Monograph

Soapnut

[*Sapindus mukorossi*]

Punam, Rameshwar, AK Handa, A Arunachalam

All India Coordinated Research Project on Agroforestry
Department of Horticulture & Agroforestry
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Citation :
Punam, Rameshwar, Handa, AK and Arunachalam, A (2024) Soapnut (*Sapindus mukorossi*) [Monograph] Department of Horticulture & Agroforestry, CSKHPKV Palampur, Himachal Pradesh, India; 114 pp.

Front cover page Photo :
AICRP-Agroforestry, CSKHPKV, Palampur

Back cover page Photo :
Different phenological stages in *S. mukorossi* (Source: Zhao *et al*., 2019)

Year of Publication : 2024

Disclaimer :
The document has been prepared for educational purposes which provide the well compiled information on history, origin, importance, latest production technology and utilization of Soapnut (*Sapindus mukorossi*). The information in the document is based on primary observation and secondary information from published sources.

Published by :
CSKHPKV, Palampur 176062, Himachal Pradesh, India

Printed at :
Ved Enterprises, Aima Palampur # 8894255909

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Foreword

Soapnut (*Sapindus mukorossi*) is an important multi-functional agroforestry tree of tropical and sub-tropical regions of Asia. In India, it is primarily known as tree of North India, a deciduous tree, known to the common man as '*ritha*'. Since ancient times, it is being used in Ayurvedic medicine system mainly as cleansing agent and for treatment of skin diseases. The plant is now gaining importance due to the presence of many phytochemical compounds of potential value.

Sapindus mukorossi is still in a state of low yield with elite varieties lacking and severe germplasm destruction ongoing. It is suspected that the diversity and habitat of *Sapindus* will suffer further damage in the background of future climate change. Therefore, the protection and management of the core *Sapindus* distribution areas should be strengthened, and natural populations at risk of destruction should be protected through in situ or ex situ conservation efforts. In this regard, this monograph on *Sapindus* a comprehensive biological descript on this species. It not only covers all phytochemical, bio-medical and economical aspects of the species but also highlights its ecological role in prevention of degradation of habitats and latest breeding and bio-technological interventions for conservation of its germplasm resources.

As we delve deeper, we uncover the ecological significance of this remarkable tree. With its ability to thrive in diverse climates and its minimal impact on the environment, the soapnut tree stands as a beacon of sustainable living. Its fruits offer a renewable alternative to synthetic detergents, reducing pollution and promoting harmony between humanity and nature.

I am confident that this monograph will make a significant contribution to researchers, growers, industrialists and health people who are captivated by this tree- "No worries" and will refer to this single -window reference material on the species.

I appreciate the efforts of scientists of this university working in All India Coordinated Research Project on Agroforestry under ICAR-CAFRI for coming out with this valuable document.

6/3/2024
(D K Vatsa)

Preface

It is our delightful pleasure to present this monograph on Sapindus mukorossi to our readers. S. mukorossi Gaertn. is the second most widespread species of Sapindus and is found in East and Southeast Asia. It is regarded as an economically important source of biodiesel, as well as biomedical and multi-functional products. The main objective of this monograph is to provide all the scattered relevant scientific information available on this multipurpose tree in the form of a holistic narration. Despite of Sapindus mukorossi being regarded as a potential ecologically and economically important tree, its cultivation has only begun recently.

This monograph attempts to present the detailed information about the social, ecological, spiritual, economical and photochemical aspects of this species which suffers natural regeneration due to poor seed germination. Propagation techniques and use of bio-technological interventions have been discussed in an elaborate manner. ICAR-Central Agroforestry Research Institute has been exploring as well as carrying out research on Sapindus mukorossi under All India Coordinated Research Project on Agroforestry and has ultimately compiled all the information in the form of this monograph.

We hope that this monograph will be beneficial not only to different categories of the stakeholders but will also be able to inspire other readers about this species. Special care has been taken to include the latest scientific technical information whilst using a simple language. All secondary information used in this book is duly acknowledged and nothing has been excluded intentionally.

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Acknowledgement

We are extremely thankful to the Director, ICAR-CAFRI for not only initiating this novel work of compilation of a monograph on selected and prioritized tree species of this AICRP centre – Sapindus mukorossi but also providing sufficient financial grant to publish the same. The work could be achieved only through his continuous inspiration and constructive suggestions. Without his guidance, compilation of this monograph would not have been possible.

We wish to extend our deep sense of gratitude and sincere thanks to Prof. (Dr) Atul, Ex-Director Extension Education, CSKHPKV, Palampur for his critical, valuable suggestions and for shaping the document to its present form.

We are also thankful to all researchers , scientists across the world and internet sites of other organizations working on different aspects of Sapindus mukorossi , who have displayed their findings in public domain.

We are thankful to Mr. Arjun (Ph.D Scholar) Department of Agronomy for his kind help during compilation of this monograph.

We would also like to express our gratitude to our Head of the Department and Director Research, CSKHPKV, Palampur for providing congenial working environment and for unwavering support of the administration throughout this whole process of publication.

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List of Acronyms

$	Dollars
%	Per cent
&	And
@	At the rate of
°C	Degree Celsius
°N	Degree North
°S	Degree South
a. i.	Active ingredient
AD	Anno Domini
AICRP	All India Coordinated Research Project
APRC/ZSI	Arunachal Pradesh Regional Centre of Zoological Survey of India
ASTM	American Society for Testing and Material
BAP	6-Benzytlamino purine
BBCH	Biologische Bundesanstalt, undessortenamt und CHemische Industrie
BC	Before Christ
CABI	Centre for Agriculture and Bioscience International
CAFRI	Central Agroforestry Research Institute
CCA	Canonical Correspondence Analysis
CFUG	Community Forest User Group
cm	Centimeter
CMC	Critical Micelle Concentration
CNPVP	China Protection of New Varieties of Plants
CSKHPKV	Choudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalya
d	Day
et al	And others
etc	Et cetera
Fig	Figure
ft	Feet
FYM	Farm Yard Manure
g	Grams
GA	Gibbrellic acid
ha	Hectare
hrs	Hours
i.e.	That is
IBA	Indole -3- Butyric acid

ICAR	Indian Council of Agriculture Research
ICIMOD	International Centre for Integrated Mountain Development
IPM	Integrated Pest Management
ISSR	Inter Simple Sequence Repeats
ITKs	Indigenous Technical Knowledge
IUCN	International Union for Conservation of Nature
kg	Kilogram
KSLCDI	Kailash Sacred Landscape Conservation and Development Initiative
LC_{50}	Lethal Concentration
LD	Lethal Dose
m	Meters
Mar	March
mg	Milligram
mgl^{-1}	Milligrams per liters
ml	Milliliter
mm	Millimeter
MT	Metric ton
N	Nitrogen
NA	Not Applicable
NPR	Nepalese Rupee
NPs	Nanoparticles
PDA	Potato Dextrose Agar
pH	Potential of Hydrogen
ppm	Parts Per Million
PTC	Plant Tissue Culture
QPM	Quality Planting Material
RAPD	Random Amplified Polymorphic DNA
RSM	Response Surface Methodology
SPF	Specific Pathogen Free
sq.m.	Square meter
SWF	Fermentation liquid of *Sapindus* saponins
t	Tonnes
UHF	University of Horticulture and Forestry
v/v	Volume per volume
VDCs	Village Development Committees
µg	Microgram
µM	Micrometre

1. Introduction

Sapindus is a genus of about five to twelve species of shrubs and small trees in the lychee family, Sapindaceae, native to warm temperate to tropical regions of the world. The genus includes both deciduous and evergreen species. Members of the genus are commonly known as soapberries as their botany is similar to berries or soapnuts because when these berries are dried, the shell crinkles up and looks just like nuts and the fruit pulp is used to make soap. The generic name is derived from the Latin words sapo, meaning "soap", and indicus, meaning "of India". Soap Nuts are an allergy-friendly and eco-friendly alternative to washing detergents. Also known as Soap Berries or Indian Wash Nuts, they have been used for thousands of years by native people in the Americas and Asia. They grow on trees and contain saponin, which cleans clothes naturally and gently

1.1. History

Sapindus mukorossi is an ancient tree found in China and India. The book titled "Saint Heritage of India" points out that "Matsyendra Nath", who was the founder of "Hatha Yoga" was enlightened below a soapnut tree in 9th to 10th century. The "Historical Dictionary of Ancient India" mentioned that soapnuts were found in 6th century BC. Some notes on the history of soapnut, soap and washerman of India between 300 BC and 1900 AD indicate its use even at earlier than 6th century (http://theindianvegan.blogspot.com - The Earth of India, 2013). The species is a bodhi tree, according to Chinese Buddhism, and its Chinese vernacular name means "no worries".

Thus, *S. mukorossi* has ecological functional, aesthetic, and spiritual attributes that make it attractive to urban dwellers. Modern cultivation of *Sapindus* species has only begun recently and lacks support from relevant research. It is still in a state of low yield with elite varieties lacking and severe germplasm destruction ongoing. Mass scale plantation needs to be encouraged, population status is not yet assessed for determining sustainable harvesting.

2. Botany of *Sapindus mukorossi*

2.1. Taxonomy and distribution

Classification and organization of living species based on the information of their shared characteristics. provides an opportunity for tracing back the origin and relationship of a particular species with its wild relatives. *Sapindus* is a genus of about five to twelve species of shrubs and small trees (Anjali *et al.*, 2018). The genus includes both deciduous and evergreen species.

Table 1. List of species under the genus *Sapindus*

Species	Common Name	Geographical Region
Sapindus mukorossi	Chinese Soapberry	India, Southern China
Sapindus emarginatus	NA	Southern Asia
Sapindus trifoliatus	South India Soapnut, Three-leaf Soapberry	Southern India, Pakistan
Sapindus delavayi	NA	India, China
Sapindus oahuensis	Hawaii Soapberry, Lonomea	Hawaii
Sapindus rarak	NA	Southeast Asia
Sapindus saponaria	Wingleaf Soapberry	Caribbean, Central & South America, Island of Hawaii
Sapindus saponaria var. drummondi (H.& Arn.)	Western Soapberry	Southwestern United States, Mexico
Sapindus marginatus	Florida Soapberry	Florida
Sapindus tomentosus	NA	China
Sapindus drummondii	Western Soapberry	Southern United States, Mexico
Sapindus vitiensis	NA	Fiji

Source: Anjali *et al.*, 2018

Table 2. Taxonomic position of *Sapindus mukorossi*

Kingdom	:	Plantae (plants)
Subkingdom	:	Tracheobionta (Vascular plants)
Superdivision	:	Spermatophyta (seed plants)
Division	:	Magnoliophyta (Flowering plants)
Class	:	Magnoliopsida (Dicotlyedons)
Subclass	:	Rosidae
Order	:	Sapindales
Family	:	Sapindaceae
Genus:		*Sapindus* L (Soapberry)
Species	:	*Sapindus mukorossi* Geartn(Chinese soapberry)

2.2. History of its cultivation

2.2.1. Native/Exotic:

Sapindus mukorossi is an ancient fruit, leaving some to claim the origin in China, while other states in India. Though the *indus* in *sapindicus* may infer India, it's disputed as to whether this term connotes the West Indies or even Native Americans, given its widespread use amongst this group in parts of North America. Ancient Indian texts make references to soapberries. The book, "Saint Heritage of India" points out the Hatha yoga Founder Machindranath was converted under a soapnut tree some time during his life in the 9th to 10th century. The "Chronological Dictionary of Prehistoric India" explains that the soapnuts were found in a monastic complex dating back to the 6th century BC, and a paper titled "Some Notes on the History of Soapnut, Soap and Washermen of India-between 300 BC and AD 1900" hints at even earlier roots.

Table 3. Vernacular names of *Sapindus mukorossi* Gaertn. in India and abroad

In India:		
Hindi	:	Phenil, Risht,Rishtak, Ritha, Dodan, Doadni, Doda, Kanmar
Manipuri	:	Hai Kya Kekru
Marathi	:	Phenil
Assamese	:	Haithaguti
Mizo	:	Hlingsi
Sanskrit	:	Urdhvashodhanah, Phenaka, Phenil, Rishtak, Rishtah, Rita, Sarishta, Hrishtah
Urdu	:	Phenil, Ritha
Telegu	:	Kunkudi
Outside India:		
English	:	Soapnut, Chinese Soap Berry, Wash Berry, Washnut
Chinese	:	Wuhuanzi
Nepali	:	Rittha
Italian	:	Uriya
Sherpa	:	Chhopra
Tamang	:	Gundarasi
Newari	:	Hathan
Tharu	:	Ritha

Source: IUCN,2004; Suhagia *et al*., 2011, Upadhyay and Singh, 2012, Hong *et al*., 2015.

Table 4. Synonyms of *Sapindus mukorossi L.*

***Sapindus mukorossi* L.,**	:	*Sapindus abruptus* Lour.,
	:	*Sapindus acuminatus* Wall.
	:	*Sapindus balicus* Radlk.
	:	*Sapindus boninensis* Tuyama,
	:	*Sapindus detergens* Roxb.,
	:	*Sapindus indicus* Poir.,
	:	*Sapindus utilis* Trab

This species is closely related to *Sapindus saponaria* (a species restricted to the Americas) and is treated as no more than a synonym of *Sapindus saponaria* by the Flora of China.

2.3. IUCN Status

The conservation status of an organism or a group of organisms (for instance, a species) indicates whether the species still exists and how likely it is to become extinct in the near future. *Sapindus mukorossi* falls in the category of Least Concern (IUCN 3.1) as per Plummer (2021).

2.4. Origin and Distribution

Sapindus mukorossi is a member of Sapindaceae family also known as soapberry family which comprises of 138 genera and 1,858 accepted species. *Sapindus mukorossi* is native to warm temperate to tropical regions of the world. It is the second most widespread species of *Sapindus* and is found in East and Southeast Asia (Table 5).

In East Asia, the plant is found in China, Japan, Korea, India, Nepal, Bhutan, Myanmar, Thailand, Cambodia, Vietnam as well as Southern China, and Taiwan. It prefers tropical and sub-tropical regions of the Indian Continent, where it can be found at elevations up to 1,500 metres. In India, this deciduous tree grows in the upper reaches of Indo-Gangetic plains, lower foothills and midhills of the Himalayas at an elevation of 200-1500 m. This plant is quite common in Shivaliks and the outer Himalayas of Uttar Pradesh, Uttranchal, Himachal Pradesh and Jammu and Kashmir. It is also native to western coastal Karnataka, Maharashtra and Goa in India. In Himachal Pradesh, it is found in Chamba, Kangra, Una, Hamirpur, Bilaspur, Solan, Sirmour, Mandi and Shimla districts.

Table5. Documented distribution of *Sapindus mukorossi* in different parts of the world and in different states of India

Country /Region	Distribution	Planted	Reference
China	Present		CABI (Undated)*
- Anhui	Present	Planted	Tang *et al.* (2016)
- Fujian	Present	Planted	CABI (Undated a)
- Guangdong	Present	Planted	CABI (Undated a)
- Guangxi	Present	Planted	CABI (Undated a)
- Guizhou	Present	Planted	CABI (Undated a)
- Hubei	Present		CABI (Undated a)
- Hunan	Present	Planted	CABI (Undated a)
- Jiangsu	Present		CABI (Undated a)
- Jiangxi	Present	Planted	CABI (Undated a)
- Sichuan	Present	Planted	CABI (Undated a)
- Yunnan	Present	Planted	CABI (Undated a)
- Zhejiang	Present	Planted	CABI (Undated a)
India	Present		CABI (Undated a)
- Assam	Present	Planted	CABI (Undated a)
- Himachal Pradesh	Present	Planted	CABI (Undated a)
- Nagaland	Present	Planted	CABI (Undated a)
- Sikkim	Present	Planted	CABI (Undated a)
- West Bengal	Present	Planted	CABI (Undated a)
Japan	Present		CABI (Undated)*
- Kyushu	Present		CABI (Undated a)
Laos	Present		CABI (Undated a)
Nepal	Present		CABI (Undated a)
North Korea	Present		CABI (Undated a)
South Korea	Present		CABI (Undated a)
Taiwan	Present	Planted	CABI (Undated a)
Vietnam	Present		CABI (Undated a)
- Socotra	Present Introduced First Reported 2001		Seebens *et al*. (2017)

*Present based on regional distribution

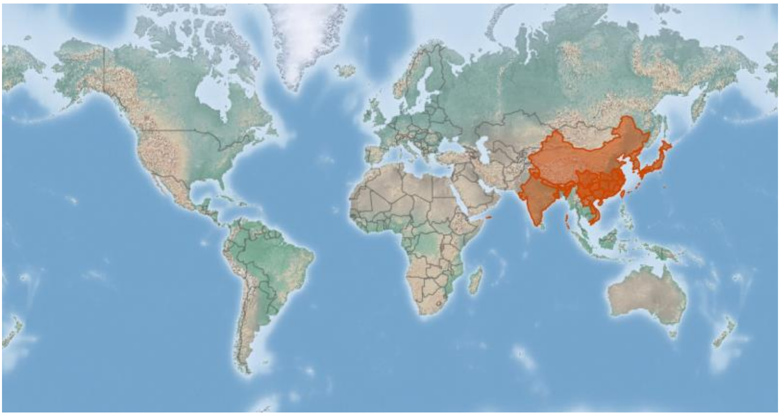
2.5. Latitude/Altitude Ranges

Latitude North (°N)	Latitude South (°S)	Altitude lower (m)	Altitude upper (m)
35	22	0	2000

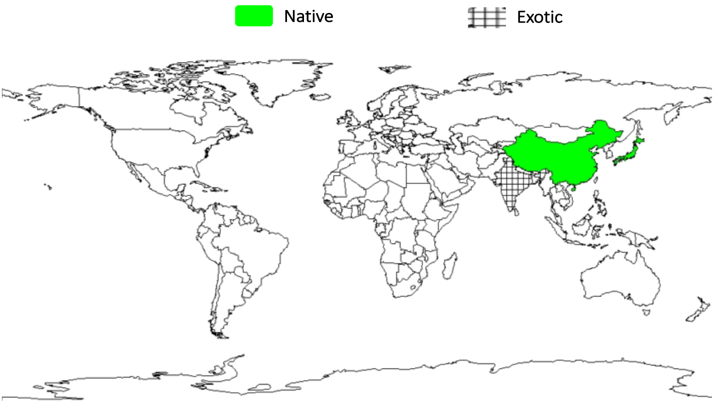
2.6. Documented species distribution

Native range : China, Japan,

Exotic range : India, Singapore



Source: https://doi.org/10.1079/cabicompendium.48326



Source: Agroforestry Database 4.0 (Orwa *et al*.,2009)

Fig 1. Documented species distribution of *Sapindus mukorossi*

2.7. Ecological niche

Niches are habitats with the minimum thresholds necessary for survival. The forest niche is strongly affected by the environment, and it changes or moves with environmental change. *Sapindus* is a biodiesel, biomedical, and multifunctional economic forest species Precipitation is the crucial factor to the habitats of *Sapindus*.

Sapindus mukorossi is a deciduous tree that grows in the lower foothills and midhills of the Himalayas at altitudes of up to 1,200 metres (4,000 ft). In India, it is also found in western coastal Karnataka, Maharashtra, and Goa. It is tolerant to reasonably poor soil, can be planted around farmers' homes. The tree is often cultivated in gardens, by temples and along roadsides in China and the Indian subcontinent, both as an ornament and also as a source of soap and as a medicine. In Indian Province of Nagaland, it is cultivated extensively for supplying raw material for small scale industries. This species has also been grown along agricultural fields for fuel, timber and medicinal requirements. It is found growing at open rocky places at elevations of 600 - 1,300 metres in Nepal This species is also native to Southern China and Taiwan as known by its many indigenous peoples. Significantly distinct ecological adaptations have been reported among *Sapindus mukorossi*, *Sapindus delavayi*, and *Sapindus rarak.*

2.8. Biophysical limitations

Though *Sapindus mukorossi* is a widely distributed economic forest genus of Sapindaceae family yet it has got few biophysical limitations such as:

- **Altitude:** Distribution is mostly confined to an altitude of upto 1500 m.
- **Mean annual temperature:** Tolerate a mean annual temperature of 15 to 25^{o} Celsius. It cannot grow in the shade and succeeds in a sunny position.
- **Mean annual rainfall:** It grows well in areas receiving a mean annual precipitation (rainfall) of 1750 mm
- **Soil type:** The tree prefers a deep, fertile, moist soil, but plants are very tolerant to wide range of soils, including those that are dry, stony or nutrient deficient. Grows well in light (sandy), medium (loamy) and heavy (clay) soils, prefers well-drained soil and can grow in nutritionally poor soil also.
- **Suitable pH:** mildly acidic, neutral and basic (mildly alkaline) soils.

