevan NP, et al. High–frequency Polyembryony in *Saraca asoca* (Roxb.) De Wilde (Caesalpiniaceae) – A red–listed medicinal tree. *My Forest*. 2005*;* 41(4):573–582.

42. Mokat DN, Desai JS, Bhave SG. Albino mutants and polyembryony in *Saraca asoca* (Roxb.) de Wilde. *Journal of Non-Timber Forest Products*. 2011;18(1):67–70.

43. Wanage SS, Mirgal AB, Naik MM, et al. Twin seedlings in *Saraca asoca* (Roxb.) De Wilde: a commercially important and critically endangered tree species. *Journal of Non-Timber Forest Products*. 2012;19(1):77–78.

44. Prajith TM, Anilkumar C, Kumar KA. Changes in abscisic acid levels in embryonic axis of *Saraca asoca* seeds during maturation and artificial dehydration. *Indian Journal of Plant Physiology*. 2017 Sep;22(3):354–357.

45. Mirgal AB, Gunaga RP, Salunkhe CB. Seed size and its influence on germination, seedling growth and biomass in *Saraca asoca* (Roxb). De Wilde, critically endangered tree species of Western ghats, India. *Journal of Applied and Natural Science*. 2016;8(3):1599–1602.

46. Prachi S, Pramod K. Evaluation on the seed germination rate of Ashoka (*Saraca asoca* (Roxb.) de Wilde) with special reference to Vrikshayurveda. *International Journal of Ayurveda and Pharma Research*. 2016.

47. Singh BG, Warrier RR, Anandalakshmi R, et al. Seed germination studies in *Saraca asoca* (Roxb.) de Wilde. *Indian Forester*. 2005;131(6):841–843.

48. Nair KKN, Pandalai RC, Chandrashekhara UM. Generation and transfer of silviculture and harvesting technology of selected medicinal plants for the sustained utilization of the wastelands of Kerala. *KFRI Research Report*. 2000;178:1–52.

49. Warrier RR, Anandalakshmi R, Singh BG. Establishment of Seed Production Systems for Non–Timber Forest Products – An approach to sustainable utilization. *Forestry Bulletin*. 2012;12(2):29–32.

50. Mathew S, Mathew G, Joy PP, et al. Differentiation of *Saraca asoca* crude drug from its adulterant. *Ancient Science of Life*. 2005;24(4):174–178.

51. Khan F, Yousaf Z, Ahmed HS, et al. Stomatal patterning: an important taxonomic tool for systematical studies of tree species of angiosperm. *Annual Research & Review in Biology*. 2014:4034–4053.

52. Kailas JG, Ramakrishna H, Prabhakar R. Palynodiversity of arborescent plants of Caesalpiniaceae family of Karimnagar district, Telangana state, India. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2014;5(6):349–353.

53. Deepa KS, Santoshkumar AV, Rekha K, Joseph J. Karyotype analysis in *Saraca asoca* (Roxb.) de Wilde. *Journal of Tropical Agriculture*. 2013;51(1):135–139.

54. Beena C, Radhakrishnan VV. Haemagglutination as a rapid tool to differentiate *Saraca asoca* bark from the adulterant Polyalthia longifolia. *Journal of Progressive Agriculture*. 2010;1(1):1–3.

55. Saha J, Mitra T, Gupta K, et al. Phytoconstituents and HPTLC analysis in *Saraca asoca* (Roxb.) Wilde. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2012;4(1):96–99.

56. Divya KR, Anjali AR, Kumar R. Phytochemical screening of *Saraca asoca* (Roxb.), De. Wild. *Journal of Pharmacognosy and Phytochemistry*. 2017;6(3):518–521.

57. Manohar VR. Chandrashekar R, Rao SN. Phytochemical Analysis of Saraca asoka Bark Extracts. *Int J Pharm Sci Rev Res*. 2012;17(2):101–103.

58. Shurjeel FS, Shakir M, Khan NJ. Phytochemical analysis of aqueous leaf extracts of some medicinal herbs, shrubs and trees from New Delhi, India. International *Journal of Innovative Research in Science, Engineering and Technology*. 2016;5(5):7869–7876.

