

**REPRODUCTIVE AND SEED BIOLOGY OF *GARCINIA*
LANCEIFOLIA ROXB. IN MIZORAM**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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**REPRODUCTIVE AND SEED BIOLOGY OF *GARCINIA LANCEIFOLIA*
ROXB. IN MIZORAM**

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**In partial fulfillment of the requirement of the Degree of Doctor of Philosophy
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CERTIFICATE

This is to certify that the thesis entitled “**Reproductive and seed biology of *Garcinia lanceifolia* Roxb. in Mizoram**” submitted by **Mr. H. Vanlalmalsawmropuia** for the award of the degree of **Doctor of Philosophy in Forestry** of Mizoram University, Aizawl, embodies the record of original investigation carried out by him under my supervision. He has duly registered and the thesis presented is worth of being considered for the award of the Ph.D. degree. The work has not been submitted for any degree to any other University.

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LIST OF ABBREVIATIONS AND SYMBOLS

Acronym/symbol	Full form/meaning
°C	Degree Celcius
%	Percentage
<,>	Less and greater than
'	Minutes
"	Seconds
µg	Microgram
&	And
ABA	Abscisic Acid
AMSL	Above Mean Sea Level
ANoFB	Average Number of Flowers per Branch
ANOVA	Analysis of Variance
AOCS	American Oil Chemists' Society
ARF	Auxin Response Factor
BT	Average Number of Branches per Tree
CdCl ₃	Deuterated Chloroform
CDS	Coding DNA Sequence
cDNA	Complementary DNA
cm	Centimeter
COX-2	Cyclooxygenase-2
CPM	Counts Per Million
CV	Coefficient of Variation
DEGs	Differentially Expressed Genes
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DQI	Dickson Quality Index
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic Acid
e.g.	Exempli gratia (for example)
EI	Emergence Index

ERF	Ethylene Response Factor
EST	Expressed Sequence Tag
FAMEs	Fatty Acid Methyl Esters
FBP	Floral Binding Protein
FCR	Fluorochromatic Reaction
FDA	Fluorescein Diacetate
FDR	False Discovery Rate
FTIR	Fourier Transform Infra-Red
FYM	Farmyard Manure
g	Gram
GA ₃	Gibberellic Acid
GC	Germination Capacity
GC-MS	Gas Chromatography-Mass Spectrometry
GE	Germination Energy
GI	Germination Index
GO	Gene Ontology
GP	Germination Percentage
GV	Germination Value
H ₂ O ₂	Hydrogen Peroxide
H ₂ SO ₄	Sulphuric Acid
HCA	Hydroxycitric Acid
HPLC	High-Performance Liquid Chromatography
IAA	Indole-3-Acetic Acid
IC ₅₀	Half Maximal Inhibitory Concentration
IICT	Indian Institute of Chemical Technology
IISR	Indian Institute of Spices Research
IMD	India Meteorological Department
ISFR	India State of Forest Report
ITS	Internal Transcribed Spacer
KEGG	Kyoto Encyclopedia of Genes and Genomes
KNO ₃	Potassium Nitrate

KOG	Eukaryotic Orthologous Groups
L.	Linnaeus
m	Meter
M→F	Male to Female (Hermaphrodite)
MDG	Mean Daily Germination
mg	Milligram
ml	Milliliter
mm	Millimeter
m/z	Mass-to-Charge Ratio
NAA	1-Naphthaleneacetic Acid
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NoB	Number of Branches
NR Database	Non-Redundant Protein Database
ORF	Open Reading Frame
P/O Ratio	Pollen–Ovule Ratio
PCA	Principal Component Analysis
PCV	Phenotypic Coefficient of Variation
Pfam	Protein Family Database
ppm	Parts Per Million
PV	Peak Value
RAPD	Random Amplification of Polymorphic DNA
RHS	Royal Horticultural Society
RIN	RNA Integrity Number
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
Roxb.	Roxburgh
rpm	Revolutions Per Minute
rRNA	Ribosomal RNA

SD	Standard Deviation
SE	Standard Error
SLA	Specific Leaf Area
SPF	Sun Protection Factor
SRAP	Sequence-Related Amplified Polymorphism
SS	Sum of Squares
SSR	Simple Sequence Repeat
Std. Dev.	Standard Deviation
TCA Cycle	Tricarboxylic Acid Cycle (Krebs Cycle)
TG	Tree Girth
TH	Tree Height
TNoFT	Total Number of Flowers per Tree
TS	Transverse Section
TSM	Tetramethylsilane
UDP	Uridine Diphosphate
UV	Ultraviolet
UV-A	Ultraviolet A
UV-B	Ultraviolet B
UV-C	Ultraviolet C
VEGF	Vascular Endothelial Growth Factor
VIF	Variance Inflation Factor
Viz.	Videlicet (namely)
w/v	Weight per Volume
β	Beta
°C	Degrees Celsius



CHAPTER - I

INTRODUCTION



CHAPTER 1

INTRODUCTION

The Indo-Burma area is part of the Earth's biodiversity hotspots, which is famous for its enormous number of species as well as the very high percentage of endemic species. The hotspot extends to Northeast India, Myanmar, Thailand, Laos, Vietnam, southern China, and Bangladesh, where approximately 13,500 species of plants exist, with about 7,000 of them regarded as endemic (Huang et al., 2023). The Northeast Indian region, particularly Mizoram, not only belongs to this biogeographic zone but also is indicated time and again for its extraordinary diversity of flowering plants and the presence of specific types of forests (Jain and Das, 2022). Mizoram is the home to over 2,500 species of flowering plants which include a variety of orchids, rhododendrons, and medicinal plants among them (Rai and Lalramnghinglova, 2010). The combination of different altitudes, the mixing of tropical wet and semi-evergreen forests, and the support of the community for conservation efforts over a long time have all been factors that allowed this variety of flora and fauna to survive. Nevertheless, the same area is also subjected to very serious dangers. Tree cutting, changing land use, partitioning of land and the rapid onset of climate change are all factors that continue to push the habitats and species closer to extinction (Hughes, 2017). In this difficult situation, there are a few tree genera that are of ecological and cultural importance, and one of them is *Garcinia*, which is still poorly documented. This group of trees supports forest Ecosystem, local nutrition, and traditional medicines, but their research and planning for conservation are commonly neglected thus leading to lack of information resulting in non-documented in the area.

The ecological and the cultural fabric of Northeast India are intertwined and complementary for the comprehension of the whole process of diversity maintenance. Over the centuries, the communities have been practicing jhum (shifting) cultivation along with other agroforestry traditions, which, although nowadays they are being debated for their sustainability, have actually led to the retention and domestication of wild edible and medicinal plants in home gardens and secondary forests (Sati and Vangchhia, 2016). Ethnobotanical studies conducted in

Mizoram and its adjacent regions like Assam have provided a record of countless relatively lesser known fruits and herbs that Indigenous tribes such as Mizo, Garo, and Khasi, still utilize them, share with mainstream agriculture and pharmacology (Dasgupta, 2023; Bora et al., 2020). An example of the ongoing conflict between use and preservation is the case of wild sour-fruited trees: the demand for the fruit in the market can lead to overharvesting, and if the collection of the fruit is not properly managed, then the reproduction of the trees will not be able to catch up with the extraction (Kumatso and Sigge, 2024). These situations highlight how conservation difficulties are linked with the survival of people and how methods of the future will have to mix scientific research in the environment with local knowledge and value chains.

Garcinia (Clusiaceae; formerly Guttiferae) remarkably stands out as a taxonomically huge, biogeographically extensive and rich in economically important species (Gaudeul et al., 2024). The global recognition includes approximately 250 species, with the greatest number of species occurring in tropical Southeast Asia (Dasgupta, 2023). Some of the species have already been assigned the roles of culinary and medicinal for a long time. *Garcinia mangostana* (mangosteen) belongs to the group of fruits that has the most popular edible part, the arils, and the pigment-rich pericarp; *Garcinia indica* (kokum) is the one that produces the sour rind which is very rich in garcinol, hydroxycitric acid (HCA), and anthocyanins; and *Garcinia cambogia* (syn. *G. gummi-gutta*) is recognized in the nutraceutical industry for its HCA content (Desai et al., 2022). Among the genus, xanthenes, benzophenones, flavonoids, and organic acids have allured biomedicine, demonstrating the continuity of traditional uses and modern pharmacological screening. The presence of edible, sour fruits along with a variety of phytochemicals makes *Garcinia* a typical “bridge genus” that connects forest ecology, household cuisine, health practices, and potential bioprospecting (Paul and Zaman, 2022).

India is home to around 43 *Garcinia* species which are mainly distributed in three large phytogeographical regions, namely the Andaman and Nicobar Islands, the Western Ghats, and Northeast India (Dasgupta, 2023). The humid, shady, and thermally stable environments of the evergreen and semi-evergreen forests in these

regions provide an ideal habitat for many genera species. In the Northeast region, *G. pedunculata*, *G. cowa*, *G. morella*, and *G. lanceifolia* are the most significant ones; all these species occupy different habitats and have different uses. Records indicate *G. lanceifolia*'s distribution extends from Assam, across to Nagaland, and then down to Meghalaya, with bits invading into Bangladesh (Chittagong Hill Tracts), Myanmar, and maybe reaching even further to Thailand, Laos, and Vietnam, hence supporting its placement in the Indo–Burma biogeographic realm (Dasgupta, 2023; Tripathi et al., 2025). The species is greatly diminishing despite its original inhabitants is in the moist forests of Assam and the nearby hills. The range of *G. lanceifolia* extends from Assam, Nagaland, and Meghalaya to Bangladesh (Chittagong Hill Tracts), Myanmar, and even further to and Thailand, Laos, and Vietnam which shows its strong connection with the Indo–Burma biogeographic region (Dasgupta, 2023; Tripathi et al., 2025). Although the first specimen of the species was found in the damp forest of Assam and its adjacent hill areas, the species has almost disappeared due to which the species has technically got extinct only in the past few years. Nowadays, *G. lanceifolia* can be found more often in village compounds or regrowth areas than in untouched primary forests—this is a sign of both habitat loss and the species' ability to survive in areas modified by human activities (Baruah et al., 2021). eFlora sources also note occurrences in Assam, Nagaland, and Meghalaya as well as the South Andamans, which underline its fragmented, disjointed distribution in humid habitats suitable for it (Garge, 2025).

Botanically, *G. lanceifolia* Roxb is classed as a small to medium-sized evergreen tree or shrub with a height of 1–6 m. It has glossy dark green lanceolate leaves and highly noticeable latex which varies from orange to yellow. Such features are very prevalent in the Clusiaceae family. The *Garcinia* genus primarily consists of dioecious plants, which means there are separate male and female plants for the species, although polygamic-dioecious forms are sometimes encountered. The flowers, which are tiny, measure around 5–8 mm across, are some shade of orange to red, and either occur singly or in small clusters on the woody stems of the plant. The fruits are globose, shallowly lobed, and measure 2.2–4.5 cm in diameter and length, turning bright orange or red when completely ripe. Each fruit has a number of

comparatively big seeds hanging in a clear, sour pulp. In terms of phenology, the flowering takes place typically from October to April, while the fruits are matured from January to June (Bora et al., 2018; Dasgupta, 2023). The species, ecologically, is found in wet tropical lowland and lower montane forests at an altitude of approximately 300–700 m where it is usually in partial shade under the closed canopies—characteristic of forest understory trees that are adapted to stable microclimates (Ngernsaengsarua et al., 2024).

The species is deeply embedded in local food cultures. In Mizoram it is widely known as “chengkek” (also *Pelh* or *Pelte*), while Assamese communities call it “Rupohi Thekera.” Ripe fruits possess acidity and are consumed in both fresh and processed forms. One typical practice is sun-drying salted ripe fruits' slices that result in a shelf-stable souring agent which is used in curries and chutneys; this is almost the same as the culinary function of kokum in West Indian cuisines. Fresh fruits can be made into juice, cured, or the mild sourness can be imparted to soups. The tender leaves are also edible and are commonly cooked as green vegetables or pickled. These different uses suggest that *G. lanceifolia* is placed next to other sour *Garcinia* species not only as an important seasonal flavor and micronutrient source in household diets but also as a reflection of both subsistence use and an emerging market potential when products can be dried, packaged, or otherwise stabilized (Dasgupta, 2023).

Ethnomedical documentation indicates that the species has numerous practical uses. In Assam and Mizoram, the indigenous medical practitioners make use of bark and leaves for various digestive disorders—dyspepsia, ulcers, diarrhea, and dysentery—as well as for diabetes (Bora et al., 2020). The fruit has been differentially used as an anti-diarrheal and anti-dysenteric remedy and is spoken of with the properties of hepatoprotective and uroprotective; the leaves are said to be stomachic and diuretic (Dasgupta, 2023). These laboratory investigations support the mentioned practices: Wistar rats fed with hydroalcoholic extracts from the bark displayed remarkable reduction of ulcer and diabetic lactate activity, while interventions to test for anti-microorganism activity suggest the availability of bioactive molecules that are in line with xanthone- and benzophenone-containing Clusiaceae (Bora et al.,

2020; Policegoudra et al., 2012; Selvaraj, 2013). *G. lanceifolia* shows through this dual perspective of ethnobotany and pharmacology a remarkable possibility for bioprospecting, despite being an underappreciated tree of fruits in local agroecosystems (Dasgupta, 2023; Dubey et al., 2023).

Apart from the medical benefits the species has, it is also potential as a functional food ingredient. Antioxidants and organic acids retention was shown in the studies on some *Garcinia* fruits to depend on the processing methods; in the case of kokum, the use of standardized extracts of garcinol and HCA has supported the nutraceutical applications (Desai et al., 2022). A preliminary study on *G. lanceifolia* indicates that freeze-drying can not only preserve but also enhance nutrient and antioxidant profiles, hence giving rise to value addition if postharvest handling is optimized (Baruah et al., 2025). Setting such processing baselines could facilitate the transition of the species from household use to artisanal and eventually commercial production, assuming that harvests are sustainable and that cultivation is expanded to reduce pressure on remnant wild stands.

Reproductive biology within *Garcinia* is notably diverse and has direct implications for conservation and domestication. Within the whole genus, sexual systems vary from strict dioecy to polygamodioecy and there are a number of species, that could either be an obligate or a facultative case that show apomixis (agamospermy), among others (Barrett, 2002; Richards, 1990). Mangosteen is a traditional case of obligate apomixis coming along with male sterility that frequently leads to the formation of more than one embryo (polyembryony) in one seed; on the contrary, in the case of the genus, facultative apomixis permits the occurrence of both sexual and asexual reproduction (Yao et al. 2023). The entire range has an impact on fruit set, genetic diversity, and breeding prospects. Insect-rich tropical forests are normally characterized by a pollination process involving bees, flies, and other floral visitors; flowers with sweet scent and modest corolla sizes like those of *G. brasiliensis* can be considered as typical examples of generalist insect pollination syndromes (Leal et al., 2012). The prevailing sexual system in the *Garcinia* genus being dioecy means that the ecological factors that determine the distribution of male and female trees, the activity of pollinators, and synchrony in flowering, will be

critical for successful sexual reproduction. The timing of fruit ripening is usually associated with the rain season which not only guarantees higher seeds survival but also provides the seeds with better chances to reach the moist microsites that are ideal for germination and early growth.

Seed biology is a central challenge in the study and management of *Garcinia*. The seeds of nearly all species studied to this date have been found to be recalcitrant: they are huge, have a high water and lipid content, maintain their metabolism during shedding, and are highly vulnerable to drying and low-temperature storage (Noor et al., 2016). Typical seeds enter a dormant state that is tolerant to desiccation and thus survive drying and cold storage, whereas the recalcitrant seeds rapidly lose their viability as soon as they are taken out of the protective environment provided by the fruit (Pammenter and Berjak, 2000; 2014). This physiological state restricts options for ex situ conservation via conventional seed banks. Research has indicated that quick and meticulously monitored removal of water from excised embryonic axes can occasionally maintain short-term viability and provide limited paths for cryopreservation, yet the reactions differ considerably among species and drying rate, thus, “critical water content” becomes a fluctuating parameter instead of a constant one (Pammenter and Berjak, 1999; 2014). In the case of tropical trees such as *Garcinia*, this implies that the conservation and propagation methods must frequently favor fresh seed sowing, vegetative propagation or advanced cryo-based methods over long-term seed storage at standard refrigerated conditions (Magnitskiy and Plaza, 2007).

Germination behavior in *Garcinia* is equally distinctive. The germination method referred to as “*Garcinia*-type” germination, means that the embryo first elongates and then both the root and the shoot grow from its tips, with the help of vascular tissue connecting those (Noor et al., 2016). The factors like seed size, moisture content, and testa thickness affect the germination rate and success in all species. Generally, big seeds like those of *G. mangostana* and *G. hombroniana*, are preferred over the smaller ones in terms of germination and water holding capacity; *G. atroviridis* and *G. prainiana* have very thick seed coats which also delay germination considerably (Normah et al., 1997; Noor et al., 2016). The

environmental situation also plays a role. Usually understory factors, namely moderate temperatures, high humidity, and indirect light, are the ones that support both seed germination and seedling vigor. To illustrate, the seedlings of *G. brasiliensis* thrive in the setting of low light and warm temperature, which goes along with the extinction-tolerant, mid-successional niche (Cardoso et al., 2021). Through ecological analogy, microhabitat preferences of *G. lanceifolia* are likely to be similar because this species is most frequently found under the forest canopy or in the partially shaded areas of gardens.

The interventions before sowing can be helpful in the emergence and early growth of plants, even though the seed coat's structural features and recalcitrance impose limitations. Simple mechanical decoating—partial or complete removal of the testa—can increase germination percentage and speed in *G. brasiliensis* and *G. gummi-gutta* (Anilkumar et al., 2007). Chemical priming with gibberellic acid (GA_3) is another widely used approach that enhances germination and seedling vigor in many horticultural and forestry species. In *Garcinia*, GA_3 treatments have induced earlier and higher germination in *G. tinctoria* and substantially improved outcomes in *G. kola*, where potassium nitrate (KNO_3) has also proven beneficial (Joseph et al., 2011; Asomaning, 2019). The generality of hormonal priming in the metabolic activation and reserve mobilization is supported by comparable findings in other crops, for instance, in the case of Kagzi lime, where GA_3 priming raised the germination rate to about 95% and also improved the vigor of the seedlings (Dilip et al., 2017; Chaudhary et al., 2019). Hydro-priming (also known as water soaking) is a low-cost alternative; in *G. kola*, germination percentage close to 97% was achieved by soaking the seeds for 72 hours after removing the seed coat, and similar studies have reported quicker emergence and more uniform seedlings for simple water soak methods (Dadjo et al., 2019; Salimata et al., 2024; Nzegbule and Mbakwe, 2001). In summary, these treatments help to overcome partially the physiological and structural barriers and facilitate the fast transition of recalcitrant seeds to autotrophic growth.

Temperature and storage regimes further modulate germination success and seedling performance. For *G. brasiliensis*, the optimal germination window lies between 25–35 °C, with especially rapid emergence at the upper end; fruit storage at ~25 °C also maintained short-term seed viability better than cooler conditions (Cardoso et al., 2021). The mentioned results concur with the overall tendency of recalcitrant seeds to be sensitive to low temperatures: the viability in the case of cold storage at 5°C is reduced to a very small percentage, and the same works for *G. kola* already (Barbosa et al., 2008; Dadjo et al., 2019). Here, however, the temperature that is best for germination may be different for different species (Oliveira et al., 2014), pointing out the importance of developing species-specific protocols instead of using the same non-adapted recommendations for all. In addition to temperature, the form of storage matters. When seeds remain within intact fruits, short-term storage can sometimes preserve emergence percentage and emergence index (EI) at levels comparable to freshly harvested material for weeks to months; by contrast, once seeds are extracted and stored separately, both emergence and EI tend to decline sharply, with refrigeration the most damaging option (Cardoso et al., 2021). For research and nursery practice, the implication is clear: sow promptly from freshly collected fruits whenever possible, and avoid cold storage of extracted seeds.

Potting media and nursery substrates are practical levers for improving emergence, rooting, and early growth. Media that combine good aeration with reliable water-holding capacity such as cocopeat (coir dust) and vermiculite—often perform well with tropical tree seedlings. For *G. indica*, cocopeat supported higher germination (81%) than several alternative substrates, while in *G. brasiliensis* vermiculite at ~25 °C yielded strong root and shoot development (Swamy et al., 2025; Cardoso et al., 2021). Experiments conducted on various tropical species have shown that coir-based substrates can equal or even surpass sphagnum peat in terms of plant growth, along with better physical properties stability than sedge peat (Meerow, 1995). The addition of organic manures like vermicompost and farmyard manure can not only improve moisture retention but also increase nutrient supply along with the activity of roots. The application of soil mixes containing both vermicompost and cocopeat has been the main factor for seedlings to be more

vigorous, however, 100% pure cocopeat does not always prove to be the best and in certain cases can even have a negative impact on growth (Kukal et al., 2012; Maynard et al., 2024). Crop-specific formulations are a great example of the principle: in tomato, a 1:1 cocopeat-soil mixture gave the best results for germination and seedling development; in paddy nurseries, a blend of 50% vermicompost, 30% farmyard manure, and 20% soil was the best for quality and downstream performance (Panthi et al., 2023; Choudhary et al., 2023). It is very likely that tailoring the substrate composition for drainage without desiccation and steady nutrient supply will especially be important because recalcitrant seeds and tender seedlings are the ones that suffer most from both waterlogging and rapid moisture loss.

The oil from seeds of many *Garcinia* species (Clusiaceae family) represents a rich resources in bioactive oil endowed with considerable medicinal as well as commercial potential. Their kernels yield fatty oils that are widely used in traditional medicine, food products, and industrial formulations. For example, the seeds of *G. kola* (bitter kola) are used as stimulants and recommended in West African folk medicine for the treatment of coughs, infections, and liver diseases (Stephen et al., 2017; Tauchen et al., 2023). In the same vein, the fat from *G. indica* (kokum) seeds has always been recognized as a good alternative to cocoa butter and a valuable skin softer, and its peel has garcinol which is a benzophenone with anti-inflammatory property (Lakshmi et al., 2011). In South and Southeast Asia, seeds of *G. gummi-gutta* (syn. *G. cambogia*, Malabar tamarind) yield an edible oil enriched in stearic acid that has been suggested for use in chocolate manufacture (Rani et al., 2022). *G. mangostana* (mangosteen) seeds, similarly, provide stearic-rich fats suitable for confectionery and cosmetic applications (Stephen et al., 2017; Tauchen et al., 2023).

Phytochemical analyses consistently reveal that *Garcinia* seed oils are dominated by The consistent results of phytochemical analyses point to the fact that *Garcinia* seed oils are composed mainly of long-chain fatty acids, among which stearic, oleic, palmitic, and linoleic acids are the most prominent, and that they also contain vitamin and polyphenolic compounds in small amounts. For instance, *G. gummi-gutta* seed oil was analyzed using GC-MS, and the results indicated that

around 55% of its composition was stearic acid, and 40% oleic acid (the latter as methyl esters) (Rani et al., 2022). In the same manner, *G. mangostana* seed fat has about 50-60% stearic acid, while oleic and linoleic acids are present in lesser amounts. Other species oils like *G. indica* and *G. cambogia* have comparable high contents of stearic acid making them suitable to be used as cocoa-butter alternatives (Ananthakrishnan and Rameshkumar, 2016). Besides triglycerides, those oils composed of the minor and pharmacologically important compounds such as tocopherols, phytosterols, and aromatic lipids. For instance, tocopherol levels of as much as 110 mg/kg have been documented in *Garcinia* seed oils and the oils are credited with their antioxidant and anti-inflammatory activities (Doan et al., 2018).

Gas chromatography–mass spectrometry (GC-MS) has been a powerful tool in unmasking the complex chemical composition of *Garcinia* seed oils, indicating the presence of both predominant and minor fatty acids along with a large number of volatile and semi-volatile constituents. GC-MS revealed five fatty acid methyl esters in *G. cambogia*, where methyl 16-methylheptadecanoate (C18 branched fatty acid) was the largest contributor to the profile, accounting for 54.6% (Choppa et al., 2015). On the other hand, *G. kola* seed oil presented an incredible range of chemicals with 18 different compounds being recognized, including alkanes (29.5%), aromatics (40.2%), and alcohols, sesquiterpenes, and fatty acid esters (27.2%). To mention a few, decane was the most prevalent (14.7%) and oleic as well as palmitic acid esters were the next ones (Stephen et al., 2017). Thus, the intricate chemical makeup of the oils confirms their multi-functionality, as they not only consist of stable edible fats but also of numerous bioactive compounds.