2.9. Macroscopic characteristics

2.9.1. Tree height:

Sapindus mukorossi is a fairly large, handsome tree, green in summers and deciduous in winters with a straight trunk up to 12 meters in height, sometimes attaining a height of 20 m and a girth of 1.8 m.

2.9.2. Tree trunk, branches, bark and canopy:

Plant growth habits show vigorous growth and high rooting ability. It has an ability to coppice also. The annual shoot length varies between 41 and 59 cm, and shoot width is less than 14 mm. The bark of *ritha* is shining grey and fairly smooth when the plant is young. It is dark grey when the plant approaches maturity, with many vertical lines of lenticels and fine fissures exfoliating in irregular wood scales. The blaze is 0.8-1.3 cm, hard, not fibrous, pale orange brown, brittle and granular. The tree has a globose crown and rather fine leathery foliage.

2.9.3. Leaves:

Leaves are 30-50 cm long, alternate, paripinnate with 14-30 leaflets, the terminal leaflet often absent; common petiole very narrowly bordered, glabrous; leaflets 5-10 pairs, opposite or alternate, 5-18 by 2.5-5 cm, lanceolate, acuminate, entire, glabrous, often slightly falcate or oblique; petioles 2-5 m long (Fig 2).

2.9.4. Flowers:

The inflorescence is a large terminal panicle growing in a sequence of 1, 2, 3 side axes on the primary axis, 30 cm or more in length, with pubescent branches. Flowers are about 5 mm across, small, terminal, polygamous, greenish white, subsessile, numerous, mostly bisexual. Functional male flowers usually bloom before females although this sequence is sometimes reversed.

2.9.1. Fruits:

The fruit is green and smooth; bract 1 mm long, subulate, margin glandular., white spots appear on the pericarp surface before maturity. These are globose, fleshy, saponaceous and at maturity the fruit is an almost-round drupe, mostly 1-seeded and sometimes two drupes together, about 1.8-2.5 cm across. The pericarp becomes yellow-brown and develops a rough surface texture. During its development and maturation period, the fruit enlarges, the seed coat hardens, the fruit discolors and, along with several times of

Well matured tree of *Sapindus mukorossi* at fruiting stage (left) and shape of leaf (top centre), inflorescence(top right),flower(bottom centre) and fruit formation stage (bottom right)

Source: Singh and Sharma, 2019

Fig 2. Different macroscopic characteristics of *S. mukorossi*

physiological fruit drops. Fruits are inedible but have high values in the bioenergy industry, medicine, and domestic chemistry engineering.

2.9.2. Seeds:

Seeds are solitary, round nuts 2-2.5 cm in diameter (Fig 3), globose, smooth, black and loosely placed in dry fruit.

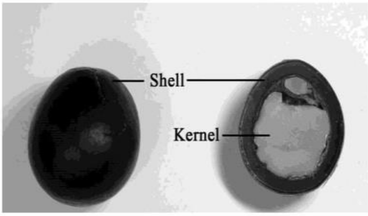


Fig 3. Seed structure

2.9.3. Roots:

Root is tap root and goes deep.

2.10. Reproductive biology

2.10.1. Phenology

Sapindus trees bloom at 4–5 years after seedlings have been planted. Flowering time is late spring/early summer (early summer, mid summer, late summer). The fruit appears in July-August and ripens by November-December. The leaves turn yellow in December before being shed in December-January (Table 6). The tree is leafless until March-April when the new leaves appear. The panicles of white or purplish bisexual flowers appear in May-June, with the green fruits ripening in October-November. These remain on the tree till January or later, the bunches of round brown or orange coloured fruits being conspicuous when the tree is leafless. 'Yue Shuo Bodhi' has been identified since the date of bud burst (1–3 March). The florescence is 25 ± 1 d (May–June). The fruit development period is 130 ± 8 d (June–October). The fruit matures in late October, and the defoliation stage occurs relatively late (December).

Table 6. Phenological stages of a typical *Sapindus mukorossi* tree.

Stage	Season/months	Typical characteristics
1st	December to January	Leaves turn yellow
2nd	January to March	Leafless tree
3rd	April	Growth and Flowering starts
4th	May	Fruit development
5th	June-July	Fruit maturity
6th	October-November	Fruit colour changes

The phenological growth stages (eight principal growth stages) of *S. mukorissi* have also been described (Fig 4) using the BBCH scale (Biologische Bundesanstalt, undessortenamt und CHemische Industrie) (Table 7).

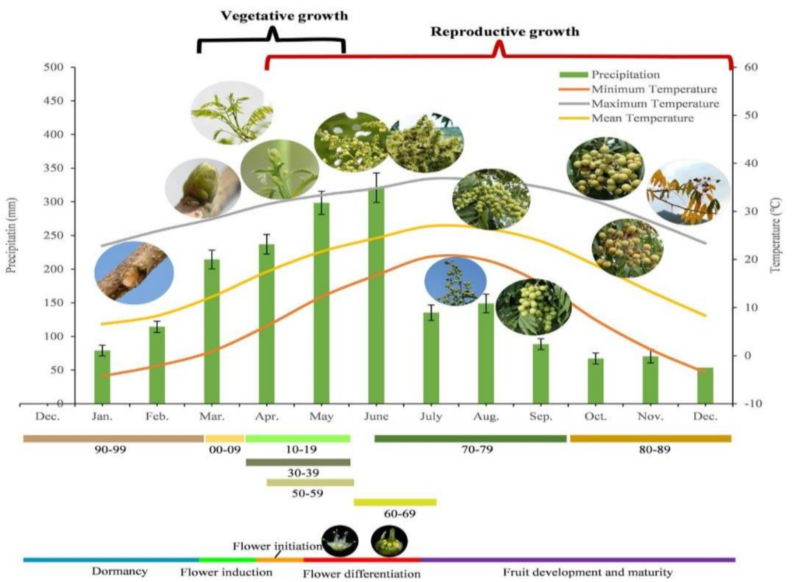
Table 7. Phenological growth stages of *S. mukorossi* according to the BBCH scale.

BBCH Code	Description
Principal growth Stage 0: Bud development	
00	Bud Dormancy
01	Beginning of bud swelling
03	End of bud swelling
05	First green bud scale visible
07	Beginning of bud burst
09	End of bud burst
Principal growth stage 1: Leaf development	

10	First compound leaves separate
11	First leaves unfold and petiole elongate
12	More leaves unfold: First compound leaves at 20% of their final area
13	More leaves unfold: First compound leaves at 30% of their final area
14	More leaves unfold: First compound leaves at 40% of their final area
16	More leaves unfold: First compound leaves at 60% of their final area
19	All leaves unfold and petiole elongates to final size
Principal growth stage 3: Shoot development	
30	Beginning of shoot elongation: Shoot axis visible
31	10% of final shoot length
32	20% of final shoot length
33	30% of final shoot length
34	40% of final shoot length
35	50% of final shoot length
36	60% of final shoot length
38	80% of final shoot length
39	90% or more of final shoot length
Principal Growth stage 5: Inflorescence emergence	
50	Beginning of reproductive bud swelling
51	End of bud swelling and bud burst
52	Primary axis elongation
53	First side axes elongate about 30% of their final length
54	First side axes elongate about 40% of their final length
56	First side axes elongate about 60% of their final length
57	First side axes elongate about 70% of their final length
58	First side axes elongate about 80% of their final length
59	First side axes elongate about 90% or more of their final length
Principal Growth stage 6: Flowering	
59	First flowers bloom
60	Beginning of flowering
63	Early flowering: 30% of flowers bloom
65	Full flowering
69	End of flowering
Principal Growth stage 7: Fruit development	
70	No ovary development
71	Early ovary growing
72	20% of the biggest fruit size
75	50% of the biggest fruit size
76	60% of the biggest fruit size
77	70% of the biggest fruit size; the second physiological fruit drop occurs

78	80% of the biggest fruit size
79	90% of the biggest fruit size
Principal growth stage 8: Maturity of fruit and seed	
81	Beginning of maturity
82	Pericarp becomes wrinkled
85	Pericarp turns a little golden yellow and transparent
87	Advanced maturity
89	Fruits are fully developed and mature
Principal growth stage 9 : Senescence and beginning of dormancy	
89	Shoots and leaves stop development
90	Beginning of leaf discoloration
91	Leaves begin to fall
92	Most leaves turn yellow: 30% leaves fall
93	40% leaves fall
94	50% leaves fall
97	All leaves fall
99	Dormancy

Source : Zhao *et al.*, 2019



Time elapsedin each stage (horizontal bars), 50 years' average data of precipitation and mean, maximum, and minimum temperatures.

Source: Zhao *et al.*, 2019

Fig 4. Main phenological growth stages of *S. mukorossi* according to the BBCH scale.

2.10.2. Flower structure

Each inflorescence contains small functional male and female flowers, which are light yellow to white—and each has five calyxes, five petals, eight stamens, and an ovary comprising 3–5 carpels. Functional male flowers usually bloom before females, although this sequence is sometimes reversed. Development is arrested in one or two carpels in each flower and in most cases only a single carpel develops a fruit after fertilization

- **Calyx:** Sepals 5, unequal, about 2 mm long, elliptic-oblong to ovate, margin ciliate, glandular at the tip
- **Corolla:** Petals 5, 3 mm long, lanceolate-ovate, clawed, ciliate, with 2 woolly scales on the claw. Disc 5-ridged, glabrous
- **Androecium:** Stamens 8, free; filament about 3 mm long, lower half pubescent; anthers 0.5 mm long, oblong, non-functional in the female.
- **Gynoecium:** Ovary sessile, 2–4 mm long, obovate, 3-locular, glabrous, rudimentary in the male flower; stigma 3-lobed.

2.10.3. Pollination biology:

The flowers are mainly pollinated by bees, flies and butterflies (Fig 5).

Source: Zhao *et al.*, 2019

Fig 5. Pollination by bee & butterfly

2.11. Cytogenetics

Sapindus mukorossi is an autodiploid with **2n = 2x = 28** chromosomes based on the results obtained for the molecular karyotype and K-mer analysis, differing significantly from the chromosome numbers of previously reported Sapindaceae species

3. Variants of *Sapindus mukorossi*

Sapindus mukorossi is widely distributed in warm temperate to tropical regions in Asia, especially China and Southeast Asian countries. Because of the high yield of its seed oil (26.15% to 44.69%) (Liu *et al.*, 2017; Sun *et al.*, 2017), it is suitable for biodiesel production according to American Society for Testing and Materials (ASTM) D6751 and European Standards (EN) 14214 (Chakraborty and Baruah, 2013; Sun *et al.*, 2019). Crude extracts from the fruit pericarps of *S. mukorossi* are rich in triterpenoid saponins (4.14% to 27.04%) and sesquiterpenoids (Liu *et al.*, 2017; Liu *et al.*, 2019), which exhibit excellent surface activity as well as antibacterial, elution, pharmacological, and physiological effects (Xu *et al.*, 2018). Therefore, *S. mukorossi* is an ideal tree species for developing forestry bioenergy and multiple other products.

'Yue Shuo Bodhi' was released as a new *S. mukorossi* cultivar in China on 21 Dec. 2020. Yue Shuo Bodhi' is a clone of elite germplasm with high yield and high quality that comes from Meilinghe Forest Farm, Guanyu Town, Deqing City, Guangdong Province in China. Field investigation, collection, and evaluation of *Sapindus* germplasms worldwide were undertaken from 2014 to 2020, in 16 provinces in the south of China that represent the natural distribution of *Sapindus* (Liu *et al.*, 2021a).

Based on the clonal test and raw material forest cultivation, 'Yue Shuo Bodhi' has been found to be productive and performing well. This variety is a strong, light-loving, deep-rooted, drought-tolerant tree species, but it is not resistant to excessive humidity. It is suitable for cultivation in fertile, well-drained, acidic or slightly alkaline sandy soil. It is suggested to popularize and cultivate in subtropical areas such as Fujian Province and Guangdong Province. The cultivar produces larger fruit with good fruit quality that is consumed extensively, especially by biochemistry and biodiesel companies.

'Yue Shuo Bodhi' has been identified since the date of bud burst (1–3 March). The florescence is 25 ± 1 d (May–June). The fruit development period is 130 ± 8 d (June–October). The fruit matures in late October, and the defoliation stage occurs relatively late (December) (Fig 6).

(A) Leaf extension stage in March. (B) Inflorescence emergence stage in April. (C) Flowering stage in May and June. (D) Primary bearing period in June. (E) Fruit expanding stage in July. (F) Fruit expanding stage in August. (G) Fruit expanding stage in September. (H) Full bearing period in October. (I) Frutescence in November.

Source: Liu *et al*., 2021a

Fig 6. Phenology of 'Yue shuo Bodhi' cultivar.

3.1. Tree and leaf characteristics

'Yue Shuo Bodhi' is a deciduous tree with an umbrella crown. Plant growth habits include a spreading cultivar with trees 3 to 4 m high and 5 to 6 m wide that show vigorous growth and high rooting ability. The annual shoot length varies between 41 and 59 cm, and shoot width is less than 14 mm. The winter buds are grayish brown and ovoid, and the outside is smooth and glabrous. Leaves are even-pinnately compound, opposite or subopposite, with six to eight pairs of leaflets. Leaflets are strip-lanceolate, tip acuminate, base cuneate, and slightly asymmetric, with an entire leaf margin. Mature leaflets are dark green, smooth on both sides, and glabrous. The inflorescence is terminal and actinomorphic. There are five petals that are light yellow and oval. The infructescence is short, the fruit is densely distributed, and the posture of the frutescence is erect or suberect.

3.2. Fruit characteristics

Fruit yield and the quality of high-value products are crucial to the *Sapindus* industry. The fruit color of 'Yue Shuo Bodhi' is yellow, the shape of the fruit is nearly spherical, and the fruit is large (Fig. 7). The fruit horizontal, vertical, and lateral diameters are 25.25, 22.12, and 20.23 mm, respectively. The 100-fruit weight and 100-fruit pericarp weight are 454.0 ± 10.22 and 279.72 ± 5.19 g, respectively, which are 164% and 171% heavier than those of the local cultivated *Sapindus* fruits. Pericarp thickness and pericarp saponin content are important traits that indicate the production capacity of saponin. The pericarp thickness and pericarp saponin content of 'Yue Shuo Bodhi' are 2.15 ± 0.36 mm and 13.76 ± 0.67%, respectively. The seed is spherical and black, with horizontal, vertical, and lateral diameters of 14.40, 15.15, and 12.32 mm, respectively. The 100-seed weight is 172.86 ± 4.39 g,

Source: Liu *et al.*, 2021

Fig 7. 'Yue Shuo Bodhi' (top) and local cultivated *Sapindus*

which is 153% heavier than that of the local cultivated *Sapindus*. The 100-seed kernel weight and seed kernel oil content are important traits that indicate the production capacity of oil. The 100-seed kernel weight and seed kernel oil content of 'Yue Shuo Bodhi' are 2.15 ± 0.36 mm and 34.50 ± 0.91%, respectively. 'Yue Shuo Bodhi' can blossom, yielding fruit within 1 to 2 years after grafting. The yield per plant of the 5- to 6-year-old grafted seedlings is 5 to 10 kg. This plant shows strong high-yield habits during successive years.

3.3. Availability

'Yue Shuo Bodhi' is protected by the China protection of new varieties of plants (CNPVP; registration number 20200312). Propagating materials are available at the *Sapindus* germplasm resources nursery in Jianning County, Sanming City and Fujian Province.

4. Ethnobotany & Ethnomedicinal Relevance

The basic aim of ethnobotany is to undertake the scientific investigation of plants used by primitive societies and to explore how these plants are used as food, clothing, shelter, fodder, fuel, furniture and medicines. In other words ,it implies the study of indigenous or traditional knowledge of plants. *Sapindus mukorossi* is an extremely valuable medicinal plant, distributed in tropical and sub-tropical regions of Asia .

Sapindus mukorossi is well known for its folk medicinal values (Sharma *et al.*, 2011). The use of *Sapindus mukorossi* in folk medicine worldwide is validated by scientific studies that have demonstrated the efficacy of the extracts in various experimental models. Documentation of ethno-medicinal information have substantial role in illuminating folk knowledge, which facilitates the discovery of modern allopathic drugs. Some of the major ethnobotanical and ethnomedicinal applications of different parts of *Sapindus mukorossi* are summarized below

4.1. Fruit

It is a psycho-medicinal plant. Pericarp of *S. mukorossi* fruit in Japan is known as "enmei-h" means life prolonging pericarp, whereas in China it is called "Wu-humnan-zi", the "non illness fruit".

- Fruits (Fig 8) are used against a number of common health problems, such as excessive salivation, pimples, chlorosis, migranes, eczema and psoriasis and as contraceptive. The lather is also used as a soap to wash the hair and rid it of lice (Kirtikar and Basu, 1991).
- The fruits of *S. mukorossi* are also used in treatment

Fig 8. Fruit, seeds & pericarp

of snake bite, scorpion stings and dandruff. (Burlakoti and Kuwar, 2008; Kunwar *et al*., 2019)

- The fruit and seeds are regarded as a cure for epilepsy in northern India.
- A decoction of the fruit is used as an expectorant (Acharya, 2012)
- A lather of the fruit is used to treat burns.
- In Indian state of Assam, fruit paste of *S. mukorossi* is used as febrifuge.
- Juice of pericarp is poured inside the ostium during the early stages of pregnancy as an abortifacient (Bhattarai, 1993).
- Soapy water prepared from the fruits is poured inside the vagina during labor pain to accelerate as well as to facilitate parturition (Bhattarai, 1994).
- Fruit juice applied in earache (Uprety *et al*., 2011).
- Fruits are used in pained parts (Thapamagar and Neupane, 2016). Fruits are also used to wash wounds.
- The fruit of *Sapindus mukorossi* are utilized by Indian jewellers for restoring the brightness of tarnished ornaments made of gold, silver and other precious metals (Singh *et al*.,2010).
- According to the book, "The Healing Power of Gemstones," jewellers used to clean delicate pearls in a mixture of water and soapnuts.
- The fruit is rich in saponins and is used as a soap substitute. It is squeezed, mixed with water, then used to clean clothes etc. Particularly useful for natural fibres and delicate materials. That perhaps is the reason that some scientists have given the species the alternate as *Sapindus detergens* also. A few businesses in India offer ready-made soapberry products: Krya is one example of eco-friendly organization in Chennai dedicated to selling all-natural laundry detergent made from soap nuts (Fig 9).

- It is considered a valuable cleaning agent. According to the book, "Life Science: Vol. 2," the photo film industry uses 6 tonnes of the fruit every year.

Fig 9. Krya laundry detergent

- *Sapindus mukorossi* fruit pericarp can be used as a selective eradicant for horny fish like *Heteropneustes fossilis* and *Channa punctata*. Hunting tribes have traditionally put large quantities of them in streams, lakes etc in order to stupefy or kill the fish
- Fruits are useful in snake bite, scorpion sting and dermatological problems (Burlakoti and Kuwar, 2008; Kunwar *et al*., 2019).

4.2. Seeds

The seeds of *Sapindus mukorossi* are used in Ayurvedic medicine to remove tan and freckles from the skin.

- It cleanses the skin of oily secretion. The application of *S. mukorossi* (Wu Huan Zi in Chinese) seed kernel for antimicrobial and skin care is recorded in China's traditional pharmaceutical book, Compendium of Materia Medica (Bencao Gangmu in Mandarin) some 500 years ago.
- The powdered seeds are employed in the treatment of dental caries, arthritis, common colds, constipation and nausea. (Gairola *et al*., 2014; Joshi *et al*., 2010).
- Seeds (Fig 10) are used to treat respiratory and urinary system disorders, to treat diphtheria, vesicula seminalis disease, and frequent urination (Zhao *et al*., 2019).
- The decoction of seed is taken orally for tuberculosis and pertussis cough by the Maonan people in China (Hong *et al*., 2015).

