A Training Manual on the Conservation of Biodiversity in Sacred Groves

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CONTENT

PREFACE

Conservation of nature has been considered as a magnificent piece of social ethos in several indigenous communities habited in various parts of the world, including India. The venerate for nature began in pre-vedic period (5000 B.C) leading to the emergence of Sacred groves and *Sthala-vrikshas*. Tamilians in particular considered trees as holy, believing the residence of spirits, divine beings. Sacred groves symbolize a harmonious amalgam of spiritual, cultural, and environmental stewardship, rendering them a distinctive and crucial part of mankind. They are also regarded as rich biodiverse pockets and the best network situated case of biodiversity preservation, showing the way how community is attached to nature. The sacred groves are a socio-ecological production landscape (SEPL), a repository for various ayurvedic medicines and enriched with replenishable resources like fruits and honey. Unfortunately, a serious erosion of the canopy in the groves of Tamil Nadu has been observed for the past 20 years. The rapid modernization and rampant encroachments have significantly impacted religious beliefs and social values which led to the unfettered human interference of these sacred places for wood, agricultural land, and other resources. The need of the hour is to conserve and restore these resources in cooperation with the local community.

The Malaiyali Sacred Groves in Kalrayan hills of Tamil Nadu are currently in the verge of disruption due to the unhindered human interference for land, wood, and many other resources. The sacred groves in Kalrayan Hills are rich in biodiversity with several medicinal and edible flora. As per the reports, a total of 2064 stems (mean 516 ha^{-1}) covering 89 species (in 74 genera and 39 families) were present in Kalrayan hills. Species richness varied from 42 to 47 species ha^{-1}, Shannon index from 2.31 to 2.87, and stand density from 367 to 667 stems ha^{-1} was also stated. Massive deforestation has abridged the forest area from 481.7 and 266.5 sq.km during 2000s. The illegal felling, forest fire, colossal exploitation of farmlands, and forest resources were speculated as the reasons behind the decline in forest area.

It is also foremost to consider the livelihood of the hundreds of Malaiyali community people residing in Kalrayan Hills. Tribal groups in Kalrayan Hills are mainly involved in economic pursuits ranging from hunting to farming and other industrial daily wage work. Forest and the resources build and nurture their spiritual, economic, and social development. Unfortunately, the socioeconomic condition of the tribal groups is critically low with poor literacy rates, infrastructural and social services including road, communication, sanitation, and health amenities (Jayakumar et al., 2016). Hence a collaborative initiative was adopted for the conservation of these valuable resources along with sustainable development of the Malaiyali tribes through our IPSI-SDM project. We developed a holistic approach involving the mapping and estimation of threatened species in the Kalrayan hills, followed by their in-vitro and in-situ conservation. The strategies pursued are presented in the training manual to aid botanists, environmentalists, conservation biologists, plant biotechnology experts.

The module 1 titled ‘Mapping medicinal plant diversity in sacred groves’ discusses the approaches to document and map the diversity of medicinal plants within the sacred groves, highlighting their ecological, cultural, and economic significance. The module 2 called ‘Determining Threatened Medicinal Plants In Sacred Groves Using The Localized Conservation Priority Index (Lcpi)’ put forward the strategies to determine the identity of threatened plants at the local level and their levels of priority and how to geotag the locally threatened plants in their localities of occurrences. The third module ‘*In Situ* Conservation Techniques for Threatened Medicinal Plants in Sacred Groves’ discourses the strategies to conserve the identified threatened species in their sites with the aid of local community. The final module titled ‘In vitro conservation techniques for threatened medicinal plants’ explains the scientific practices to conserve medicinal plants in optimized laboratory conditions.

This manual can be revised timely to suit the geological contexts. Students, researchers, and practitioners are welcome to provide their constructive insights to improve the manual. I hope to train as many professionals as possible to help in the study of treasured sacred groves in the world.