GC-MS detected many constituents that were reported to have pharmacological activities. Methyl palmitate and methyl oleate are examples of fatty acid esters presenting with antioxidant and anti-inflammatory activities. Long-chain alkanes and fatty esters could possibly have antimicrobial action through the disruption of the microbial membranes, whereas the tocopherols are pronounced as strong antioxidants (Doan et al., 2018). The polyphenols that are specifically discovered in *Garcinia*, including kolaviron (a biflavonoid complex originating from *G. kola*) and garcinol, not only increase their importance in biochemical terms but

also control oxidative stress, inflammation, and even have a possibility of inducing apoptosis in cancer cells (Tauchen et al., 2023; Lakshmi et al., 2011). These activities have been empirically certified by the researchers, for example, *G. kola* seed oil has been identified to contain a very high amount of phenolics and flavonoids, and its DPPH radical-scavenging activity has been noted as quite significant ($IC_{50} < 1$ mg/mL), and the oil has been noted to have antimicrobial activity against bacteria such as *Salmonella* and *Staphylococcus* (Stephen et al., 2017).

Against this technical background, the case for focused study on *G. lanceifolia* becomes compelling. This tree is still not sufficiently exploited although being an endemic fruit tree with great ethnobotanical significance and an impressive phytochemical profile (Dasgupta, 2023; Policegoudra et al., 2012). It is partly endangered by changing habitats and uncontrolled collecting (Baruah et al., 2021), but still grows near human settlements, showing that there is a possibility for community-based growing and in situ, ex situ mixing of the same genus. The prevailing dioecy with its potential for plasticity makes the tree very dependent on pollinating agents, the distance between the sexes, and the timing of flowering and fruiting; however, these very same characteristics pose problems for breeding and plantation design and at the same time present research opportunities on pollination networks and gene flow in fragmented habitats (Barrett, 2002; Leal et al., 2012). Seed biology, characterized by such factors as recalcitrance, large size, and lipid-rich tissues, along with sensitivity to cooling, will demand specific propagation protocols and thus probably will be the reason for the non-applicability of the standard seed banking method. This has pushed the conservation efforts towards timely sowing, vegetative propagation or advanced cryogenic techniques (Noor et al., 2016; Pammenter and Berjak, 2014; Magnitskiy and Plaza, 2007). On the other hand, the medicinal and culinary uses of the species encourage the development of postharvest handling (e.g., freeze-drying) and small-scale processing, which, when coupled with the increase in price, can support cultivation and reduce the burden on wild populations (Desai et al., 2022; Baruah et al., 2025).

From the perspective of the Indo-Burma hotspot, the research on *G. lanceifolia* is an important part of a larger conservation effort. The species represents the main characteristics of biodiversity-rich but resource-limited areas: it is an ecological and cultural icon as a shade-tolerant, fruit-bearing understorey tree; it is an environmental and cultural icon via food and medicine; it has economic potential through small-scale processing; and it is biologically challenging because of dioecy, recalcitrant seeds, and storage sensitivity. The understanding obtained from the reproductive and seed biology of this species will not be limited to one species' benefit, but in addition, it will be applicable to the related *Garcinia* species like *G. pedunculata*, *G. morella*, and *G. cowa*, also, it will help to tropical trees which are of economic worth and have intricate life-history traits. In practical terms, the future depends on coupling field ecology, pollination biology, and seed science with community knowledge and market realities. This would lead to the development of propagation protocols, which are not only scientifically valid but also acceptable and easy-to-handle for the local nursery and farmer groups; besides, the value-addition paths that help to improve the livelihoods; and the conservation plans that, while requiring the use of the maintained genetic diversity, protect it at the same time.



CHAPTER - II

REVIEW OF LITERATURE



CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction to *Garcinia* Species and Its Importance

The genus *Garcinia* belongs to the family Clusiaceae (Guttiferae) and includes roughly 400 species of flowering plants distributed throughout tropical Asia, America, Australia, Africa and Polynesia (Tan et al., 2023). *Garcinia lanceifolia* Roxb. is an underexplored species within this genus and is native to the north-eastern region of India, particularly Assam and nearby areas, including Mizoram (Gupta et al., 2019). This species is particularly valued among the indigenous population due to its significantly high ethnobotanical importance, as it has several traditional uses, ranging from the culinary to medicinal applications. Even today, it is consumed raw or as pickles and is used to treat dysentery, dyspepsia, and biliousness (Baruah et al., 2021). The bark of the plant shows good antibacterial activity, suggesting its possible legitimacy in the treatment of various diseases caused by gastrointestinal disorders (Bora et al., 2014). *G. lanceifolia* is, therefore, an understudied plant having potential as a promising drug with bridging traditional knowledge and modern scientific research (Dasgupta, 2023).

The ethnobotanical significance of the species was extended beyond the Indian landscape to numerous other regions of China, where the genus *Garcinia* has been used historically for food, health, building, and cultural purposes (Lin et al., 2021). The plant is commonly referred to as "Rupohi thekera" in Assam; according to pharmacological studies, it also demonstrates potent analgesic properties along with anti-inflammatory properties (Bora et al., 2014). A variety of *Garcinia* species found in Assam contain bioactive compounds, including hydroxycitric acid, xanthenes, and flavonoids, which exhibit cardio-protective, antioxidant, antibacterial, anti-hypolipidemic, and anti-malarial properties (Brahma et al., 2022). *Garcinia pedunculata*, another species found in Assam, possesses anti-oxidative, antibacterial, anti-fungal, anthelmintic, hypolipidemic, antidiabetic, hepatoprotective, neuroprotective, and nephroprotective properties (Bhattacharjee and Devi, 2021). Because of both ecological and economic relevance, *G. lanceifolia* has not been

utilized significantly, and further studies are needed of its reproductive biology and seed ecology.

Taxonomic classification of species within the genus *Garcinia* presents challenges due to morphological similarities, followed by the complex reproductive systems within the genus. RNA phylogenetic analysis shows the monophyletic clades within these species (Oktafia and Badruszaufari, 2021). Several species of *Garcinia* exhibit apomixis, a form of asexual reproduction through seeds, thereby contributing to genetic diversity within *Garcinia* species (Baskaware and Deodhar, 2023). Apomicts such as *G. mangostana* are obligatory among the species, while others, such as *G. celebica*, and *G. cowa*, are facultative ones (Emlee et al., 2025). A consensus view is obtained with Nuclear ITS and chloroplast intergenic spacers-based molecular phylogenetic analyses, which provided a revised infrageneric classification in *Garcinia* for the presence of New Caledonian endemic species; its results have further supported the extensive circumscription of the genus (Gaudeul et al., 2024). Morphological characters used for the identification of *G. lanceifolia* include those of lanceolate leaves with an acuminate apex, paracytic stomata, and yellow latex (Gupta et al., 2019). Specific leaf area (SLA) has been considered to be a quantitative morphological trait for many other species showing differences between sexes, shoot types, and canopy portions, such as in *Ginkgo biloba* (Christianson and Niklas, 2011). However, no such work has been done for *G. lanceifolia*. The presence of latex in plants underscores their phylogenetic importance; this evident is in the Malpighiaceae family (Vega et al., 2002). The fruits are eaten fresh or processed into traditional condiments, chutneys, or pickles, while most parts are used in indigenous medicine for treating digestive ailments, pain, and inflammation.

2.2 Global Distribution and Intra-Species Variability of *Garcinia* Species

The genus *Garcinia* has a pantropical distribution with Southeast Asia and Africa as its centers of diversity. The genus displays impressive floral variation and can be commonly found in lowland tropical forests (Sweeney, 2008). According to recent phylogenetic studies, a broadly circumscribed *Garcinia* would encompass all

previously segregated genera and indicate two large lineages within the genus, based on floral characteristics (Sweeney, 2008; Gaudeul et al., 2024). The genus *Garcinia* comprises over 250 species distributed in the tropical regions of the world. India is home to approximately 43 species of *Garcinia*, primarily distributed in the Western Ghats, Eastern Himalayas, and Northeast regions. The northeastern states offer favorable ecological conditions for various species of *Garcinia*, owing their tropical climate, heavy rainfall, and varied forest ecosystems. The Indian Institute of Spices Research (IISR), Calicut, possesses a collection of *Garcinia* genetic resources comprising 15 species from the Western Ghats and the Eastern Himalaya species. Morphological characterization of the species of these two different ecosystems indicates that variations exist within species of the same ecosystem, while similarities persist among species of two different ecosystems (Parthasarathy and Nandakishore, 2014; Tripathi et al., 2025).

Considerable intra-species variation in vegetative and reproductive traits in *Garcinia* species has been observed and reported. Leaf morphology was found to be a key distinguishing factor between species regarding size, shape, color, texture, and venation (Amron, 2020; Nimanthika and Kaththriarachchi, 2010). Flowers vary across species in terms of size, color, and sexual expression, with observations of both male and female flowers. Characteristics of the fruit, such as size, shape, color, and pulp quality, vary depending on the species, ranging from yellow to dark purple (Te-chato, 2007). Reproductive biology variability has some species showing obligate apomixis (e.g., *G. mangostana* var. *mangostana*), while others display facultative apomixis (e.g., *G. celebica* and *G. prainiana*) (Emlee et al., 2025). Environmental factors exert their effects on phenotypic plasticity, whereby some variations occur within the same species in the same ecosystem, while other variations exist between species inhabiting two different ecosystems (Parthasarathy and Nandakishore, 2014). Studies on the genetic relationships of *Garcinia* species using molecular markers have revealed clusters that are governed by floral morphology and (-) hydroxycitric acid content in the fruit rind (Parthasarathy et al., 2016). Such morphological and reproductive variations facilitate adaptation to local conditions. The variety within the *Garcinia* species underscores the need for

comprehensive studies that integrate vegetative, reproductive, and genetic data for accurate species identification and conservation (Nimanthika and Kaththriarachchi, 2010; Emlee et al., 2025).

Ecological factors influencing the spatial distribution of *Garcinia* species are altitude, rainfall, soil, and vegetation. Topography and soil properties are essential for the distribution of *Garcinia* species, with most of these tree species typically preferring flatland and well-drained alluvial soils in lowland forests (Nazre et al., 2009). Altitude also influences growth patterns, where higher altitudes favor more favorable conditions for tree height, girth, and crown diameter (Aravind et al., 2004). Most species grow in evergreen or semi-evergreen forests and occur at elevations ranging from sea level to as high as 1,500 meters (Ngernsaengsaruary et al., 2004). In Mizoram, varying forest types from tropical wet evergreen to montane subtropical to temperate forests have created a mosaic of habitats that most likely support multiple *Garcinia* species (Sati and Vangchhia, 2016). The Northeast part of India, particularly Mizoram, has been described as one of the most important transition zones in terms of biogeography between Southeast Asian and Indian biodiversity. High richness of species and endemism has been recorded in this region. It is one of the priority areas for conservation in the Indo-Burma biodiversity hotspot because a large number of endemic species also exist in this region (Sharma and Sharma, 2015).

Table 2.1: Morphological Variations in some *Garcinia* Species

Species	Leaf Characteristics	Flower Traits	Fruit Features	Distribution	Reference
<i>G. lanceifolia</i>	Lanceolate, coriaceous	Small, yellowish	Small, ovoid	Northeast India	Gupta et al., 2019; Dasgupta, 2023
<i>G. mangostana</i>	Elliptic-oblong, thick	Large, reddish	Large, purple	Cultivated tropics	Yuniastuti, 2014; Ketsa & Paull, 2011
<i>G. gummi-gutta</i>	Elliptic, glossy	Small, yellow	Yellow, grooved	Western Ghats	Nair et al., 2005; Shameer et al., 2021
<i>G. indica</i>	Oblanceolate, red tint	Small, pink	Purple, juicy	Western Ghats	Desai et al., 2022
<i>G. xanthochymus</i>	Oblong-lanceolate	Yellowish-white	Yellow, pointed	Eastern Himalayas	Ngernsaengsaruy et al., 2023
<i>G. cowa</i>	Elliptic, acute tip	Yellow,	Orange-yellow, juicy	Northeast India	Hossain et al., 2023

Species	Leaf Characteristics	Flower Traits	Fruit Features	Distribution	Reference
<i>G. brasiliensis</i>	Elliptic, glossy green	White to greenish	Yellow-orange	South America (Brazil)	Santa-Cecília et al., 2013; Cardoso et al., 2013
<i>G. morella</i>	Elliptic, dark green	Small, white	Yellow, small, acidic	India, Sri Lanka	Murthy et al., 2020
<i>G. pedunculata</i>	Obovate, leathery	White, large	Large, yellow, acidic	Northeast India, Bangladesh	Bhattacharjee and Devi, 2021
<i>G. spicata</i>	Elliptic, acute base	White, in clusters	Orange-yellow, small	India, Sri Lanka	Memon et al., 2025

2.3 Pharmacological and Therapeutic Significance

2.3.1 Bioactive Compounds and Traditional Medicine

It is well-demonstrated that the genus *Garcinia* is an eminent natural biomedical pharmacy and has been the subject of unfaltering interest in the past few decades due to the fact, that as a plant, it is endowed with the availability of and an abundance of biologically active metabolites. The most common of these are in polyisoprenylated benzophenones, flavonoids, xanthenes, and biflavonoids. They have wide-range of beneficial effects in treating multiple diseases (Kumar et al., 2013; Brito et al., 2022). In response to various infections, among others, studies on *Garcinia* extracts and most of its isolated compounds have revealed antioxidant, anticancer, anti-inflammatory, antimicrobial, and cardioprotective effects. More significantly, research on this genus has focused on the treatment of cancers, as recent studies stress its potential anticancer activity. Besides, economically, some notable *Garcinia* plants have ethnobotanical and nutritional significance; their fruits and by-products yield high levels of fiber, protein, and bioactive metabolites (Bruto et al., 2022; Paul and Zaman, 2022). *Garcinia*'s medicinal history can be traced back to traditional healing systems across its indigenous habitat, where various parts of the plant have long been employed to treat various disorders.

Garcinia species are traditionally known for their medicinal properties, used to treat skin disorders and inflammatory diseases, and are believed to provide relief from for pain and infections. The specific healing properties of different species have been widely demonstrated: *G. brasiliensis*, a plant native to the Brazilian Amazon, has indeed been recently reported to exhibit significant therapeutic potential for a variety of diseases. Some researchers have already demonstrated that the aqueous extract from *G. brasiliensis* exhibits potent anti-inflammatory and antinociceptive effects in animal models, thereby substantiating its use as an anti-inflammatory and analgesic medication (Santa-Cecília et al., 2011). This plant contains active ingredients, primarily bioflavonoids, which specifically inhibit the burst of neutrophils and are also protective against oxidative damage in human erythrocytes. It stands to reason, therefore, that they possess antioxidant and, thereby, anti-

inflammatory attributes (Arwa et al., 2015); *G. mangostana*, particularly its pericarp, has demonstrated significant medicinal properties.

Research has shown its efficiency in treating skin infections, inflammation, and wound conditions. Traditionally, it has also been used to relieve abdominal pain, diarrhea, dysentery, and chronic ulcers. (Tatiya-aphiradee et al., 2019; Pedraza-Chaverri et al., 2008; Obolskiy et al., 2009). The therapeutic potential of this plant is extensive, and that extends to being antimicrobial, antioxidant, anticancer, antidiabetic, and neuroprotective-all properties which are mostly linked with xanthenes, particularly α -mangostin, found in the pericarp. (Pedraza-Chaverri et al., 2008; Anis et al., 2025). Extracts obtained from *G. mangostana* pericarp have high anti-inflammatory and wound-healing effects as compared to that of α -mangostin alone in the treatment of MRSA- induced skin infections. Studies have also shown that *G. kola*, which is commonly known as bitter kola, is a medicinal plant native to West and Central Africa, prized for its therapeutic properties. The seeds of this plant are widely used in the treatment of various diseases, which include gastric disorders, coughs, liver diseases, and as an aphrodisiac (Tatiya-aphiradee et al., 2019).

The bioactive compounds- like biflavanoids, benzophenones, and xanthenes- are a typical trait of the plant's pharmacological activities (Tauchen et al., 2023; Gogo et al., 2025). It has also been reported that *G. kola* exerts its antioxidant, anti-inflammatory, and antimicrobial activities. *G. kola* seeds exhibit significant lack of harmful effects on the liver, testes, and spermatozoa in animal studies due to long-term consumption; thus, they enhance antioxidant status and levels of testosterone (Farombi et al., 2013; Anyanwu et al., 2025). There are about 418 species comprised under the genus *Garcinia*, roughly 60 of which have become widely useful in medicine, food, cosmetics, and industry. The high demand for plant-derived starting materials due to their bioactivities and clinical applications has increasingly drawn attention towards species of *Garcinia*, leading to a surge in scientific and applied interest concerning their utilization as resources (Guedje, 2019; Paul & Zaman, 2022). Such substantial traditional use underscores that these plants are culturally important and effective in terms of indigenous healthcare remedies.

2.3.2 Demonstrated Biological Activities

According to modern pharmacology, the most recent studies have confirmed the potential of these medicinal tree species in therapy, demonstrating significant bioactivity across multiple dimensions in the tests performed in in-vitro and in-vivo. *Garcinia* contains a wide array of biologically active metabolites, such as xanthenes and benzophenones, which possess strong antioxidant potential (Kazimierczak et al., 2023; Espirito Santo et al., 2020). These compounds showed significant beneficial effects in multiple diseases, including antinociceptive, antitumoral, antimicrobial, antidiabetic and cardioprotective properties (Espirito Santo et al., 2020). Antioxidant activities have been described in *Garcinia* fruits, including certain phenolic compounds, which protect against most of the current life style diseases, such as diabetes, obesity, and cardiovascular diseases. Studies also showed that the genus *Garcinia* possesses anti-inflammatory, anti-diabetic, neuroprotective, and hepatoprotective properties (Paul and Zaman, 2022).

The antioxidant action prevents oxidative stress-related illnesses from mutating cells. *G. brasiliensis* and its compounds, particularly 7-epiclusianone, exert very potent anti-inflammatory and antioxidative actions. 7-Epiclusianone inhibits carrageenan-induced paw edema and leukocyte recruitment in rats, whereas it also has antinociceptive effects in several pain models (Santa-Cecília et al., 2011). It also inhibits the generation of superoxide anions by neutrophils, which may be the mechanism through which it exerts its anti-inflammatory actions (Santa-Cecília et al., 2012). Garcinol represents a polyisoprenylated benzophenone of *G. indica* proven for anticancer potential among several cancer arrays. Tumor cell proliferation is inhibited by it, apoptosis is induced, and the cell cycle is arrested in oral and pancreatic cancer cells. Its anticancer activities are accomplished through a variety of mechanisms, including inhibition of NF- κ B and COX-2 expression, and suppression of angiogenic factors like VEGF, as well as modulating apoptosis-related proteins (Aggarwal and Das, 2016; Parasramka and Gupta, 2011). Moreover, garcinol possesses antioxidant, anti-inflammatory, as well as anti-epigenetic properties by inhibiting histone acetyltransferases (Saadat and Gupta, 2012).

There are many activities through which this oncogenic capability acts in different cancer types, such as breast, colon, hepatocellular carcinoma, and others (Aggarwal et al., 2020). Such natural compounds, when tested against human cancer cell lines, show significant anticancer potential, as xanthones are types of phytochemicals found in *G. mangostana* plants. One type of xanthone, garcinone E, is claimed to be cytotoxic in hepatocellular and certain other cancers (Ho et al., 2002). Garcinia xanthone, extracted from *G. xanthochymus*, induces apoptosis in HepG2 through the mitochondrial pathway and inhibits cell migration (Jin et al., 2019). It has very strong antimicrobial activity against almost every pathogen, be they Gram-positive or Gram-negative bacteria, like *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as fungi like *Candida albicans* or *Aspergillus niger*, according to the results of the studies (Arekemase et al., 2012; Akerele et al., 2008).

Antimicrobial activities are imparted by compounds such as kolanone and hydroxybiflavanonols. GB1 is especially active (Madubunyi, 1995). *G. Kola* extracts have been demonstrated to exhibit antimicrobial effects comparable to hops when tested on beer spoilage microbes like *Lactobacillus delbruckii* and *Candida vini*. Hence, their possible use as a substitute for hops in beer brewing (Aniche and Uwakwe, 1990). Kolaviron, a biflavonoid complex obtained from the seeds of *G. kola*, possesses hepatoprotective activities against chemical-induced liver damage. Kolaviron has been reported to reduce the hepatotoxicity induced by carbon tetrachloride and ethanol via several mechanisms. These mechanisms include free radical scavenging, evidenced in decreased lipid peroxidation and enhanced activities of antioxidant enzymes. Kolaviron also enhances the activities of drug-detoxifying enzymes such as UDP-glucuronosyl transferase and glutathione S-transferase (Farombi, 2000; Braide, 1991; Adaramoye et al., 2009).

**Table 2.2: Some bioactive compounds and their therapeutic effects
in *Garcinia* species**

Compound Class	Compounds	Biological Activities	Sources	References
Xanthones	α -mangostin, γ -mangostin	Antioxidant, anti-inflammatory, anticancer, antimicrobial	<i>G. mangostana</i> , <i>G. xanthochymus</i>	Jindarat, 2014; Saraswathy et al., 2022
Benzophenones	Garcinol, 7-epiclusianone	Anticancer, anti-inflammatory, antimicrobial	<i>G. brasiliensis</i> , <i>G. indica</i>	Santa-Cecília et al., 2011; <u>Ananthakrishnan</u> and <u>Rameshkumar</u> , 2016
Biflavonoids	Kolaviron, fukugetin	Hepatoprotective, antioxidant, anti-inflammatory	<i>G. kola</i> , <i>G. brasiliensis</i>	Arwa et al., 2015
Organic Acids	Hydroxycitric acid (HCA)	Anti-obesity, appetite suppression	<i>G. cambogia</i> , <i>G. indica</i>	Chuah et al., 2013; Noreen et al., 2023

2.4 Reproductive Biology in *Garcinia* Species

The reproductive biology of the *Garcinia* species exhibits an incredible range of mechanisms, from hermaphroditism and dioecy to polygamodioecy, apomixis, and agamospermy. These strategies also affect the reproductive success, ecological adaptation, genetic variability, and conservation potential of species.

The majority of the species in the genus exhibit apomixis, some of which are obligate apomicts, while others are facultative. *G. mangostana* var. *mangostana* (mangosteen) exemplifies as a classic case of obligatory apomixis, characterised by male sterility, with the result that reproduction takes place solely through asexual seed formation (Richards, 1990; Emlee et al., 2025). The majority of the mangosteen trees are females, reproducing clonally through seeds. This genotype has maintained

the limitation of extreme phenotypic as well as genetic diversity, while characterized by dioecious ancestry (Sando et al., 2005; Emlee et al., 2025). It is hypothesized that mangosteen originated as an allopolyploid because it is believed to be developed from *G. hombroniana* and *G. malaccensis* through single hybridization events. Neither of the parent species is like the mangosteen, which clearly is an obligate agamosperm because it can reproduce both sexually and asexually (Richards, 1990; Emlee et al., 2025). The genus also affords a certain degree of obligate and facultative apomixis in its other members; *G. celebica* and *G. prainiana* are facultative apomicts (Emlee et al., 2025), while *G. atroviridis* has a mixed system whereby both sexual and asexual reproduction types are achievable. Unfertilized flowers of *G. atroviridis* can bear fruits, but, it's important to note that better fruit and seed viability are generally produced through cross-pollination. This species demonstrates gynodioecy, with female trees producing larger fruits and seeds than hermaphroditic trees (Pangsuban et al., 2007, 2009).