- The seed oil of *S. mukorossi* is found to be effective in healing skin wounds . The study done by Chen *et al*. (2020) on seed oil extract of *S. mukorossi* confirms antibacterial, anti-inflammatory, antioxidant, cell proliferation, and skin wound healing properties.

Fig 10. Soapnut powder

- Moreover, seed oil possesses anti-inflammatory and antimicrobial activities (Upadhyay and Singh, 2012).

4.3. Leaves

- The leaves are used in baths to relieve joint pains (Upadhyay and Singh, 2012)
- Mostly fruit extract and leaf extract are used to treat diseases (Singh and Kumari, 2015).

4.4. Roots

- Root and fruit is used to remove heat and phlegm, check diarrhoea, and blood stasis (Zhao *et al.*, 2019).
- Roots are used in the treatment of gout and rheumatism.

5. Phytochemistry and Pharmacological Importance

From ancient times, botanicals are used as main medicinal sources to treat the various health problems. Medicine systems of Ayurveda, Unani and Chinese are rich sources of plant derived products. In developing countries, still today plant based medicines are frequently used in the treatment of various diseases. Eighty percent world population in the poor countries uses herbal medicine in primary health care (Vines, 2004). People, in different parts of the world, have trust on traditional plant based medicines, particularly in Africa, part of Asia and Central South America (Allkin, 2017). Secondary plant metabolites are a rich source of many substances that manifest biological activities . Contemporary economic development places particular emphasis on pro-ecological activities, including the preference for technological solutions based on natural, renewable material sources, especially using plant sources for this purpose (Khan, 2018; Kharissova *et.al.*, 2019; Hojnik *et al.*, 2019).

Sapindus mukorossi Gaertn, distributed in tropical and sub-tropical region of Asian continent, is an important medicinal plant. The major phytochemicals isolated and identified from the different parts of the *S. mukorossi* are triterpenoids, saponins of oleanane, dammaranes, tirucullanes. Phytochemicals such as flavonoids, carbohydrates, fatty acids, phenols and fixed oils are also found in fruit, seed and leaves of *S. mukorossi*. All these phytochemicals, found in different parts of *S. mukorossi*, are potential sources of different medicines. Its medicinal properties are based on traditional experiences passed from generation to generation. Scientists from various disciplines are now working on different aspects of medicinal properties of *S. mukorossi*. They have reported that crude as well as isolated phytochemicals possess pesticidal, herbicidal, piscicidal, anti-microbial, anti-cancer and free radical scavenging, skin wound healing, detergent, anti-gonorrheal, spermicidal, anti-platelet aggregation, anti- diabetic, anti-melanogenesis, neuritogenic, hepatoprotective, anti-inflammatory, anti- asthmatic, anti-protozonal, anti-lipid peroxidation activity. Recently, role of *S. mukorossi* on dental pulp mesenchymal stem cells, as renewable bio-surfactants of cotton fabrics and its role in nanoparticle synthesis of gold, silver and platinum are reported by different researchers.

5.1. Major chemical phytoconstituents

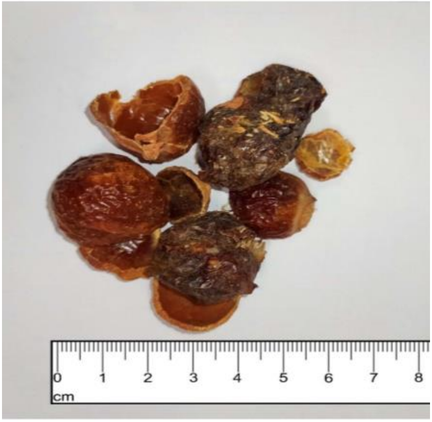
The major constituents of *Sapindus mukorossi* fruit are saponins (10%-11.5%), sugars (10%) and mucilage (Francis *et al.*, 2002). Saponins are secondary plant metabolites with divergent biological activities. *Sapindus* saponins are a mixture of six sapindosides (sapindosides A, B, C, D and mukorozi saponins (E1 and Y1), with sapindoside B as one of the major constituents, isolated by n-butanol extraction of the ethanolic extract of fruit pericarp of *Sapindus mukorossi* and identified by liquid chromatography and mass spectroscopy (Saxena *et al.*, 2004). Different types of triterpene, saponins of oleanane, dammarane and tirucullane type have been isolated from the galls, fruits and roots of *Sapindus mukorossi* (Table 8). Oleanane type triterpenoid saponins named Sapindoside A&B have been reported from the fruits of *Sapindus mukorossi* (Chirva *et al.*, 1970). Sapindoside C, Sapindoside D, which is a hexaoside of hederagenin, and Sapindoside E, a nonaoside of hederagenin, have been isolated and identified from the methanolic extract of the fruits of *Sapindus mukorossi*. Seeds of *Sapindus mukorossi* contain 23% oil of which 92 % is triglycerides; the triglyceride fraction contain 30 % oleo-palmito-arachidin glyceride, 13.3 % oleo-diarachidin glyceride and 56.7 % di-olein type glycerides such as dioleo-palmitin, dioleo-stearin and dioleo-arachidin. The non-glyceridic component of the seed oil is a cyanolipid (1-cyano-2-hydroxymethyl prop-1-ene-3-ol).

Table 8. Major phytochemical compounds present in different plant parts of *Sapindus mukorossi*

S. No.	Chemical constituent	Part of the plant
1	Triglyceride Oleo-palmito-arachidin glyceride Oleo-di-arachidin glyceride Di-olein	Seed
2	Lipid	Seed
3	Sesquiterpeneoidal gtlycosides	Fruits
4	Flavanoids Quercetin, Apigenin, Kaempferol, Rutin	Leaf
5	Saponin Triterpene Oleanane (sapindosideA & B) Dammarane(sapinmusaponin A-E) tricullane (sapinmusaponin F-K)	Gall, fruit & root Fruit gall gall & root

Source:Anjali et al., 2018

Fruits of *Sapindus mukorossi* are reported to contain sesquiterpenoidal glycosides and six different fatty ester of tetracyclic triterpenoids . Its leaf extract contains different type of flavanoids like quercetin, apigenin, kaempferol and rutin. Various types of triterpene, saponins of oleanane, dammarane and tirucullane type have been isolated from the galls, fruits and roots of *Sapindus mukorossi*. Oleanane type triterpenoid saponins named Sapindoside A&B have been reported from the fruits of *Sapindus mukorossi* . Sapindoside C, Sapindoside D, which is a hexaoside of hederagenin, and Sapindoside E, a nonaoside of hederagenin, have also been isolated and identified from the methanolic extract of the fruits of *Sapindus mukorossi* (Fig11). Dammarane-type saponins, named Sapinmusaponins A & B , C-E, together with three known phenylpropanoid glycosides, have also been isolated from the galls of *Sapindus mukorossi* (Sochacki and Vogt, 2022)



Source: Sochacki and Vogt, 2022

Fig 11. Commercially available, dry washnut pericarps.

5.2. Major pharmaceutical properties

The biological activity of saponins is linked to their role in plant organisms . Although the role of saponins is not fully understood, it can be indicated that their function in plants is primarily protective (Lorent *et al*., 2014). The major pharmaceutical activities of *S.mukorossi* are listed in Table 9.

Table 9. Major pharmaceutical activities of different parts of *Sapindus mukorossi*

S. No	Activity	Methods used	Part used	References
1	Anti-Bacterial activity	Ethanolic and chloroform extracts	Leaf	Singh and Ali, 2019

2	Spermicidal Activity	Saponins	Fruit pericarp	Saxena *et al.*, 2004; Shiau *et al.*, 2009
3	Anti-Trichomonas Activity	Mixing of *Sapindus* and saponin		Garg *et al.*,1993
4	Insecticidal Activity	Ethanolic extract		Rahman *et al.*,2007
5	Anxiolytic Activity	Methanolic extract		Chakraborty *et al.*, 2010
6	Anticancer Activity	Saponin from galls extracts	Galls	Man *et al.*, 2010
7	Hepatoprotective Activity	Fruit pericarp extract	Fruit	Ibrahim *et al.*, 2008
8	Molluscicidal Activity	Extract	Fruit	Upadhyay and Singh, 2011
9	Piscicidal Activity		Fruit Pericarp	Virdi,1982
10	Fungicidal Activity	Crude extract	Pericarp	Tsuzuki *et al.*, 2007
11	Anti-Inflammatory Activity	Crude extract / isolated saponin and hederagenin	Plant	Takagi *et al.*, 1980
12	Anti-Platelet Aggregation Activity	Isolation of compounds	Gall	Ni *et al.*, 2004
13	Tyrosinase Inhibition and Free Radical Scavenging	Methanolic extract	Seed	Chen *et al.*,2010
14	Antidiabetic and Anti-hyper-lipidaemic Activity	Hydroalcoholic extract	Fruits	Verma *et al.*, 2012
15	Anti-oxidant Activity	Response Surface Methodology (RSM).	Stem Bark Fruit extract	Shah *et al.*, 2017 Chen *et al.*, 2020

5.2.1. Anti-bacterial activity:

Ethanolic and chloroform extracts of *Sapindus mukorossi* have been reported to inhibit the growth of *Helicobacter pylori* (both sensitive and resistant), at very low concentrations, when given orally for seven days to male wister rats. In the *in vitro* study, the isolates show a considerable zone of inhibition at very low concentration (10 µg/mL) and in the *in vivo* study the *Helicobacter pylori* infection was found to be clear with minimal dose extracts of 2.5 mg/mL (Singh and Ali, 2019).

5.2.2. Spermicidal activity:

Saponins from *Sapindus mukorossi* are known to be spermicidal. (Garg *et al.*, 1993, Rastogi, 1999). Morphological changes in human ejaculated spermatozoa after exposure to this saponin were evaluated under scanning electron microscopy. The minimum effective concentration (0.05% in spot test) did not affect the surface topography after exposure for one minute. However, incubation of spermatozoa for 10 minutes resulted in extensive vesiculation and a disruption of the plasma membrane in the head region. Higher concentrations (0.1%, 1.25%, 2.5% and 5.0%) caused more or less similar changes which included vesiculation, vacuolation, disruption or erosion of membranes in the head region. These findings suggest that the morphological changes observed are due to alterations in the glycoproteins associated with the lipid bilayer of the plasma membrane of spermatozoa (Dhar *et al.*, 1989). This spermicidal property has been used in contraceptive cream (Dwivedi *et al.*, 1990).

5.2.3. Anti-*Trichomonas* activity:

Sapindus saponin mixture has been found to show anti-Trichomonas activity at a 10-fold lower concentration (0.005%) than its minimal effective spermicidal concentration against human spermatozoa (0.05%) (Garg *et al.*,1993) Saponins produced no adverse effect on host cells in the mitochondrial reduction potential measurement assay. Saponin disrupts the actin cytoskeleton network beneath the cell membrane and affects membrane-mediated adherence of Trichomonas to the host cells.

5.2.4. Insecticidal activity:

The powdered seeds have insecticidal properties due to saponins present in it (Rahman *et al.*, 2007). They have been reported to cause mortality and/or growth inhibition in the insects tested, the cotton leaf worm *Spodoptera littoralis* caterpillars and the pea aphid *Acyrthosiphon pisum*. In the experiments with *Acyrthosiphon pisum*, 0.1% saponin killed all aphids, whereas with *Spodoptera* some caterpillars were still able to develop into apparently normal adults on food containing 7% saponin. Saponins can be employed as novel natural tactics in integrated pest management (IPM) to control pest insects, which fit in modern agriculture and horticulture (Geyter *et al.*,2007). Ethanolic extract of *Sapindus mukorossi* has also been found to have repelling and insecticidal activity against *Sitophilus oryzae* and *Pediculus humanus*. It is also used as an emulsifier in insecticides.

5.2.5. Anxiolytic activity:

Methanolic extracts of *Sapindus mukorossi* (200 and 40 mg/L) have been reported to have significant anxiolytic activity as compared to standard anxiolytics Diazepam (2 mg/Kg) and Fluoxetine (10 mg/Kg) (Chakraborty *et al.*, 2010).

5.2.6. Anti-cancerous activity:

Due to the great variability in saponin structure, saponins always display anti-tumorigenic effect through varieties of anti-tumor pathways. They have been found to be beneficial in the inhibition of tumor angiogenesis by suppressing its inducer in the endothelial cells of blood vessels, and then in the prevention of adhering, invasion and metastasis of tumor (Man *et al.*, 2010).

5.2.7. Hepatoprotective activity:

Extracts of *Sapindus mukorossi* have been reported to have a protective capacity both *in vitro* on primary hepatocytes cultures and in *in vivo* in a rat model of CCl_4 mediated liver injury as judged from serum marker enzyme activities (Ibrahim *et al.*, 2008).

5.2.8. Molluscicidal activity:

Extracts of *Sapindus mukorossi* have also been reported to show molluscicidal effect against the golden apple snail, *Pomacea canaliculate* Lamarck. (Ampullariidae) with LC_{50} values of 85, 22 and 17 ppm at 24, 48 and 72h exposure period, respectively. It has been reported that *Sapindus mukorossi* fruit pericarp is a potential source of botanical molluscicides against *Lymnaea acuminata.* These snails are the intermediate host of liver fluke *Fasciola gigantica*, which causes 94% fascioliasis in the buffalo population of northern India. The active molluscicidal component of *Sapindus mukorossi* fruit is soluble in chloroform, ether, acetone and ethanol (Upadhyay and Singh, 2011).

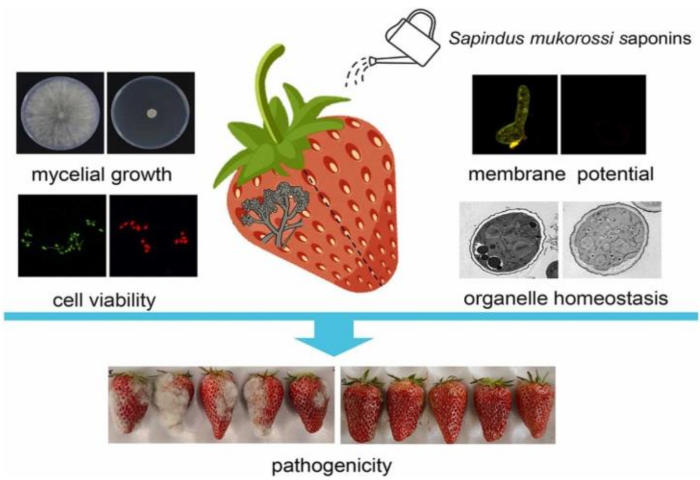
5.2.9. Piscicidal activity:

Effects of *Sapindus mukorossi* have been studied on fish. Pericarp of *Sapindus mukorossi* is the most toxic part yielding 100% mortality within 12 hours and mean survival time was found to be 1.18 hours.

LD10, LD50, LD100 ranging between 3.5 ppm and 10 ppm at 48 hrs and possess high potential for fish eradication. *Sapindus mukorossi* fruit pericarp can be used as a selective eradicant for horny fish like *Heteropneustes fossilis* and *Channa punctata* (Virdi, 1982).

5.2.10. Fungicidal activity:

The crude extract of *Sapindus mukorossi* is also reported to exhibit a strong growth inhibition against the pathogenic yeast *Candida albicans*, which causes cutaneous candidiasis. Extracts from the dried pericarp of fruits were investigated for their antifungal activity against clinical isolates of yeasts *Candida albicans* and *Candida non-albicans* from vaginal secretions of women with Vulvovaginal Candidiasis (Tsuzuki *et al*., 2007)



Source: Huang *et al*., 2023

Fig 12. Fungicidal effect of *S. mukorossi* saponins on gray mold of strawberry

In an another study by Huang *et al*. (2023), it was found that *Sapindus mukorossi* saponins inhibited in vitro mycelial growth of *B. cinerea* and gray mold on strawberry fruit in a dose-dependent manner (Fig 12). This inhibitory effect was attributed to the impairment in membrane potential and integrity, induction of autophagic-like vacuoles, and disturbance in organellar homeostasis of *B. cinerea*, as revealed by fluorescence labeling and ultrastructural observations. Moreover, *S. mukorossi* saponins damaged normal structures of mitochondria and induced oxidative bursts in the cytoplasm, thereby contributing to the abolished cell vitality. These results suggest that *S. mukorossi* saponins may be utilized as safe fungicide alternatives to control gray mold on postharvest fruit.

5.2.11. Anti-inflammatory activity

It has been reported that crude saponin and hederagenin isolated from *Sapindus mukorossi* inhibited the development of carrageen-induced edema in the rat hind paw as well as on granuloma and exudates formations induced by croton oil in rats. Anti-inflammatory activity on carrageenin edema was observed after intraperitoneal and oral administration of crude saponin, whilst hederagenin and the other agents showed activity only when administered (Takagi *et al.*, 1980).

5.2.12. Anti-platelet aggregation activity:

Five new tirucallane type saponins, sapinmusa saponins isolated from the galls of *Sapindus mukorossi,* were found to show moderate activity in a 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation assay (Ni *et al.*, 2004).

5.2.13. Tyrosinase inhibition and free radical scavenging:

Extracts of *Sapindus mukorossi* seeds have been found to show tyrosinase inhibition and free radical scavenging properties. They show strong specific inhibition activities on the proliferation of human melanoma and lung cell lines and thus hold a very high potential of applying *Sapindus mukorossi* extracts in medical cosmetology, food supplementation, antibiotics and chemotherapy (Chen *et al.*, 2010)

5.2.14. Antidiabetic and Anti-hyperlipidaemic Activity :

Antihyperglycaemic and anti-hyperlipidaemic activity has been reported from the hydroalcoholic extract of *S. mukorossi* fruits (Verma *et al.*, 2012). Streptozotocin was used to induce the diabetic condition in Wistar albino male rats. Extracts were given through oral administration to treat the diabetes. The diabetic rats were treated for 20 days from the extracts with 250 mg/kg and 500 mg/kg and glybenclamide as standard. The daily administration of this extract significantly decreased the blood glucose level and lipid level. This extract decreased the elevated glucose level in diabetic animals.

5.2.15. Antioxidant activity:

Natural antioxidant properties are highly valued due to the damaging role of free radicals on health and the quality of everyday products (Chen *et al.*, 2010). Extracts from *S. mukorossi* exhibit antioxidant properties. Shah *et al.*, 2017 performed antioxidant assays of the stem bark extract. In the studies of the Chen *et al.* (2020), the antioxidant effect of saponin extract obtained from washnut fruits was presented. The best percentage result of the DPPH radical sweeping activity was 91.56%, obtained by the process optimization using Response Surface Methodology (RSM).

5.2.16. Surfactant Activity

Saponins are characterized by surfactant properties that allow them to lower the surface tension of aqueous solutions (Rai *et al.*, 2021). This feature is a direct result of the saponin structure, since they consist of two parts with different solubility in water, which in effect form an amphiphilic molecule (Goral and Wojciechowski, 2020; Aziz *et al.*, 2019) . Saponins in aqueous solutions assume the form of monomers, adsorbing at the water-air interface (Lorent *et al.*, 2014; Penfold *et al.*, 2018) , and after reaching critical micelle concentration (CMC), they begin to aggregate into micelles (Myers, 2020). Saponins exhibit many functional properties, such as foam formation (Meshram *et al.*, 2021), as well as wetting (Yekeen *et al.*, 2020), emulsifying (Yang *et al.*, 2010) , solubilizing (Samal *et al.*, 2017) , adsorptive (Bottcher and Drusch, 2017) and detergent properties (Pradhan and Bhattacharyya, 2017).

5.3. Safety and Toxicity assessment

Although poisonous, saponins are poorly absorbed by the human body and so most pass through without harm. Saponins are quite bitter and can be found in many common foods such as some beans. They can be removed by carefully leaching in running water. Thorough cooking, and perhaps changing the cooking water once, will also normally remove most of them. However, it is not advisable to eat large quantities of raw foods that contain saponins. Saponins are much more toxic to some creatures, such as fish, and hunting tribes have traditionally put large quantities of them in streams, lakes etc in order to stupefy or kill the fish.