I would like to convey my gratitude to Institute for Global Environmental Strategies (IGES), the United Nations University Institute for the Advanced Study of Sustainability (UNU-IAS) and the Ministry of the Environment of Japan (MOEJ) for funding the project entitled ’Developing a sustainable socio economy through the restoration of Sacred groves of Malaiyali community in Kalrayan hills of South India’. I acknowledge the management of Vellore Institute of Technology for their immense support in the implementation of the project and the preparation of modules. I thank and appreciate the co-authors Dr. Inocencio E Buot, Jr., University of the Philippines Los Banos, Dr. Usha S, SSL, Vellore Institute of Technology and Dr. Rajasekaran C, SBST, Vellore Institute of Technology for their invaluable contributions in devising the training manual.

Dr. Siva R
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GENERAL INTRODUCTION

Sacred groves are ecologically rich forest patches, traditionally conserved by local communities through deep cultural, spiritual, and ecological significance (Kumar et al., 2022). These groves are often linked to religious beliefs, myths, and rituals, which have played a pivotal role in their preservation for centuries (Kent, 2009; Mequanint et al., 2020; Kossi et al., 2021). Considered sacred and protected from exploitation, they serve as invaluable reservoirs of biodiversity, providing habitat for a wide variety of flora and fauna, including many species with significant medicinal properties. The traditional healthcare systems of many Indigenous communities heavily rely on the medicinal plants found within these groves, highlighting their crucial role in sustaining human health and well-being (Imarhiagbe & Ogwu, 2022).

Studies across the globe have demonstrated that sacred groves often harbour greater floral diversity compared to managed forests, playing a crucial role in biodiversity conservation and contributing to the ecological stability of their surroundings (Khumbongmayum et al., 2006; Pradhan et al., 2019). These groves serve as refugia for indigenous, rare, and medicinally significant plant species, as well as diverse fauna, providing a rich source of wild cultivars and genetic diversity essential for ecological resilience (Aboli Kulkarni et al., 2018; Khumbongmayum et al., 2006). Moreover, the widespread distribution of sacred groves, ranging from micro-sized patches of 0.0001 hectares to expansive areas exceeding 26,326 hectares, underscores their critical role in conserving biodiversity across diverse ecosystems (Bhagwat & Rutte, 2006; Khan et al., 2008). The biodiversity within these groves is shaped by a range of microenvironmental factors such as soil composition (Arunachalam et al., 1999; Arunachalam & Arunachalam, 2000), litter dynamics (Rajendraprasad et al., 2000), habitat variability, geographic location (Jamir & Pandey, 2003), and traditional conservation practices (Kossi et al., 2021).

The Kalrayan Hills, part of the Eastern Ghats in Tamil Nadu, India, exemplify the ecological and cultural significance of such sacred landscapes (Kent, 2009). This region is a hotspot of biodiversity, harbouring a wide array of flora and fauna, including numerous endemic, rare, and medicinally important plant species. These hills are particularly noted for their rich repository of medicinal plants, which are integral to traditional healthcare practices. Conservation of the sacred groves of Kalrayan Hills is vital for protecting their unique biodiversity and preserving the traditional knowledge systems and cultural heritage of the indigenous communities that depend on them.

Fig. 1 Medicinal plant diversity in Sacred groves

TRAINING MODULE 1

MAPPING MEDICINAL PLANT DIVERSITY IN SACRED GROVES

Introduction

Mapping medicinal plant diversity in sacred groves is a crucial step toward understanding and conserving plant resources that hold ecological, cultural, and economic significance (Batame et al., 2023). While global assessments like those by the IUCN and national Red List committees provide valuable insights, they often do not capture localized biodiversity patterns (Díaz et al., 2019). Thus, community-based approaches that focus on sacred groves are known to harbor rare, endemic, and culturally significant species are essential for conservation planning at the local level (Parthasarathy et al., 2020).

Sacred groves, deeply rooted in cultural and spiritual traditions, serve as natural reservoirs of biodiversity and are often rich in medicinal plants used by indigenous and local communities (Imarhiagbe & Ogwu, 2022). Identifying and mapping these plant species can provide essential data for conservation initiatives, local resource management, and sustainable utilization. By engaging local communities in mapping medicinal plants, this module seeks to empower them with knowledge on their biological heritage while supporting conservation efforts (Chathavong and Buout, 2019).