Diversity can also be observed among different species such as *G. indica*, a polygamo-dioecious species, reflecting diversity in floral types and also in pollen traits (Dike and Deodhar, 2015). Critically endangered species like *G. imberti* which is strictly dioecious and self-incompatible, need pollination for fruiting and to produce seeds (Kandasamy et al., 2015). Studies on seed morphology undertaken on *G. indica*, *G. cambogia* and *G. xanthochymus* have corroboratively indicated that so-called 'seeds of these genera lack separated embryos and endosperm "thus generally are not true seeds' (Malik et al., 2005).

It has been observed that there is agamospermy in *Garcinia* and can occur via multiple pathways, including adventitious embryony and gametophytic agamospermy. Furthermore, it increases a new level of complexity in the reproductive biology of the genus (Richards 1990). Due to the variety of reproductive systems extending from obligatory apomixis to sexual dimorphism and facultative reproductive strategies, there are significant implications for *Garcinia* species which is essential for fruit production, crop improvement, and conservation of genetic resources (Baskware and Deodhar 2023; Kandasamy et al. 2015).

Garcinia's floral biology is highly diversified in terms of morphology, seasonality, and functionality as reproductive structures, indicating that the genus is complex and well-adapted to ecological change. Its flowers are generally small and inconspicuous, colors ranging from white to pink, or red and are most commonly comprised of four sepals and four petals (Te-chato, 2007). The genus is divided into two basic evolutionary lines, consequentially, the presence or absence of nectaries, which forms a basis, being the most important pollinator attractant structure. It has nectaries derived from receptacular or staminodial origin, which determine the pattern of development across species (Sweeney, 2008; Leal et al. 2012).

Sexual systems within the *Garcinia* are also quite diverse. The majority of species are dioecious, bearing male and female flowers on separate individuals, which differs from others that exhibit monoecy, gynodioecy, or polygamodioecy. (Baskaware and Deodhar, 2022). The variability of this structure is attributable to differences in reproductive strategies and has considerable implications in pollination ecology and conservation of endemics. The flowering phenology of species varies greatly within and between species; for example, some can bear flowers in as few as five years, while others require a longer duration of 10-15 years to mature (Mujadadi et al., 2024). There is even a seasonal variation in most of cases where *G. hombroniana* flowers from January to June in most of the years. Altogether male and female flowers manifest reproductive alternatives. Male flowers follow a “steady-state” approach by producing pollen over an extended period, with female flowers adopting a “big-bang” strategy through producing numerous receptive flowers in a short period (Richards, 1990).

Research on pollen helps in understanding the plants' reproductive biology. There is an ample interspecific variation within pollen, in the sense of its viability, germination, and functionality. As an illustration, *G. celebica* is found to have a moderately high proportion of viable pollen (68%) and germination, and this confirms the functional production of male fertility in the species (Sutthinon et al., 2018). In a similar way, different floral types in *G. indica* are found to have fertile or functional pollen (Dike and Deodhar, 2019). *G. mangostana* var. *mangostana*, on the other hand, exhibits complete male sterility and abortion of pollen grains due to

an interruption in its process of microgametogenesis (Sutthinon et al., 2019). In some facultative apomicts, such as *G. atroviridis*, *G. cowa*, and *G. parvifolia*, male trees produce fertile pollen, allowing for both sexual and asexual reproduction (Emlee et al., 2025). Various studies have shown total pollen viability of 100% in *G. atroviridis*, *G. prainiana*, and *G. hombroniana*, while *G. schomburgkiana* was recorded with zero viability, thereby displaying the extremes of reproductive capacity within the genus (Te-chato, 2007).

Male sterility has been studied quite extensively in mangosteen, being a model plant that has apomixes, an obligate type of reproduction. The study of *G. mangostana*, together with its fertile relative *G. celebica*, indicates that dysfunctions such as the early degeneration of the tapetum, anomalous callose wall deposition, and insufficient starch accumulation in microspore mother cells are related to the abortion of pollen in mangosteen (Sutthinon et al., 2019). In mangosteen, these abnormalities prevent pollen maturation and cause male sterility. On the contrary, the tapetum of *G. celebica* functions properly, generating healthy, viable, and germinable pollen, supporting the hypothesis that it is one of the progenitors of *G. mangostana* (Sutthinon et al., 2018). Male sterility in more generalized plant species is predominantly caused by a mutation in the gene that is crucial for the development of anthers and pollen (Yang, 2013).

In general, the flowers of *Garcinia* show great variability in floral structures, pollen functionality, and the sexual system. This variability is especially troublesome for reproductive and breeding studies, given *Garcinia*'s long juvenile phase and its flowering window, which is often very narrow. Similarly, these features highlight the evolutionary adaptations that underpin *Garcinia*'s reproductive ecology defining its ecological achievements and its relevance for ecological and genetic conservation, as well as for the improvement of *Garcinia* as a crop.

A research study on the pollination ecology of *Garcinia* species is still lacking. Despite of this, available studies suggest that several insects, such as bees, flies, and ants, play roles. Trigona bees are the pollinator of *G. hombroniana*, which has a 'wet' stigma and binucleate pollen (Richards, 1990). Most of the *Garcinia*

species are dioecious and facultatively apomictic; however, *G. mangostana* var. *mangostana*, is unique, obligate apomict as a result of male sterility (Emlee et al., 2025). Pollen viability and germination studies in *G. indica* reveal variability in floral types and reproductive behaviour (Deodhar and Dike 2019). Tropical dioecious species typically have flowers that are specific to particular pollinators, among which the most common are bees, beetles, moths, flies, and wasps (Renner and Feil, 1993). The environmental conditions significantly dictate the timing of flowering and fruiting in tropical species, including *Garcinia*. In *G. kola*, rain is a significant flowering cue; however, tree gender determines the flowering intensity, as rainfall patterns during different seasons have a varying impacts (Okonkwo et al. 2024). Temperature, relative humidity, and rainfall are also significantly related to flowering and fruiting times for some Annonaceae species, with flowering generally occurring in wet months and fruiting in dry periods (Lestari and Fiqa, 2020). Flowering of *G. mangostana* is influenced by rainfall, temperature, relative humidity, and dry spells preceding flowering, allowing dates to be predicted based on these parameters (Ounlert et al., 2017). In southern Thailand, long-term climate changes have led to delayed flowering of mangosteen, with increasing annual rainfall, more rainy days, higher humidity, and higher temperatures observed over a 33-year period (Apiratikorn et al., 2014). The reproductive biology of the species remains complicated, with apomixis and some very specific pollination execution constituting important components of their ecology and conservation.

Different species of *Garcinia* exhibit a wide range of reproductive strategies contributing to the varying reproductive successes and genetic variability. An example of such divergence is facultative apomixis imposed by these plants, where asexual seed production coincides with sexual reproduction (Baskaware and Deodhar, 2022; Emlee et al., 2025). As illustrated with *G. atroviridis*, studies on such species, using manually controlled pollination, revealed that apomictic reproduction is dominant in the process. Yet, when it comes to fruit set, fruit size, and good seed quality, sexual reproduction played an active role, significantly improving all these attributes. Molecular evidence from RAPD analysis further justified this dual system, in which approximately 58% of the progeny were apomicts, while the rest were

products of sexual reproduction, further enforcing the facultative characteristic of the reproductive system (Pangsuban et al., 2009).

On the contrary, a few species indeed exhibit obligate apomixis, such as *G. mangostana*, which arises from male sterility, whereas the others retain their functional male individuals and, are therefore termed as facultative apomicts (Emlee et al., 2025). It is indeed surprising that in the presence of considerable morphological variation in species like *G. indica*, molecular markers reveal very low genetic polymorphism (3–12%), leading, in some cases, to high endemism and limited genetic diversity within the genus (Dike and Deodhar, 2019). At the same time, the findings importantly stress the difficult equilibrium between asexuality and sexuality in determining success in reproduction as well as creating highly stable gene pool longevity in populations of *Garcinia*.

2.5 Seed Biology and Germination Ecology in *Garcinia* Species

Seed biology reveals a fundamental aspect of the plant life cycle, and furthermore, influences the population's dynamics, the distribution of species, and the success of regeneration. Besides, seeds have become an important source of spermatophyte colonization and human food (North et al., 2010). Both temperature and water availability induce variations in seed dormancy and germination. Hence, climate change has become an important contributor to the modifications that will lead to changes in regenerating patterns and shifted germination phenologies, eventually affecting population and community dynamics (Walck et al., 2011). Progress in seed biology has been to view the seed not as a single individual, but to acknowledge its ecological individuality. Thus, it has been integrating that molecular genetics and ecology can look into developing an understanding of how the environmental signals influence development, dormancy, and germination (Penfield, 2017).

The fruit of *Garcinia* species is fleshy and mainly dispersed by animals, such as mammals and birds, in a manner referred to as animal-mediated seed dispersal. Fruit of *G. cowa* plants are large (5.5 cm in diameter and 73 g in weight), with a sweetish-sour succulent pulp, generally eaten by animals such as monkeys, raccoon

dogs, and squirrels - arboreal frugivores, with 86.5-91.4% of fruit being removed by these animals. The multi-step dispersal comprises primary removal of the fruits by primary frugivores, followed by separate secondary dispersal and seed predation activities by such terrestrial predators as rodents (Liu, 2002). Broad cross-species comparison across angiosperms reveals that fleshy fruit characteristics, which facilitate animal dispersal, are subjected to strong phylogenetic constraints, with an evolutionary relationship between disperser type and fruit traits being primarily confined to fruit size (Jordano, 1995)

These species produce seeds within fleshy, edible fruits that are unique due to their biological characteristics. The seeds are relatively recalcitrant, have low desiccation resistance, and their size and moisture content, which greatly influence their germination ability (Emlee et al., 2025). The seeds are recalcitrant, meaning they are mostly desiccation and cold-sensitive, making long-term storage a problem (Noor et al., 2016; Emlee et al., 2025). Distinct among *Garcinia* seeds is the fact that they do not have a separate embryo, endosperm, or embryonic axis, meaning they are not actual seeds in botanical terms (Malik et al., 2005). *Garcinia* species are known for most of their species being apomictic, especially agamospermic or asexual reproduction, which could be facultative or obligate depending on the species (Emlee et al., 2025; Baskaware and Deodhar, 2023). Most *Garcinia* species exhibit typical *Garcinia*-type germination, characterized by the emergence of radicles and shoots from opposite ends during germination (Noor et al., 2016).

Garcinia species present complex seed dormancy mechanisms, influencing germination timing in the tropical environment. Seed size can show considerable variation among species, with larger seeds, such as those of *G. hombroniana*, retaining moisture for prolonged durations and having higher germination percentages. Germination success, in turn, depends on seed size, as larger seeds, such as those of *G. hombroniana* and *G. mangostana*, retain moisture for longer and germinate at a higher percentage than smaller seeds (Noor et al., 2016). Germination in seeds of *G. gummi-gutta* is a true physiological shallow type of dormancy, where seeds with intact seed coats germinated 90% after 24 weeks of warm stratification, and de-coated seeds germinated within four weeks of storage (Joshi and Phartyal,

2017). Dormancy is mainly due to a thick seed coat rather than to chemicals, as revealed by germination success after mechanical scarification or treatment with acid (Anilkumar et al., 2007). These seeds are recalcitrant and sensitive to drying and chilling, with a critical seed moisture content of approximately 34% in the seed of *G. gummi-gutta*. It is evident that these dormancy mechanisms have evolved toward matching dormancy breaking with seasonal conditions in the tropical areas (Joshi and Phartyal, 2017; Emlee et al., 2025).

Research has shown that physical dormancy in response to pre-sowing treatments is likely to improve germination in most tree species, but limited studies have been conducted specifically for *Garcinia*. In *G. gummi-gutta*, mechanically scarified seeds showed 100% germination within three months, while nicked shade-dried seeds also resulted in uniform seedling growth (Anilkumar et al., 2007). In *G. kola*, germination rate was fairly improved with cold water pretreatment and later on incubated on polyethylene bags to 62% from 28% and decreased time of germination from 71.2 to 25.8 days (Nzegbule and Mbakwe, 2001). Similar successes have been recorded on other species with physical dormancy, where treatments such as acid scarification, mechanical scarification, and seed nicking proved useful towards increasing germination percentages and reducing mean germination time in arid land species (Jaishwal, 2020) and multipurpose tree species (Airi et al., 1998).

Among different species, varying degrees of achievement have been observed through treatment. For example, it improved germination in *G. gummi-gutta* (60.05%), *G. cowa* (65.33%), and *G. tinctoria* (78.6%) when treated with 30% hydrogen peroxide for 30 minutes. Growth hormone treatments also promoted germination, with kinetin (500 ppm) and gibberellic acid (500 ppm) as the main agents that produced better results. Kinetin treatment, in particular, led to 93.3% germination of *G. tinctoria* (Joseph et al., 2007). There is a general assumption that seed coat impermeability can be overcome through physical, chemical, or hormonal procedures to increase the uniform rate of germination in *Garcinia* species.



CHAPTER - III

MATERIALS AND METHODS



CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

Mizoram, the study site for this research, situated in the northeastern part of India, is a designated part of the Indo-Myanmar biodiversity hotspot, one of the richest areas with distinct endemic flora and fauna diversity (Barman et al., 2018; Sati and Vangchhia, 2016). It has been found that community conservation efforts have helped in maintenance of plant species diversity in community-conserved areas as compared to formal protected areas; nevertheless, they are facing the decline of endemic species and threatened species (Lalzarzovi and Lalnuntluanga, 2022). The Eastern Himalayan extension covers diverse types of forests that serve as a vital source of livelihood, and Mizoram is distinct for showing a 1.4% increase in forest cover over three decades despite the national deforestation trends (Sati et al., 2014). With a total geographic area of approximately 21081 sq km, it accounts for about 0.64% of India's total land area. (Directorate of Economics and Statistics, Mizoram, 2018). In the northeastern part of India, Mizoram falls between 21°56'-24°35' N latitudes and 92°16'-93°26'E longitudes. The Tropic of Cancer passes through the state at 23°30'N, significantly influencing its climatic and ecological conditions (Vabeikhokhei et al., 2019). Myanmar surrounds to its eastern boundary, while Bangladesh aligns westward and southward. Furthermore, it is connected through territorial boundaries with Assam, Manipur, and Tripura. This mountainous region is covered by evergreen rainforests that provide essential resources to indigenous communities through jhum cultivation, hunting, and gathering (Purkayastha, 2019; Vanlalruati and Thlanga, 2024).

Most parts of the state are mountainous with rough terrain. The state covers an area of 21,081 km², with north-south hill ranges separated by narrow valleys and deep river gorges. Elevation ranges from below 100 m along the Assam and Bangladesh borders to 2,157 m at Phawngpui Peak, the highest point in the area. The eastern region includes other prominent highlands such as Lengteng (2,141 m). The elevation ranges from below 100 m along the Assam and Bangladesh borders to

2,157 m at Phawngpui Peak, the highest point above sea level. In the east and southeast, other high mountain ranges; such as the Lengteng, which is 2,141 m (Choudhury, 2006; Beingachhi and Zothansanga, 2019).

Mizoram falls within the tropical monsoon and sub-tropical moist region, where rainfall is high, with distinct seasonal variations. From May to September, four months are considered as the rainy months with an annual rainfall of 2,000 mm to 3,500 mm. The average maximum and minimum temperatures in summer generally range between 14°C and 30°C, while those in winter are mild and ranging between 6°C and 24°C (Forest Survey of India, 2021). The soils are generally acidic, rich in organic matter, and mostly have a sandy loam to loam texture, which allows various types of vegetation to thrive.

Among the states in India, Mizoram particularly has a very high percentage of forest cover. As per the records of the India State of Forest Report (ISFR 2021), it has forest cover of 84.53% of its total geographical area, amounting to 17,820 km². The percentage of forest cover has reduced substantially compared to the recorded assessment from ISFR 2015, which was noted at 88.93% (18,748 km²), and a similar reduction has been observed in ISFR 2019. The decline showcases the impact of anthropogenic stress, which is mostly becoming evident from shifting cultivation and frequent habitat conversion. Traditional cultivation practices such as jhum cultivation and home gardening still serve as the major aspects of agrarian life and reflect the deep-rooted power in the socio-economic fabric of the people of Mizoram.

Ecologically, Mizoram possesses diverse types of forests, and these are found to range widely with respect to altitudes, rainfall regimes, and species composition. The factors of the above have been recognized and classified into six major categories, Viz. Tropical Wet Evergreen Forests, Montane Subtropical Forest, Temperate forests, Bamboo forests, Quercus forests, and Jhum fallow land (Gogoi et al., 2022). This ecological and climatic diversity, coupled with its status as part of a global biodiversity hotspot, makes Mizoram an important site for studying underexplored plant taxa such as *Garcinia lanceifolia* Roxb. The region's unique landform and mild yet well-circulated climate are favorable features for the study of

reproductive biology, seed ecology, and conservation strategies of this hardly known but botanically and ethnologically significant species.

3.2 Study Sites

Two field sites were selected in Mizoram for this investigation (Figure 1): Thenzawl in Serchhip District and Thingdawl in Kolasib District. Both differ in their ecological characteristics, thereby providing complementary habitats for the study of *Garcinia lanceifolia* Roxb.

3.2.1 Thenzawl

Thenzawl (23°17'08" N, 92°46'51" E) lies in the heart of Mizoram at an elevation of about 786 m above sea level. The area lies within the subtropical evergreen forest zone, with dense natural vegetation thriving under limited human interference. A humid climate has an average rainfall of almost 2299 mm with, the western or southwest monsoon bringing the majority. Seasonal temperatures range from as low as 11 degrees Celsius in winter to about 28 degrees Celsius in summer. The soil is generally well-drained, varying from loamy to sandy-loam in texture and rich in organic matter, making it very suitable for forest growth. The landscape is dominated by evergreen stands that remain largely undisturbed, marking it as one of the relatively pristine forest habitats in Mizoram.

3.2.2 Thingdawl

Thingdawl (24°11'04" N, 92°41'39" E) is located in northern Mizoram at an altitude of about 714 m above sea level. The area falls within a semi-evergreen forest zone where the vegetation is a mixture of natural forest patches and landscapes altered by human activities. With an average annual rainfall of nearly 2,540 mm, the regional temperatures usually oscillate between 13°C in winter and 30°C in summer. The soils are mostly lateritic to red loamy in texture, moderately fertile, with the ability to sustain different types of vegetation. Unlike Thenzawl, human consequences in the form of shifting cultivation and settlement expansion are much more severe at Thingdawl, a disturbed forest habitat scenario in the state.

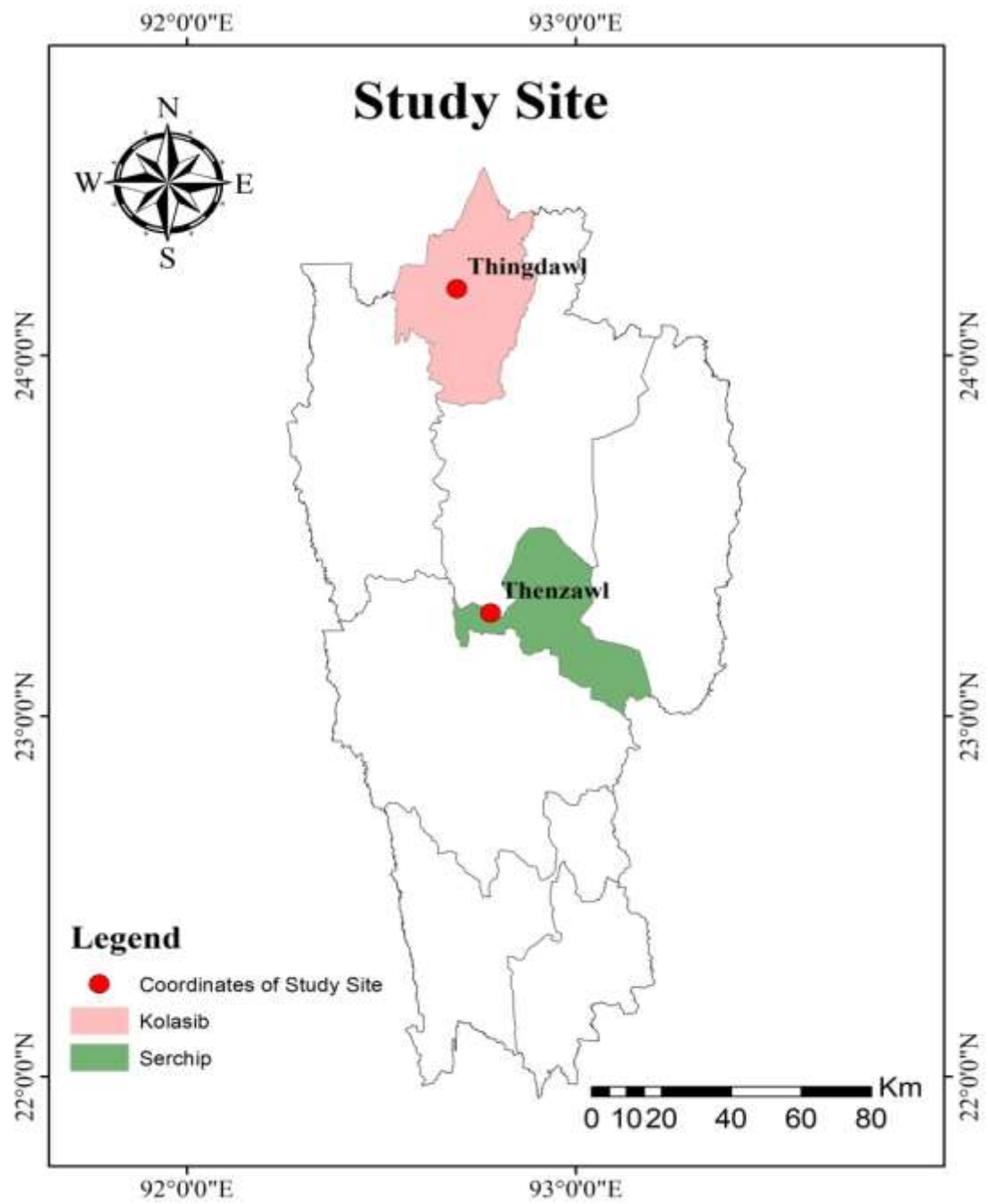


Figure 3.1: Map showing study sites

3.3 Meteorological Data

Meteorological data on rainfall and temperature were collected for the two study sites, for Thenzawl (Serchhip District) and Thingdawl (Kolasib District), between the 1st of January 2021 and the 31st of December 2022. These data were downloaded from IMD through the IMDLib Python library, which allows access to quality-controlled, high-resolution NetCDF files. Daily rainfall data in grids and daily temperature data for the Thenzawl (23°17'08" N, 92°46'51" E) and Thingdawl (24°11'04" N, 92°41'39" E) locations were extracted, processed, and aggregated for analysis purposes. A summary of the results is presented in Figure 2.

The average rainfall in Thenzawl in 2021 was roughly 2,310 mm, and it was about 2,285 mm in 2022, with maximum annual rainfall occurring during the peak of monsoon months from June to September. The average monthly temperature oscillated between 11.4°C in January and 27.6°C in July, describing the climate particular to that of the subtropical evergreen forest zone.

On the contrary, Thingdawl received slightly higher annual rainfall than Thenzawl, with about 2,525 mm of rainfall in 2021 and about 2,548 mm in 2022. Seasonality would generally show more or less similarity with Thenzawl, but with comparatively higher temperatures having an average minimum temperature of approximately of 12.8°C in winter and soaring to nearly 29.8°C in summer, relatively warm values concurrent with its semi-evergreen forest environment.