Wei *et al*. (2021) evaluated the toxicity of saponins present in the F4 fraction extracted from the washnut fruit by a multi-step purification process. A toxicity assay was performed on three organisms using the T.E.S.T. software. Acute toxicity (oral LD_{50}on rats, LC_{50} on fathead minnow after 96 h, and LC_{50} on *Daphnia magna* after 48 h), mutagenicity, developmental toxicity, and bioaccumulation factors of compounds present in the F4 fraction was evaluated. The compounds showed no harm to rats for oral LD_{50} between 115.58 and 238.76 mg/kg, indicating that the same substances exhibit different toxicity to different organisms. The reason for the high toxicity of aquatic organisms tested may be due to the piscicidal activity of *S. mukorossi* (Upadhyay and Singh, 2012).

Another evaluation of the toxicity of saponins was undertaken by the team of Du *et al*., 2015. The saponins were extracted from the pulp of *S. mukorossi* in 85% ethanol, followed by washing with chloroform and ethyl acetate. The dry matter was re-dissolved in 85% ethanol and centrifuged. The separated supernatant was used as the saponin extract. Toxicity evaluation was carried out against SPF Wistar rats. Acute oral toxicity study showed that LD_{50} of soapnut saponins after administration was equal to 9260 mg/kg and 7940 mg/kg for female and male rats, respectively. An acute dermal toxicity study showed that the LD_{50} of saponins is greater than 5000 mg/kg in both male and female Wistar rats. A dermal irritation test indicated an average irritation score per day of each rabbit equal to zero after 14 days of continuous irritation. On the basis of the results and the cosmetic toxicity classification standard, it was concluded that saponins from *S. mukorossi* are safe for use in cosmetics. A skin irritation study of saponins was also conducted by Wei *et al*., 2021. Two fractions of F4 and SWF extracted from *S. mukorossi* fruit by the multi-step purification were tested with a 4-hr human patch test. Among the 30 subjects tested, a concentration of 25 mg/mL of the F4 fraction and SWF did not cause swelling or allergic reaction in any of the test subjects. Thus, the toxicity of the saponins should not be ignored and further toxicological evaluation is required.

6. Propagation techniques

As the domestication and large scale cultivation of *S.mukorossi* is being started, the demand of quality seedling in proper planting time is very high. Low, delayed and uneven germination have created problem in raising seedlings in nursery.

6.1. Nursery raising techniques

6.1.1. Through seeds:

Seed collection:.

Soapberries are harvested and collected during the winter months in India. Fruits may be harvested from 8 to 10 years old trees. Harvesting the fruits usually entails climbing up the trunk and beating the soapberry branches with a long stick—the ripe ones will fall to the ground, ready for collection. Once harvested in this ripened state, the fruits must be de-seeded and dried. Nuts are sometimes pulverized into a powder, depending on its end use. It's a highly labor-intensive, incredible collective effort. Soap nuts absorb moisture quite easily, and should therefore be kept in a dry, airtight container. This is true of any soapberry derivatives such as dried fruit and powders. Dried soapberries can be kept for several months. It is possible to forage for these fruits during the winter months, as they are ornamental trees in several urban cities and household gardens. Gentle cracking of the pericarp releases the seeds. There are 700-800 seeds/kg.

Seed Storage and Viability:

Storage may be defined as the preservation of viable seeds from the time of collection until they are required for sowing. The period for which seed can remain viable without germinating is greatly affected by its quality at the time of collection, its treatment between collection and storage and the conditions in which it is stored.

In case of *Sapindus mukorossi*, seeds being orthodox are successfully stored at low or sub-freezing temperatures for long periods. Seeds retain viability for 1-2 years.

Seed treatment:

According to some researchers, the improvement in germination of *Sapindus* seeds can be achieved by using pre-sowing treatments, cow dung slurry, acid and hot water treatment etc. Generally, acid scarification is the most effective pre-treatment for quicker and higher germination of this species. The two acid treatments (75 and 90 minutes in concentrated hydrochloric acid) can be used to have higher and quicker germination. In case of unavailability of acid and problem in using acid properly, the four pre-treatments, 3 minutes in boiled water and 48 hours in cold water, 5-minutes in boiled water and 120 hours in cold water, 144 hours in cold water and 120 hours in cow dung slurry are suggested as an alternative to acid treatments.

Seed and Nursery:

In seed germination process, seed mass is considerable and significant factor and also in growth of the plant in early stage. Due to the hard seed coat, *Sapindus mukorossi* seeds usually exhibit slow and poor germination rate. Seed mass and pre-sowing treatment of seed influence the germination percentage under nursery conditions. Studies on seed germination (Bisht *et al.*, 2016) in relation to the seeds collected from trees varying in age at different sites and seeds from identical trees varying in sizes revealed that, the seeds from mature trees (>30-years old; mean seed weight 1.72 ± 0.13–2.26 ± 0.14 g) germinated better as compared to the seeds from young trees (10–12 years old; mean seed weight 1.01 ± 0.17–1.97 ± 0.14 g). This study has indicated that, the seeds from mature trees having relatively better seed weight germinate more as compared to the seeds collected from young trees.

Seeds are sown either in polythene bags filled with clayey loam soil mixed with farmyard manure (Fig 13) or similarly prepared nursery beds. Raised nursery is prepared for sowing the seeds. A spacing of 25 x 10-15cm (row to row x seed to seed) is kept.

Fig 13. ***Sapindus mukorossi*** **seedlings in the nursery**

It has been recorded that the seeds start germination after 44 days of sowing and complete within 80 days. Maximum and faster germination is recorded in seed class I (SC I) with 80 % and completed within 60 days and the seed class III (SC III) records least rate of germination with 65 % and completes within 80 days.

Regular irrigation ensures good response of germination. Weeding should be done at an interval of 15 days. Urea @ 200g/100sq.m. should be applied for better growth.

6.1.2. Vegetative propagation:

The problem with *S.mukorossi* is its regeneration through seeds which is quite low due to its poor or slow rate of germination (Parkash, 1991). The vegetative propagation through cuttings therefore offers an alternative to mass propagate the species to meet large scale planting stock requirement under agroforestry/ plantation programmes. The other advantages of this method include faster growth rate, uniform stock, true-to type, higher production rate and quality stock production (Ooyama and Toyashima,1965). In spite of voluminous literature published on vegetative propagation, very few reports are available on vegetative propagation of social forestry/agroforestry species (Khosla *et al*., 1982; Nagpal and Singh, 1986; Puri and Nagpal, 1988; Puri and Shamet, 1988).

In one of the study conducted at UHF, Solan (Mundhe *et al*., 2011), cuttings of soapnut were tested for their rooting response as affected by donor stage, pre-conditioning (girdling) and auxin treatments during monsoon (July-August) and spring (March-April) seasons. It was found that the species can be propagated through stem cuttings preferably in monsoon. It was evident that girdled cuttings of seedling origin material exhibited significantly higher sprouting, rooting and root characteristics in the species. A maximum up to 90 per cent rooting was achieved when girdled cuttings from seedling donors were treated with 1.0% IBA+ 2% captan + 2% sucrose-talc during monsoon season. The technique had potential to mass propagate species to produce uniform and quality planting stock for agroforestry and plantation forestry programme. The technique has a potential for mass multiplication of the species for direct planting or raising clonal seed orchards.

Sharma *et al*. (1999) reported for the first time vegetative propagation of soapnut through Chip Budding. However, vegetative propagation of soapnut had not been very satisfactory.

6.2. Transplantation of seedlings

- 3-4 months old seedlings are ready for planting out .
- The best time of planting is June-July.
- For planting, pits of 60cmx60cm are dug and in June, these pits are filled with a mixture of soil and FYM.
- The transplanting is done on rainy day or irrigation is ensured for better establishment.

6.3. Basic maintenance tips for young plants

For successful establishment of any plant, following points (Anuragi *et al*., 2023) have to be well taken care of :

- Plants need six hours a day of direct sunlight.
- These plants require fertile, organic, well-drained soil to thrive.
- Once a week, when watering the plants, water should be kept off the leaves
- Water the plant from the base up and avoid overwatering it.
  In the summer, watering should be done more frequently whereas in the wet and winter months, frequency should be less.
- Apply nitrogen fertilizer to the soil before planting the seeds. After applying the fertilizer, soil should be watered immediately.
- When repotting a plant, it is advisable to do so at night and to leave the plant in a shady place for at least two to three days before moving it to the right location .
- Spray an infestation with citrus oil, eucalyptus oil, or neem oil as a first line of defence.

7. Plant Tissue culture techniques for QPM production

7.1. Basics of PTC concepts for woody perennial

Plant tissue culture (PTC) of micropropagation technique refers to the cultivation of undifferentiated plant cells, tissues or organs on synthetic media under aseptic environment and suitable controlled physical conditions. It is an important tool for both basic research as well as commercial applications. Plant tissue culture is based upon the unique characteristic of a plant cell i.e., totipotency which is the ability of a vegetative cell to divide and differentiate into any type of specialised cell or to regenerate into a whole plant.

Traditional procedures of propagation are laborious, dependent on environmental conditions and the success rate is also low. Micropropagation can be used to address the above mentioned problems. It results in rapid multiplication of plants within a short period of time in a small space. Since it is performed under controlled environmental conditions, micropropagation is not season dependent. Virtually any part of the plant like leaf, apical meristem, embryo, cotyledon, hypocotyl, etc., can be used as a starting material called explant. These explants are transferred on to the nutrient media and whole plants can be regenerated through in vitro culture..

A variety of nutrients and suitable environmental conditions are required for optimal growth and development of an explant. Depending upon the type of plant species, like monocot or dicot; domesticated or wild, etc., composition of the culture media varies. Even different tissues from the same plant may have different nutritional requirements for optimal growth. Therefore, success of in vitro culture of plants mainly depends upon the selection of the right composition of culture medium. A wide array of synthetic nutrient media extensively used for this purpose are MS media (Murashige and Skoog medium), B5 media (Gamborg medium), LS media (Linsmaier-Skoog medium), SH media (Schenk- Hilderbrandt), QL media (Quoirin & Lepoivre medium), WPM media (woody plant medium), NN media (Nitsch and Nitsch medium), etc. These media are reservoir of carbohydrates as energy source, proteins, essential macro, and trace elements and buffering agents to support the growth and development of the plant part. Also, a suitable proportion of auxin and cytokinine is used for organogenesis. Root development is promoted through auxin are Indole-3-butyric acid (IBA), Naphthalene acetic acid (NAA) and indole acetic acid (IAA) whereas, 6-Benzylaminopurine (BAP) is used for shoot development.

A lot of research efforts are being made to develop and refine micropropagation methods and culture media for large-scale plant multiplication of several number of forest plant species. Few tree species also face the issue of seed dormancy and seed sterility which directly reduce the multiplication rate and the speed. Further, in medicinally important species, the extraction of secondary metabolites particularly in terms of quality and quantity is a very crucial mechanism for optimum utilization in medicines. Tissue culture techniques solve these problems through micropropagation, synthetic seed formation, protoplast culture, haploid or triploid culture, virus free plants production, secondary metabolites production, etc.

7.2. Usefulness of PTC for *Sapindus mukorossi* QPM production

Sapindus mukorossi is a slow growing perennial tree. Owing to its rich medicinal and commercial properties, the plant has been exploited commercially in the past few decades, resulting in the decline of this plant from its natural habitat. Seed germination is poor and erratic, thereby creating problems in raising seedlings (Parkash, 1991). New plants could also be developed from stem cuttings, yet the cuttings did not develop a taproot necessary for tree stability, thus survival in the field was low (Bhardwaj *et al.*, 1985). Micropropagation of shoot tips excised from seedlings was shown by Philomina and Rao (2000), with the same taproot problem.

Tissue culture methods could be a promising modern technique offering several advantages over traditional techniques in *Sapindus mukorossi* as follows,

- Require small propagule/explant: Even a small plant tissue or explant like cotyledons, embryo, leaf or nodal tissues would be sufficient for mass propagation of plant.
- Large scale production of QPM: A large number of fresh plants can be produced even from a small explant.
- True-to type QPM: Newly generated plants are genetically true-to type to their mother plant.

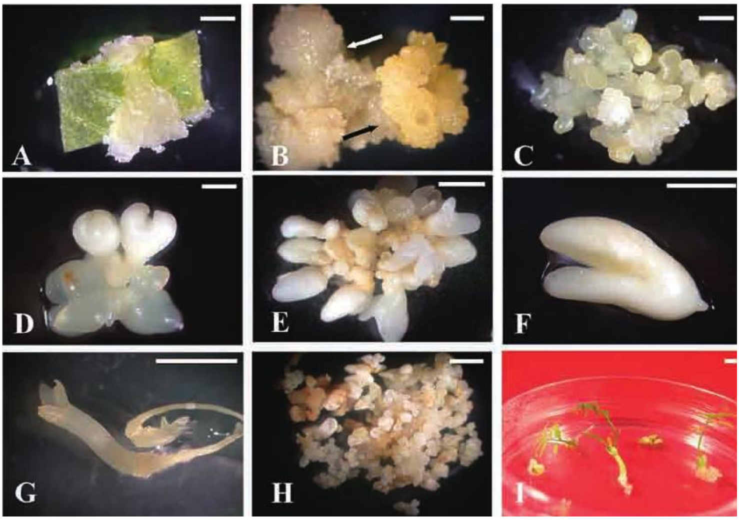
- Virus and disease free QPM: Tissue culture technique do not transmit the seed-borne diseases thus newly generated plants are virus and disease free in nature.
- Year-round production of QPM: In-vitro propagation is not dependent on the season and thus uninterrupted, round the year production of plants are possible.
- Quicker production of QPM: In-vitro propagation shortens the production cycle therefore reduces the long juvenile phases of perennial trees.
- Require lesser space: Micropropagation is performed under controlled laboratory conditions followed by green houses, therefore does not require much space.
- Reduced cost of QPM production: Production cost per plant is quite less when QPM produced at large scale.
- New somaclonal variations: A somaclonal variants with unique characteristics can be expected during the process which could of significant importance to breeding programs.
- Enhanced yield of secondary metabolite production: a small plant tissue or explant would be sufficient for mass propagation of plant.
- Support research activities: Tissue culture offers many opportunities to accelerate genetic improvement and other research activities.

7.3. Status of PTC research in *Sapindus mukorossi*

Studies have been conducted both at the global and national level in the use of somatic embryogenesis technique in *S. mukorossi*. Somatic embryogenesis is considered to be an efficient clonal propagation method that can ensure a taproot system.

7.3.1. Global status :

In a study carried out at Forest Environment Conservation Division of Korea (Fig 14), leaf explants formed embryogenic calluses at a frequency of 53.9% when cultured on B5 media supplemented with 0.1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-d) and 0.01 mg l^{-1} 6-benzyladenine (BA) for 6 weeks (Kim *et al*., 2012). Upon transfer onto media with 5mgl^{-1} abscisic acid, embryogenic calluses yielded somatic embryos at 73%. Somatic embryos developed into plantlets



The above figure shows the Callus formation on leaf tissue (A) and (B) embryogenic (closed arrow) and non-embryogenic calluses (open arrow); (C) globular shaped embryos; (D, E) heart-shaped embryos; (F) torpedo-shaped embryos; (G) plantlet developed from somatic embryo; (H) proliferation of embryogenic calluses with somatic embryos; (I) plantlets regenerated from somatic embryos. Bar=5 mm.

Source: Kim *et al.*, 2012

Fig 14. Somatic embryogenesis in leaf tissue culture of *S.*

on media without plant growth regulators at 90%. Embryogenic calluses proliferated and maintained embryogenic capacity when subcultured on media with 0.1mgl^{-1} 2,4-d and 0.01mgl^{-1} BA at 4-week intervals. This culture system was found to be an effective means for clonal propagation and genetic manipulation of soapberry because it ensured taproot development required for tree stability.

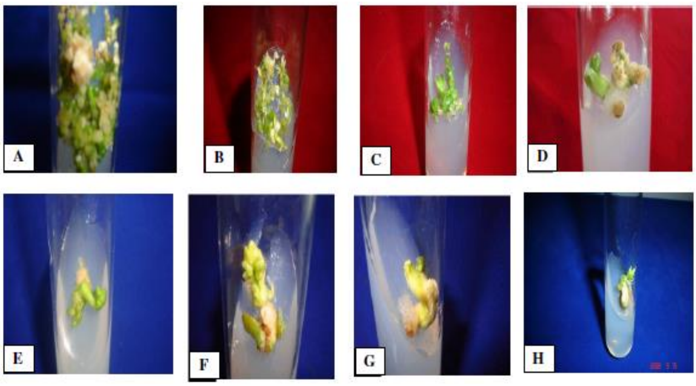
7.3.2. Indian status:

Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell, tissue and organ culture on well-defined tissue culture media under aseptic conditions. Micropropagation of soapnut tree is at a stage of infancy in this forest tree species which has great importance in the soap industry and social forestry programmes. A number of workers established the effective regeneration protocol of *Sapindus* species using the various explants through either organogenesis or somatic embryogenesis.

First report of organogenesis was made by Philomina and Rao (2000). Seedlings were raised aseptically by inoculating seeds on agar-water medium. They took apical and axillary meristems from in vitro raised seedlings as explants and successfully induced organogenesis. Maximum shoot formation in both explants was observed on MS medium supplemented with BAP (0.4 µM) and GA_3 (2.8 µM), and rooting of shoots was observed on MS medium containing IBA. The seedling-derived explants are considered as unproven materials, and eliteness of plant can be checked only after occurrence of flowering and fruit setting. Nodal explants from field grown plants were used for indirect organogenesis by Singh *et al*. (2010).

In one of the study conducted, a reliable protocol (Fig 15) was established for propagation of *Sapindus mukorossi* through somatic embryogenesis (Dhobal *et al*., 2012). Somatic embryogenesis is a process by which somatic cells undergo differentiation to form a bipolar structure containing both root and shoot axes. Multiplication of plants from cell culture by somatic embryogenesis has a potential for highest rate of plant production. Thousands of somatic embryos can be produced in a single flask. Callus derived from the leaf and petiole explants were cultured on MS medium supplemented with different range and combination of Kn (0.1-0.4 mgl-1) and KNO_3 (500 – 1500 mgl-1). The embryogenic calli thus formed were separated from non-embryogenic calli and were sub-cultured. Globular and heart shaped somatic embryos thus formed were transferred to MS medium containing high level of KNO_3, with varying combinations of casein hydrolysate CH (500 & 1000 mgl-1) and L-glutamate (50 mgl-1) for further proliferation. Addition of GA_3, lead to the maturation of somatic embryos. Matured cotyledonary embryos were germinated on fresh MS medium supplemented with Kn (1.0 & 2.0 mgl-1), NH_4Cl (50 and 100 mgl-1) and coconut milk CM (10%) for further growth and germination. The fully developed plantlets were successfully transferred to soil.

In another study, in vitro micropropagation method for *Sapindus mukorossi* tree was developed using apical and axillary meristem explants (Philomina and Rao , 2000). Bud break and multiple shoots were induced in apical and axillary meristems derived from one month old seedlings of *S. mukorossi* on Murashigc and Skoog (MS) medium supplemented with benzytamino purine (BAP) alone. A combination of BAP and gibbcrellic acid (GA) produced elongated multiple shoots from both types of explants. Excised shoots were rooted on MS medium respectively with indole-3-butyric acid (IBA).



[A] Embryogenic callus, [B] Somatic Embryos, [C-G] Development stages of somatic embryos, [H] Plantlet Regeneration.

Source: Dhobal *et al.*, 2012

Fig 15. Somatic embryogenesis in leaf and petiole explants of *S. mukorossi*

The regenerated plantlets were successfully acclimatized and transferred to soil.