Few studies have systematically documented medicinal plant diversity within sacred groves. Through a participatory and scientific approach, this module will guide trainees in conducting a structured inventory and mapping of medicinal plant species in sacred groves, ensuring that conservation efforts are based on ecological and socio-cultural priorities. By integrating traditional ecological knowledge with scientific mapping techniques, this module aims to enhance conservation strategies and ensure the sustainable use of medicinal plant resources in sacred groves.

Objectives

1. To document and map the diversity of medicinal plants within the sacred groves, highlighting their ecological, cultural, and economic significance.
2. To map the locations of medicinal plants in sacred groves to help with conservation and management.

Activity

1. **Identifying and documenting the medicinal plants in the sacred groves**
    - 1.1. Get hold of the list of plant species found in the sacred grove.
    - 1.2. Identify medicinal plants from the list and assess their significance based on ecological importance (rarity, role in the ecosystem), cultural value (traditional uses, spiritual significance) and economic value (medicinal trade, local livelihoods).
    - 1.3. Record the observations in a data sheet, noting plant names, characteristics, and uses.
2. **Mapping medicinal plant diversity in sacred groves**

    The following method provides a structured approach from mapping medicinal plant diversity in sacred groves (Adapted from Rawat et al., 2004) . This process ensures a systematic inventory and spatial documentation of medicinal plants for conservation and management.

2.1 Reconnaissance Survey

2.1.1 Identify the administrative and ecological zones within the sacred grove.

2.1.2 Develop a preliminary checklist of medicinal plants used locally and commercially with the help of local communities or forest staff.

2.1.3 Record key environmental features such as forest types, existing trails, and overall grove size.

2.2 Stratification of the Sacred Grove

2.2.1 Divide the survey area into smaller natural units (strata) based on key factors such as Landform and terrain, altitude and vegetation type and human disturbances (e.g., grazing, collection, fire). This stratification helps to ensure a balanced and representative sampling process.

2.3 Selecting and marking of the Transect/Trail

2.3.1 Establish the sample locations along trails or transects within each stratum. In rugged or hilly terrains, use existing paths or the least disturbed trails to ensure accessibility.

2.4 Laying out sample plots

2.4.1 The length of transects depends on the size of the grove. Typically, a 1 km transect is laid out within a 5 km^2 forest patch.

2.4.2 Circular plots (10m radius) are marked at 50m intervals on either side of the transect, avoiding 10-15m buffer zones to minimize disturbance.

2.4.3 Ensure that plots are placed systematically to cover different habitat types within the grove.

2.4.4 Record the total number of plots for reference.

2.5 Collection of Data

2.5.1 Record all medicinal plants (trees, shrubs, climbers, and herbs) within each sample plot.

2.5.2 Collect additional environmental data including

- Altitude and aspect
- Associated plant species
- Topography and presence of perennial water sources
- Human pressure (e.g., grazing, uprooting, fire incidents)

2.5.3 Measuring Frequency (How often a species appears in plots)

Record Presence per Plot

- For each sample plot, list down the medicinal plant species found.
- Mark the species as “present” or “absent” in each plot.

Calculate Frequency (%)

$$Frequency\ (\%) = \frac{(Number\ of\ plot\ where\ species\ is\ present)}{Total\ number\ of\ sample\ plots} \times 100$$

2.5.4 Measuring Density (Number of individuals per plot)

Count Individuals in Each Plot

- Count all individual plants of the target species within each sample plot.
- Record the total number of individuals for each species.