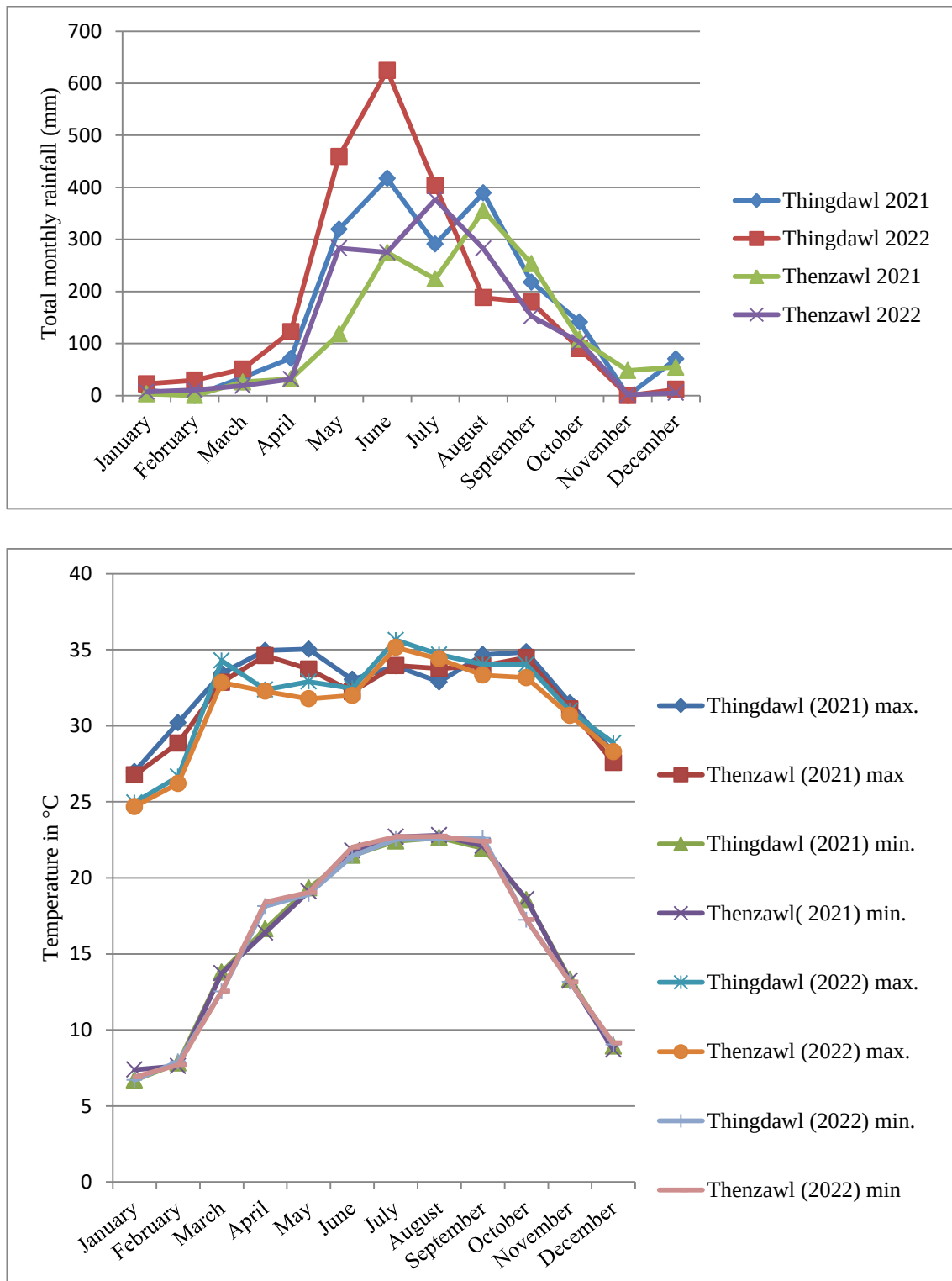


Figure 3.2: Weather of the study sites for the year 2021 and 2022

3.4 Morphological Characteristics of *Garcinia lanceifolia*

All morphological characters were assessed following the procedure described by Kumari (2020) with slight modifications. The morphological traits of *Garcinia lanceifolia* were described by documenting natural variability in different attributes. Observations were carried out on different plants, following standardized procedures and visual observations. Measurements of tree height, leaf size, and assessments of leaf, and tree form, along with qualitative categories of traits such as foliage density, shape of the leaves, type of apex, texture on surface, and colour of trunk were also conducted during the investigation.

3.4.1 Tree Height (m)

The height of the tree was measured using a measuring tape

3.4.2 Tree Shape

The tree was visually observed and categorized as Erect, Spreading, Semi-spreading, or Drooping.

3.4.3 Foliage

The foliage of the tree was visually observed and categorized as Sparse or Dense.

3.4.4 Leaf Size

The size of the leaf was measured with the help of a Measuring Scale.

3.4.5 Leaf Shape

The leaf shape was visually observed and categorized as Elliptical, Oblong, Oval-Oblong, or Oval.

3.4.6 Leaf Apex

The leaf apex was visually observed and categorized as Obtuse or Acute.

3.4.7 Leaf Surface

The surface of the leaf was also observed visually and categorized as Glabrous, Moderately glabrous, or Non-glabrous.

3.4.8 Trunk Colour of the Tree

The trunk colour was assessed with the help of the Royal Horticultural Society (RHS) colour chart.

3.5 Floral biology, flowering Phenology and Pollen Production

A study had been carried out following the methodology for floral biology, flowering phenology, and pollen production with necessary modifications from Kumari (2020), Mujadadi et al. (2024), and Shivanna & Rangaswamy (2012). Systematic field observations made to document flowering patterns, floral traits, and pollen output are critical to understanding reproductive biology and thus reproductive success in *Garcinia lanceifolia*. Standardized procedures ensure consistency in data recording for the determination of flowering time, peak flowering, inflorescences, and flower size, as well as anthesis timing, pollen production, and female flower production.

3.5.1 Duration of Flowering

The opening of 50 fresh flowers per tree on a given day was kept as the criterion for the beginning of the flowering season. The dates for the first and last appearance of flowers were recorded to determine the flowering season.

3.5.2 Time of Peak Flowering

The peak flowering time was recorded when 50% of the flowers on the tree had opened.

3.5.3 Length of Inflorescence

The length of the inflorescence was measured in centimeters from the base of the flower to the tip of the inflorescence.

3.5.4 Size of the Flower

The size of individual flowers was measured in centimeters using a measuring scale.

3.5.5 Anthesis

About 50 flower buds expected to open the next day were tagged and observed the following day for the time of anthesis, with counts taken at half-hour intervals. The time of anthesis was recorded over a period of five days during peak flowering.

3.5.6 Pollen Production

3.5.6.1 Pollen production per anther

Pollen production per anther was calculated using the formula

$$P = \frac{TpA}{N}$$

Where,

TpA = Total number of pollen grains counted in all anthers

N = Total number of anthers (slides) used for counting

3.5.6.2 Pollen per Flower (PF)

Pollen production per flower was estimated by multiplying the number of pollen grains per anther by the total number of anthers per flower.

$$PF = PA \times AF$$

Where:

PA = Mean pollens per anther

AF = Average number of anthers per flower

3.5.6.3 Pollen Production per Tree (TP)

The total pollen production per tree was estimated using the formula:

$$TP = PA \times AF \times FB \times BT$$

Where:

PA = Average number of pollens per anther

AF = Average number of anthers per flower

FB = Average number of flowers per branch

BT = Average number of branches per tree

3.5.7 Female flower production

Total flower production per tree was estimated by multiplying the average number of branch per tree by average number of flower per branch

3.5.8 Pollen viability

The viability of the pollen grains has a critical influence on reproductive success, and it is an essential factor that affects fruit set and seed production in angiosperms (Dafni and Firmage, 2000). Pollen viability was determined using the Fluorochromatic reaction (FCR) based on the method given by Heslop-Harrison and Heslop-Harrison (1970), and Heslop-Harrison et al. (1984) with required modifications. FDA stock ($2 \text{ mg} \cdot \text{mL}^{-1}$ in acetone) was prepared and stored at -20°C . A working solution ($20 \text{ } \mu\text{g} \cdot \text{mL}^{-1}$) was made by diluting stock 1:100 into 10% (w/v) sucrose. Fresh pollen was mounted in a drop of the working FDA solution, incubated in the dark for 5–15 min, and observed with a fluorescence microscope (FITC filter). Viable pollen grains exhibited bright green fluorescence and were counted; viability was expressed as percent fluorescing grains.

3.6 Transcriptome Sequencing of *G. lanceifolia* Flowers

Transcriptome sequencing was conducted to compare gene expression profiles of male and hermaphrodite flowers of *Garcinia lanceifolia*. Fresh floral tissues at anthesis were collected, frozen in liquid nitrogen, and stored at -80°C until RNA extraction. High-quality RNA ($\text{RIN} \geq 7.0$) was isolated, checked on an Agilent 2100 Bioanalyzer, and used for cDNA library preparation with the IlluminaTruSeq RNA Kit. Libraries were sequenced on an Illumina HiSeq platform to generate paired-end reads (Mortazavi et al., 2008).

Raw reads were filtered with Trimmomatic (Bolger et al., 2014) and assembled de novo using Trinity (Grabherr et al., 2011). Functional annotation of the unigenes was performed against the NR, Uniprot, KOG, KEGG, and Pfam databases. Transcript quantification was performed using RSEM (Li & Dewey, 2011), and differential expression analysis between flower types was conducted with DESeq2 (Love et al., 2014), with $|\log_2 \text{fold change}| \geq 1$ and $\text{FDR} \leq 0.05$ considered significant.

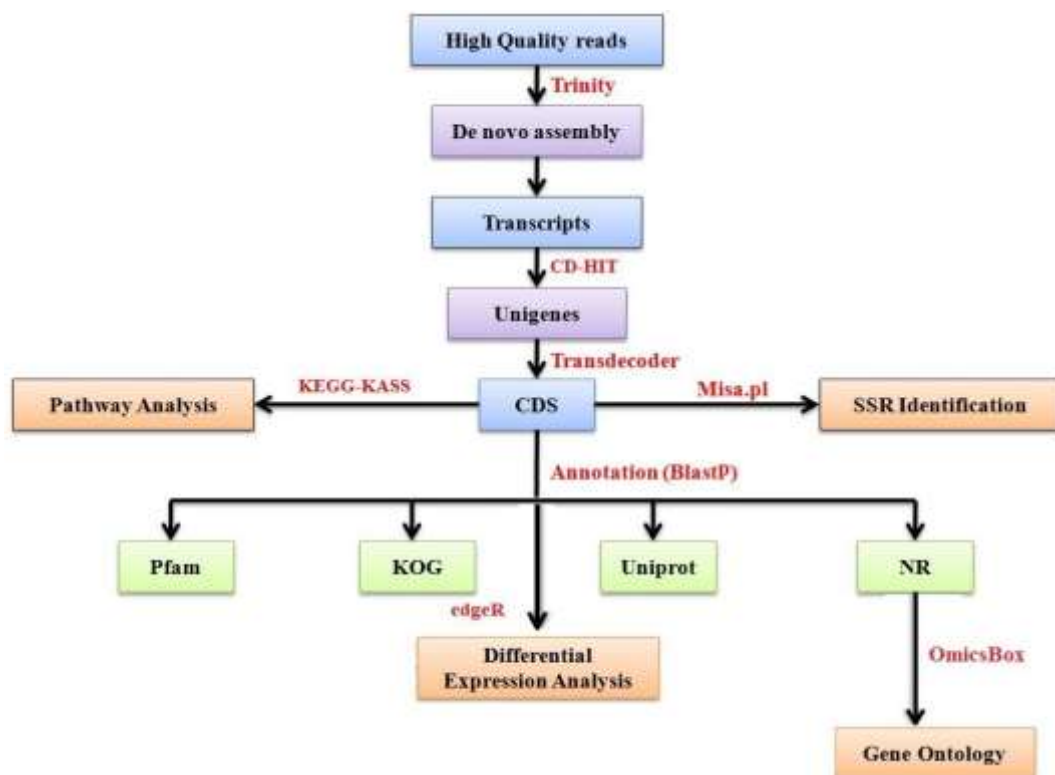


Figure 3.3: Bioinformatics workflow

3.7 Physical Parameters of the Fruits

The assessment of fruit physical parameters provides essential information on the variability, structural composition, and potential utilization of *Garcinia lanceifolia*. An important attribute of a fruit is its morphological characteristics, which are necessary for taxonomic identification, evaluation of genetic resources, and understanding the ecological adaptation of a species. Methodologies were standardized for recording fresh and dry weights, dimensions, rind, and seed characteristics, along with colour attributes of the fruit at maturity (Tripathi et al., 2024). Mature and healthy fruits were randomly collected from selected trees to ensure representative sampling, and twenty fruits were used for detailed measurements of morphological attributes.

3.7.1 Fresh Fruit Weight

The fresh weight of each fruit was measured using an electronic top-pan balance and expressed in grams (g).

3.7.2 Fruit Length

The length of the fruit was measured from the base (pedicel attachment point) to the tip using a digital Vernier caliper (mm).

3.7.3 Fruit Diameter

The diameter was measured at the widest part and near the shoulder of the fruit using a digital Vernier caliper (mm).

3.7.4 Fruit Shape

The shape was visually observed and categorized as conical, oblong, pear-shaped, spherical, or spherical with a pointed end.

3.7.5 Rind Thickness

The rind thickness of fresh fruits was measured at two points using a digital Vernier caliper (mm).

3.7.6 Fruit Rind Dry Weight

The rind was separated, dried in an oven at 50°C for one week, and weighed using an electronic top-pan balance (g).

3.7.7 Fresh Rind Weight

The fresh rind weight was measured immediately after separation using an electronic top-pan balance (g).

3.7.8 Fruit Colour at Ripe Stage

The mature fruit colour was assessed visually using a standard colour chart (e.g., RHS Colour Chart).

3.7.9 Number of Seeds per Fruit

Ten representative fruits were dissected, and the seeds were counted manually.

3.7.10 Seed and Pulp Weight

The weight of seeds and pulp was measured using an electronic top-pan balance (g).

3.7.11 Seed Dry Weight per Fruit

Seeds (with thick coat) were dried in an oven at 50°C for one week, and their weight was measured using an electronic top-pan balance (g).

3.8 Seed Germination Study

Seed germination experiments were conducted to investigate the effectiveness of various pre-sowing treatment methods on improving seed germination performance in *Garcinia lanceifolia*. Since the species itself is bearing recalcitrant seeds characterized by a relatively limited storability, germination boost is declared imperative in this species of plant to propagate and conserve (Noor et al., 2016; Emlee et al., 2025). Seeds collected from various sites of Mizoram were

combined and used to obtain several replications under controlled polyhouse conditions.

Various pre-sowing treatments used are as follows:

3.8.1 Dist. Water (18 hrs)

3.8.2 Sulphuric acid 30% (30 min)

3.8.3 KNO₃ (Potassium Nitrate) (0.2% 18 hrs)

3.8.4 Kinetin (500 ppm 18 hrs)

3.8.5 Gibberellic acid (300 and 500ppm 18 hr

3.8.6 Thiourea (2% 18hrs)

3.9 Germination and Seedling Emergence Parameters

The phase of seed germination is the most crucial stage in the life cycle of forest tree species, as it determines seedling establishment and future existence. It is necessary to assess parameters such as seed vigor, seed viability, and efficiency of different pre-sowing treatments on germination attributes. Seeds of *Garcinia lanceifolia* with different pre-sowing treatments were sown in mist chamber seed bed conditions, which provided suitable humidity and temperature. Thus, the rate and degree of germination are optimally detected after measurement of almost all germination parameters and seedling emergence.

3.9.1 Germination Percentage (GP)

The percentage of germination was calculated by dividing the number of seeds germinated by the total number of seeds sown and expressed as a percentage (Tanaka-Oda et al., 2009).

$$\text{GP} = (\text{Germinated seeds} / \text{Total seeds sown}) \times 100$$

3.9.2 Germination Capacity (GC %)

Germination capacity was determined as the cumulative number of seeds that germinated during the 28-day test period, plus the number of seeds that remained viable but ungerminated at the end of the test (Noor et al., 2016).

$$\text{GC (\%)} = ((\text{Cumulative germinated seeds} + \text{Viable un-germinated seeds}) / \text{Seed sown}) \times 100$$

3.9.3 Germination Energy (GE %)

Germination energy, which reflects the speed and uniformity of germination, was expressed as the percentage of seeds that germinated up to the time of peak germination, usually the highest number of germinations recorded in a 24-hour period (Abdul-Baki & Anderson, 1973).

$$\text{GE (\%)} = \times 100 (\text{Seeds germinated during peak 24-hour period} / \text{Total Seeds})$$

3.9.4 Germination Value (GV)

As defined by Czabator (1962), germination value integrates both the total germination and germination speed into a single index. It is calculated as:

$$\text{Germination value} = \text{PV} \times \text{MDG}$$

Where, PV = Peak value of germination

MDG = Mean daily germination

3.9.5 Germination Index (GI)

The germination index, which combines germination rate and capacity, was calculated following Maguire (1962):

$$\text{GI} = \text{Total germinated seeds} / \text{Time to 50\% germination (days)}.$$

3.10 Potting Mixtures

The effect of different substrate compositions on establishment of seedlings was investigated using various potting mixtures necessarily materials available locally. It is essential to find the more suitable medium to promote growth and vigor in *G. lanceifolia* seedlings in controlled conditions. Forest soil was considered the base medium since it closely resembles the natural habitat of the species and provides inherent fertility. Sand was incorporated for the absorbent purposes to increase drainage, aeration, and prevent waterlogging, which is detrimental to seedling survival. Most importantly, organic amendments such as farmyard manure (FYM) and vermicompost are introduced for supplying essential nutrients to the overall substrate, to enhance microbial activity and overall improvement of the soil structure (Mechergui et al., 2022; Tiwari and Saxena, 2003). The choice of these combinations has been rationalized by their connection and significance in tropical nursery practices, as well as the cost-effectiveness and potential benefits in the elicitation of the physiological and morphological activities of seedlings. The ratio of treatments (various potting mixtures) is as follows:

3.10.1 Control (Forest soil) – P0

3.10.2 Forest soil: Sand: FYM (3:2:1) – P1

3.10.3 Forest soil: Sand: Vermicompost (3:2:1) – P2

3.10.4 Forest soil: Sand: FYM: Vermicompost (3:2:1:1) – P3

3.11 Growth Parameters

One of the most critical factors for assessing seedling acquisition parameters is the comparison between the pre-sowing preparation and the potting mix on the performance of seedlings. Growth analysis provides valuable insight into seedling vigor, biomass allocation, and overall quality, which are essential for making decisions in nursery practices and subsequent field establishment. Parameters such as height, collar diameter, and root development are crucial morphological and physiological traits, indicating healthy plant growth, while the biomass-related traits

reflect nutrient utilization and carbon distribution patterns (Pinto, 2011). For example, the root/shoot ratio and DQI-Dickson Quality Index have been used most frequently as indicators of plant quality and use values for seedlings, incorporating a wide range of growth attributes into a single index of quality (Dickson et al., 1960). It is necessary to record all the parameters in a controlled environment to compare treatments and establish a common framework for the most appropriate nursery activity for propagation at a larger scale. The following parameters were recorded under poly-house conditions:

3.11.1 Plant Height

The height of the shoot was measured using a scale from the ground up to the tip of the stem and recorded in centimeters (cm).

3.11.2 Collar Diameter

The diameter of the seedling collar was measured using an electronic vernier caliper and expressed in millimeters (mm).

3.11.3 Root Length

The length of the tap root was measured by placing it horizontally on the ground and using a scale; measurements were recorded in centimeters (cm).

3.11.4 Root–Shoot Ratio

The root–shoot ratio was calculated on a dry weight basis for each plant by dividing the dry weight of the root by the dry weight of the shoot

3.11.5 Total Dry Weight of Seedling

This was determined by adding the dry weight of the root to the dry weight of the shoot and was expressed in grams (g).

3.11.6 Dickson Quality Index (DQI)

The seedling quality index was calculated using the formula given:

DQI = Total dry weight / [(Shoot height / Collar diameter) + (Shoot dry weight / Root dry weight)]

3.12 Seed oil extraction

Extraction of seed oil from collected seeds of *G. lanceifolia* was done through the following procedure at the laboratory. The collected fruits were cut, and the seeds were removed and air-dried. Seeds were cleaned and ground into a fine powder by a mixer grinder. The seed oil was extracted through a cold percolation method; the fine-powdered seed material was soaked in hexane solvent for four days with intermittent flask shaking. Further, the seed oil was separated from hexane by using a rotary vacuum evaporator (Büchi Rotavapor R-300), dried at 60 °C, and weighed (Kalia et al., 2002; Mohammed et al., 2016).

3.13 Physicochemical properties of seed oil

Acid value, saponification value, peroxide value, and iodine value of extracted seed oil of *G. lanceifolia* were determined according to "Fats and Oils Handbook" AOCS (American Oil Chemists' Society) Press, referencing to the method standard number for acid value (AOCS: Cd 3a-63); saponification value (AOCS: Cd 3-25, Cd 3b-76); peroxide value (AOCS: Cd 8-53 and Cd 8b-90) and iodine value (AOCS: Cd 1-25, Cd 1b-87 and Cd 1d-92). Specific gravity of seed oil was determined by dry pycnometer, and the viscosity of the seed oil sample was estimated as per (Zahir et al., 2017). The brief protocols of the above methods are given below.

3.13.1 Peroxide Value

About 30 ml of acetic acid–chloroform solution was poured into a conical flask containing 5 g of the oil sample. To this, 1 ml of potassium iodide was added, followed by 30 ml of distilled water, and shaken thoroughly. Then 0.5 ml of 1% starch solution was added as an indicator, and the mixture was titrated against 0.1 N sodium thiosulphate until the endpoint was reached (disappearance of blue color).

3.13.2 Acid Value

About 2.5 g of oil sample was taken in a volumetric flask, and 50 ml of neutralized ethanol was added. Then, 2–3 drops of phenolphthalein indicator were introduced and titrated against 0.1 N sodium hydroxide until a faint pink color appeared, indicating the endpoint.

3.13.3 Saponification Value

To 2 g of the oil sample, 50 ml of 4% ethanolic KOH was added and heated in a heating mantle for 30 minutes. A blank containing 50 ml of 4% ethanolic KOH was also heated simultaneously. After cooling, a few drops of phenolphthalein indicator were added and titrated against 0.5 N HCl with vigorous agitation until the pink color disappeared, marking the endpoint.

3.13.4 Iodine Value

An accurately weighed 0.52 g of oil sample was transferred into a glass-stoppered flask, followed by the addition of 25 ml of carbon tetrachloride. Both the sample and blank flasks were swirled gently and treated with 25 ml of Wijs solution. Potassium iodide crystals were placed around the stopper, and the flasks were kept in the dark for 30 minutes. After incubation, 100 ml of distilled water was added, and titration was performed with standardized sodium thiosulfate solution using starch as an indicator until the disappearance of the blue coloration.

3.13.5 Specific Gravity

Specific gravity was determined using a pycnometer. First, the empty, dry pycnometer was weighed, then filled with oil and weighed again to obtain the oil's mass. The volume was calculated based on the displaced liquid volume, and the specific gravity was expressed as the ratio of the oil's mass to its volume, corrected by the density of the reference liquid.

3.13.6 Viscosity

Viscosity was measured using a viscometer. The oil sample was maintained at a uniform temperature, and the spindle was immersed in the oil. Viscosity readings were taken and analyzed to assess the oil's flow behavior. After measurement, the equipment was thoroughly cleaned to maintain accuracy.

3.14 UV-Visible spectroscopy analysis

The UV-visible spectroscopy scan of the seed oil was conducted by preparing a clean sample, and dissolving it in a concentration of 5000ppm in DMSO (Dimethyl sulfoxide) solvent. A cuvette was filled with the sample solution, and a baseline correction was performed using a DMSO-filled cuvette as a reference. The cuvette containing the sample solution was then subjected to scanning in a UV-visible spectrophotometer (Hitachi U-4100) across a wavelength range of 200 to 900nm. The resulting absorption spectrum was obtained, allowing for the analysis of the seed oil's composition and molecular characteristics (Tissue, 2002; Mohammed et al., 2019).

3.15 Gas chromatography—mass spectrometry (GC-MS)

The FAMES composition was assessed by transforming the oil into fatty acid methyl esters (FAMES). This involved adding 4 ml of 0.5M methanolic KOH into 200 µl of seed oil in a flask, followed by heating the flask at boiling temperature for 15 min. 1.6ml of methanolic HCl solution was then added and heated for another 25 min; after cooling at room temperature, 8ml of deionized water and 6ml of n-hexane were added, rotated, and shaken for 1min, the upper layer was collected and filtered using 0.45µm syringe. 1ml of the filtrate was subsequently introduced into a gas chromatograph (GC) for analysis (Negash et al., 2019).