Somatic embryogenesis and plantlet formation were achieved from leaf and leaf-derived callus culture of *Sapindus mukorossi* Gaertn. (Sinha *et al.*, 2000) Callus subcultured on MS medium containing lower levels of kinetin (0.2-0.5 mg l^{-1}) and higher levels of potassium nitrate (2900 mg I^{-1}) resulted in embryogenic callas often with globular embryos. Rapid and enhanced rate of embryo formation was achieved on transfer of embryogenic callus to an embryo proliferation medium containing kinetin (0.5 mg I^{-1} and a different source of nitrogen with or without gibberellic acid (0.2 mg I^{-1}). Increased level of gibberellic acid restricted embryo multiplication and led to embryo maturation. Use of kinetin (2.0 mg I^{-1}) along with ammonium chloride (50 mg l^{-1}) and coconut milk, 10% (v/v) in the medium proved to be effective for embryo germination and plantlet formation. This report described the protocol for somatic embryogenesis and plant regeneration from leaf explant of *S. mukorossi* without any exogenous supply of auxin. Philomina (2010) also reported somatic embryogenesis from leaf explants of soapberry

Somatic embryogenic system was also developed using rachis as explants from a mature tree (Singh *et al*., 2016). Explants showed callus initiation on Murashige and Skoog medium supplemented with TDZ (1-Phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea), zeatin or 6-benzylaminopurine. Induction of somatic embryogenesis was achieved on both MS basal medium and MS medium supplemented with 8.88 μM 6-benzylaminopurine. Hundred percent embryogenesis was observed on MS medium supplemented with 8.88 μM 6-benzylaminopurine with maximum intensity of embryogenesis (51.92 ± 0.40 a). Maximum maturation of somatic embryos (92.86 ± 0.34 a) was observed on induction medium supplemented with 0.0378 μM abscisic and treated for 21 days. Germination of somatic embryos was maximum (77.33 ± 0.58 a) on MS medium supplemented with 8.88 μM 6-benzylaminopurine. In vitro raised plantlets were hardened, acclimatized and transferred to the field. Survival frequency of plantlets was 80 % in field conditions. The genetic fidelity of in vitro regenerated plants was also evaluated and compared with mother plant using random amplified polymorphic DNA and inter simple sequence repeat. Both markers showed similarity in molecular profile of mother plant and in vitro regenerated plants.

7.4. Scope of PTC in *S. mukorossi* research and development

As *Sapindus mukorossi* biotechnology is in the beginning stage, extensive research is required for the large-scale production of QPM and high- value secondary metabolites for pharmaceutical exploitation.

A thorough optimization of tissue culture conditions using different explants still requires extensive research for effective genetic improvement of *Sapindus mukorossi* species for futuristic medicinal exploitation, ecological sustainability and other social utilities.

8. Major silvicultural practices

Silvics forms the foundation of silviculture and is concerned with the development and growth of trees and forests with particular reference to environmental influences. The silvics of *S.mukorossi* can be described as

- Strong
- Light-loving
- Deep-rooted
- Drought-tolerant
- Able to coppice
- Not resistant to excessive humidity.
- Prone to frost

8.1. Natural regeneration

Generally, *S. mukorossi* in mid hill areas exists as planted trees and beneath mature trees, it is exceptional to find its seedlings. Fruiting in planted trees of *S. mukorossi* starts after 10 years of plantation and onward next year of fruiting, fruits yield and seeds size improves gradually. Majority of soap nuts are collected for commercial purposes and whatever quantity of seeds remains as leftover may not be able to germinate due to the inhibitory effect of pericarp.

Seeds bearing hard seed coat retain viability for 1-2 years but their germination is very low and erratic.

8.2. Artificial regeneration

The artificial regeneration through vegetative propagation means has already been discussed in Chapter 7. However, there had been studies indicating the relationship of leaf traits in fruit quality and oil content in the seed.

Leaf size and shape can predict single fruit weight and other qualitative traits and can therefore be used as indicators for evaluating fruit quality (Jin *et al.*, 2016; Long *et al.*, 2017; Rowland *et al.*, 2020). The saponins yield and oil content are important fruit traits. Studies have shown that the effect of plant size on the seed oil content of *S. mukorossi* was in the order of TH>DBH>CW, and the effect of plant size factor on the saponin content of *S. mukorossi* decreased in the order of DBH>TH>CW (Fan *et al.*, 2013, 2014). The size of an oleaginous tree affects its fruit yield and oil quality to a certain extent (Wu *et al.*, 2012; Guo *et al.*, 2014). The inherent association between leaf traits and fruit characteristics has been explored in some studies (Wang *et al.*, 2014; Zhang *et al.*, 2020a). Although leaf traits have laid a foundation for the selection of excellent fruit quality traits, a study was conducted to assess the leaf structural traits of plants of different sizes in an even- aged forest of *S. mukorossi*. The covariation indicated that *S. mukorossi* adjusted and balanced the combination of leaf structural traits in response to environmental changes. Considering that climate, soil, and topographic variables can influence leaf traits, future progress requires more research on the effects of environmental factors on leaf traits. The influence of leaf traits on fruit characteristics also differs among various species. Further studies are needed to investigate the inherent association between leaf traits and fruit characteristics of *S. mukorossi* to predict and increase fruit yield.

8.3. Wood properties of *Sapindus mukorossi*

The wood is moderately hard, compact and close-grained, weighing nearly 28 to 30 kg per cubic foot. The heartwood is yellowish grey and the sapwood is yellowish white. Although there is little documentary evidence to suggest conversion of *Sapindus* tree into timber, the wood is utilised for modest rural building construction, oil and sugar presses, agricultural implements, etc.

In a study conducted in China, fifteen kinds of young plantation wood, commonly used for hardware tool handle from Yongkang in central Zhejiang Province, were used to compare and comprehensive evaluate physical and mechanical properties (Li *et al.*, 2010). The result showed that each timber trait was significantly different among tree species. The difference of full-dry density was most than that of other physical properties. The oven-dry density of *Sapindus mukorossi* was 1.99 times higher than that of *Alniphyllum fortunei* . Processing applicability of fifteen young tree species were comprehensively evaluated by coordinate synthesis method. The result showed that wood processing adaptability of *Sapindus mukorossi*, *Schima superba* and *Cyclobalanopsis glauca* were the best.

9. *Sapindus mukorossi* based agroforestry practices

Agroforestry involves the deliberate growing of trees and shrubs with crops, sometimes with animals, in interacting combinations for a variety of objectives. Agroforestry farming practices have been used throughout the world for millenia but have only attracted scientific attention and attained prominence as relevant land-use practices since the late 1970s. Today, acting as an interface between agriculture and forestry, agroforestry is considered to be a promising and sustainable approach to land use. Improvement and utilization of multipurpose trees that produce food, fodder, timber, and fuelwood in integrated farming systems offer sustainable solutions to several serious land management issues such as food security, environmental protection, and climate change, in both developing countries and the industrialized world (Thevathasan *et al.*,2023).

Like many other woody perennials, *Sapindus mukorossi* is a multipurpose tree species having a great agroforestry potential. In addition to its socio-economic benefits to the rural community, it supports various agro-ecological services . *S. mukorossi* trees are grown in the form of agrisilviculture system on the field bunds or boundary of agriculture fields having traditional crops or plantations are raised in tea orchards, grasslands and waste lands.

A spacing of 5m between plant to plant is recommended for plantation on field boundaries with a density of 400 plants/ha. Seedlings are ready for planting out in 3-4 months. The best time of planting is June-July. For planting, pits of 60cmx60cm are dug. In June, these pits are filled with a mixture of soil and FYM. The transplanting is done on rainy day or watering is ensured for better establishment.

Though the tree is not affected by the weeds yet regular weeding and basin preparation is required in the initial years of establishment with ensured irrigation.

Organic manures like Farmyard Manure / Vermicompost have been recommended for application@10t/ha at the time of transplantation along with NPK(60N: 40 P_2O_5: 40K_2O). After establishment of 1 year in the field, for better growth 50kgN/ha in three split doses in the months of first week of June, July and third week of August are recommended.

The tree is ever-growing and trees with a height of 9 m and diameter of 10 cm have been recorded in 5 years in Pakistan. Total expected average yield/annum (from 5th year onward) is 10 kg/tree (approx.) which on the minimum market price of Rs.5,000/quintal may give annual return of Rs.150,000/ha. However, the average yield/annum may increase up to 2 quintal/tree after 15 years of propagation under ideal conditions.

Fruits of *S. mukorossi* is one of the most traded medicinal plant products from Nepal (Olsen and Helles, 2009). The annual trade range and annual value range exported from Nepal is 1,465,000 kg – 4,956,000 kg and 655,000 US$-2,216,000 US$ respectively (Olsen, 2005a, b). The major destination of this trade is in India for several years and transported via Kathmandu (Ghimire *et al.*, 2016; Pyakurel *et al.*, 2017). It is an indigenous plant in the western region of Nepal being traded in the largest volume (Kunwar *et al.*, 2015). Because of its high market value, the plant is also cultivated in private farmlands (Kunwar *et al.*, 2013).

9.1. Ecological services

In urban settings, trees make a significant contribution to improving the environment and help in managing urban emerging

Source: Zhou *et al.*, 2019

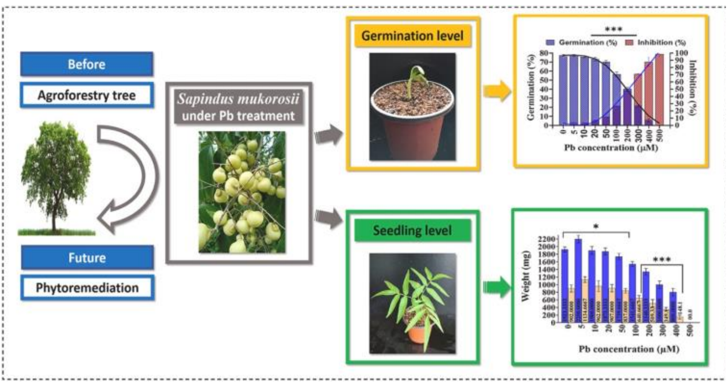
Fig 16. Hilly landscape and Road side Plantation in China

issues (Georgi and Dimitriou, 2010; Cavender and Donnelly, 2019). *Sapindus mukorossi* is a multi- functional species with ecological, environmental, social, and economic benefits. It is widely grown as a native ornamental landscaping species in tropical and subtropical regions worldwide (Jia and Sun, 2012; Liu *et al.*, 2017). The extended summer flowering period, yellow leaves, and golden fruits that are borne in fall and winter make this species an attractive choice for urban forestry establishment in southern China (Fig 16), where trees with leaf colors other than green are rare. *S. mukorossi* trees planted for greening in urban landscapes have strong carbon sequestration abilities, and they are salt- and alkali-resistant (Jim and Chen, 2008; Wan *et al*., 2018). Thus, *S.mukorossi* has ecological, functional, aesthetic, and spiritual attributes that make it attractive to urban dwellers. The trees are planted in courtyards, forest parks, along city streets, and in university campuses, parks, popular tourist areas, and temples.

S. mukorossi like other agroforestry trees provide ecological services like improved microclimate, reduced runoff, carbon storage and sequestration and purification of air by fixing contaminants into their above-ground tissues (Liu *et al*., 2013; Selmi *et al*., 2016).

9.1.1. Phytoremediation:

Heavy metal pollution in metropolitan soils poses significant risks to human health and the entire ecosystem. Effective mitigation strategies and technologies are crucial for addressing these



Source: Sahito *et al*., 2023

Fig 17. Remediation property of *S. mukorossi* plant

environmental issues. Fast-growing trees are an essential part of phytoremediation projects all over the world and provide long-term ecological benefits to mankind. *S.mukorossi* exhibits lead tolerance (Fig 17) and has a long term potential as a phytoremediation plant (Sahito *et al*., 2023) and features in afforestation and soil reclamation. Mukhopadhyay *et al*.(2018) have established the usefulness of *S.*

mukorossi as a zinc washing agent from iron-rich soil with minimal damage to the soil.

9.1.2. Reclamation:

The tree has proved successful in the afforestation of eroded hill slopes at elevations below 900 m in the western Himalayas . The species is extensively planted in Uttarakhand hills on the agricultural field bunds. Seed kernels which are a by-product of the oil extraction from the pericarp and shells can be used as fertilizer (https://apps.worldagroforestry.org/treedb/AFTPDFS /*Sapindus*_mukoross)

9.1.3. Pollution control:

A surfactant obtained from the fruit pericarp of *S. mukorossi* has proved effective in the remediation of contaminated soils. Mondal *et al*. (2017) have assessed the uptake-reduction capabilities of hexavalent chromium contained in contaminated water, using saponins from the fruit pulp of *S. mukorossi*.

9.1.4. Others:

S. mukorossi saponins have been evaluated as environmentally friendly coal dust suppressants (Lu *et al*., 2021), renewable latex polymerization additives (Schmitt *et al*., 2014) , and foam-stabilizing agents in fire extinguishers (Yang *et al*.,2010).

The ecological services of the tree are restricted to terrestrial ecosystems, while the fruit is toxic to aquatic animals. More research is needed to establish the permissible limits for soapnut saponins in wastewater and their biodegradability before soapnuts can be accepted as a bio-based surfactant. Nevertheless, research indicates that it will be beneficial to propagate soapnuts as a sustainable supplement to petroleum-based surfactants and fuels. With many by-products from soapnuts, it is possible to attain zero wastage. Propagation techniques, including natural regeneration, selective crop breeding, vegetative propagation, and tissue culture, have been explored to promote high-quality crops. Planting the appropriate variety of soapnuts could provide a sustainable agroforestry crop that is resilient to climate change.

9.2. Socio-economic services

Soap nut tree being a multi-purpose tree has a great potential in uplifting the socio-economic conditions of any community or an area.

Being a widely distributed economically important tree, this species provides biodiesel, biomedical and biochemical products.

As described in earlier chapters, its fruit rind contains up to 13.26% saponins ; therefore, it is commonly known as soap nut. Saponins are high-quality and pure natural detergents and are effective for heavy metal removal. In addition, saponins have a variety of physiological activities and curative effects on a variety of diseases. The roots, rind, seeds, and flowers of *S. mukorossi* are all medicinal materials and are used in traditional medicine. The *S. mukorossi* seed kernel contains up to 44.69% oil , has good oxidative stability, and is a high-quality raw biomass material (Wang and Wu, 2010). The oil residue can be made into protein cakes, and the seed shell can be made into activated carbon. Moreover, *S. mukorossi* trees, which have a high comprehensive utilization rate, can easily form a complete industrial chain and are, therefore, an emerging new energy source (Song *et al.*, 2023). Initiatives have to be taken especially in India for mass propagation of high yielding cultivars and making QPM available to the farming community for plantation under different agroforestry systems.

9.3. Allelopathy

Allelopathy is an ecological phenomenon which affects directly or indirectly the normal activities of neighbouring plants through the release of allelochemicals in environment. The effects of these allelochemicals on other plants may be detrimental or beneficial (Rice, 1979; Peneva, 2007; Zeng *et al.*, 2008). The detrimental potential of allelopathic substances can be utilized for weeds and pest management. These allelochemicals may inhibit germination rate, overall growth and nutrients uptake of susceptible species (Rizvi *et al.*, 1999; Marwat and Khan, 2006). The absence of allelopathy effect in the tree species is one of the important criteria for inclusion of the species in agroforestry.

Preliminary study conducted by Ullah *et al.* (2015) revealed that aqueous extracts and soil intoxicated with litter of *S. mukorossi* possess strong allelopathic potential towards the test species *Pennisetum americanum*(L.) leeke, *Setaria italica* (L.) Beauv. and *Lactuca sativa* (L.). The results recommended their use as bioherbicides against these weeds on commercial scale and also suggested isolating and characterizing the active constituents of the plant responsible for inhibitory effects.

Protection of field crops from weeds, diseases, and other crop pests are part of the critical factors for sustainable crop production. Weed management strategy plays a vital role and directly influences food security and productivity worldwide. In managing weed interference, the extensive use of synthetic herbicides have led to the emergence of herbicide resistant/tolerant weeds. In an important study conducted at Bangladesh, focus was on identifying new volatile allelochemical containing species that have the potential to suppress weeds in agricultural farming. Identified allelopathic species can be utilized in sustainable weed management, to minimize any adverse effects of synthetic herbicides . Identification of the new volatile allelopathic species can contribute to the prospect of finding novel volatile allelochemicals, which may promote the development of new natural herbicides for weed management in agriculture. A total of 103 plant samples from 40 different families were assessed with the dish pack (DP) method. About 25% of the evaluated plant samples influenced (inhibited or stimulated) the growth of lettuce, due to the presence of potentially volatile allelochemicals. The pericarp of *Sapindus mukorossi* Gaertn. caused the lowest radicle elongation (3% of control) of the lettuce. This was the first report (Begum *et al.*, 2020) of the pericarp of *S. mukorossi* as a potential volatile allelopathic candidate for weed management in agroecosystems.

There are several studies about the herbicidal potential of the extract from *S. mukorossi*. The leaf and bark extracts of *S. mukorossi* reveal both inhibitory and stimulatory effects on the germination and root growth of test crops (Thapaliyal *et al.*, 2008). The leaf extract of *S. mukorossi* has an inhibitory effect on seed germination and root growth in crop pea owing to the presence of allelochemicals (Siddiqui, *et al.*, 2018). The allelopathic effects may be due to the presence of saponins in *S. mukorossi*.

Study conducted in China by Dai *et al.* (2021) also verified that the pulp extract of *S. mukorossi* had an inhibition effect on the root growth of *Trifolium pratense* L. and *Cynodon dactylon* L. with an 50% inhibitory concentration (IC50) value of 35.13 mg/L. An active compound was isolated from the 70% ethanol fraction and identified as hederagenin 3-o-β-D-xylopyranosyl-(1→3)-α-l-rhamnopyranosyl-(1→2)-α-l-arabinopyranoside (Compound A). Compound A had an IC_{50} value of 16.64 mg/L. Finally, a new formulation was prepared based on the 70% ethanol fraction, which exhibited good efficacy against broadleaf weeds in a carrot field. The fresh weight control efficacy was 78.7% by

45 days after treatment at the dose of 1500 g a. i./ha. Hence, the extract of *S. mukorossi* pulp could be a promising supplement to the synthesized herbicides. Furthermore, compound A from *S. mukorossi* may be responsible for its phytotoxic activity. Nevertheless, the safety and the herbicidal action mechanism of the developed formulation needs to be further studied.

10. Major socio-cultural and economic utilities

10.1. Socio-cultural importance

Sapindus mukorossi Gaertn. is a multi-purpose tree used for landscaping and ecological protection, and in the chemical and wood oil industries in China. It is a common garden tree in southern China; in addition, *S. mukorossi* has golden-yellow leaves in the fall and is a famous landscaping species (Jia and Sun, 2012). It has been recorded in early Chinese literature, such as "The Classic of Mountains and Seas" and "Compendium of Materia Medica." It is cultivated throughout northern India as an ornamental plant. Seeds of *Sapindus* are also made into rosaries (Chadha, 1972).

Owing to rich medicinal properties of *Sapindus mukorossi*, the plant has found mention in ancient literature. Various parts of the plant have been used in ayurvedic preparations by Vaidyas for curing various ailments discussed in earlier chapters.

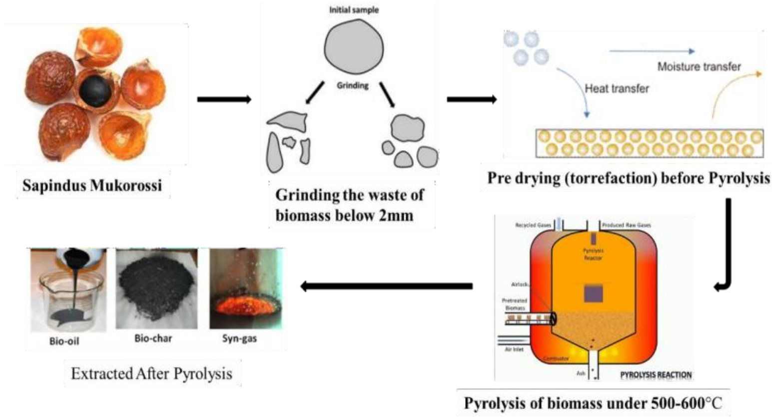
10.2. Economic importance

S. mukorossi is considered as one of the major commercial medicinal and aromatic plant species (MAPs) and plant of national priority in Nepal (Bhattarai and Ghimire, 2006; Baral and Kurmi, 2006; Gewali and Awale, 2008). Government of Nepal has also identified this plant as one of the 30 medicinal plant species of national priority for development, research, and cultivation (MFSC, 2006). The list is prepared on the basis of national and international commercial demand, distribution, use, and medicinal properties.

S. mukorossi can be used for economic upliftment of the local inhabitants as its fruits are being sold at high price inthe market for preparing medicine/ detergent. Annual demand of fruits is 200–500 MT and annual consumption by Indian domestic herbal market is around 182 MT.