Calculate Density (Individuals/m^2)

$$Density\ (Individuals/m^2) = \frac{(Total\ number\ of\ individuals\ of\ the\ species)}{Total\ sample\ plot\ area}$$

2.5.5 Measuring Cover Percentage (For spreading or mat-forming species)

Estimate the Area Covered by the Species

- Identify all patches of the species within the plot.
- Measure the total area occupied by the species using quadrats or visual estimation.
- Calculate Cover Percentage

$$Cover\ Percentage = \frac{(Total\ Area\ Covered\ by\ Species)}{Total\ Sample\ Plot\ Area} \times 100$$

2.6 Data Analysis and Interpretation

2.6.1 Assess medicinal plant presence, abundance, and distribution using the following measures:

- Frequency (how often a species appears in plots)
- Density (number of individuals per plot)
- Cover percentage (for spreading or mat-forming species)

2.6.2 Use collected data to map and monitor medicinal plant populations within sacred groves over time.

2.6.3 Mean cover values of individual species per transect can be used for spatial mapping and conservation planning.

2.6.4 Interpretation of Data

- Identify high-frequency species (indicating widespread distribution).
- Compare density values to assess species abundance.
- Use cover percentage to determine the extent of ground coverage by mat-forming species.

2.6.5 Use data for Conservation Planning

- Prioritize species with low frequency, low density, or declining cover for conservation efforts.
- Identify species under threat from overharvesting or habitat disturbance.
- Map species distribution using GIS tools.

2.7 **Reporting and discussion**

2.7.1 Prepare a Summary Report

- Include key findings on medicinal plant distribution and abundance.
- Highlight species that need urgent conservation measures.

3. Mapping the locations of medicinal plants

3.1 Setting up the GPS Device

3.1.1. Ensure your GPS device is turned on and ready to record locations. Ensure it is functioning properly. Devices like Garmin GPS (2025) or Magellan GPS (2025) are popular for field use (https://ph.garmin.com) (https://www.magellangps.com)

3.2 Geotagging the medicinal plants

3.2.1 Walk to the medicinal plants and stand at its base. Record the latitude and longitude displayed on the GPS screen. Save the coordinates as a waypoint or write them down in a field notebook.

3.2.2 Move to the next medicinal plant and repeat the geotagging process. Ensure all the selected plants in the sacred groves are recorded.

3.3 Transferring and Visualizing the data

3.3.1 Transfer the GPS coordinates to a computer using a USB cable or GPS software. Input the data into a spreadsheet or mapping tool like Google Earth or GIS software.

3.3.2 Create a digital map to visualize the distribution of medicinal plants in the sacred grove (Optional).

The frequency and density of each medicinal plant species are calculated, while for spreading and mat-forming species, percent area coverage on the ground is a more effective measure of availability. Mapping and monitoring are more reliable when these measurements are taken within fixed plots. The mean cover of individual species is estimated for each transect to support spatial mapping and conservation efforts.

TRAINING MODULE 2

DETERMINING THREATENED MEDICINAL PLANTS IN SACRED GROVES USING THE LOCALIZED CONSERVATION PRIORITY INDEX (LCPI)

Introduction

Setting conservation priorities with village communities is essential and imperative since not all plant species had been assessed by IUCN and the national committee on Red List. Setting priority plant species for localized conservation, helps local communities in deciding which species are to be given priority attention. It can give the local planners, resource managers, and local people essential information on local biological diversity of cultural and economic importance (Brehm et al., 2010). Further, this approach can also be used in identifying the priority areas for conservation (Chanthavong and Buot, 2019) such as sacred groves. Known to harbor rare and community important species, sacred groves in India and around the world, need an urgent attention for local species conservation.

There are only few papers dealing with localized conservation priority index (Buot et al., 2024a, Buot et al., 2024b, Sumabat et al 2024, Villanueva and Buot, 2020). Briefly, the Localized Conservation Priority Index (LCPI) is a point scoring method used to rank species by the level of priority (Buot et al. 2024a). It is composed of five criteria: harvesting risk, economic use, cultural use, species distribution, and frequency value, which can be categorized as ecological and socio-cultural indicators. Determining the prioritization will revolve around these important indicators.

This module would help trainees determine priority medicinal plants for conservation using localized conservation priority index (LCPI) especially customized for the locality. This undertaking hopes to contribute to SDGs 5 (Gender equality), 6 (Clean water), 11 (Sustainable cities and communities), 12 (Responsible consumption and production), 13 (Climate Action), 15 (Life on land) and 17 (Partnership to achieve goal).