A GC-MS system consisting of a Perkin Elmer Technologies Model Clarus 680 GC equipped with Clarus 600 (EI) was used for qualitative and quantitative analyses. Separations of the volatile components were performed on a column. One microliter of extract was injected at split (20:1) mode with an injector temperature of 200 °C. Helium gas (ultra-high purity grade, 99.99 %) was used as a carrier gas. GC

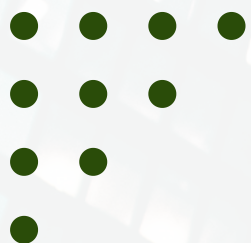
oven conditions were initially at 40 °C and held for 5 min, then programmed at 10 °C/min until 250 °C, finally held for 10 min. Helium carrier gas flow was set at 30 cm/s. The MS conditions were as follows: ion source temperature, 250 °C; MS transfer temperature, 250 °C; electron multiplier, 1400V; mass range, 25–500 Da; and scan rate, 2.91scans/s. The MS was run in the electron ionization (EI) full-scan mode, m/z 25–500, at the Department of HAMP, Mizoram University.

3.16 FTIR (Fourier transform infra-red) spectra

FTIR analysis was carried out on an IR Affinity-1S FTIR spectrometer. The spectrum of the sample was recorded in the range of 4000–500 cm⁻¹ at a resolution of 4 cm⁻¹. The spectrum data was collected and treated using the LabSolutions IR software at the Department of Chemistry, Mizoram University.

3.17 NMR analysis

¹H NMR and ¹³C spectrum of *G. lanceifolia* seed oil were obtained on an Avance 300-MHz NMR Spectrometer using a 5-mm broadband inverse probe head equipped with shielded z-gradient accessories. The samples to be analyzed were dissolved in deuterated chloroform (CDCl₃) and TSM as an internal reference at the Indian Institute of Chemical Technology (IICT), Hyderabad.



CHAPTER - IV

RESULTS AND DISCUSSIONS



CHAPTER 4

RESULTS AND DISCUSSION

4.1 General Morphological Description of *G. lanceifolia*

The species under consideration was identified as *G. lanceaefolia* Roxb. based on the uniform suite of distinctive and consistent morphological characters observed across all sampled populations (Table 4.1). The tree appears straight and pyramidal in habit (Figure 4.1). The branching is opposite-decussate, with emerging nodes being almost right-angled symmetrical pairs above and below. The leaves are placed diametrically opposite each other and are oblong-elliptic or elliptic-lanceolate, with an apiculate acute point at the apex, with a coherent margin (Figure 4.1). The leaf sheet is glabrous, smooth, hairless, and dark green, lustrous, and the flowers are noticeable because they are deep red in colour with a fleshy, hard perianth, but in most cases, there are greenish hints (Figure 4.2).

The above characteristics are not just valuable for determination, but also manifestations of adaptations in *G. lanceifolia*. Opposite-decussate leaf arrangement and dense, pyramidal crown may serve an important trait in terms of maximizing light capture in the forest understorey (Fritz et al., 2018). Thick, leathery leaves with entire margins suggest better tolerance to humid tropical environments, thus reducing water loss and herbivorous damage. This is a common feature in many *Garcinia* species (opposite, coriaceous, shiny leaves), indicating a high level of conservation within the genus; possibly due to the evergreen nature and resource-conservation strategies (Pringle et al., 2011; Amron et al., 2020). The color of the flower and the bright red color of ripe fruit indicate biotic interactions: these probably help in attracting specific pollinating insects as well as frugivores. Such is the case in *Garcinia*; female (or hermaphroditic) flowers tend to be larger or much larger than the male flowers to attract more effective pollination (Ushimaru et al., 2023). It is observed that the hermaphrodite blossoms indeed are conspicuously larger and brighter. The presence of multiple stigmatic rays on the ovary is one of the characteristics of such a section of *Garcinia*, to which *G. lanceifolia* belongs. Altogether, these morphological details are well-aligned with published botanical

revisions. *G. lanceifolia* is described as a dioecious shrub or small tree 1-6 m tall with opposite, elliptic-lanceolate, shiny leaves (4.5-10 × 1.5-3 cm) and small reddish-orange flowers about 5-8 mm in diameter (Ngernsaengsaruy, 2022), consistent with our observation in most cases. The observations are congruent with the literature and provide an accurate determination of the specimens, adding confidence that any such differences being interpreted as biological variability, rather than a case of misidentification.

Table 4.1: Morphological characteristics of *G. lanceifolia*

Characteristic	Description
Habit	Tree
Tree Shape	Erect and pyramidal
Branching Type	Opposite decussate
Leaf Arrangement	Opposite decussate
Leaf Shape	Lanceolate to elliptic lanceolate
Leaf Density	Sparse to medium
Leaf Apex Shape	Acuminate
Leaf Blade Margin	Entire
Leaf Surface	Green, lustrous, and glabrous
Flower Description	Reddish with a fleshy thick perianth



Figure 4.1: Tree and leave of *G. lanceifolia* showing branching and leave pattern

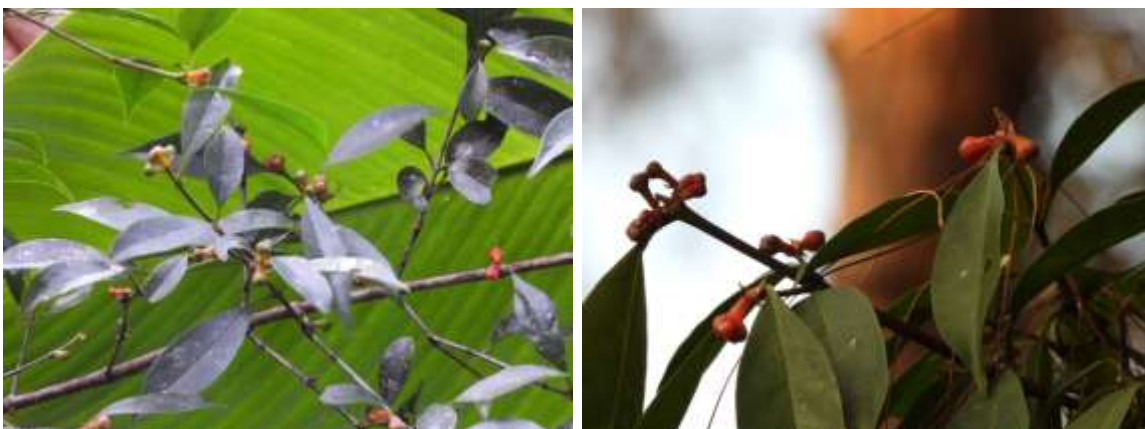


Figure 4.2: Flowers of *G. lanceifolia*

4.2 Phenotypic Variability in Tree Architecture

Phenotypic coefficient of variation (PCV) accounted for the extent of variability in key architectural traits across different populations by defining differences (Table 4.2), and the results showed a gradient of variability usually distinguishable by both site and sexual form of the trees.

In Thingdawl, a hermaphrodite population showed 14.23 % moderate phenotypic variability in girth and 11.28 % in height. Much higher variability was observed in the total number of branches (16.26%). On the other hand, the Thenzawl populations showed a very different pattern: both the hermaphrodite and the male trees had a high to very high level of variability in all the traits recorded. For example, tree girth and height showed high variability ($PCV > 22\%$) while the number of branches ranged from 6.47% in hermaphrodite trees up to 39.61% in male trees; thus, the mean overall phenotypic differentiation between the two populations was ordered by a higher plasticity in respect to growth form of the population from both hermaphrodite and male trees as to the lesser population from Thingdawl.

Quartile analysis (Figure 4.3) clearly reveals wider interquartile ranges in the Thenzawl population, especially for branch number, highlighting morphological heterogeneity. Thenzawl males exhibit extremely high fourth-quartile and maximum branch numbers, indicating higher phenotypic plasticity and reproductive potential, which tallies with the elevated PCV values.

The variation in branching architecture, and in particular, branching pattern is indicative of tropical tree phenotypic plasticity in variable light and moisture conditions, and is greater in Thenzawl because of more heterogeneous terrain and microclimatic gradients (Das et al., 2025). Other than variation in stem diameter and branch number, male trees differ from females in their amount of branching flexibility. Staminate trees, which do not have to invest their resources in the production of reproductive biomass, can thus spend more on the vegetative part of the tree, which is a great help in colonizing forest fragments (Rathcke and Lacey 1985; Valladares et al., 2014). The results of this study indicated that the G.

lanceifolia population remains highly variable in terms of intra-specific differences. Consequently, this provides a basis for local adaptation and resilience.

Table 4.2: Phenotypic Coefficient of Variation (PCV) for tree architectural traits

Population	Trait	Mean	Std. Dev	PCV (%)	Interpretation
Thingdawl Hermaphrodite	Tree Girth (cm)	32.81	4.67	14.23	Moderate variability
	Tree Height (m)	3.99	0.45	11.28	Moderate variability
	Total Branches	21.9	3.56	16.26	High variability
Thenzawl Hermaphrodite	Tree Girth (cm)	30.18	7.42	24.59	High variability
	Tree Height (m)	3.43	0.84	24.49	High variability
	Total Branches	24.2	9.07	37.48	Very high variability
Thenzawl Male	Tree Girth (cm)	32.64	7.34	22.49	High variability
	Tree Height (m)	3.83	0.96	25.07	High variability
	Total Branches	22.8	9.03	39.61	Very high variability

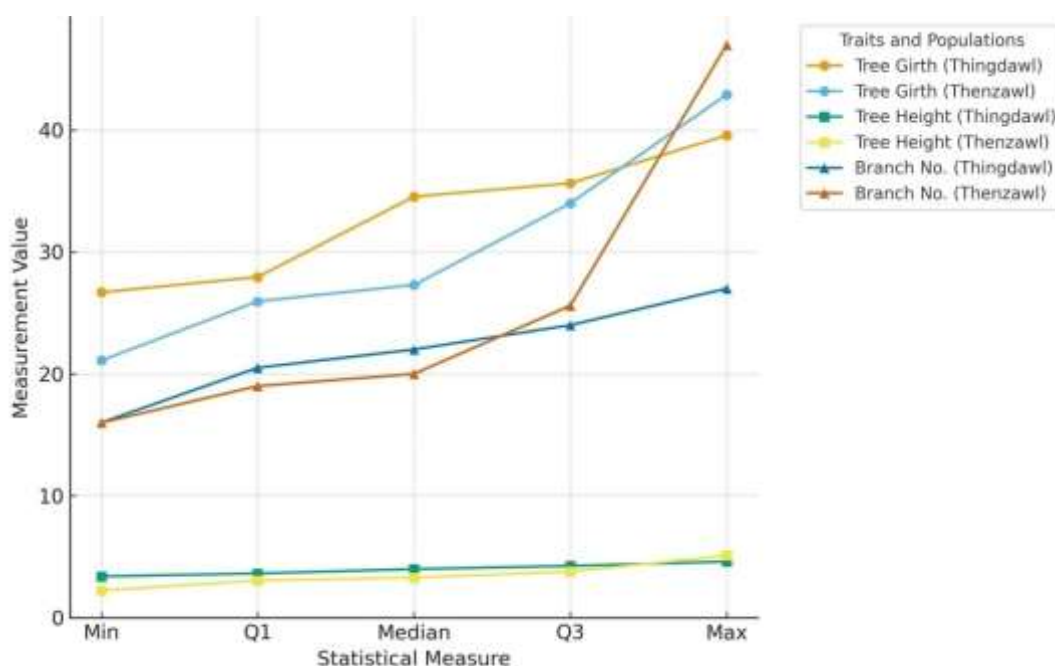


Figure 4.3: Variation in tree girth (cm), height (m) and number of branch across population

4.3 Variation in Leaf Traits

To examine the impact of sexual dimorphism and site variation, a comparative analysis of leaf morphometric traits was done. The one-way ANOVA and post-hoc Tukey's HSD tests used to analyze the data showed numerous significant differences ($p < 0.05$), which are presented in Table 4.3 and Figure 4.4.

4.3.1 Sexual Dimorphism in Leaf Traits

There was a noticeable and distinct sexual dimorphism. The male trees were significantly different in leaf weight from the female trees, and all trees with a mix of genders (0.35 ± 0.09 g and 0.34 ± 0.09 g, respectively; $p = 0.002$). On the other hand, the male trees had petioles (leaf stalks) that were shorter than the hermaphrodites (0.80 ± 0.11 cm) ($p = 0.019$). In addition, it was found that the trees with mixed sexes had leaves that were significantly longer (8.31 cm to 8.57 cm) than the shorter leaves of the male trees (7.28 cm; $p < 0.001$). The clear sexual dimorphism observed in leaf morphometry further underscores the role of genetic factors. The significantly heavier leaves in male trees, compared with the longer leaves and petioles in

hermaphrodite trees, suggest divergent evolutionary strategies potentially related to reproductive investment and efficiency. These findings align with modern genetic studies, which show that traits such as leaf morphology and canopy surface temperature are largely genotype-dependent, with robust genomic associations revealing the genetic control of these functional traits (Coupel-Ledru et al., 2022).

4.3.2 Site-Specific Variation in Leaf Traits

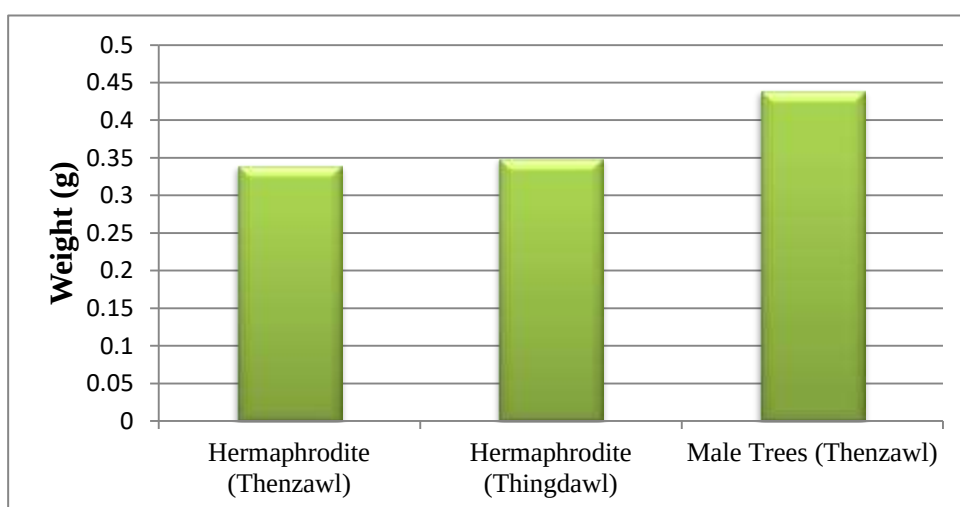
The site-related differences were clearly seen. Although the weight of leaves was similar among the two hermaphrodite populations ($p > 0.05$), leaf width differed. In particular, the hermaphrodite leaves from Thingdawl were significantly narrower (2.53 ± 0.41 cm) than the male tree leaves from Thenzawl (3.04 ± 0.31 cm; $p < 0.001$). It implies that the combination of genetic (sex) and environmental or epigenetic (site) factors plays a role in the variation in leaf morphology observed.

The narrower leaves in Thingdawl hermaphrodites and the wider leaves in Thenzawl males are examples of site-specific variation, suggesting that environmental or epigenetic factors are also important in shaping morphology. This is the same for other plant species, where diversity in critical traits for adaptation, such as fruit morphology in chile peppers, has been reported to show significant phenotypic diversity among populations and under different growing conditions (Khokhar et al., 2025). The interaction between the genetic makeup (including sex) and local environmental factors (e.g., light, water) eventually leads to the formation of different phenotypes, which are then available for natural selection to act upon (Kolluru, 2014; Coupel-Ledru et al., 2022).

Table 4.3: Comparative analysis of leaf traits across sexes and sites (Mean $\pm$ SD)

Population	Weight (g)	Petiole Length (cm)	Leaf Length (cm)	Leaf Width (cm)
Hermaphrodite (Thenzawl)	0.34 $\pm$ 0.09a	0.94 $\pm$ 0.18b	8.31 $\pm$ 1.01b	2.74 $\pm$ 0.38a
Hermaphrodite (Thingdawl)	0.35 $\pm$ 0.09a	0.87 $\pm$ 0.13ab	8.57 $\pm$ 1.12b	2.53 $\pm$ 0.41a
Male Trees (Thenzawl)	0.44 $\pm$ 0.08b	0.80 $\pm$ 0.11a	7.28 $\pm$ 0.59a	3.04 $\pm$ 0.31b
ANOVA p-value	0.002	0.019	<0.001	<0.001

Different superscript letters (a, b) within a column indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.



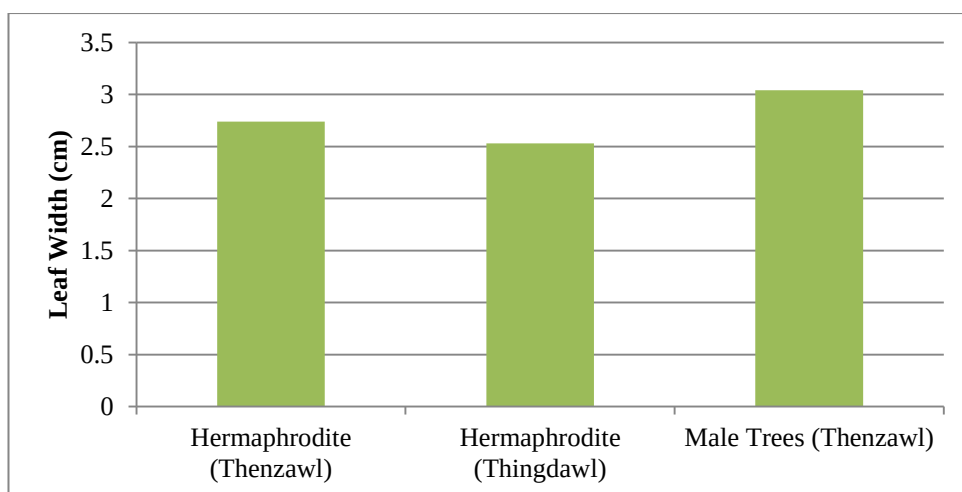
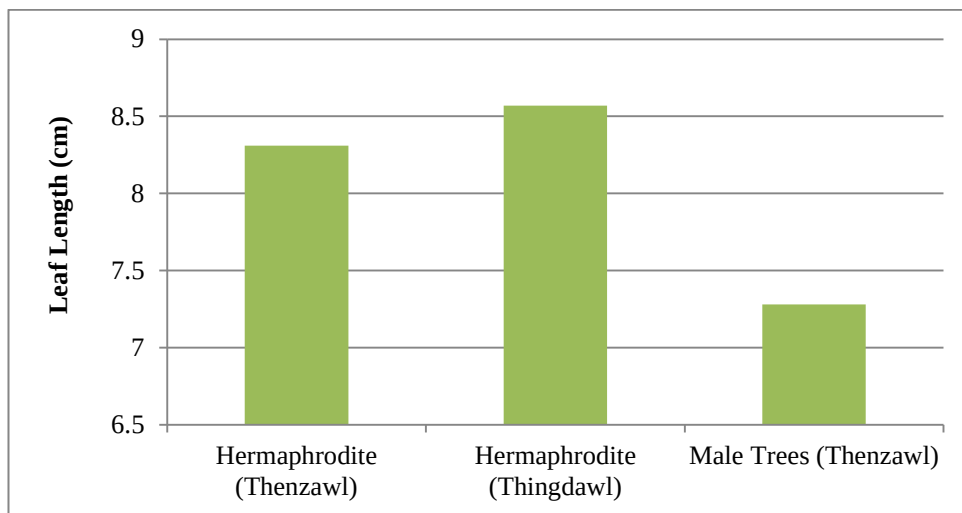
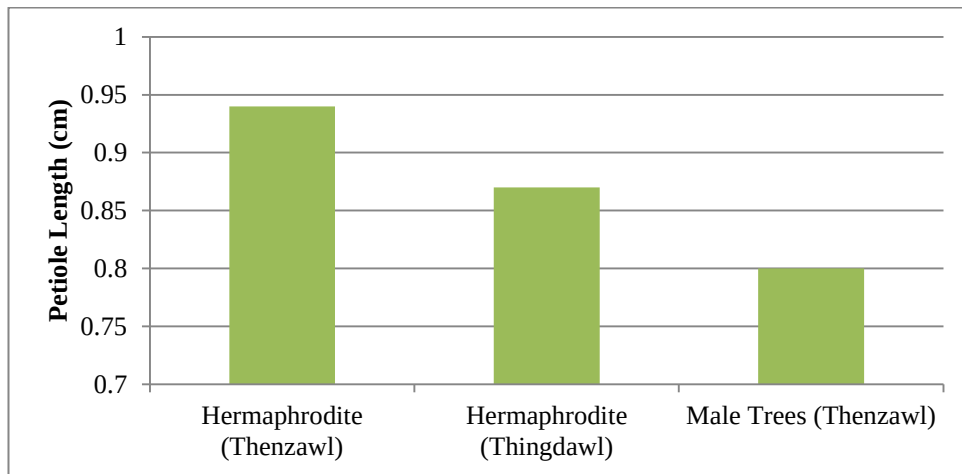


Figure 4.4: Leaf traits across population

4.4 Floral Morphometry and Dimorphism

4.4.1 Inter-Population Variation in Floral Traits

The differences in floral characteristics across all three groups were evident in the results (Table 4.4). One-way ANOVA showed that differences in floral traits among the groups were significant ($p < 0.001$). Post-hoc Tukey's tests placed the populations into the following categories: Thenzawl hermaphrodite flowers (TH) were always the largest, being considerably heavier and having a larger diameter than both Thenzawl male (TM) and Thingdawl hermaphrodite (TDH) flowers. Thenzawl male flowers were generally of intermediate size but were statistically distinct, thereby forming separate groups for weight and diameter. Thingdawl hermaphrodite flowers were the smallest in all respects. For instance, flower opening size was equal in Thenzawl hermaphrodite and male flowers, but much smaller in the Thingdawl population.

The comprehensive study of floral traits in *G. lanceifolia* has revealed a complex diversity of patterns, the result of the combined effects of sexual dimorphism and site-specific environmental factors. The significant differences in flower size and weight between the Thenzawl and Thingdawl groups, along with the conspicuous sexual dimorphism in the Thenzawl group, give important indications of the species' reproduction method and adaptive capability.

The evident inter-population variation, in which Thenzawl hermaphrodites (TH) had the biggest and heaviest flowers while Thingdawl hermaphrodites (TDH) showed the opposite case, highlighting that the phenotype is not solely determined by genetic sex. Such geographical variation in flower traits is a normal occurrence and may result from various selection pressures acting in the respective environments. Thenzawl's diverse landscape and microclimatic gradients, which were mentioned as factors shaping architectural traits, likely exert a variety of selective pressures (Barrett and Hough, 2013; Wallace and Bowles, 2023). One example is the climate that can have a direct impact on resources such as light and water, which in turn decide the amount of energy a plant can devote to its reproduction structures (Correia et al., 2025; Plendl et al., 2024). Consequently, the larger flowers of Thenzawl might

be an indication of a more conducive environment or of the large and attractive flowers being subjected to stronger selection in such an ecological context.

Moreover, it is observed that the size and length of flower opening were comparable in both sexual morphs in Thenzawl, but they differed greatly in hermaphrodite populations (TH vs. TDH), which indicates that some floral traits are more prone to environmental modification or local adaptation than others. This is also the case with studies on other species, for example, *Rhododendron*, where nectar traits and corolla length were found to have a very strong phylogenetic signal, meaning that some traits are evolutionarily constrained while others are more flexible (Basnett et al., 2025).