10.2.1. Bio-oil:

Soap nuts are the main waste materials produced from the soap production areas. Soap production factories are using the flesh of the soap nut fruits and the inlet nuts they throw as garbage. Soap nuts can also be used for the thermal degradation to produce bio-oil, biochar and syngas, that can be converted by a fast pyrolysis process (Ashraf *et al*., 2020). A small amount of "Erucic" acid is present in soap nuts which is absent to bio-oil produced by another biomass. This "Erucic" acid improves the quality and distinguishes it from others as unique characteristic. During the pyrolysis process (Fig 18), the maximum amount of moisture extracted from the feedstock gives a higher amount of bio-oil. Grinding the size of the feedstock plays a very important role in the optimum quantity of bio-oil extraction. The studies indicate that the 0.5-1.4 mm range is a better range for maximizing the output of bio-oil while maintaining the moisture content low.



Source: Ashraf *et al*., 2020

Fig 18. Pyrolysis process using soap-nuts (*Sapindus mukorossi*)

10.2.2. Fodder:

Ritha foliage is useful as fodder for cattle, especially during grass famines (Kirtikar and Basu, 1991).

10.2.3. Sweetener:

Decoction of fruit is added in certain Indian milk sweet "Rasogullas" (Facciola, 1998).

10.2.4. Quality enhancer:

Seeds are used to bleach cardamom seeds to improve flavours and colour of spices (Facciola, 1998).

10.2.5. Apiculture:

Honey water-white (also described as light golden), of mild flavour and good aroma is obtained from *Sapindus mukorossi* based apiculture.

10.2.6. Essential Oils:

Seeds contain 23 % oil of which 92 % is triglycerides; the triglyceride fraction contained 30 % oleo- palmito-arachidin glyceride, 13.3 % oleo-diarachidin glyceride and 56.7 % di-olein type glycerides such as dioleo- palmitin, dioleo-stearin and dioleo-arachidin.

10.2.7. Medicinal :

Plants of the genus *Sapindus* have been used in traditional medicine for the treatment of ulcers, external wounds, inflammation, epilepsy, dental caries, arthritis, joint pain, gout and rheumatism (Goyal *et al.*, 2014). Phytochemical studies of this genus have identified more than 103 compounds. Of these compounds, triterpenoidal saponins of oleanane, dammarane and tirucullane are regarded as the active group that is most likely to be responsible for the observed biological activities. The crude extracts, as well as the isolated compounds, from the genus possess antimicrobial, anticancer, spermicidal, hepatoprotective, antioxidant, anti-inflammatory, anti-platelet aggregation, anti-hyperlipidemic, anti-migraine, anti-diabetic, anti-ulcerogenic and analgesic properties.

Dental caries are treated with *S. mukorossi* seeds (www.worldagroforestry.org). The fruits of *S. mukorossi* are used in treatment of snake bite, scorpion stings and dandruff (Kunwar *et al.*, 2009). The fruit is used as expectorant, demulcent, emetic, anthelmintic, purgative, and in treating epilepsy/cholera (Baral and Kurmi, 2006). In Indian state Assam, fruit paste of *S. mukorossi* is used as febrifuge (Chadha, 1972). In addition to the earlier listed medicinal uses under Chapter 5, Central Drug Research Institute, Lucknow, has recently developed a contraceptive cream out of *Sapindus* fruit. The same is being marketed under the trade name "Consap".

10.2.8. Timber:

The wood is light yellow, compact, close-grained and fairly hard, weighing 750 kg/m3 at 12 % moisture content. In Vietnam, *S. mukorossi* wood is used for making furniture, sawing board, plywood etc. (Hoang *et al.*, 2004).

10.2.9. Fuelwood and Charcoal:

The wood is also used for fuel and to make charcoal.

10.2.10. Detergent:

Soapnuts are used as detergent for washing clothes. In Kashmir soapnuts are the best soap for washing woollen shawls (Chadha, 1972).

10.2.11. Feed:

Dietary supplementation of 0.026% SSP (saponin equivalent 75 ppm) obtained from powder shell of soapnut has been found to improve the reproductive performance of broiler breeders.

10.2.12. Fertlizer:

The seed kernels, which are a by-product of the oil extraction from the pericarp and shells, can be used as fertilizer.

10.2.13. Surfactant:

Methods of extracting the maximum amount of oil from existing oil reserves has become a scientific focus in a world that has become dependent on fossil fuels. Researchers have found that the *Sapindus* fruit can be used in an enhanced oil recovery technique. More specifically, Chhetri *et al*., (2009) found that extracts from the soapnut can be used as an organic surfactant to increase the mobility of oil from the fields. In addition, researchers have demonstrated the potential for the soapnut to be used as a natural surfactant for washing arsenic from soils that are rich in iron.

11. Value addition and value chain development

A value-added product is a saleable commodity that has been enhanced with additional qualities that make it worth a higher price than the raw materials used to make it. It may be made more convenient, more attractive, more palatable, or easier to use than its raw ingredients.

Every part of *Sapindus mukorossi* is of immense economic importance as described in earlier chapters. A typical case study of value chain development in *Sapindus* fruit has been reported by ICIMOD from Nepal.

11.1. Case study of value chain

In Nepal, KSLCDI (Kailash Sacred Landscape Conservation and Development Initiative) identified potential value chains in selected pilot sites. For 2015 and 2016, the Kailash Nepal Chapter prioritized upgrading and promoting these value chains through product development and improving market linkages . KSLCDI included remote portions of the Tibet Autonomous region of China and contiguous areas of Nepal and India. The landscape is environmentally fragile and its people are highly vulnerable to climate change and environmental degradation (ICIMOD, 2015).

Ritha commonly known as soap nut, is a deciduous tree widely grown on farms in the hilly far-western districts of Nepal as a non-timber forest product. It contains saponin – a good substitute for washing soaps and shampoo and is commonly used among women in rural areas for washing hair and clothes. It is a major component in the large-scale manufacture of soap, shampoos, and detergents. The tree is found in Nepal between altitudes of 1,000 and 1,400 m. It flowers from July to August, and its fruits are harvested in November and December.

11.2. Reasons for choosing the *Ritha* value chain

Ritha produced from Baitadi and Darchula districts in far-western Nepal is either used to manufacture soap in domestic industries or exported, primarily to India, China, and Germany. Annually about 450 tonnes of *ritha* is exported to India from different village development committees (VDCs) in Darchula District (Gwani, Ranisikhar, Deitala, Boharigaun, Gokuleshwor) and in Baitadi District (Dalisani, Rudreshwor, Gokuleshwor, Kotpetara) .

One of the main reasons for choosing the *ritha* value chain was the high availability of the *ritha* plants in the area, which has favourable geoclimatic conditions.

The plant grows on community marginal lands and on farmlands, and is especially prevalent in the KSLCDI pilot districts. There are already several established *ritha* cooperatives, its national and international demand is rising, and there are numerous possibilities for value additions to powder and herbal soaps (Fig 18).

The lack of processing, quality control, storage, and modern packaging, as well as the need for organic certification, in combination with market price fluctuations and disease and pest infestations were the challenges faced by this value chain.

11.2.1. About the KSL *Ritha* pilot site

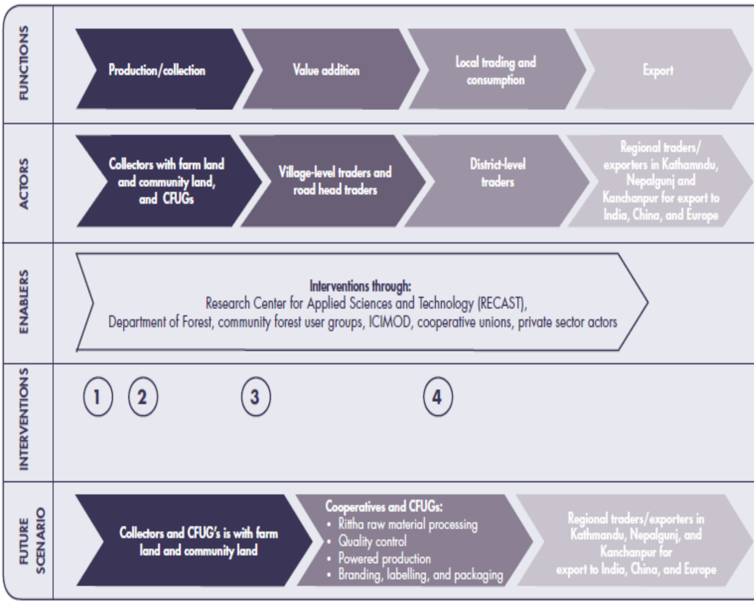
Gokuleshwor VDC of Baitadi had 771 households with a total population of 4,073. Almost 80% of the households in the village (606 total) were involved in the *ritha* trade, which made it as one of the most important additional income generating activities in the village. *Ritha* was also widely available in Gokhuleshwor, and most households were owning one or two *ritha* trees. Timber from the *ritha* tree was used in rural building construction, as fuelwood, and as agricultural equipment. The plant's foliage was being used as cattle fodder during the dry season. In terms of volume, *ritha* is one of the top export products from far-western Nepal.

The average per capita income in Gokuleshwor was NPR 17,230 (USD 290), which placed many people below the poverty line of USD 1.25 per person per day. Wage labour, primarily across the border in India, was a major source of household income for people in Gokuleshwor. Analysis showed that non-timber forest products, medicinal and aromatic plants, and vegetable cash crops were major livelihood options that improve food security.

11.3. Steps in the *Ritha* value chain

Farmers collected *ritha* from trees grown on their own land, which they sold in their village as well as to the road head trader or to cooperative outlets. These traders and cooperatives supplied raw material to regional traders based in Nepalgunj and Kanchanpur, who then exported the material, primarily to India and occasionally to China and Europe (Table 10). In 2014–2015, about 233 tonnes of *ritha* from Gokuleshwor and its neighbouring VDCs were traded, with farmers earning nearly NPR 3.5 million (USD 58,000) at the existing price of NPR 15 (USD 0.25) per kilogramme.

The Department of Forests and its partners had mobilized farmers in the area to establish business entities, like Malika Arujun Natural Product Pvt. Ltd., and cooperatives, like the Kaflansaini–Betaleshawor Agriculture Cooperative. These local groups were



Source: ICIMOD, 2015

Fig 19. *Ritha* value chain interventions

primarily engaged in the *ritha* trade, but also worked with other varieties of medicinal and aromatic plants and agricultural commodities.

These organizations were established within the framework of community forest user group (CFUG) guidelines. Malika Arjun Natural Product Pvt Ltd had 1,462 members from nine CFUGs of adjacent VDCs in Darchula and Baitadi districts. The Kaflansaini-

Betaleshawor Agriculture Cooperative was formed by members from Gokuleshwor, Dilasani, Rudreshwor, and Kotpetara VDCs of Baitadi District. They had established market linkages with the Kathmandu-based company Discover Nepal.

In 2015, two other private entrepreneurs – Mahalaximi Jadibuti Suppliers and Kedear Bhumiraj Traders and Suppliers – started to work with *ritha* marketing.

Table 10. *Ritha* value chain upgrading strategies

1	**Strengthened existing community forest user groups (CFUGs) and cooperatives**:
	Existing groups engaged in the collection and trade of *Ritha* needed support to develop their knowledge and skills on better business planning and instituting strong governance mechanisms. Among these groups, the sustainable use of resources were encouraged through the promotion of nurseries, high-quality seedlings, and scaling up of plantations, both in private land and community forests.
2	**Supported cooperative storage facilities:**
	A substantial amount of *Ritha* was produced each season, and cooperatives currently lacked the infrastructure to store surplus *ritha*. By supporting the construction of collection centres in the villages and a larger storage facility in the market hub of Gokuleshwor, damage and wastage of *ritha* crop was largely minimized.
3	**Introduced grading and quality certification system**:
	Ritha came in different qualities due to the lack of appropriate harvesting practices and periodic infestation by pest and diseases. This affected the marketability of the product, thus reducing its overall price. Potential interventions included capacity building of collectors on quality segregation, grading, packaging, and branding and providing support for the development of certification systems. Good quality soap nuts were traded directly, whereas moderate quality soap nuts were used for powder making and poor-quality soap nuts for saponin use in soaps, shampoos, and detergents.
4	**Supported product diversification, packaging, and branding**:
	Ritha was earlier traded from Nepal in the raw form. Opportunities existed for value addition, such as making powder, extracting oil, or making herbal soaps and detergents. Interventions were also required for improving packaging and developing the *ritha* brand.

Source: ICIMOD, 2015

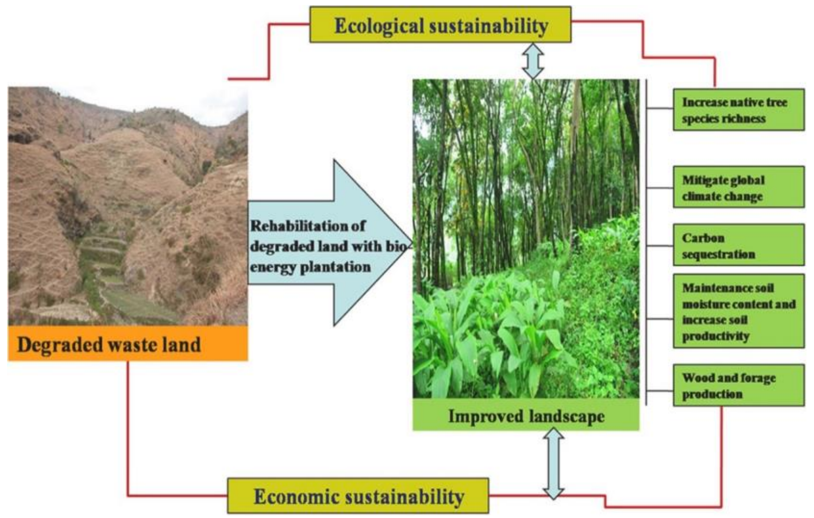
12. Implications on ecological restoration

The Himalayan ecosystem has global significance for supporting unique and rich ecological and socio-cultural diversity as well as a range of ecosystem services important for the very survival of human beings. However, constant anthropogenic pressure, unsustainable developmental practices and climate change led to the transformation of irrational land-use practices that have seriously compromised the ecosystem productivity and livelihood of the mountain communities. Regaining the functionality of degraded ecosystem with adaptive management requires interlinking of different land uses, such as agroforestry (Fig 20), agriculture, waste and degraded land, protected landscapes and policy implementation (Schlaepfer *et al*., 2005, Berendse *et al*., 2015, Gibbs and Salmon, 2015).

Fig 20 Wasteland reclamation through *Sapindus* in HP mid-hills

Conversion of degraded land through mixed tree plantation rather than monoculture plantation may be better for meeting the diverse products needs of local people and environmental amelioration (Lin *et al*., 2012). Tree planting in degraded lands, apart from enhancing ecosystem functions of the treated areas, contributes to conservation of the remaining forest (Semwal *et al*., 2013).

In one of the case study from Uttrakhand (Rawat *et al*., 2022), realising the increasing trend of degradation of natural resources and associated livelihood challenges for rural communities, a comprehensive scientific framework was developed for improving the functionality of marginal degraded land. Three study sites viz., Jaminikhal village cluster (JVC), Manjgaon village cluster (MVC) and Hadiya village cluster (HVC) in the hilly terrain of Tehri Garhwal district of Uttarakhand were selected for tree plantation .A total of twelve tree species (including *Sapindus mukorossi*), used for a variety of purposes prioritised by local communities based on ecological, social and economic value, were selected for plantation on marginal degraded land belonging to the village community. Significant improvement in survival rate, growth, and circumference of planted tree species was observed across the sites after ten years of the plantation (Fig 21). Among the species, *Sapindus mukorossi* displayed the highest survival (98.5%) in JVC whereas, *Grewia oppositifolia* exhibited a maximum (92.3%) survival rate in MVC while minimum (83.6%) at HVC. An average survival rate of 68% was observed at the age of ten years of the plantation in all clusters without further mortality .



Source: Rawat *et al*., 2022

Fig 21. Ecological restoration through trees including *S. mukorossi* plantation

The plantation activities enhanced the net productivity of degraded landscape while increasing characteristics of soil leading to increment in water percolation, improve soil moisture and decreased runoff. Total biomass accumulated by tree species planted in three selected sites within the period significantly contributed to sequestration thus reducing the adverse impact of changing climate. The provisioning services in the form of fuel, fodder, and grasses obtained from developed landscapes reduced the drudgery and workload of marginal communities.

Another study conducted in China on ecological implications of *S.mukorossi* (Liu *et al.*,2021b) revealed that classified management, increasing the yield, reducing the inputs of chemicals and decreasing the unproductive years are the key ways to improve the environmental performance of soapberry cultivation in Southeast China. Woody biomass carbon should be included in LCAs, and 3.71-5.11 t CO2 can be fixed by soapberry plantations per ha year, indicating that soapberry cultivation provides a net carbon sink (Table 11).

Table 11. Carbon contents in different plant parts of soapberry.

Item	**Stem**	**Branch**	**Root (3 cm)**	**Root (1–3 cm)**	**Root (0.2-1 cm)**	**Root (0.2 cm)**	**Wood**
Carbon content %	46.78 ± 0.912	46.94 ± 1.209	43.69 ± 1.587	45.68 ± 3.984	45.62 ± 6.978	46.57 ± 6.114	46.39 ± 0.099

Source: Liu *et al.*,2021b

Role of *Sapindus mukorossi* in soil reclamation especially in problematic soils has already been discussed in earlier chapters.

13. Indigenous traditional knowledge

Indigenous knowledge refers to the unique, traditional, local knowledge existing within and developed around the specific conditions of women and men indigenous to a particular geographic area. It is the local knowledge - knowledge that is unique to a given culture or society. *Sapindus mukorossi* has been used by local peoples for thousands of years as a source of saponins that can be used for cleaning clothes etc. (Dutta, 2007). It is also harvested from the wild for local use as a food, medicine and source of other materials. Every part of this plant has got one or the other kind of phytochemical compounds which have been traditionally used (Table 12).

Table 12. List of *Sapindus mukorossi* based ITKs.

S.No.	ITK	Description
1.	Leaf bath	Leaves are used in baths to relieve joint pains (Upadhyay and Singh 2012)
2.	Use of fruits by Jewellers	The fruit of *Sapindus mukorossi* are utilized by Indian jewellers for restoring the brightness of tarnished ornaments made of gold, silver and other precious metals (Singh *et al.*,2010).
3.	Fruit paste	In Indian state Assam, fruit paste of *S. mukorossi* is used as febrifuge (Chadha, 1972)
3	Use of seeds for curing common ailments	The powdered seeds are employed in the treatment of dental caries, arthritis, common colds, constipation and nausea. (Gairola *et al.*, 2014; Joshi *et al.*, 2010).
4	Use of Seeds for curing respiratory disorders	The decoction of seed is taken orally for tuberculosis and pertussis cough by the Maonan people in China (Hong *et al.*, 2015).
5.	Use of seeds for religious purposes	In some parts of the world, the seeds are drilled for use as beads and are also made into rosaries (Chadha, 1972) Seeds are also carved as buttons.
6 .	Seeds as ornaments	In parts of eastern Nepal, children use soapberries as marble and jack substitutes; and in the West Himalayas, children wear ankle bracelets made from seeds and encrusted in gold and silver to ward off the evil eye

7.	Seed powder as sweetener	The seeds are crushed and boiled to make a liquid that is added to certain kinds of Indian milk sweets, known as 'rasgullas'. This adds a frothy quality to the dessert (Facciola, 1998).
8.	Seeds as quality enhancer in Cardamom	The seeds have been used to bleach cardamom seeds and this treatment is reported to improve the flavour as well as the colour of the spice (Facciola, 1998)
9.	Fruits to cure poisonous bites	Fruits are useful in snake bite, scorpion sting and dermatological problems (Burlakoti and Kuwar 2008; Kunwar *et al*., 2019).
10.	Fruits as soap substitute	Fruits are squeezed, mixed with water, then used to clean clothes etc. Particularly useful for natural fibres and delicate materials. That perhaps is the reason that some scientists have given the species the alternate as *Sapindus detergens* also.
11.	Fruit pericarp as selective eradicant for fish hunters	*Sapindus mukorossi* fruit pericarp is used as a selective eradicant by hunting tribes for horny fish like *Heteropneustes fossilis* and *Channa punctata*. These tribes traditionally put large quantities of them in streams, lakes etc in order to stupefy or kill the fish
12	Fruits for well being	The fruit and seeds are regarded as a cure for epilepsy in northern India. A decoction of the fruit is used as an expectorant (Acharya, 2012) Fruits are also used to wash wounds
13	Wood for furniture	In Vietnam, *S. mukorossi* wood is used for making furniture, sawing board, plywood etc. (Hoang *et al*., 2004).