Objectives

1. To determine the identity of threatened plants at the local level and their levels of priority
2. To geotag locally threatened plants in their localities of occurrences to enhance in situ conservation

Activity

1. Determining the identity of threatened plants in the sacred groves

   1.1 Get hold of the list of plants in a particular sacred grove. Or using the data from Training Module 1, indicate the scores for each of the following indicators with full guidance from **Table 1.1**:

   - harvesting risk,
   - economic use,
   - cultural use,
   - species distribution, and
   - frequency value

Table 1.1. **Criteria and scores used in calculating conservation priority scores of the useful plants using point scoring (From Buot et al. 2024c, 2024d).**

CRITERIA	CATEGORIES	SCORE	Sources of Information
Harvesting Risk	Harvesting represents the removal of the whole plant (includes all the basic parts: root, stem, leaf, flower, and fruit).	5	Interview (Key informants)
	Harvesting of four out of the five basic parts	4	
	Harvesting of three out of the five basic parts	3	
	Harvesting of two out of the five basic parts	2	
	Harvesting of at least one of the five basic parts	1	
Economic Use	Five or more economic uses	5	Interview (Key informants); Ethnobotanical survey from CONserve-KAIGANGAN Project 2; Published literature
	Four economic uses	4	
	Three economic uses	3	
	Two economic uses	2	
	One economic use	1	
Cultural Use	Five or more cultural uses	5	Interview (Key informants); Published literature
	Four cultural uses	4	
	Three cultural uses	3	
	Two cultural uses	2	
	One cultural use	1	
Species Distribution	Within SINP/GMRPLS	5	Merrill; Catalogue of Life (2020); IPNI; Pelser et al. (2011-)
	Within Samar Island	4	
	Within Visayas	3	
	Within the Philippines	2	
	Cosmopolitan	1	
Frequency Value (%)	Not recorded (0) - 20	5	Vegetation sampling in three municipalities (CONserve-KAIGANGAN Project 1)
	21-40	4	
	41-60	3	
	61-80	2	
	81-100	1	

Another option would be to conduct a Plant Interview with the local people. This is done by simply asking the locals direct questions regarding harvesting risk, economic use, and cultural use. Species distribution data can be obtained from flora and other handbooks. Frequency value can be taken from the field data from Training Module 1

1.2 Each criterion in the LCPI may score ranging from 1 (lowest) to 5 (highest), where a higher score could be an indication of higher danger and hence, stricter or higher conservation priority. The scores of the criteria can be summed up using the formula:

Localized Conservation Priority Index = Harvesting Risk + Economic Use + Cultural Use + Species Distribution + Frequency Value

1.3 Each plant is scored according to these criteria. The scores will be summed up to identify the priority level (**Table 1.2**). Based on the formula above, the plant can have a maximum of 25 points. A higher score indicates a higher conservation priority.

1.4 Once a priority level has been assigned to a plant in **Table 1.2**, a community or village decision can be made following **Table 1.3** (Villanueva and Buot, 2020a).

LCPI is the beginning of our operationalizing localized prioritization in biodiversity conservation. LCPI recognizes the fact that there is ongoing nature and culture interaction (Buot et al., 2024a, Buot et al., 2024b, Villanueva and Buot, 2020). This is quite common in the discipline of ethnobotany and ethnobiology (Buot 2009, Pretty et al., 2009Villanueva and Buot, 2020a, 2020b, Caringal et al., 2020, Chanthavong and Buot, 2019, Sopsop and Buot, 2009). And this is very evident in sacred groves worldwide. LCPI integrates the perspectives of local villages and global conservation authorities, which are essential in conservation management. Our current LCPI concept is congruent with the ideals of the new conservation science, advocated by Karieva (2012, 2014) and Karieva and Marvier (2012). In the new conservation science concept, humans and nature are of equal importance and on equal footing. Though this was opposed by Soule (2013), we believe that the concept of the new conservation science is the way to go (Buot, 2008a, 2008b). While there is an urgent need to conserve biodiversity, there is also a need to consider the traditions and values of the local people (Villanueva and Buot, 2020a and 2020b). As the direct users of these resources, local communities have a critical role in conservation (Engels et al., 2011; Caringal et al., 2020).