**Table 4.4: Floral characteristics and comparative analysis across populations
(Mean $\pm$ SD)**

Population	Weight (g)	Diameter (mm)	Stalk Length (mm)	Flower Opening (mm)	Flower Length (mm)
Thenzawl Hermaphrodite (TH)	0.43 $\pm$ 0.07	10.24 $\pm$ 0.68	6.83 $\pm$ 0.81	4.96 $\pm$ 0.66	6.57 $\pm$ 0.62
Thenzawl Male (TM)	0.25 $\pm$ 0.03	8.81 $\pm$ 0.38	4.82 $\pm$ 0.58	4.88 $\pm$ 0.41	6.24 $\pm$ 0.28
Thingdawl Hermaphrodite (TDH)	0.21 $\pm$ 0.06	7.12 $\pm$ 0.82	5.73 $\pm$ 1.60	2.59 $\pm$ 0.44	5.30 $\pm$ 0.61
Tukey's Groupings	TH > TM > TDH	TH > TM > TDH	TH > (TM, TDH)	(TH, TM) > TDH	(TH, TM) > TDH

4.4.2 Morphometry and Allometry of Thenzawl Flowers

For Segregation of sexual dimorphism from possible site-effects, a thorough study was performed on Thenzawl population. The descriptive statistics for male and hermaphrodite flowers have been given in Tables 4.5 and 4.6, respectively. Hermaphrodite flowers expressed a significant number of variability, especially in stalk length (CV = 12.7%) and flower opening size (CV = 13.7%). On the other hand, male flowers exhibited less variation, especially in diameter (CV = 4.7%) and flower length (CV = 4.8%), which are structural traits.

The results of Pearson correlation analysis, as shown in Figure 4.5 and figure 4.6, exhibited strong allometric relationships among the different flower types. For hermaphrodite flowers, the strongest positive correlation among all measured factors was flower weight with diameter ($r = 0.83$, $p = 0.003$), while there was also a very noticeable positive correlation between weight and flower length ($r = 0.64$, $p = 0.046$). Just like with the female flowers, the same trend could be seen in male flowers where the weight was highly correlated with the diameter ($r = 0.72$, $p = 0.019$) and also with the length ($r = 0.69$, $p = 0.027$) to a lesser extent. This means that the heavier flowers are always those that are bigger in size (diameter and length) no matter the sex of the flower.

Furthermore, Predictive models for flower weight based on other morphological traits were developed using multiple linear regression (Table 4.7). For the hermaphrodite and male flowers, the combination of flower diameter and flower length was found to be a major factor determining weight. The hermaphrodite flower model was a great success, as it could elucidate 72% of the weight variance (Adjusted $R^2 = 0.72$, $p = 0.005$). The male flower model was also significant, offering up to 65% of variance explanation (Adjusted $R^2 = 0.65$, $p = 0.009$). The VIFs (Variance of Inflation Factor) of the predictor variables were all less than 5 in both models, thus ruling out significant multicollinearity.

The remarkable sexual dimorphism in the Thenzawl population is indicative of different selection pressures on male and female reproductive functions. The significant difference in the weights and dimensions of the hermaphrodite flowers

compared to male flowers is indicative of a radical difference in the reproductive investment made by the two sexes. This can be considered from the perspective of sexual selection and resource allocation theory (Li et al., 2024; Chen, et al., 2021)

Hermaphrodite flowers, that produce seeds, usually need to invest more in structures that secure the growing ovules and draw in pollinators to have successful fruit set. Studies on sexual allocation in flowering plants show different investment strategies between flower types. In andromonoecious species such as *Solanum carolinense*, the flowers weigh 11-16% more than those of males, containing more nitrogen and having bigger reproductive parts. In contrast, the flowers of males conserve up to 86% of the investment in non-functional female organs (Solomon, 1986). The phenomenon of male-biased larger hermaphrodite flowers that invest a lot in pollination via petals and female-biased smaller flowers with more sepal investment for seed protection is common among the angiosperms (Paterno et al., 2020). According to theoretical models, if the sex ratio is biased towards females at the flower level, plants would slowly adapt to the production of female and male flowers and eventually change to monoecious through the andromonoecious stage particularly in case of the species that have expensive fruits (Jong et al., 2008). Nevertheless, empirical data demonstrate that gender-specific selection patterns are heavily influenced by the context rather than strictly adhering to simple male-function or gender-balance hypotheses (Ashman and Morgan, 2004). According to the transcriptomic studies carried out in dioecious species such as *Vernicia montana*, this concept is confirmed, as it indicates that the male-biased genes are frequently enriched for the functions associated with the viability and production of pollen (Li et al., 2024). The lower coefficient of variation (CV) in male floral traits seen in the observation can be interpreted as a further indication of a more stabilized and optimized phenotype for their particular function.

Table 4.5: Morphometric traits of Thenzawl hermaphrodite flowers

Trait	Mean $\pm$ SD	Range	CV (%)
Weight (g)	0.432 $\pm$ 0.073	0.308 – 0.569	16.9
Diameter (mm)	10.215 $\pm$ 0.647	9.220 – 11.257	6.3
Stalk Length (mm)	6.832 $\pm$ 0.869	5.035 – 7.720	12.7
Flower Opening (mm)	4.951 $\pm$ 0.677	3.880 – 5.920	13.7
Flower Length (mm)	6.531 $\pm$ 0.627	5.390 – 7.480	9.6

Table 4.6: Morphometric traits of Thenzawl male flowers

Trait	Mean $\pm$ SD	Range	CV (%)
Weight (g)	0.253 $\pm$ 0.026	0.205 – 0.291	10.3
Diameter (mm)	8.713 $\pm$ 0.407	8.11 – 9.29	4.7
Stalk Length (mm)	4.819 $\pm$ 0.627	3.93 – 5.60	13.0
Flower Opening (mm)	4.883 $\pm$ 0.356	4.20 – 5.56	7.3
Flower Length (mm)	6.243 $\pm$ 0.302	5.91 – 6.70	4.8

Table 4.7: Multiple linear regression models for predicting flower weight

Model Parameter	Hermaphrodite Flowers	Male Flowers
Model Equation	Weight = $-0.681 + 0.071 \times$ Diameter + $0.062 \times$ Length	Weight = $-0.192 + 0.038 \times$ Diameter + $0.049 \times$ Length
Adjusted R ²	0.72	0.65
Model F-statistic (p-value)	F=11.6 (p=0.005)	F=8.94 (p=0.009)
Predictor: Diameter (p-value)	0.071 (p=0.008)	0.038 (p=0.020)
Predictor: Flower Length (p-value)	0.062 (p=0.028)	0.049 (p=0.028)
VIF (for both predictors)	1.37	1.35

4.4.3 Comparison of Sexual Dimorphism in Flowers

The independent samples t-tests performed on Thenzawl hermaphrodite and male flowers resulted in a direct statistical comparison, which confirmed the presence of sexual dimorphism (Table 4.10). In fact, hermaphrodite flowers were much larger than male flowers in a few dimensions such as weight (70% heavier, $p < 0.001$), diameter (17% larger, $p < 0.001$), and stalk length (42% longer, $p < 0.001$). On the other hand, flower opening size ($p = 0.804$) and overall flower length ($p = 0.241$) showed no significant differences, which indicates that these specific traits have been retained across the sexual morphs.

The powerful allometric links (like those between weight, diameter, and length) in the two flower types point to simultaneous growth and give dependable models for estimating flower investment. The greater phenotypic variability (CV) found in hermaphrodite flowers, especially in stalk height and flower opening, implies more phenotypic plasticity in this flower type. Studies show that phenotypic plasticity in floral traits has critical adaptive roles in various plant species. In *Mercurialis annua*, hermaphrodites from populations with the presence of males exhibited higher plasticity in sex allocation, decreasing down on pollen production in low resources and on the other hand, increasing seed production in high resources, as opposed to hermaphrodites from populations with no males (Vilas and Pannell, 2014). In the same way, *Solanum hirtum* manifests developmental plasticity in sex expression that differs among genotypes and is influenced by the resource status of the individual (Diggle, 1993). This plasticity can be a benefit in changing environments, giving the plants the ability to modify their flower visuals according to the characteristics of the environment, for example, the presence of pollinators or the quantity of resources.

From the point of view of conservation, the great intra-specific variation documented in this study is an important source of the resilience. The Thenzawl population, which possesses high variability in both architectural and floral traits, is a potential source of adaptive genetic diversity. In the case of the genus *Paphiopedilum*, the knowledge of the genetic structure and the morphological variation among the different populations has been shown to be fundamental in the process of setting the conservation priorities (Tang et al., 2025). The results of this study indicate that the conservation planning of *G. lanceifolia* should give the prime importance to the protection of different populations as in Thenzawl and Thingdawl to keep the complete range of the species' adaptive capacity, particularly in the context of the environmental changes.

Table 4.8: Comparison of floral traits between Thenzawl hermaphrodite and male flowers

Trait	Hermaphrodite Mean $\pm$ SD	Male Mean $\pm$ SD	Mean Difference	p-value (t-test)
Weight (g)	0.432 $\pm$ 0.073	0.253 $\pm$ 0.026	-0.179	<0.001*
Diameter (mm)	10.215 $\pm$ 0.647	8.713 $\pm$ 0.407	-1.502	<0.001*
Stalk Length (mm)	6.832 $\pm$ 0.869	4.819 $\pm$ 0.627	-2.013	<0.001*
Flower Opening (mm)	4.951 $\pm$ 0.677	4.883 $\pm$ 0.356	-0.068	0.804
Flower Length (mm)	6.531 $\pm$ 0.627	6.243 $\pm$ 0.302	-0.288	0.241

** indicates statistical significance at $p < 0.05$ *

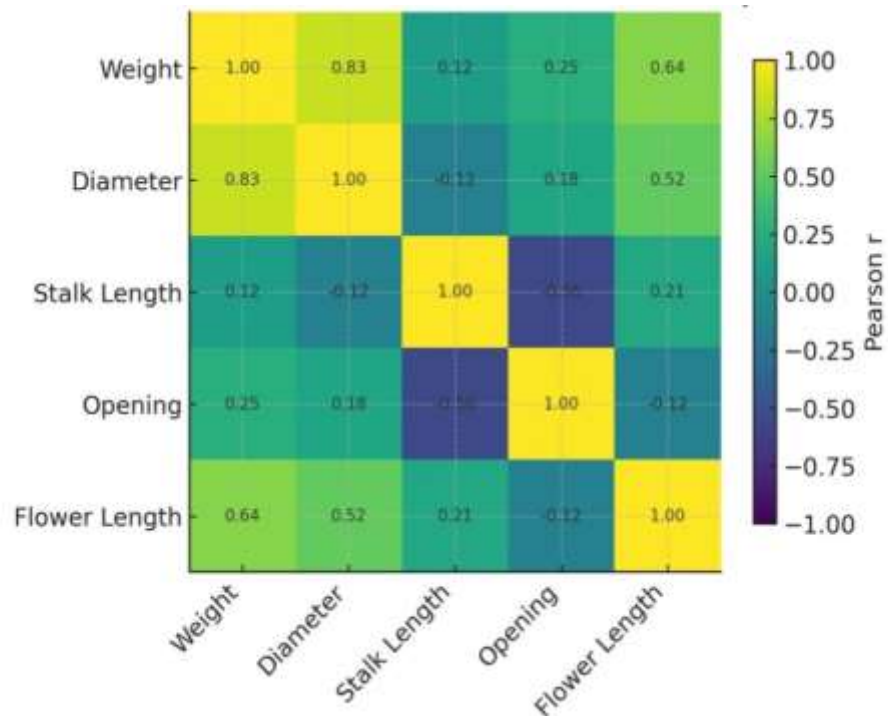


Figure 4.5: Heat map of Pearson correlation matrix (r-values) for Thenzawl hermaphrodite flowers

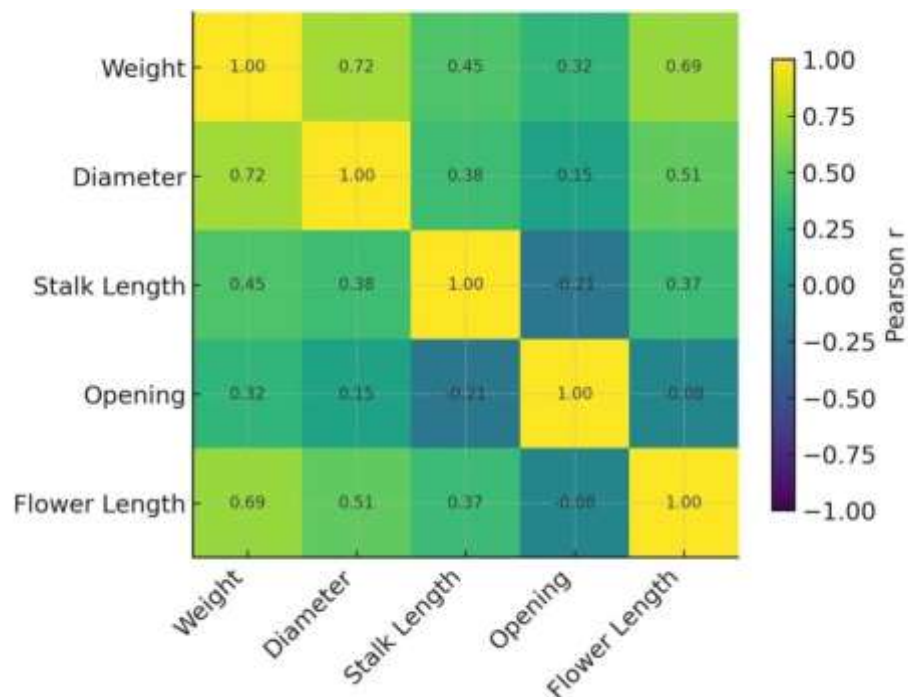


Figure 4.6: Heat map of Pearson correlation matrix (r-values) for Thenzawl male flowers

4.5. Morphological Characterization of *G. lanceifolia* Fruits

An extensive investigation was carried out to describe the morphological diversity in *G. lanceifolia* fruits from various geographical regions in the Mizoram. The fruits were divided into two principal categories: those collected from natural forest locations (Thingdawl and Thenzawl) and those sold in the markets of seven different villages (Bualpui, Bukpui, Hmuifang, Lungdai, Hmar Chaltlang, Tanhril, and Zanolawn). The metrics analyzed were fruit weight, diameter, rind thickness, seed number, rind mass as a percentage of total fruit mass, and moisture content of the rind.

4.5.1 Variation in Fruits Collected from Natural Sites

Table 4.9 presents the descriptive statistics for the fruits gathered from the two natural sites, Thingdawl (Kolasib district) and Thenzawl (Serchhip district). It can be seen that the mean values for fruit weight, diameter, and seed count were comparable for both places while the rind thickness was also not distinct. There was, however, a very significant difference with regard to the percentage of fruit made up of the rind, with the mean value for Thenzawl fruits being 67.27% as against 55.32% for Thingdawl fruits, thus showing a very sharp contrast. The analysis of variance (ANOVA) has established the difference in the proportion of fruit rind between Thingdawl and Thenzawl as highly statistically significant ($p < 0.001$). On the other hand, the differences in all the other parameters including fruit weight ($p = 0.47$), fruit diameter ($p = 0.52$), rind thickness ($p = 0.68$), seed count ($p = 0.88$), and rind moisture content ($p = 0.61$) were not significant.

Within-site analyses showed that there were positive correlations between fruit weight and diameter in both Thingdawl ($r = 0.97$) and Thenzawl ($r = 0.96$) that were not only strong but also very significant ($p < 0.001$), thus indicating that heavier fruits were consistently larger in diameter. However, fruit weight was not a significant predictor of rind thickness at either site (Thingdawl: $p = 0.12$; Thenzawl: $p = 0.78$).

Quartile analysis showed the two populations had moderate variability (Figures 4.7 to 4.13). Thenzawl fruits had a larger range of rind thickness and percentage of rind, which means that there was more variability in pericarp development than in Thingdawl fruits. On the other hand, Thingdawl fruits had higher median values for fruit weight and diameter, which is in line with the mean-based comparisons. The outliers were most noticeable in fruit weight and rind percentage while the rind moisture percentage had very minimal variability between the two locations.

4.5.2 Variation in Fruits Collected from Village Markets

A significantly broader range of morphological variability was noted in the fruits collected from the seven village markets of the Mizoram. The descriptive statistics for these locations are presented in Table 4.10. Bukpui fruits were the most vast and heaviest ones (85.9 ± 34.8 g, 59.0 ± 8.6 mm), whereas Zanlawn fruits were the lightest and the smallest ones (26.9 ± 9.4 g, 40.8 ± 6.4 mm). Hmar Chaltlang fruits had the thickest rinds (5.89 ± 0.67 mm) and the highest proportion of rind ($68.2 \pm 7.1\%$) whereas Tanhril fruits had the thinnest rinds (3.79 ± 0.71 mm). There were statistically significant differences between the evaluated traits for the following parameters: fruit weight ($p < 0.001$), fruit diameter ($p < 0.001$), fruit length ($p < 0.001$), rind thickness ($p = 0.002$), percentage of fruit rind ($p < 0.001$) and moisture content of rind ($p = 0.03$). On the other hand, the number of seeds per fruit turned out to be the least variable trait, demonstrating no significant difference among the markets locations ($p = 0.07$).

Internal relationships among various market sources were also examined (Table 4.11). The very strong and significant positive correlation between fruit weight and diameter was the main feature that characterized all seven markets, as the analysts computed the correlation coefficients (r) from 0.92 to 0.98 ($p < 0.01$ for all). Likewise, it is observed among the other three studied traits that all locations showed the same pattern of correlations with almost equal magnitudes for fruit weight and length ($r=0.75-0.95$) and for diameter and length ($r=0.73-0.95$). This reveals an allometric relationship being consistent and predictable across all three dimensions.

In addition, the studies noticed a significant positive regression between fruit weight and rind thickness in five out of the seven markets suggesting that thicker rinds are more likely to be found on larger fruits in most markets.

The post-hoc analyses (Tukey HSD) for the significant factors have shown specific pairwise differences (Table 4.12). For example, the fruits of Bukpui were significantly heavier, had bigger diameters, and were longer than the fruits of most other places, especially Zanolawn and Tanhril. In the same way, Hmar Chaltlang and Hmuifang had a common characteristic of significantly higher rind percentage compared to Bualpui and Bukpui, thus forming a distinct group.

An examination of fruit traits by quartiles showed distinct morphological differences among fruits gathered from village markets (Figures 4.14 - 4.20). The fruit of Bukpui was the largest and heaviest (73.5 g for median wt; 55.9 mm for median diam.), and Zanolawn exhibited the smallest and lightest fruits (28.1 g for median wt; 41.4 mm for median diam.). The rind of the fruits also changed, with Hmar Chaltlang and Hmuifang fruits having the highest percentage and thickest layers of rind (median: 5.8-5.9 mm), whereas Tanhril and Zanolawn had the thinnest layers of rind. However, rind moisture was fairly stable (88.6-93.5%) while seed count exhibited quite a small range of variability with medians from 3 to 4.5 seeds per fruit for all the market locations.

On the fruits collected from seven village markets, PCA was applied to the seven morphological traits measured (Fruit weight, Fruit diameter, Fruit length, Rind thickness, Seed count, % Fruit rind, and % Rind moisture) to outline and plot the connections between the sites and the traits. The two principal components (PC1 and PC2) together explained 84.80% of the total variance (PC1 = 55.27%, PC2 = 29.53%), and thus it was concluded that the majority of the morphological variation is represented by these two axes.

The variable loadings on PC1 and PC2 are given in Table 4.12a. The first principal component (PC1) was mainly responsible for the overall fruit size representation (larger fruits have more negative PC1 scores) with strong negative loadings for fruit weight (-0.490), fruit diameter (-0.489), and fruit length (-0.491).

Rind thickness (-0.357) and % rind moisture (-0.317) also negatively loaded on PC1, albeit slightly less. On the other hand, PC2 differentiated rind-associated characteristics from seed count: the % fruit rind was the strongest loading positive trait (0.608) and rind thickness contributed positively (0.438) although seed count had a strong negative loading (-0.602). This indicates that PC2 mirrors an axis of trade-off between thicker rind development (positive PC2) and fewer seeds (negative PC2). The PCA biplot (Figure 4.21) shows the different sample sites as points and the trait vectors as arrows.

The different forms of the fruits of *G. lanceifolia* in different locations prove that there is strong environmental and maybe even genetic factors influence the fruit development. Fruits that were acquired from natural forest places (Thingdawl and Thenzawl) had about the same size. Still, the difference in rind proportions was significant that it could point to pericarp growth being influenced by the specific ecological conditions of the site. The study reveals that microclimate plays a significant role in determining fruit pericarp characteristics and quality even in the case of tropical fruits. In the case of mangosteen, during the off-season, production related to lower humidity and soil moisture resulted in thicker rind compared to on-season production (Jaroensutasinee et al., 2023). This variation within a species corresponds to the wider acknowledgment of the genus *Garcinia* as a valuable source of bioactive compounds with notable morphological and genetic diversity (Lin et al., 2025; Boruah et al., 2025).

Among the fruits obtained from the market, the significant differences in size, thickness of the rind and content of moisture highlight the phenotypic plasticity of the species. The very strong positive relationships between the weight, diameter, and length of the fruits at all locations show a stable allometric relationship. PCA results indicated the first component as representing total fruit size, whereas the second component compared rind development to seed number, suggesting possible trade-offs in resource allocation during fruit maturation (Gambin and Borrás, 2010). Such variability is an important ecological and genetic factor that supports the selection of superior genotypes for propagation and domestication. A research direction that is

particularly imperative for endemic and endangered species like *G. lanceifolia* (Hazarika and Lalnunsangi, 2019).

In addition, the features of the fruit rind are very important from the aspect of phytochemicals. Research on relatives species such as *G. indica* (kokam) and *G. gummi-gutta* has revealed that the fruit rind is a major source of antioxidant activity owing to the presence of high amounts of flavonoids, tannins, and polyphenols (Sudharani et al., 2018; Mishra et al., 2006). This indicates that the variability in morphology observed in rind proportion and thickness might have a direct impact on the nutraceutical value and economic potential of fruits that come from different geographical areas.

Table 4.9: Morphological and compositional characteristics (mean $\pm$ SD) of fruits collected from two natural sites.

Location	Fruit Weight (g)	Fruit Diameter (mm)	Fruit length (mm)	Rind Thickness (mm)	Seed Count	% Fruit Rind	% Rind Moisture
Thingdawl	45.9 $\pm$ 18.9	47.3 $\pm$ 8.3	36 $\pm$ 4.2	5.63 $\pm$ 0.64	3.4 $\pm$ 1.1	55.3 $\pm$ 7.6	89.84 $\pm$ 0.48
Thenzawl	41.0 $\pm$ 16.0	45.3 $\pm$ 7.3	35.6 $\pm$ 3.4	5.50 $\pm$ 0.84	3.3 $\pm$ 1.9	67.3 $\pm$ 7.4	89.70 $\pm$ 0.89
ANOVA p-value	p = 0.47	p = 0.52	p = 0.78	p = 0.68	p = 0.88	p < 0.001	p = 0.61

Table 4.10: Morphological and compositional characteristics (mean $\pm$ SD) of fruits collected from village markets.

Location	Fruit Weight (g)	Fruit Diameter (mm)	Fruit Length (mm)	Rind Thickness (mm)	Seed Count	% Fruit Rind	% Rind Moisture
Bualpui	46.5 $\pm$ 23.0	48.1 $\pm$ 9.3	34.6 $\pm$ 4.9	5.16 $\pm$ 0.57	3.5 $\pm$ 1.3	52.2 $\pm$ 3.5	91.05 $\pm$ 0.82
Bukpui	85.9 $\pm$ 34.8	59.0 $\pm$ 8.6	42.5 $\pm$ 6.2	5.14 $\pm$ 0.86	4.8 $\pm$ 1.7	54.4 $\pm$ 4.4	92.10 $\pm$ 0.52
Hmuifang	58.5 $\pm$ 25.2	52.0 $\pm$ 9.0	41.9 $\pm$ 5.1	5.89 $\pm$ 1.22	3.2 $\pm$ 1.1	67.5 $\pm$ 9.1	92.18 $\pm$ 0.45
Lungdai	54.3 $\pm$ 19.9	49.8 $\pm$ 6.1	39.6 $\pm$ 5.8	5.11 $\pm$ 0.97	3.2 $\pm$ 1.5	58.9 $\pm$ 3.4	91.85 $\pm$ 0.64
Hmar Chaltlang	54.4 $\pm$ 18.9	50.5 $\pm$ 9.2	40.0 $\pm$ 7.4	5.89 $\pm$ 0.67	3.0 $\pm$ 1.7	68.2 $\pm$ 7.1	91.15 $\pm$ 0.65
Tanhril	35.0 $\pm$ 13.9	42.0 $\pm$ 7.0	35.5 $\pm$ 5.3	3.79 $\pm$ 0.71	4.0 $\pm$ 1.4	56.7 $\pm$ 4.0	92.20 $\pm$ 0.64
Zanlawn	26.9 $\pm$ 9.4	40.8 $\pm$ 6.4	31.0 $\pm$ 3.9	3.95 $\pm$ 0.72	3.2 $\pm$ 1.0	60.3 $\pm$ 5.9	90.77 $\pm$ 1.39
ANOVA p-value	p < 0.001	p < 0.001	p < 0.001	p = 0.002	p = 0.07	p < 0.001	p = 0.03

Table 4.11: Inferential statistics of relationships between parameters within each village market location.