59. Dash GK, Senapati SK, Rout GR. Effect of auxins on adventitious root development from nodal cuttings of Saraca asoka (Roxb.) de Wilde and associated biochemical changes. *Journal of Horticulture and Forestry*. 2011;3(10):320–326.

60. Ali MA, Kim SY, Pan TK, et al. Complete chloroplast genome of vulnerable medicinal plant *Saraca asoca* (Fabaceae). *Mitochondrial DNA Part B*. 2020;5(1):754–755.

61. Saha J, Gupta K, Gupta B. Phylogenetic analyses and evolutionary relationships of *Saraca asoca* with their allied taxa (Tribe–Detarieae) based on the chloroplast matK gene. *Journal of Plant Biochemistry and Biotechnology*. 2015;24(1):65–74.

62. Sumangala RC, Shaanker RU, Dayanandan S, et al. Identification of novel microsatellite markers for *Saraca asoca*, a medicinally important tree species in India. *J Genet*. 2016;93(2):93–95.

63. Senapati SK, Das GK, Aparajita S, Rout GR. Assessment of genetic variability in the Asoka tree of India. *Biodiversity*. 2012;13(1):16–23.

64. Mohan C, Reddy MS, Naresh B, et al. RAPD studies of *Saraca asoca* by fluorescent–labeled primers and development of micropropagation protocol for its conservation. *International Journal of Applied Agricultural Research*. 2017;12(2):137–151.

65. Sharma P, Sharma S, Yadav S, et al. Categorization of *Saraca asoca* and Polyalthia longifolia, using chemical and genetic fingerprinting associated with multivariate statistical analysis. *Journal of Applied Research on Medicinal and Aromatic Plants*. 2018;9:91–96.

66. Hegde S, Pai SR, Bhagwat RM, et al. Population genetic and phytochemical dataset of *Saraca asoca*: A traditionally important medicinal tree. *Data in Brief*. 2019;25:104173.

67. Hegde S, Pai SR, Roy S. Combination of DNA isolation and RP–HPLC analysis method for bark samples of *Saraca asoca* and its adulterant. *3 Biotech*. 2017;7(3):1–9.

68. Hegde S, Pai SR, Bhagwat RM, et al. Genetic and phytochemical investigations for understanding population variability of the medicinally important tree *Saraca asoca* to help develop conservation strategies. *Phytochemistry*. 2018;156:43–54.

69. Yadav SS, Waoo AA, Tiwari SK. Identification of genetic relationship among *Saraca asoca* genotypes using Inter Simple Sequence Repeat markers. *Biosc Biotech Res Comm*. 2020;13(4):2072–2076.

70. Rout S, Khare N, Beura S. Vegetative propagation of Ashoka (*Saraca asoca* Roxb. De Wilde.) By stem cuttings. *The Pharma Innovation Journal*. 2018;7(1):486–488.

71. Shirin F, Parihar NS, Shah SN. Effect of nutrient media and KNO3 on in vitro plant regeneration in *Saraca asoca* (Roxb.) Willd*. American Journal of Plant Sciences*. 2015;6(19):3282.

72. Nayagam JR, KI MV. Sprouting Value Index: A Mathematical Approach for Evaluation of Vegetative Propagation in Tree Species. *American Journal of Environmental Protection*. 2015;4(6):271–274.

73. Arathi S, Muthubhavani M, Sruthi S, et al. Sprouting Value Index–A Tool to Identify Shoot Initiation Ability in *Saraca asoca*. *Indian Forester*. 2020;146(11):1084–1085.

74. Rane AD, Gogate PP, Aparadh VV, et al. A note on the influence of Polythene sheet on root biomass of air–layered *Saraca asoca. Life Sciences Leaflets*. 2011;18:736–738.

75. Nayak SR, Panigrahi MR, Gupta N. Beneficial impact of phosphate solubilizing fungi on growth of *Saraca asoca* (Roxb.) de Willd. under nursery condition. *Tropical Plant Research*. 2017;4(2):242–245.

76. Paranthaman M, Kumari RU, Narayanan NL, Sivasubramaniam K. Morphological Characterization and In Vitro Callus Induction in Ashoka [*Saraca asoca* (Roxb.) De Wilde.]–A Vulnerable Medicinal Tree. *Journal of Tree Sciences*. 2017;36(1):103–109.

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