2. Geotagging locally threatened plants in sacred groves

Geotagging is to pinpoint the location of a particular plant of interest in sacred groves. This procedure will help us locate threatened plants especially if we need to protect them or need to collect samples in situ.

2.1. Set Up the GPS Device

2.1.1. Ensure your GPS device is turned on and ready to record locations. Devices like Garmin GPS (2025) or Magellan GPS (2025) are popular for field use (https://ph.garmin.com) (https://www.magellangps.com)

2.1.2. Some GPS devices may require you to select your coordinate system (WGS84 is standard for GPS).

2.2. Walk to the tree. Go to the base of the tree where you want to record the location. Record the Coordinates. Once you're at the tree, look at the GPS screen to find the coordinates (latitude and longitude). Note down the exact coordinates displayed on the GPS device.

2.3. Optional: Save the Location. Many GPS devices allow you to save the coordinates as a "waypoint" for easy reference later. Some devices also let you take a quick note or add a label to the waypoint, which can be helpful to identify the tree.

2.4. Repeat for other trees. Repeat the process for each tree, walking to each one and recording the coordinates.

2.5. Transfer data. Once you've collected the data, transfer the coordinates from the GPS device to a computer or spreadsheet using a USB cable or software (Garmin BaseCamp 2025). (https://www8.garmin.com/support/download_details.jsp?id=4435)

2.6. Plot coordinates on a map (Optional). After transfer, input your coordinates into a mapping tool like *Google Earth* or any GIS software to visualize the tree locations on a map.

Table 1.2. List of plants in sacred groves and their scores based on the indicators of the localized conservation priority index (LCPI).

Scores of various LCPI indicators

Plant species Family/genus/species (alphabetical order)	Harvesting risk	Economic use	Cultural use	Species distribution	Frequency value	Total	Priority level

Table 1.3. Conservation priority classification based on LCPI scores (modified from Villanueva and Buot 2020).

Scores	Priority level	Decision
1-8	Low	Suitable for high-impact harvesting
9-16	Medium	Can be harvested with specific quotas
17-25	High	Requires strict regulation in harvesting

TRAINING MODULE 3
IN SITU CONSERVATION TECHNIQUES FOR THREATENED MEDICINAL PLANTS IN SACRED GROVES

Introduction

While protecting the sacred groves, it is also foremost to consider the livelihood of the hundreds of Malaiyali community people residing in Kalrayan Hills. Tribal groups in Kalrayan Hills are mainly involved in economic pursuits ranging from hunting to farming and other industrial daily wage work. Forest and the resources build and nurture their spiritual, economic, and social development. Unfortunately, the socioeconomic condition of the tribal groups is critically low with poor literacy rates, infrastructural and social services including road, communication, sanitation, and health amenities (Jayakumar et al., 2016). Hence a collaborative initiative is the most appreciable approach for the conservation of threatened medicinal plants along with sustainable development of the Malaiyali tribes. Awareness should be spread among the young population regarding the conservation of medicinal plants and how they could be successfully employed for the development of the community. For instance, 1kg of *Terminalia chebula* (Haritaki) costs above Rs. 500, which is an indigenous species of the Kalrayan hills and could be commercialized on a large scale with the aid of the local community.

This module discusses the establishment of *Vrikshavalli* for the protection of threatened plants in the sacred groves. The training protocol will aid in the *in situ* conservation of threatened species in sacred groves.

Objectives

1. To plant trees and develop *Vrikshavalli* in association with the local community.

Activity

Establishment of Vrikshavalli

A training protocol is provided for the establishment of saplings in the sacred groves.

It is obligatory to attain permission from authorities and local communities for the planting of saplings since the sacred groves are highly conserved areas.