Analysis Type	Significant Findings	Notable Exceptions
Weight-Diameter Correlation	All locations showed strong positive correlations ($r=0.92-0.98$, $p<0.01$).	-
Weight-Fruit Length Correlation	All locations showed strong positive correlations ($r=0.75-0.95$, $p<0.01$).	-
Diameter-Fruit Length Correlation	All locations showed strong positive correlations ($r=0.73-0.95$, $p<0.01$).	-
Weight-Rind Thickness Regression	Significant positive relationship in 5/7 locations ($p<0.05$).	Not significant in Hmar Chaltlang ($p=0.12$) and Lungdai ($p=0.08$).
Seed Count ANOVA	No significant effect of seed count on fruit weight in any location ($p>0.05$).	Seed count is independent of fruit size.

Table 4.12: Key statistical comparisons of morphological parameters across all village market locations.

Parameter	Highest Value (Location)	Lowest Value (Location)	Statistical Test Used	p-value
Fruit Weight	85.9 g (Bukpui)	26.9g (Zanlawn)	One-way ANOVA	< 0.001
Fruit Diameter	59.0 mm (Bukpui)	40.8 mm (Zanlawn)	One-way ANOVA	< 0.001
Fruit Length	42.5 mm (Bukpui)	31.0 mm (Zanlawn)	One-way ANOVA	< 0.001
Rind Thickness	5.89 mm (Hmar Chaltlang)	3.79 mm (Tanhril)	Kruskal-Wallis Test	0.002
Seed Count	4.8 (Bukpui)	3.0 (Hmar Chaltlang)	One-way ANOVA	0.07
% Fruit Rind	68.2% (Hmar Chaltlang)	52.2% (Bualpui)	Welch's ANOVA	< 0.001
% Rind Moisture	92.20% (Tanhril)	90.77% (Zanlawn)	One-way ANOVA	0.03

Table 4.12a: PCA loadings for the first two principal components

Variable	Fruit Weight	Fruit Diameter	Fruit Length	Rind Thickness	Seed Count	% Fruit Rind	% Rind Moisture
PC1 (loading)	-0.490	-0.489	-0.491	-0.357	-0.218	-0.064	-0.317
PC2 (loading)	-0.095	-0.009	0.121	0.438	-0.602	0.608	-0.229

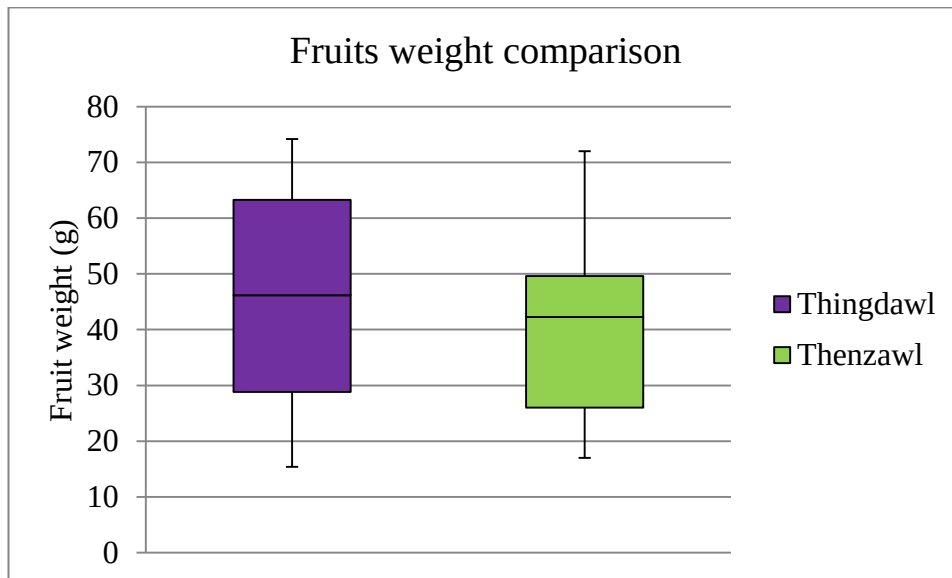


Figure 4.7: Boxplot of *G. lanceifolia* fruit weight distribution collected from natural sites

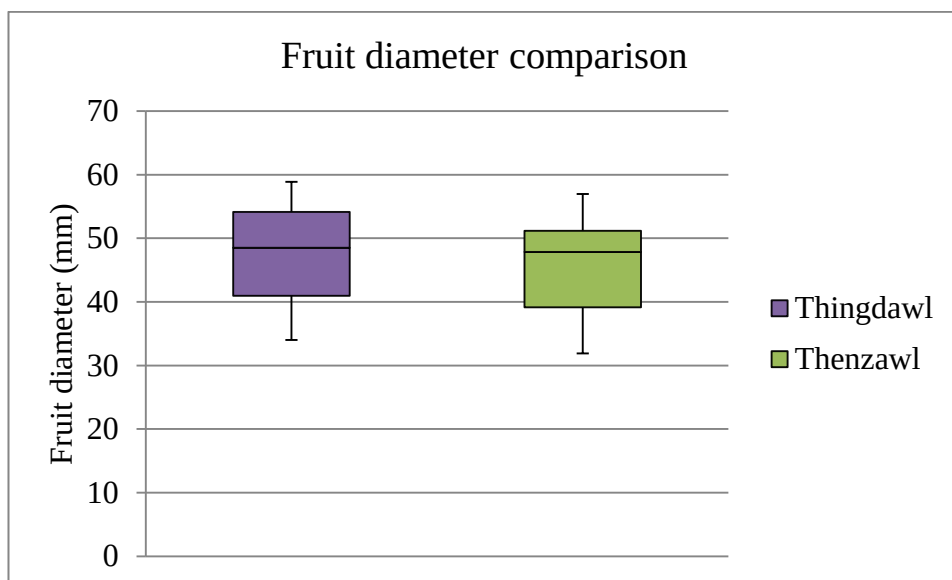


Figure 4.8: Boxplot of *G. lanceifolia* fruit diameter distribution collected from natural sites

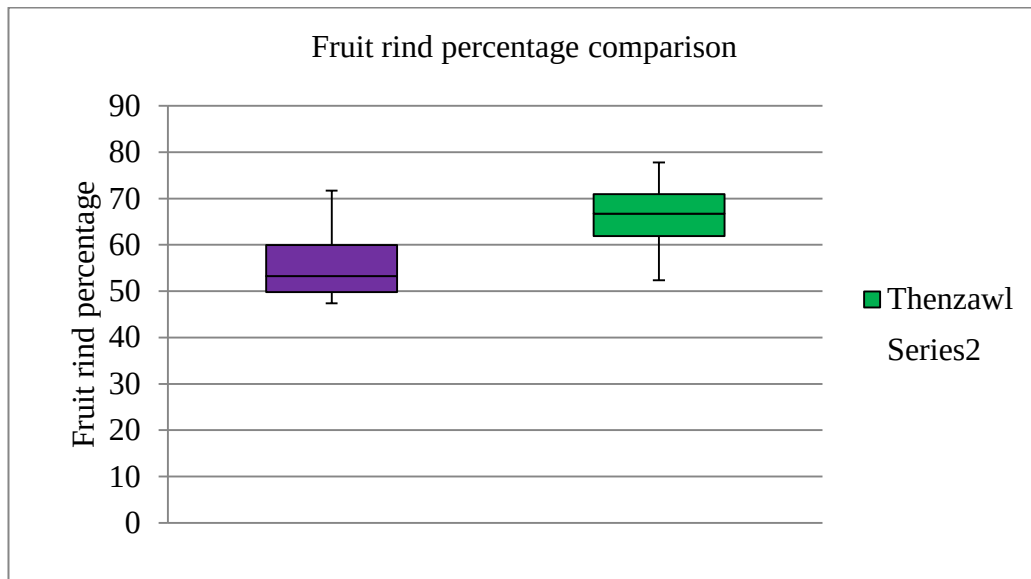


Figure 4.9: Boxplot of *G. lanceifolia* fruit rind moisture percentage distribution from natural sites

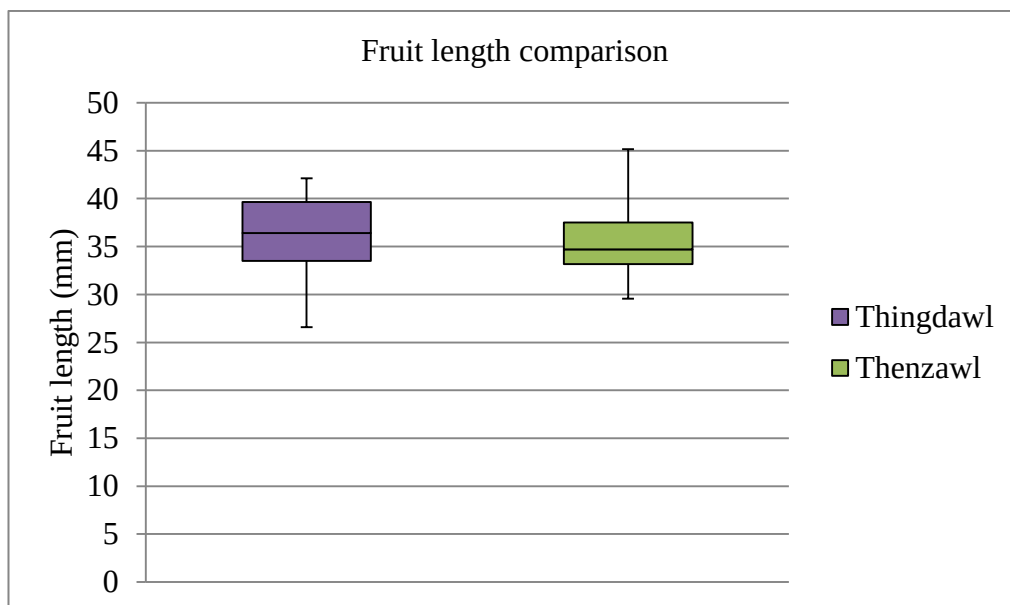


Figure 4.10: Boxplot of *G. lanceifolia* fruit length distribution collected from natural sites

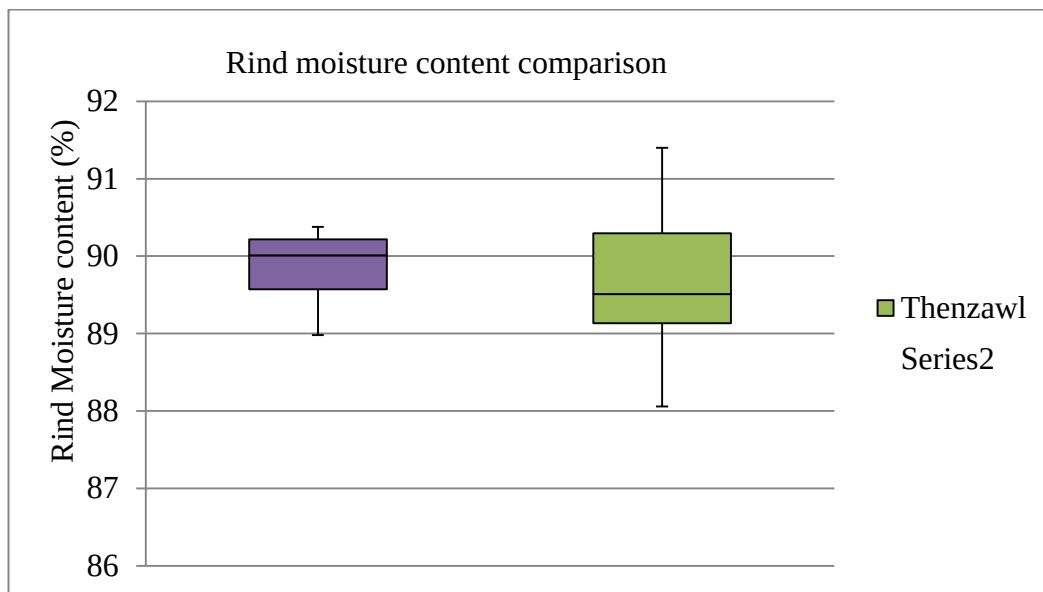


Figure 4.11: Boxplot of *G. lanceifolia* fruit rind moisture distribution collected from natural sites

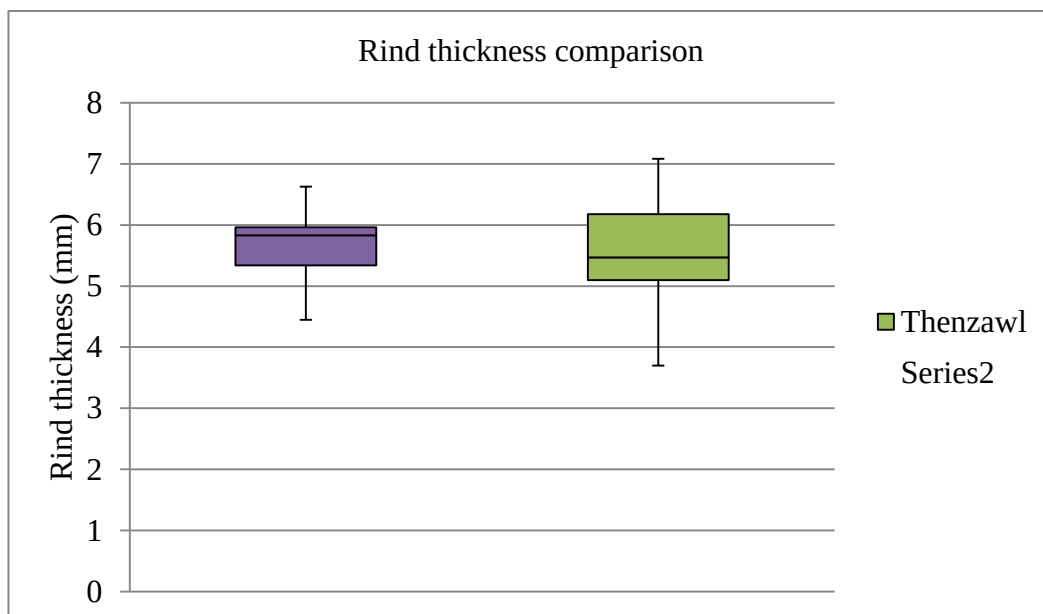


Figure 4.12: Boxplot of *G. lanceifolia* fruit rind thickness distribution collected from natural sites

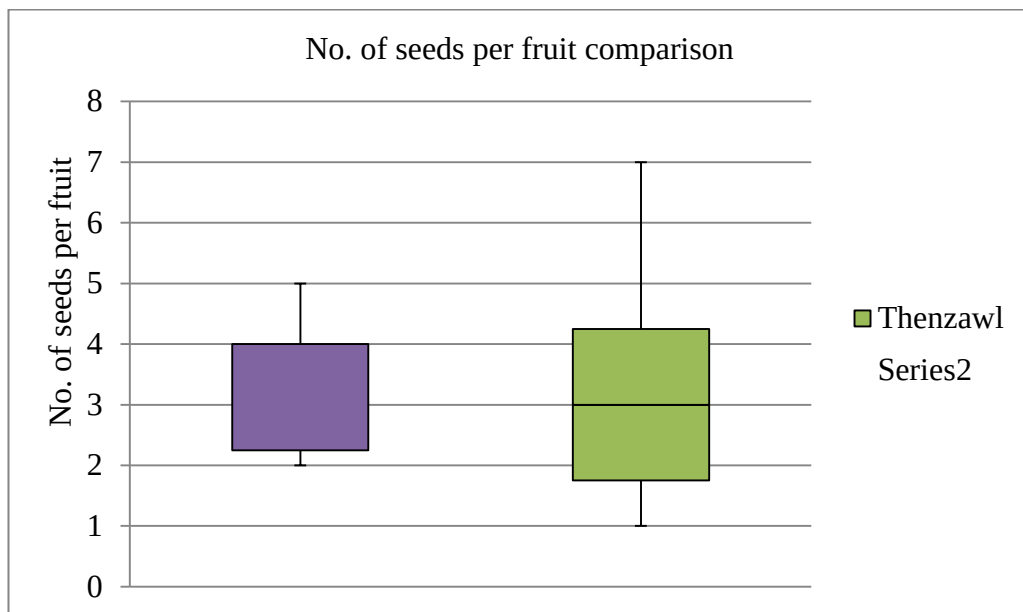


Figure 4.13: Boxplot of *G. lanceifolia* number of seeds distribution collected from natural sites

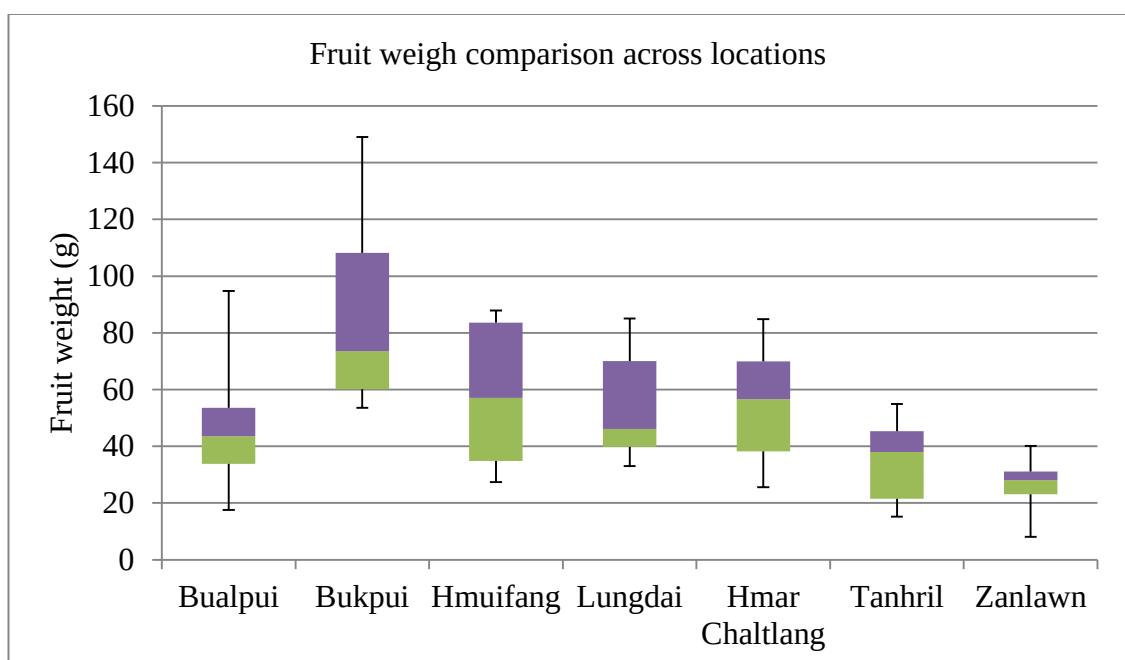


Figure 4.14: Boxplot of *G. lanceifolia* fruit weight distribution across location

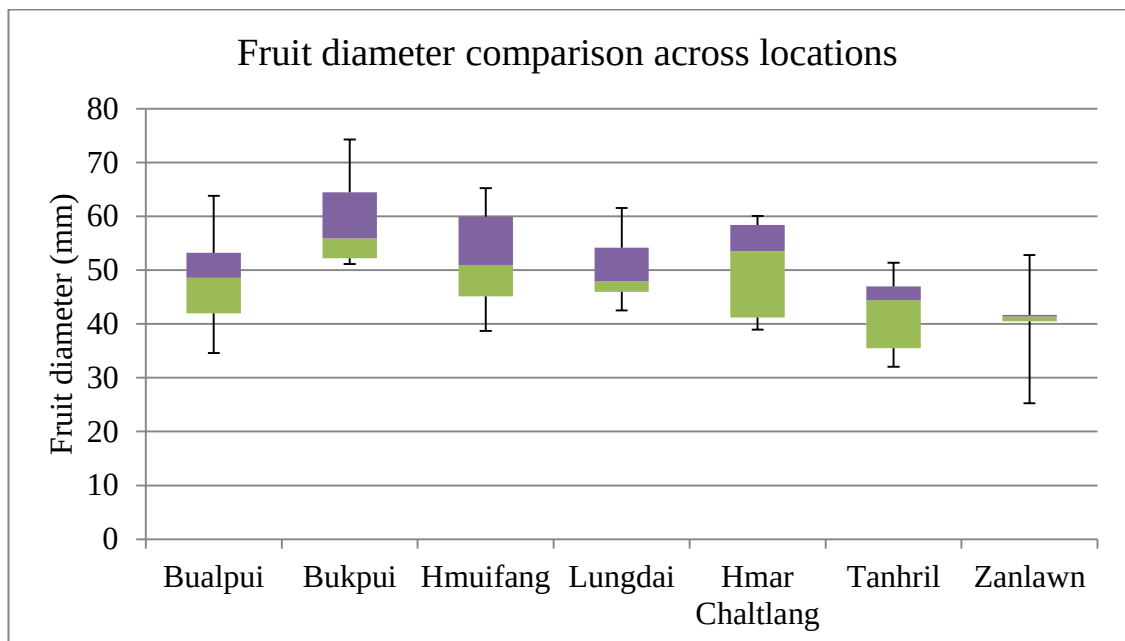


Figure 4.15: Boxplot of *G. lanceifolia* fruit diameter distribution across location

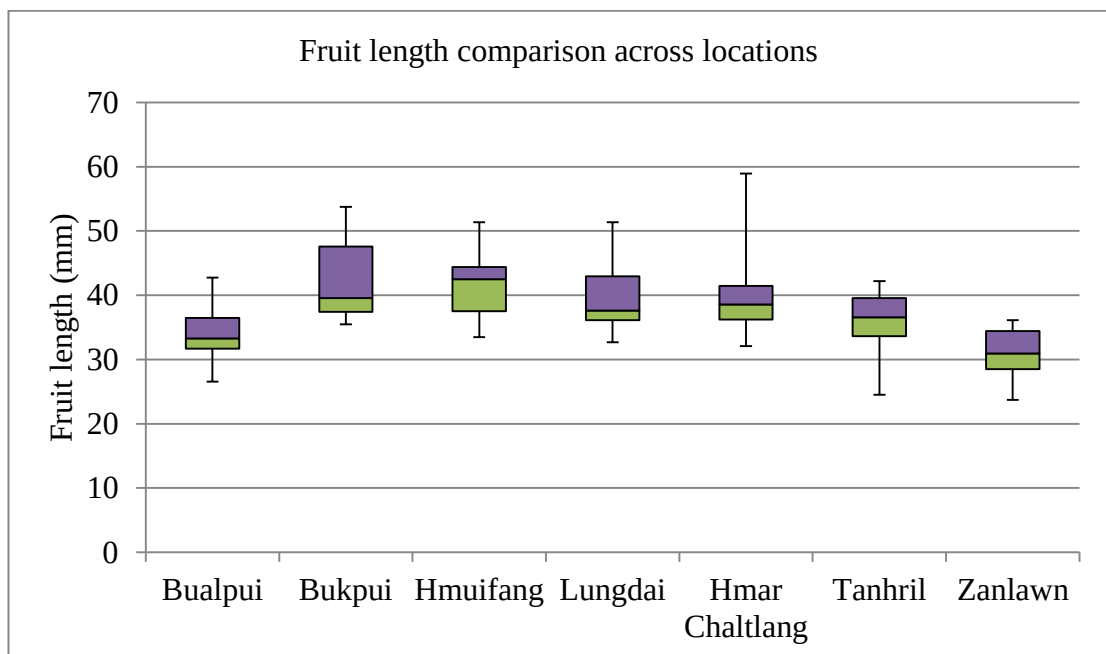


Figure 4.16: Boxplot of *G. lanceifolia* fruit length distribution across location