14. Breeding and biotechnological interventions

Germplasm resources form the foundation of forest genetic breeding, and the development of forest tree breeding and industry depends largely on the extent and diversity captured by these resources. However, redundancy in germplasm resources may lead to lower conservation and management efficiency. The construction of core germplasm collection (Fig 22) is the optimal solution to genetic redundancy. Core germplasm collection is a subset of germplasm accessions that represents the minimum repeatability and maximum genetic diversity of one species (Frankel, 1984; Brown, 1989). They have been widely used for germplasm management, conservation, and application in crop, flower, and horticultural tree species. Most core germplasm collections represent only 5–20% of the total germplasm collected (Hintum *et al*., 2000; Lv *et al*., 2020), thereby reducing conservation and management costs and improving the efficiency of germplasm utilization. However, woody plant germplasm is predominantly derived from natural populations with brief history of domestication and long generation time, therefore the accessions have a high intrinsic genetic diversity and core germplasm collections typically represent 10–45% of the complete germplasm collections within these species (Belaj *et al*., 2012; Duan *et al*., 2017; Min *et al*., 2017; Preethi *et al*., 2020). The wild *S. mukorossi* germplasm

Fig 22 Promising *Sapindus* germplasm of HP

resources have recently been of great interest, because of their importance for developing new cultivars that can be commercially used in cosmetics and their potential for bioenergy and pharmacology.

In an important study conducted in China, 42 *S. mukorossi* fruits from mother trees were collected from 39 distinct locations in 12 Chinese provinces to infer fruit and seed trait responses to environmental factors. Canonical correspondence analysis (CCA) was conducted using 21 horticultural fruit traits and 10 environmental factors that represented different climatic and geographic conditions throughout southern China. CCA revealed well-developed patterns of natural phenotypic variation, and insight into the ecological factors that are potentially important in shaping this variation. The results elucidated the natural distribution and ecological adaptations of wild *S. mukorossi* resources, which will be valuable for *S. mukorossi* cultivation by helping identify ideal planting areas. The germplasm resources with extensive morphological variation can also contribute to *S. mukorossi* breeding in the future by helping develop new cultivars with high saponin yield.(Caowen *et al.*, 2019).

Efforts have been made to develop *Sapindus* breeding programs by mostly focusing on genetic diversity, phenotypic variance, and biochemical variance analyses to identify lines that produce high seed oil yield, high saponin yield, and stronger adaptability (Brown,1989; Dervishi *et al.*,2021; Diao *et al.*, 2016 and Duan *et al.*,2017).

14.1. Status of genetic improvement research

Programs for genetic analysis and selective improvement of species varieties are currently underway, along with developments in planting techniques, studies of biological characteristics, processing and utilization, and the establishment of a sustainable industrial chain.

In China, efforts have been made to develop *Sapindus* breeding programs by mostly focusing on genetic diversity, phenotypic variance, and biochemical variance analyses to identify lines that produce high seed oil yield, high saponin yield, and stronger adaptability (Sun *et al.*, 2016; Fan *et al.*, 2013; Shao *et al.*, 2013 and Diao *et al.*, 2014). Although these studies have made progress, additional research on the natural adaptive diversification of *Sapindus* is required. Most previous studies have focused on how a single environmental factor influenced fruit trait variation. However, limited information is available on how numerous environmental factors shape morphological diversity at a large scale. The germplasm resources with extensive morphological variation can also contribute to *S. mukorossi* breeding in the future by facilitating development of new cultivars with high saponin yield (Sun *et al.*, 2017)

S.mukorossi has a long history of cultivation in China and its forestry has developed rapidly in recent years. As of 2016, *S. mukorossi* plantations covered nearly 26,000 ha in south China (e.g., Fujian, Zhejiang, and Guizhou provinces) (Gao *et al.*, 2016). However, yields fluctuate widely because of the lack of improved varieties and limitations of the technical cultivation system (Liu *et al.*, 2017). The *Sapindus* research team of Beijing Forestry University have investigated, collected, and evaluated the elite germplasm resources of *Sapindus* since 2014 and established the *Sapindus* germplasm resource nursery in Jiaming county to perform the breeding of *Sapindus* (Liu *et al.*, 2019; Wang *et al.*, 2020). 'Yue Shuo Bodhi' was released as a new *S. mukorossi* cultivar in China on 21 Dec. 2020. This new cultivar, which has a fruit weight of 4.45 ± 0.10 g, relatively large size, high yield, and excellent fruit traits, has been widely planted in the Fujian province.

However, with worldwide deforestation and the rapidly anthropogenic expanding, the habitat and populations of *Sapindus* have been severely damaged or vanished in recent centuries, and the genetic diversity of *Sapindus* faces unprecedented threats (Liu *et al.*, 2017; Liu *et al.*, 2021c). Hence, breeders have recently carried out several surveys and collections of *Sapindus* germplasm resources, and over 1,000 samples have been collected (Liu *et al.*, 2017). However, due to inconsistencies in the timing, standards, and designation of germplasm collections, there is considerable homonymy, synonymy, and genetic redundancy within the resources. Therefore, a comprehensive evaluation of the genetic diversity in *Sapindus* and the construction of a rationally designed core germplasm collection are needed.

In India, a study was conducted in Himachal Pradesh (Kairon and Sankhyan, 2017) to select better seed sources for improved genetic material and quality production of soapnut (*Sapindus mukorossi*). Genetic variability was evaluated for different traits viz., morphometric traits, germination parameters, percent seed oil and progeny performance traits were carried out among different twenty four seed sources from the state. The study revealed significant variation among different seed sources. Seed sources which have higher seed weight and seed diameter showed better performance over others, those seed having lower seed weight and seed diameter comparatively. Banjar seed source was found to be superior and best, followed by Garsa seed source, for all morphometric traits, germination parameters, percentage seed oil content and progeny performance traits over all other seed sources studied. It was revealed that high heritability with low genetic advance was associated with seed diameter.

Work on conservation, evaluation and mass multiplication of germplasm resources of *S. mukorossi* is being carried out by ICAR-Central Agroforestry Research Institute (CAFRI) , Jhansi India under its All India Coordinated Research Program on Agroforestry in .mid-hills of Himachal Pradesh by HP Agriculture University Palampur. This work on selection of superior germplasm of desired phenotypes of *S. mukorossi* for soapnut production (Fig 22) has been in progress since 2005. Twenty five seed sources collected from trees in different parts of the state and selected on the basis of phenotypic characters were firstly evaluated in the nursery and 8 promising seed sources were then transplanted in the field and have been under evaluation since 2012 (Fig 22) for the various growth parameters. Out of these 8 superior seed sources, four seed sources from Dhraman, Chuari , Mangla I and Mangla II – all from Distt. Chamba have been performing best for the last five years (Anonymous, 2022).

Biotechnological interventions in propagation of *Sapindus mukorossi* have earlier been discussed under the Chapter 7.

14.2. Genetic Fidelity in *Sapindus mukorossi*

Genetic fidelity is a useful feature when clonal stability is required to maintain the characteristics of elite genotypes, for example, for conservation of selected germplasm or mass scale propagation of improved varieties. Singh and Sharma, 2019 have done elaborate studies on three species of *Sapindus* and in case of *S. mukorossi*, the following results have been reported

The use of DNA-based markers allows efficient comparisons because genetic differences are detectable at all stages of development of the organism unlike allozymes which may show age-dependent changes. Detection of somaclonal variation at early stage is very important to avoid the economic loss (Chung *et al.*, 2009) because commercial value of plant decreases due to somaclonal variation (Oropeza *et al.*, 1995). Clonal fidelity was assessed using PCR-based RAPD and ISSR analysis.

14.3. In Vitro Raised Plantlets from Leaf Explants

A total of 16 primers (RAPD) were used for the production of scorable and reproducible bands. Amplified DNA profile of RAPD showed monomorphic banding pattern of genomic DNA of mother and micropropagated plants. All primers generated a clear, scorable and reproducible banding pattern ranging from 350 to 1250 bp in RAPD. All these RAPD primers produced a total of 84 bands, and a number of scorable bands for each primer varied from 2 (OPL-20) to 9 (OPK-03) with an average of 5.25 bands per primer

For ISSR molecular marker, 16 primers were used for generation of DNA profile. The DNA profile of ISSR showed monomorphic amplified bands of genomic DNA of both mother and regenerants. In ISSR primers the total number of scorable bands was 61 and varied for each primer from 2 (UBC-814, 842) to 6 (UBC-836, 848) with an average 3.81 bands per primer .All primers generated clear, scorable and reproducible bands ranging from 250 to 1250 base pairs.

14.4. In Vitro Raised Plantlets from Rachis Explants

A total of 16 primers (10 ISSR and 6 RAPD) were used for the production of scorable and reproducible bands. RAPD and ISSR markers were most frequently used for assessment of clonal fidelity. RAPD marker assay is technically simple, rapid and requires small amount of DNA and due to these unique features, it has been preferred among other molecular markers (Ceasar *et al.*, 2010). RAPD and ISSR gel photographs have shown monomorphic and clear DNA bands.

14.5. In Vitro Raised Plantlets from Leaf Explants

RAPD and ISSR analysis of in vitro raised plants showed similar banding pattern with mother plants that indicated the genetic integrity between them. RAPD and ISSR photographs showed clear, scorable and monomorphic bands. Amplification bands showed genetic similarity between mother and in vitro raised plants. In RAPD, a total of 27 bands were observed with 6 primers, and the average number of bands was 4.5 (Table 13). In ISSR, the total number of bands was 30 with 10 primers, and the average number of bands was 3. The presence of monomorphic bands in both RAPD and ISSR assays showed that use of in vitro leaves for regeneration purposes was safe and there was no adverse effect of in vitro conditions and culture media, as both donor plants and cultures had been maintained in in vitro conditions for longer duration

Table 13. RAPD and ISSR primers amplified for in vitro raised plants from IVL explants

RAPD primer	Amplified bands (total no.)	Scorable bands (no.)	ISSR primer	Amplified bands (total no.)	Scorable bands (no.)
OPE-03	80	8	UBC-824	20	2
OPA-06	30	3	UBC-834	40	4
OPE-12	50	5	UBC-856	20	2
OPC-07	60	6	UBC-825	40	4
OPP-10	20	2	UBC-814	30	3
OPH-04	30	3	UBC-807	40	4
			UBC-810	20	2
			UBC-840	30	3
			UBC-842	30	3
			UBC-854	30	3
Total	**270**	**27**	**Total**	**300**	**30**

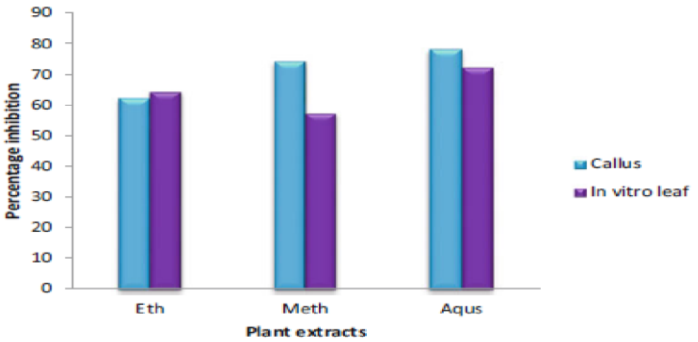
Source: Singh and Sharma, 2019

Most of the medicinal plants possess antioxidant potential and serve as good source of natural antioxidant. Therefore , pharmaceutical and scientific communities are concentrating on production of potential pharmaceuticals and natural antioxidants. Sometimes the medicinal plants fall under the category of threatened or endangered due to overexploitation of naturally grown of cultivated plants. In vitro culture can be an alternative source for the production of phytochemicals or metabolites without harming the natural population of the medicinal plants. Plant tissue culture makes the rapid multiplication of selected genotypes possible, allowing the useful metabolites to be collected in greater quantities (George *et al*., 2008).

In vitro cultures and plants are rich source of secondary metabolites. Production of secondary metabolites from in vitro cultures has a long history (Verpoorte *et al*., 2002). In vitro cultured plant cells synthesize, accumulate and sometimes exude different classes of secondary metabolites (Matkowski, 2008). Production of alkaloids, saponins, polyphenols, terpenes, anthraquinones, cadenolides, etc. have been reported from in vitro cultures and reviewed by several authors (Vanisree and Tsay, 2004; Matkowski, 2008). Although, in vitro regeneration method is costly, it offers several advantages such as simpler extraction and purification from interfering matrices, independence from climatic factors and seasons, avoiding loss of biodiversity and more control over biosynthetic routes for production of more desired metabolites (Matkowski , 2008). Various techniques of plant biotechnology such as cell culture, suspension culture, hairy root culture, etc. have also been used for the scaling-up of production of secondary metabolites, and sometimes bioreactors are also used for large-scale production of phytochemicals (Table 14). Metabolic engineering deals with the enhancement of producing certain metabolites within the cell by optimizing the genetic and regulatory processes. Some novel products have been found in in vitro culture, and quantity of compound can be increased or modified by altering the culture conditions. Various natural compounds and antioxidants have been produced from different in vitro plant materials such as leaf, callus, suspension culture, etc. in many plants .

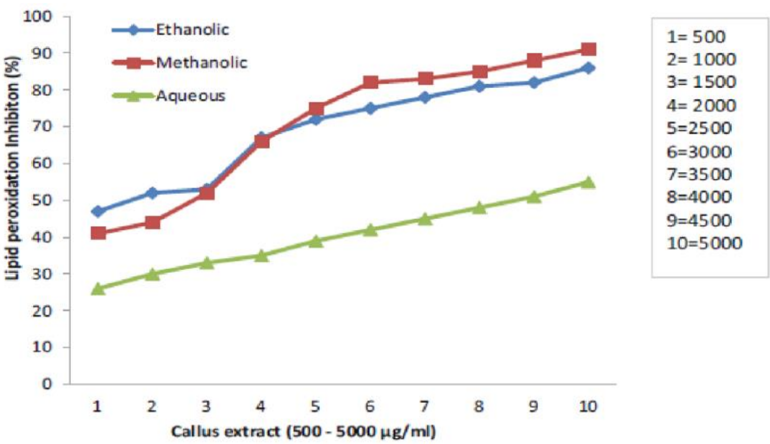
Table14. Methods used for estimation of different phytochemical activities.

Phytochemical / compound	Method	Standard used	References
Antioxidant potential	TLC	NA	Tepe *et al*. (2005)
	DPPH assay	Ascorbic acid	Yu *et al*. (2008)
	β -carotene/ linoleic acid bleaching assay	Ascorbic acid	Miller (1971)
Hydrogen peroxide scavenging capacity	Spectroscopic method	NA	Ruch *et al*. (1989)
Reducing potential	Spectroscopic method	Ascorbic acid	Nabavi *et al*. (2008)
Lipid peroxidation inhibition	TBARS	Ascorbic acid	Okhawa *et al*. (1979)
Total phenolic content	Folin-Ciocalteu assay	Gallic acid	McDonald *et al*. (2001)
Total flavonoid content	Spectroscopic method	Rutin	Zhu *et al*. (2010)



Source: Singh and Sharma, 2019

Fig 23. β - carotene / linoleic bleaching assay- based antioxidant activity

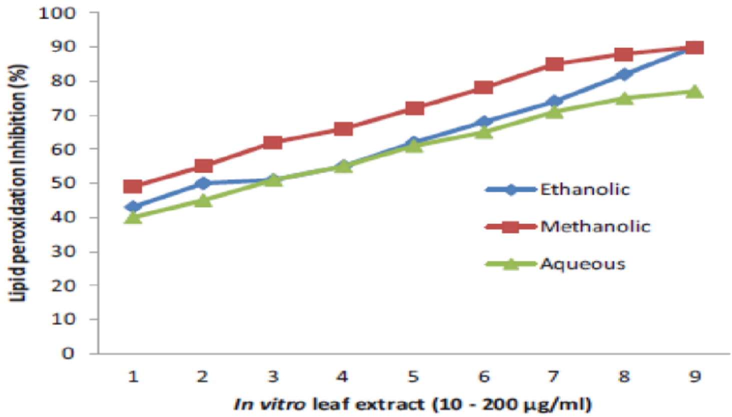


Source: Singh and Sharma,2019

Fig 24. Lipid peroxidation inhibition activity of callus and IVL extracts. (a) Callus extracts

While evaluating phytochemical activity in *S. mukorossi*, lower antioxidant potential was observed in callus extracts than in vivo material (leaf and fruit) extracts. (Singh and Sharma, 2019). In their study on *S. mukorossi*, β-carotene/linoleic acid method (Fig 23) was used to estimate the antioxidant potential to inhibit lipid peroxidation

(Lai and Lim, 2011) . Both callus and IVL extracts showed the presence of significant antioxidant activity. In callus extract (Fig 24), aqueous extract showed highest percentage inhibition (78.44%) than ethanolic (62.57%) and methanolic (74.40%). In IVL, highest percentage inhibition was shown in methanolic extract (72.84%), whereas percentage inhibition in aqueous (57.20%) and ethanolic (64.95%) was comparatively low(Fig 25). Similar experiments were carried out to determine reducing potential (RP), Lipid Peroxidation(LPO)Inhibition activity, total phenolic content and total flavonoid content of all extracts of callus and IVL.



Source: Singh and Sharma, 2019

Fig 25. Lipid peroxidation inhibition activity of callus and IVL extracts. (b) IVL extracts

14.6. Nano particles Synthesis and Nanotechnological applications

Nanotechnology promises with the synthesis and characterization of nanoparticles of desired shape and size by modulating of the materials up to the nanoscale. A wide range of applications of nanoparticles in the various fields promote the synthesis of nanoparticles (NPs). Work on this aspect with reference to *Sapindus mukorossi* has been thoroughly reviewed by Singh and Sharma, 2019 and is reproduced here in. Nanoparticles are continuously used in the field of health care, biosensors, biotechnology, electronics, etc. (Mehata, 2015; Ratnesh and Mehata, 2015). Green synthesis method is one of the most favoured methods for NP synthesis that is used in the health-care sector. Green synthesis method has been used for NP synthesis from plants (Makarov *et al*., 2014; Mittal *et al*., 2013), bacteria, fungus, yeasts (Narayanan and Sakthivel, 2010) and DNA (Sohn *et al*., 2011).

Nanoparticle characterization is a foremost stage to augment the nanoparticles synthesis. Suitable shape and size of nanoparticles improve the efficiency of activity. Several techniques are available for characterization of nanoparticles.

Different parts of *S. mukorossi* have been used for the synthesis of the NPs using various materials. Ramgopal *et al*. (2011) synthesized the silver NPs using the fruits of this plant. They evaluated the antimicrobial activity of these silver NPs against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Serratia marcescens* and found the significant antimicrobial activity. Aqueous extract of *S. mukorossi* fruit's pericarp was used as reducing agent as well as stabilizer for synthesis of silver nanoparticles. This silver colloid showed antibacterial activity against *Bacillus subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. This Ag colloid has also been evaluated for the degradation of 4-nitrophenol and Eosin blue (common environmental pollutants) (Dinda *et al*., 2017). Porchezhiyana and Noorjahan (2016) prepared the chitosan nanocomposite from silver nanoparticles of fruit pericarp of this plant. They standardized the formation of hydrogels and also found the significant antibacterial activity against *E. coli* and *S. aureus*. Reddy *et al*. (2013) synthesized gold NPs (AuNPs) using fruit pericarp extract of this species. They investigated the catalytic activity for reduction of p-nitroaniline. The fruit pericarp of *S. mukorossi* has also been used for the synthesis of platinum NPs (Kumar *et al*., 2017). Manganese NPs have been prepared using the fruits (Jassal *et al*., 2016). Palladium NPs from aqueous extract of *Sapindus mukorossi* seed have been synthesized by Borah *et al*. (2017).

14.7. Scope for future research and development

There is a long list of saponins present in *Sapindus mukorossi*. It needs individual attention so that they can be explored in different pharmacological studies. There is also a need for much additional research regarding pharmacological effects of *Sapindus mukorossi* at molecular level to explain their mode of action.

15. Major insect pests and diseases

15.1. Insect pests

Two insect species are known to attack this tree. *Aulacaspis orientalis* and *Lecanium* (*Eucalymnatus*) *tesselatum*, which feed on sap of foliage, the latter feeding on foliage of branches as well.