1. Selecting the site

- Choose the sites that faced more deforestation due to human interference and natural disturbances.
- Special attention should be given to checking the quality of oil and provisions for optimum sunlight, water availability, and space for growth.

2. Sapling preparation

- Select a sapling from the local nursery that will be appropriate for the climate and soil conditions of the area.
- The medicinal plants facing extinction should be chosen in the establishment of *Vrikshavalli.*

3. Planting the sapling

- Measure the root collar and dig a hole 2-3 times as wide as the root collar. Mount some soil at the bottom to back the roots.
- Make sure the sapling is free of contamination and clip out the damaged parts before planting.

- Keep the sapling in the center of the hole and ensure the root collar is at the same level as the soil surface. Remove the synthetic wrappings from the sapling if present.
- Add sufficient soil to remove air pockets and compost to aid the growth of the plant.

4. Watering and follow-up care of the saplings

- Make sure to water deeply and regularly. It is obligatory to water the plants every day to facilitate root establishment.
- Mulching can be performed to retain moisture and to regulate the optimum temperature for the growth of plants.
- Special care should be taken to safeguard the saplings from herbivores and check frequently for any signs of damage or stress.
- Trim the damaged or dead branches for better growth over time.

There should be a strong rapport with the local community for the maintenance of the established garden. The growth of saplings should be assessed at regular intervals.

TRAINING MODULE 4

IN VITRO CONSERVATION TECHNIQUES FOR THREATENED MEDICINAL PLANTS IN SACRED GROVES

Introduction

The sacred groves are a socio-ecological production landscape (SEPL), a repository for various medicinal plants and enriched with replenishable resources like fruits and honey. They are regarded as the repositories of primeval biodiversity, moderators of local climate, and protectors of soils and watersheds. Unfortunately, a serious erosion of the canopy in the groves of Tamil Nadu including the ones in Kalrayan hills has been observed for the past 20 years. The Kalrayan hills are the reserved area that ranges in height from 2000 feet to 3000 feet and extends over an area of 1095 sq km with inexplicably rich biodiversity. The sacred groves in Kalrayan Hills are currently on the verge of disruption due to unhindered human interference for land, wood, and many other resources. These sacred groves in Kalrayan Hills are abundant with more than 56 species of edible and medicinal plants. Some of the prominent medicinal plants in this region are *Terminalia chebula*, *Hedyotis* sps., *Toddalia asiatica*, *Santalum album*, *Mallotus philippensis*, and *Rubia cordifolia* which are exploited copiously owing to their medicinal and industrial value.

The most potent strategy for conserving the plants is the tissue culture technique which integrates traditional ecological knowledge and modern science. Growing cells, tissues, and organs of plants in artificial synthetic media under appropriate environmental conditions are termed tissue culture (Van Eck and Smith 2018). Tissue culture is employed for understanding plant developmental processes, functional gene studies, commercial plant micropropagation, development of transgenic plants with potent industrial and agronomical traits, plant breeding and crop improvement, synthesis of high-quality healthy plant material, preservation and conservation of germplasm (Loyola-Vargas and Ochoa-Alejo, 2018; Phillips et al., 2019). Tissue culture approaches stand out because of the ease of cultivation, reduced space, lowered time, substantial number of clones, and most importantly reduced biotic and abiotic stress in culture. The *in vitro* conservation of these medicinal species also opens the avenues for the sustainable production of metabolites. It provides resource material for advanced genomics research to develop potent varieties and decipher key biosynthetic pathways.

This module discusses *in-vitro* conservation protocols of priority threatened plants including *Sthala-vrikshas* as a model of integration of traditional ecological knowledge and modern. A training manual on performing plant tissue culture is provided for trainees.

Objectives

1. To develop *in vitro* cultures of threatened medicinal plants of the sacred grove.

Activity

The instructions for developing *in vitro* plant tissue cultures are provided here.