Devanda and Jayashankar (2017) observed different life stages of *Tassaratoma. Javanica* (Fig 25) , a minor pest of Litchi on two trees of *Sapindus mukorossi* , one in the APRC/ZSI (Arunachal Pradesh Regional Centre of Zoological Survey of India) office premises (Site 1) and the other tree in the APRC residential quarters (Site 2). About ten adults and three nymphs were recorded during May 2017 in Site 1 and thirty three adults and eight nymphs were counted during August 2017 in Site 2. Adults and nymphs were seen gregariously and voraciously sap-sucking from tender shoots. The bugs congregated in large numbers during breeding season on host plants. Mating pairs (males smaller than females, Fig 26) and also individuals of different nymphal stages (Fig 27) were observed on the tree.

The host *S. mukorossi* is reported to possess insect repellent properties (Rahman *et al.*, 2007). Most insect crop pests are specialists preadapted to feed on plants, often within a single plant family and feeding more widely only when preferred hosts are unavailable (Capinera, 2005). However, this aspect needs to be studied in details.

Source: Devanda and Jayashankar, 2017

Fig 26. ***Tesaratoma javanica*** **adult on** ***Sapindus mukorossi*** **and its mating pair**

Source: Devanda and Jayashankar, 2017

Fig 27.Nymphal stages and Drying of new shoots due to bug infestation

Timber is susceptible to attack by insect borers, the powder-post beetle, *Lyctus brunneus* has been identified in Korea. The tree is also attacked by a powdery mildew, *Uncinula sapinda* (Orwa *et al*., 2009)

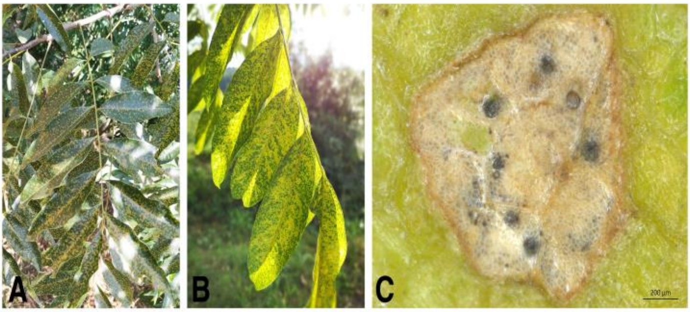
Host of Pests Recorded

Sapindus mukorossi is reported (https://doi.org/10.1079/ cabicompendium.48326) to be a host of the following pests:

Aleurocanthus woglumi (citrus blackfly), *Ceroplastes japonicus* (tortoise wax scale), *Gymnandrosoma aurantianum* (citrus fruit borer) and *Trichothecium roseum* (fruit rot of tomato)

15.2. Diseases

In recent years, leaf spot disease on soapberry has been observed (Si *et al*., 2022) frequently in a soapberry germplasm repository in Jianning County, Sanming City, Fujian province, China. Symptoms occurred from the summer to fall and initially appeared as irregular, small, yellow spots, and the centres of the lesions became dark brown with time (Fig 28). Pycnidia were observed on the lesions . The same fungus was isolated in 73% of the samples, and three isolates (FJ15, FJ16, and FJ17) were obtained. Koch's postulates were performed, and their pathogenicity was confirmed. Morphologically, α-conidia from diseased tissues were single-celled, hyaline, smooth, clavate or ellipsoidal, and biguttulate, measuring 6.2 to 7.2 × 2.3 to 2.7 µm. In addition, the three isolates in this study developed three types (α, β, and γ) of conidia on potato dextrose agar, and their morphological characteristics matched those of *Diaporthe*. A phylogenetic analysis based on internal transcribed spacer, TEF, TUB, HIS, and CAL sequence data determined that the three isolates are a new species of *Diaporthe*. Based on both morphological and phylogenetic analyses, the causal fungus, *Diaporthe sapindicola* species was described and illustrated.



Source: Si *et al*., 2022

Fig 28. Leaf spot disease on soapberry

All isolates in this study were maintained in the Forest Pathology Laboratory at Nanjing Forestry University and deposited at the China Forestry Culture Collection Center (CFCC), Chinese Academy of Forestry, Beijing, China. The fungal pathogen is new to science and described as *D. sapindicola* based on morphological and phylogenetic studies.

In another incidence of disease occurrence reported by Tang *et al*.(2016), affected trees were approximately 10 to 15 years old and approximately 20% exhibited longitudinal cracking on the trunk, with cracks ranging from 50 to 150 cm in length. Mycelia and pink spores occasionally were observed on cross sections of the trunk, while no symptoms were found on the tree branches. In most cases, the foliage of affected plants was yellowish and had fewer new shoots than the previous year. To isolate the causal organism, fragments (1 mm^2) of tissue were excised from lesions on the symptomatic trunks and surface disinfested by sequential dipping in 1.5% sodium hypochlorite for 2 min, rinsed in sterile water, and cultured on 2% potato dextrose agar (PDA) amended with streptomycin sulfate (0.1g/liter). Plates were incubated at 25°C under a 16-h/8-h light/dark cycle for 7 days. Morphologically similar fungal isolates were recovered (*n* = 16) that produced salmon-colored fungal colonies with hyaline hyphae, simple or branched conidiophores, and conidia produced in basipetal chains. Conidia were ellipsoidal or obpyriform. Almost all spores were two-celled and single-septate (Dong *et al*., 2013). Conidia measured 14.9 to 18.6 µm long × 7.2 to 11.3 µm wide (*n* = 50). These characteristics are consistent with the description of *Trichothecium roseum* (Inácio *et al*., 2011). Isolate HF-27 was selected for molecular identification by amplification and sequencing of the ribosomal DNA internal transcribed spacer (rDNA-ITS) using the universal primer pair ITS4 and ITS5. Sequencing of the PCR product revealed a 100% similarity with the ITS sequences of *T. roseum* in GenBank (EU552162.1, JQ434579.1, and JN942882.1). The sequence of isolate HF-27 was deposited in GenBank (Accession Nos. KT899886). Pathogenicity tests were performed by inoculating wounded trunks of healthy 15-year-old *S. mukorossi* with isolate HF-27. Twenty trees were inoculated by placing a 5-mm^2 agar plug from the growing margin of 5-day-old colonies upside down directly into a fresh wound made with a scalpel to a depth of 5 mm, and the inoculated wounds were sealed with Parafilm. Twenty plants that served as negative controls were mock-inoculated with a sterile PDA plug without mycelial growth. After 30 days, all of the inoculated plants developed symptoms similar to natural infections of *T. roseum*, whereas control plants remained symptomless. *T. roseum* was reisolated from pathogen-inoculated trees but not from control trees, fulfilling Koch's postulates. *T. roseum* is reported as a pathogen on apple (Žabka *et al*., 2006) and is also known as a pathogen on Chinese cantaloupe in China (Tang *et al*., 2016). This was perhaps the first report of *T. roseum* as a pathogen on *S. mukorossi* in China. Because the disease directly decreased the ornamental value of *S. mukorossi*, appropriate management measures were suggested to be taken in that region.

16. Conclusion and way forward

Sapindus mukorossi is a tropical tree widely distributed in warm temperate and tropical regions of Asia. It is an important multi-functional agroforestry tree species with ecological, economic and social benefits. This famous landscaping species has been recorded in "Compendium of Materia Medica" because of its enormous medicinal uses.

Studies on chemical investigation and pharmacological evaluation have shown that *Sapindus mukorossi* contains a number of bio-active novel compounds which need detailed chemical analysis for isolation and also for studying their biological activities. The methanolic extract of *S. mukorossi* leaves contains many bioactive compounds, including alkaloids, flavonoids, phenols, carbohydrates, terpenoids, and saponins. The stems also include flavonoid, phenolic, and polysaccharide constituents . A large amount of saponins, amounting to about 10.1–11.5% of the fruit, are present in the pericarp , where this value increases to 56.5% in the drupe . The fruit also contains about 10% sugars, mucilage , and sesquiterpene oligoglycosides. Kernel mass consists of 40% oil, which is a mixture of medium-chain monounsaturated and polyunsaturated fatty acids, mostly of oleic and linoleic acid, respectively, along with triglycerides. Roots, flowers, and galls are also a source of triterpenoid saponins . The plant is grown for its fruit, the pericarp of which is used as a natural soap. Other parts of the plant are also used for many other purpose. The seed kernel oil of *Sapindus mukorossi* is a potential biodiesel material. This plant can play an important role in modern medical system in near future. Saponins isolated from *S. mukorossi* as alternative sources of active compounds are constantly being sought as a substitute for synthetic drugs.

In India, the tree needs to be widely cultivated in most of the areas where climatic conditions favour its optimum growth. Need of the hour is to make available the quality planting material of *S. mukorossi* -a plant of numerous economic applications, for it successful inclusion in the social forestry, agroforestry or forestry plantation programs. Substantial studies are urgently required to design regeneration techniques employing various explants. Furthermore, the advanced biotechnological tools have certainly opened up a new area for advanced genetic/molecular interventions that can lead to high level of medicinal and non-medicinal applications in future. In this way, a maximum yield of its different usable parts can be achieved to derive the maximal amount of commodities of a multifarious nature for the welfare of mankind. *S. mukorossi* is an ideal tree species for developing forestry bioenergy and multiple other products. Additional research is required on the natural adaptive diversification of *Sapindus* .

17. Institutes working on *Sapindus mukorossi*

Sapindus mukorossi is an important tree species with multiple ecological, environmental, social and economic benefits and is widely distributed in warm temperate to tropical regions in Asia, especially China and Southeast Asian countries. In addition to its medicinal use, the plant has gained immense importance as an industrial raw material and forest bio-energy. Various research and developmental activities are being performed by various institutes (Table 15) across the world.

Table 15. Institutes working on *Sapindus mukorossi*

S. No.	Agency/Organization/Institute	Location
	National	
1	Arunachal Pradesh Regional Centre/Zoological Survey of India, Senki Valley, Itanagar,	Arunachal Pradesh, India
2	Central Drug Research Institute	Lucknow, India
3	Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences and Allied Hospitals, Kanchanbagh	Hyderabad, India
4	CFTRI, Mysore	India
5	Council of Scientific and Industrial Research (CSIR) New Delhi	New Delhi India
6	Cytology and plant tissue culture laboratory, Department of life science, Tripura university,	Agartala, India
7	DDU Gorakhpur University	Uttar Pradesh, India
8	Department of Atomic Energy, Board of Research in Nuclear Science	India
9	Department of Biochemistry, University of Allahabad	Allahabad, India
10	Department of Biotechnology, St. Peter's Engineering College	Chennai, India
11	Department of Botany, S. V. University, Tirupati	India

13	Department of Chemical Sciences, Tezpur University	Assam, India
14	Department of Chemistry, Government General Degree College, Singur	West Bengal, India
15	Department of Chemistry, H.N.B. Garhwal University, Srinagar	Uttarakhand, India.
16	Department of Chemistry, Hemwati Nandan Bahuguna Garhwal University	Uttarakhand, India
17	Department of Energy, Tezpur University	Napaam, Assam, India
18	Department of Food Technology & Biochemical Engineering, Jadavpur University	India
19	Department of Forestry, Garhwal University, Srinagar Garhwal,	Uttarakhand, India
20	Department of Horticulture and Agroforestry CSK HP Agriculture University,	Palampur, Himachal Pradesh, India
21	Department of Humanities & Science, Raghu Institute of Technology	Visakhapatnam, India
22	Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical Education and Research	Punjab, India
23	Department of Pharmacology, C. L. Baid Metha College of Pharmacy,	Chennai, India.
24	Department of Science and Technology, Government of India, New Delhi	India
25	Department of Science and Technology, India	Tamil Nadu, India
27	Department of Science and Technology, New Delhi	Uttar Pradesh, India
28	Department of Tree Improvement and Genetic Resources, College of Forestry, Dr Y. S. Parmar University of Horticulture and Forestry, Solan	Himachal Pradesh, India
29	G.B. Pant Institute of Himalayan Environment and Development, Pantnagar.	India
30	Himalayan Institute of Pharmacy and Research, Dehradun	Uttarakhand, India

31	ICAR-Central Agroforestry Research Institute, Jhansi	Uttar Pradesh, India
32	Indian Council of Agricultural Research	India
33	Indian Council of Forestry Research & Education	Dehradun, India
34	L.M. College of Pharmacy	Ahmedabad, Gujarat, India
35	Laser-Spectroscopy Laboratory, Department of Applied Physics, Delhi Technological University	Delhi
36	National Institute of Immunology	New Delhi, India
37	Numaligarh Refinery Ltd.	Assam, India
38	Pacific College of Pharmacy, Udaipur	Rajasthan, India
39	Plant Pests and Diseases Research Institute, Samastipur	Bihar, India
40	Sri Venkateswara University, Tirupati, India	Andhra Pradesh, India
41	UHF Regional Horticultural Research Station, Jachh	Kangra, Himachal Pradesh, India
42	Yogi Vemana University, Kadapa	Andhra Pradesh, India
43	Herbal Research and Development Institute,	Uttarakhand, India
	International	
1	Department of Biotechnology, Faculty of Natural Sciences, University of Tirana	Albania
2	Department of Biology, College of Science, King Khalid University, Abha	Saudi Arabia
3	Department of Medicinal Chemistry, Qassim University	Saudi Arabia
4	CSIRO, Plant Industry	Canberra, Australia
5	School of Science and Technology, Charles Sturt University, New South Wales.	Australia
6	Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University	Belgium
7	Universidade Estadual de Maringá	Brazil
8	Universidade de Brasília	Brazil

9	Plant Protection Institute, Kostinbrod	Bulgaria
10	Faculty of Engineering, Dalhousie University	Canada
11	College of Forestry, Beijing Forestry University, Beijing.	China
12	China Institute of Communications	China
13	Chinese Academy of Forestry	China
14	Huazhong Agricultural University	China
15	College of Light Industry and Food Sciences, South China University of Technology, Guangzhou.	China
16	Department of Life Science, Chinese Culture University, Taipei, Republic of China	China
17	Department of Science and Technology of Guangxi Zhuang Autonomous Region	China
18	Institute for Food and Drug Control (SIFDC) China	China
19	Ministry of Education Key Laboratory for Silviculture and Conservation, Beijing Forestry University, Beijing.	China
20	National Energy R & D Center for Non-Food Biomass, Beijing Forestry University	Beijing, China
21	National Natural Science Foundation of China	China
22	National Research Institute of Chinese Medicine	Taiwan, China
23	Natural Science Foundation of Jiangxi Province of China and Health Commission of Jiangxi Province	China
24	Research Institute of Subtropical Forestry,	China
25	Chinese Academy of Forestry, Fuyang	China
26	Chinese Academy of Sciences	China
27	Zhejiang Forestry Academy	China
28	Beijing Forestry University	China
29	Fujian Normal University,	China
30	Nanjing Forestry University	China
31	Wuhu Institute of Technology	China

32	Nanjing Vocational Institute of Transport Technology	China
33	College of Forestry and Biotechnology	China
34	Beijing Forestry University	China
35	SFA Key Laboratory of Bamboo and Rattan Science and Technology, International Centre for Bamboo and Rattan	China
36	State Key Laboratory of Phytochemistry and Plant Resources in West China	China
37	The School of Chinese Medicine, the Chinese University of Hong Kong.	China
38	Yuanhua Forestry Biotechnology Co., Ltd.	China
39	University of South Bohemia	Czech Republic
40	University of Copenhagen	Denmark
41	Royal Veterinary and Agricultural University, Copenhagen	Denmark
42	Senckenberg Biodiversity and Climate Research Centre	Germany
43	School of Energy, Geoscience, Infrastructure and Society, Institute for Infrastructure & Environment	Edinburgh
44	Université de Strasbourg	Strasbourg, France
45	Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences.	Iran
46	National University of Ireland	Galway, Ireland
47	Department of Biological Production Science	Japan
48	Institute of Biochemistry, Faculty of Medicine, University of Nagoya	Japan
49	Science University of Tokyo	Japan
50	TSBF-CIAT, Nairobi	Kenya
51	Center for NanoBioEngineering & SpinTronics (nBEST)	South Korea
52	Department of Chemical and Biochemical Engineering, Chosun University	Gwangju, South Korea

53	Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries	Korea
54	Julius Kühn-Institute and Luxembourg Institute of Science and Technology	Luxembourg
55	Monash University Sunway	Malaysia
56	Universiti Teknologi PETRONAS	Malaysia
57	University of Malaya, Kuala Lumpur	Malaysia
58	School of Engineering, University of Wollongong Malaysia KDU,	Malaysia
59	Faculty of Physical-Mathematical Sciences, Autonomous University of Nuevo León	Mexico
60	Centre for Genetic Resources	Netherlands
61	Division of Pharmacognosy, Leiden	Netherlands
62	Nature Conservation and Plant Ecology Group, Wageningen University	Netherlands
63	University of Toyama	Nepal
64	Central Department of Botany, Tribhuvan University	Nepal
65	National Herbarium and Plant Laboratories	Nepal
66	Center for Biological Conservation,	Nepal
67	Agriculture and Forestry University	Nepal
68	Department of Biochemistry Quaid-i-Azam University Islamabad	Pakistan.
69	Department of Botany, Islamia College Peshawaz.	Pakistan
70	Department of Weed Science, NWFP Agricultural University	Peshawar, Pakistan
71	Higher Education Commission of Pakistan	Pakistan
72	Department Pharmaceutical Biology, Medical University in Wroclaw	Poland
73	Faculty of Chemical Engineering and Technology, Cracow University of Technology, Warszawska Cracow	Poland
74	Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University	Russia

75	Nanyang technological university, Singapore	Singapore
76	Faculty of Management, University of Primorska, Cankarjeva, Koper	Slovenia
77	Spanish National Institute for Research in Agricultura	Spain
78	National Institute of Standards and Technology, Materials Measurement Science Division	United States
79	Nelson Institute for Environmental Studies	United States
80	Department of Cosmetic Science, Providence University	Taiwan
81	Institute of Biotechnology, Chaoyang University of Technology	Taiwan
82	National Science Council of Taiwan	Taiwan
83	Research Center for Circular Economy, Chung Yuan Christian University	Taiwan
84	Department of Biology, Faculty of Science and Literature, Cumhuriyet University, Sivas.	Turkey
85	Department of Medical Microbiology, Faculty of Medicine, Uludağ University	Turkey
86	Department of Medical Microbiology, Faculty of Medicine, Uludağ University	Bursa, Turkey
87	Royal Botanic Gardens, Kew	London (UK)
88	Department of Neurobiology and Behavior, Cornell University	USA
89	University of California	USA
90	Department of Pathology, Medical College of Ohio	Toledo, USA

18. Major funding agencies

Development of a species is feasible only if a detailed basic and advanced research is carried out on its different aspects and for that the foremost requirement is of the financial grant. There are many national and international agencies (Table 16) funding research and developmental activities on *Sapindus mukorossi*.

Table 16. Major funding agencies for *Sapindus mukorossi* R & D

S. No	Agency/Organization	Country
	National	
1	Central Agroforestry Research Institute, Jhansi	India
2	Herbal Research and Development Institute, Uttarakhand	India
3	Indian Council of Forestry Research & Education (ICFRE)	India
4	Ministry of Human Resource Development (MHRD)	India
5	National Medicinal Plants Board, New Delhi	India
	International	
1	Canadian Center for International Studies and Cooperation (CECI)	Montreal, Canada
2	Council of Agriculture and National Science Council of ROC	China
3	Department of Plant Resources (DPR), Ministry of Forest and Soil Conservation,	Nepal
4	Fundamental Research Funds for the Central Universities	China
5	Guangdong Provincial Science and Technology Plan Project	China
6	International Center for Integrated Mountain Development (ICIMOD),	Nepal
7	National Energy R&D Center for Non-food Biomass	China
8	National Natural Science Foundation of China	China
9	Research Committee for Development Research (FFU) of the Danish Ministry of Foreign Affairs, Denmark	Denmark
10	Special Funding Project of Fujian Provincial Department of Finance, China	China

11	The Czech Ministry of Education	Czechoslovakia
12	The Department of Plant Resources and the Nepal Country Office of the World Conservation Union	Nepal
13	The Industry-University Cooperation Project of Fujian Science and Technology Department, and the Minjiang Scholar Program of China	China

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