Methodology

1. **Plant materials and surface sterilization**

- Leaves or shoot stems can be used as an explant material

- The explants should be rinsed for 30 min under running tap water, followed by the five-minute washing with Dettol.
- Wash again with distilled water to eradicate traces of the germicidal agent.
- The subsequent surface sterilization procedures must be performed under aseptic conditions in a laminar flow hood.
- Treat the explants with 70% ethyl alcohol (70 %) for 3 min followed by incubation in mercuric chloride (0.1 % w/v) solution for 2 min.
- Rinse the treated materials repeatedly in sterile water for thorough disinfection. The disinfected explants were incised into small pieces (10−12mm) to develop in-vitro cultures.

2. Plant tissue culture media preparation

Murashige and Skoog (MS) media can be prepared for the development of *in vitro* cultures of the chosen medicinal plants.

Requirements:

1. Stock Solution of Macro salts (20X)
2. Stock Solution of Micro salts (100 X)
3. Stock solution of vitamins (100X)
4. Stock Solution of Plant Hormones (100X) as per the type of *in vitro* culture intended to develop
5. 1 N NaOH & 1 N HCl

- Prepare stock solutions of macro- and micronutrients, vitamins, and plant growth regulators as provided in Table 4.1

MACROELEMENTS	**mg/L**
Ammonium nitrate	1650.000
Calcium chloride	332.200
Magnesium sulphate	180.690
Potassium nitrate	1900.000
Potassium phosphate monobasic	170.000
MICROELEMENTS	**mg/L**
Boric acid	6.200
Cobalt chloride hexahydrate	0.025
Copper sulphate pentahydrate	0.025
EDTA disodium salt dihydrate	37.300
Ferrous sulphate heptahydrate	27.800
Manganese sulphate monohydrate	16.900
Molybdic acid (sodium salt)	0.213
Potassium Iodide	0.830

Zinc sulphate heptahydrate	8.600
VITAMINS	**mg/L**
myo-Inositol	100.000
Nicotinic acid (free acid)	0.500
Pyridoxine HCl	0.500
Thiamine hydrochloride	0.100
AMINO ACIDS	**mg/L**
Glycine	2.000
CARBOHYDRATE	**mg/L**
Sucrose	30000.000

- Mix applicable volumes of stock solutions corresponding to the recipe for MS
- Alter the pH of the medium using NaOH or HCl to the desired level (pH 5.8 for MS)
- Add agar or Gelrite (at around 0.8-1.0% w/v) to the medium to solidify it, in case of solid medium.
- Autoclave the prepared media at 103.42kPa at 121°C for 20 min.

3. Callus induction

- Transfer the disinfected explants into the phytohormone-supplemented media. Different combinations of auxins (2,4-D and NAA) and cytokinins (BAP and kinetin) are generally used for callus induction.
- Incubate at 25 ± 1°C under a photoperiod of 16h with cooling white, fluorescent tubes at a 40μmolm^{-2} s^{-1} light intensity.
- Subculture the developed callus on the optimized medium every four weeks and conserve under the above-mentioned *in vitro* conditions.

  The results can be added in Table 4.2 to study the effects of different phytohormones.

4. Multiple shoot generation

- Select nodal segments (approximately 2 cm) containing a single axillary bud.
- Sterilize the nodal segments as discussed in the previous subsections.
- Inoculate in MS (Murashige and Skoog, 1962) basal medium of pH 5.8 supplemented with phytohormones, solidified with agar/gelrite. Cytokinins are largely utilized in tissue culture to promote adventitious shoot formation.
- Incubate at 25 ± 2°C under a 16-h photoperiod with a light intensity of 2,000 lux under white, fluorescent tubes.
- Subculture the developed shoots on the optimized medium every four weeks.

The results can be added in Table 4.3 to analyze the effects of different phytohormones on callus induction.

Table 4.2. The effect of phytohormones in inducing callus of threatened plants of sacred groves.

Phytohormone	Concentration (µM)	Response (%)	Callus morphology	Fresh weight of callus (g)	Dry weight of callus (g)

Table 4.3. The impact of phytohormones in promoting multiple shoots of threatened plants of sacred groves.

Phytohormone	Concentration (µM)	Explant response (%)	No. of shoots/ explant	Mean shoot length(cm)

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