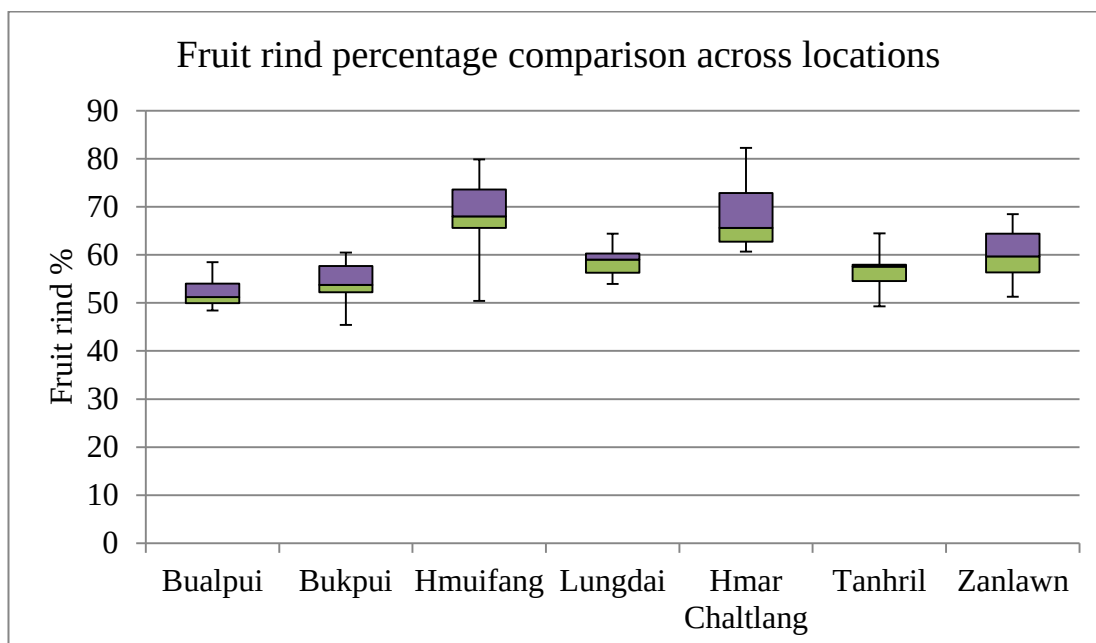


Figure 4.17: Boxplot of *G. lanceifolia* fruit rind percentage distribution across location

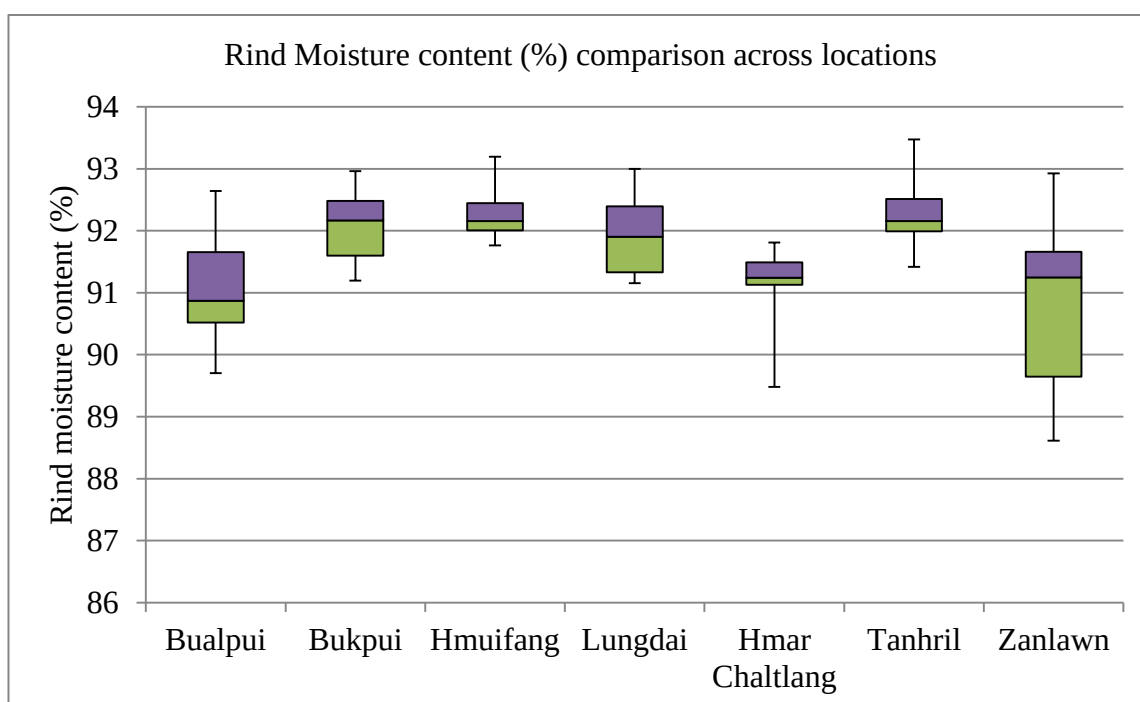


Figure 4.18: Boxplot of *G. lanceifolia* fruit rind moisture percentage distribution across location

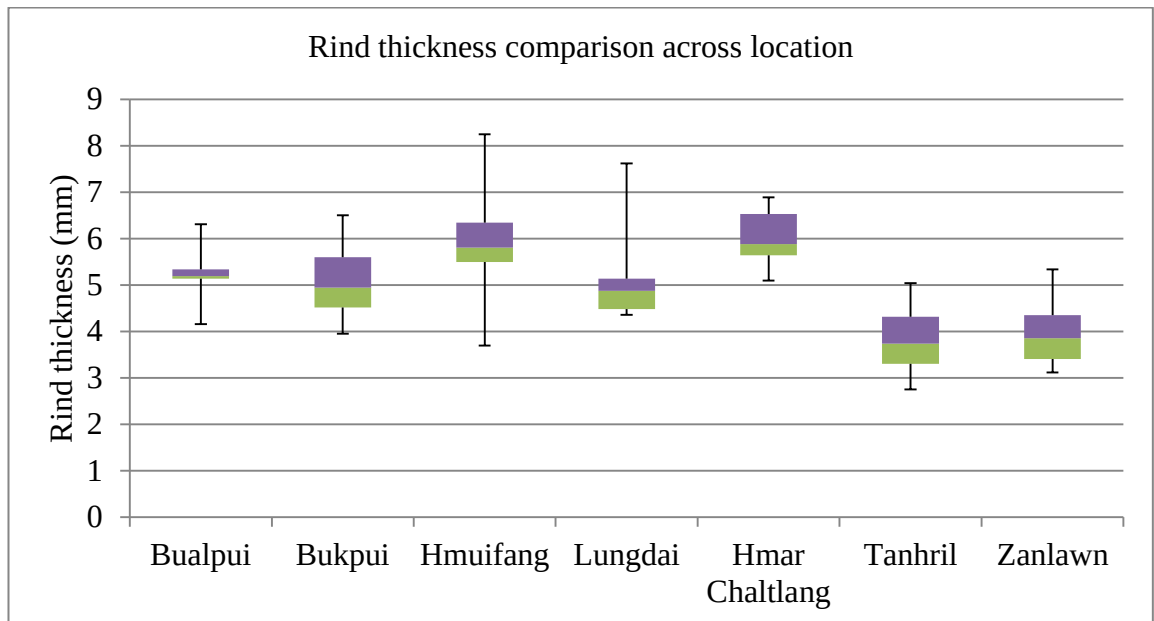


Figure 4.19: Boxplot of *G. lanceifolia* fruit rind thickness distribution across location

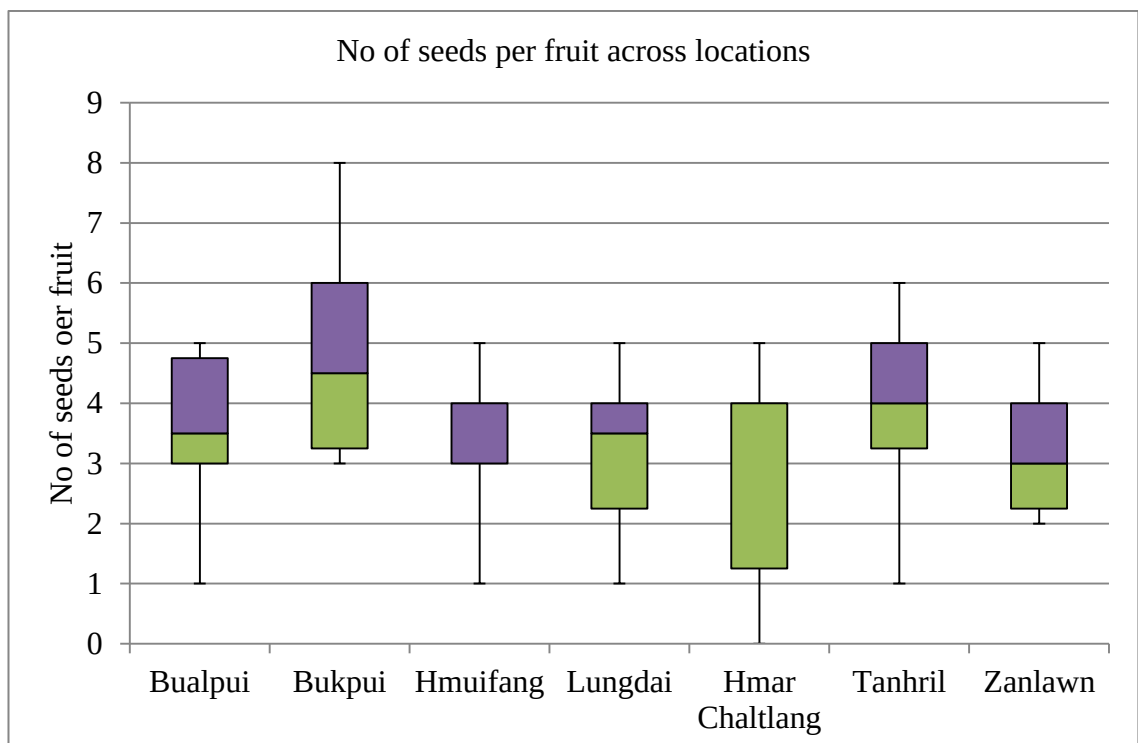


Figure 4.20: Boxplot of *G. lanceifolia* number of seeds per fruit distribution across location

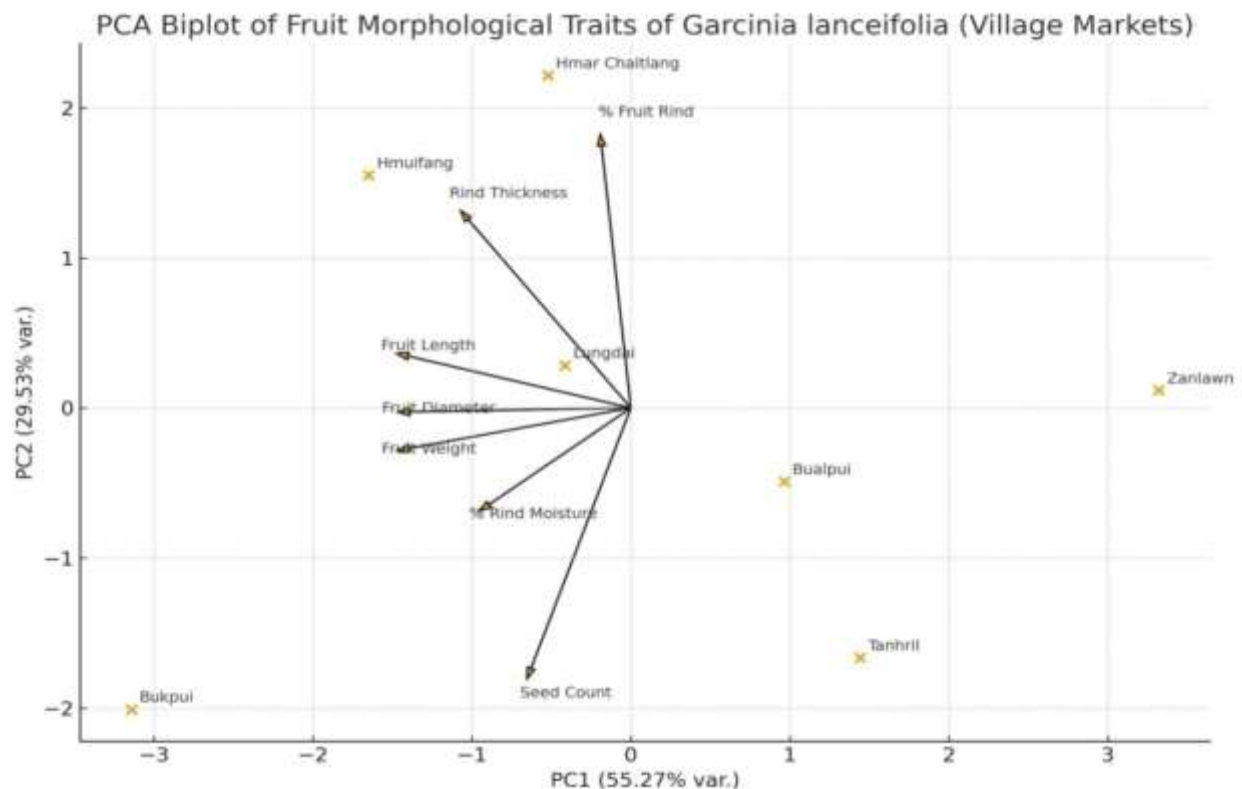


Figure 4.21: PCA biplot (PC1 vs PC2) of fruit morphological traits for *G. lanceifolia* from seven village markets (PC1 = 55.27% variance; PC2 = 29.53% variance).

4.6 Flowering Phenology

The flowering phenology of *G. lanceifolia* was carefully observed throughout the study period with the aim of determining the timing, length and order of reproductive events under natural environmental conditions in Mizoram. Flowering and fruiting phases of the species as shown in the Table 4.13 were established by collecting data from the different populations located at different altitude gradients.

The findings indicated that *G. lanceifolia*'s flowering season typically starts in the third week of October, which could be recognized as the switch from the late monsoon period to the cold season in Mizoram. The most prominent blooming interval was tracked from the second week of December to January, when most of the trees were at their optimal in the blooming period that varied from 120 to 145

days at the population level. The transition from the development of floral buds to anthesis took about 13 to 18 days, during which several morphological changes such as bud swelling, color changing, and bract dehiscence, could be seen. The time between flower opening and full anthesis was recorded ranging from 8 to 12 days, and fruit initiation took place approximately 28 to 35 days after flower opening, visible enlargement of the ovary marking the beginning of fruit set. The phase of development from fruit setting to ripening was about 55 to 65 days, which depended on the type of climatic conditions, especially temperature and humidity.

Therefore, the duration of the entire process from flower bud initiation to fruit ripening was between 125 and 150 days, which is almost five months of uninterrupted reproductive activity. All these findings imply that *G. lanceifolia* has a long annual reproductive cycle with distinct phases of bud initiation, flowering, fruit set, and maturation occurring one after another. The timing indicates adaptation to the climatic rhythm of subtropical Mizoram, where the fruit ripens in the pre-monsoon season (March to April), which may be advantageous for seed dispersal efficiency as it will be before the rains, which are usually extremely heavy.

The flowering phenology of *G. lanceifolia*, indicates a reproductive strategy that is very much in tune with the subtropical climatic rhythm of the region. By starting flowering in the third week of October, this species is among those that bloom during the post-monsoon and early winter period. This timing has a purpose, as it corresponded with a gradual decrease of rainfall and humidity, thus possibly avoiding the disruptive effects of the moist monsoon rains on delicate floral structures and pollinator activity while utilizing the leftover soil moisture for the resource-intensive bud formation (Okonkwo et al., 2024).

The flowering duration at the population level (120-145 days) being observed is a risk mitigation strategy that is very much characteristic of the tropics tree species. The prolonged flowering period thus secures that the occasional non-appearance of pollinators and the short, unfavorable weather conditions, in fact, will not affect the reproductive success so severely as to cause destruction, thus

increasing the chance of cross-pollination and preserving the genetic diversity of the populations (Richards, 1990; Mujadadi et al., 2024).

The finding that the peak flowering occurs from December to January indicates a relatively synchronized flowering behaviour. This synchrony, while variable across microclimates, is crucial for successful reproduction. Furthermore, the strong influence of environmental factors such as temperature is evident across developmental phases (Singh and Singh, 1992). The period from flower opening to fruit setting (28-35 days) and from fruit setting to ripening (55-65 days) is highly dependent on climatic conditions, particularly temperature and humidity. In Jamun (*Syzygium cumini*), the flowering period usually lasts from the end of February to the middle of March, while the whole reproductive cycle needs 119-126 days in total (Bajpai et al., 2012). Temperature and rainfall are the main factors affecting the timing of flowering and fruiting, with flowering being closely related to these climatic variables (Mohandass et al., 2018). The timing of fruit ripening is such that it often occurs either before or with the onset of the rains, ensuring that soil moisture is plentiful in the soil for seed germination and seedling establishment (Sundarapandian et al., 2005). This phenomenon of synchronizing different stages in the life cycle is an adaptive strategy by which trees adjust their reproductive cycles so that they take full advantage of the environment. This is particularly the case with the transition from dry to wet seasons, which is the main environmental condition favoring their growth.

From the standpoint of conservation and domestication, the phenological data for *G. lanceifolia* are indispensable. The unambiguous pointing out of the peak flowering period is of utmost importance for pollination services management, as well as for the controlled collection of germplasm for breeding programs. As an endemic medicinal plant of Assam, with its pharmacological properties already known, the knowledge of its reproductive cycle is a prerequisite for its sustainable use and conservation (Gupta et al., 2019; Bora et al., 2014). The documented phenological events along with their durations provide vital support for future monitoring, particularly in the case of determining the species' susceptibility to phenological shifts as a result of global warming. Changes in the timing of the

monsoon or in temperature regimes could very likely unbalance this carefully set cycle, thus affecting fruit set and the long-term regeneration of this species, which is quite important from the perspective of biodiversity.

4.6.1 Anthesis, Anther dehiscence and stigma receptivity

Anthesis, anther dehiscence and stigma receptivity study was done in hermaphrodite flower of *G. lanceifolia*. The anthesis process in *G. lanceifolia* was discovered to be a lengthy and gradual process (Table 4.14). It was discovered that the interval between the emergence of the initial bud and the starting of anthesis was takes from 13 to 18 days; the mean duration was 15.5 ± 1.72 days. Further to this, the interval between the appearance of anthesis and the bloom's full opening lasted between 8 and 12 days, having a mean duration of 9.9 ± 1.46 days. The increase flower-opening size from study recorded to be 0.199 ± 0.04 mm (Figure 4.22). The process of prolonged, staggered anthesis is a complete opposite of other *Garcinia* species. *G. indica* (kokum), for example, has a rapid blooming process that begins in the early morning (around 6:00–8:00 AM) and ends within a few hours only. On the opposite end of the spectrum, *G. gummi-gutta* (syn. *G. cambogia*, Malabar tamarind) has flowers that fully open around 4:30–6:30 PM which means that the anthesis is completed during a single evening (George et al., 2002; Pooja and Narayanapur, 2002)

The anther walls of the hermaphrodite flower of *G. lanceifolia* do not open during anthesis while the rest of the genus usually opens within 24 hours. In the case of *G. lanceifolia*, the anther dehiscence occurs 3-5 days after anthesis. Anther dehiscence takes place by longitudinal splitting (Table 4.4 and Figure 4.23). **Stigma receptivity** test was carried out with the help of hydrogen peroxide and discovered that even closed flowers were receptive. The receptivity was observed to be at its peak when the flowers were half open i.e. 4-6 days after anthesis which coincided with the dehiscence of the anther (Figure 4.24). Receptivity on the stigmas then decreases as the flowers approach maturity or full bloom.

Unlike *G. lanceifolia*, in most of the other *Garcinia* species such *G. gummi-gutta*, *G. indica* (Kokum), *G. morella*, *G. imberti*, the anthers dehiscence occurs just

before or at anthesis via longitudinal slits, releasing pollen almost immediately as the flower opens. *G. morella* (Indian gamboge), maximum stigma receptivity is reached about one day after anthesis. *G. imberti* stigmas are receptive for only a limited window (up to ~12 hours after anthesis). The nearly simultaneous anther dehiscence and stigma activity in hermaphroditic flowers of *G. indica* doesn't exhibit pronounced dichogamy; nonetheless, cross-fertilization is promoted by its dioecious tendency and multi-day stigma receptivity allowing pollen arrival from other individuals (Gawas et al., 2024; Kandhasamy et al., 2015; George et al., 2002; Pooja and Narayanapur, 2002)

The phenomenon of protogyny in *G. lanceifolia* increases the likelihood that the pollen will be from a separate flower or tree which in turn facilitates the process of genetic recombination. This can be a benefit to nature as it results in more genetically fit and diverse young ones. The prolonged life of the flowers that comes with the late opening of the anthers keeps the flower alive and attractive for many days. Nevertheless, the delayed of pollination brings about a reduction in fitness through a decrease in both the number and quality of seeds. This is a consequence of the aging of the flowers and is not affected by the inbreeding depression (Richards, 1990; Hildesheim et al., 2019).

Table 4.13: Flowering phenology

Phenological Stage	Observation/Duration
Beginning of flowering season	3rd week of October
Peak flowering	2nd week of December to January
Duration of flowering (population)	120–145 days
Bud formation to flower opening	13–18 days
Flower opening to fully open	8–12 days
Flower opening to fruit setting	28–35 days
Fruit setting to ripening	55–65 days
Flower bud to fruit ripening	125–150 days

Table 4.14: Timeline of anthesis, anther dehiscence and stigma receptivity

Phenological Stage	Range	Mean $\pm$ SD (Days)
Bud formation to Anthesis (Days)	13 - 18	15.5 $\pm$ 1.72
Anthesis to Full Bloom (Days)	8 - 12	9.9 $\pm$ 1.46
Increment of flower opening per day (mm)	---	0.199 $\pm$ 0.04
Anther dehiscence	3 - 5	
Stigma receptivity	Prior opening to full open	

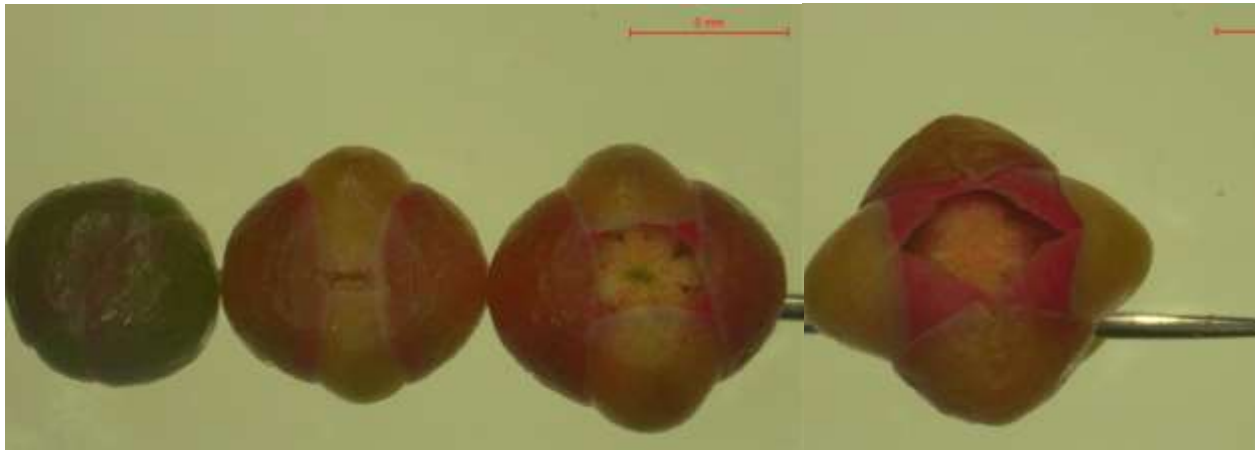


Figure 4.22: Flower showing different stages of opening from unopen to complete open



Figure 4.23: Anthers showing different stage of anther dehiscence (3- 4 days after dehiscence), (0 -2 days) and un-dehisce

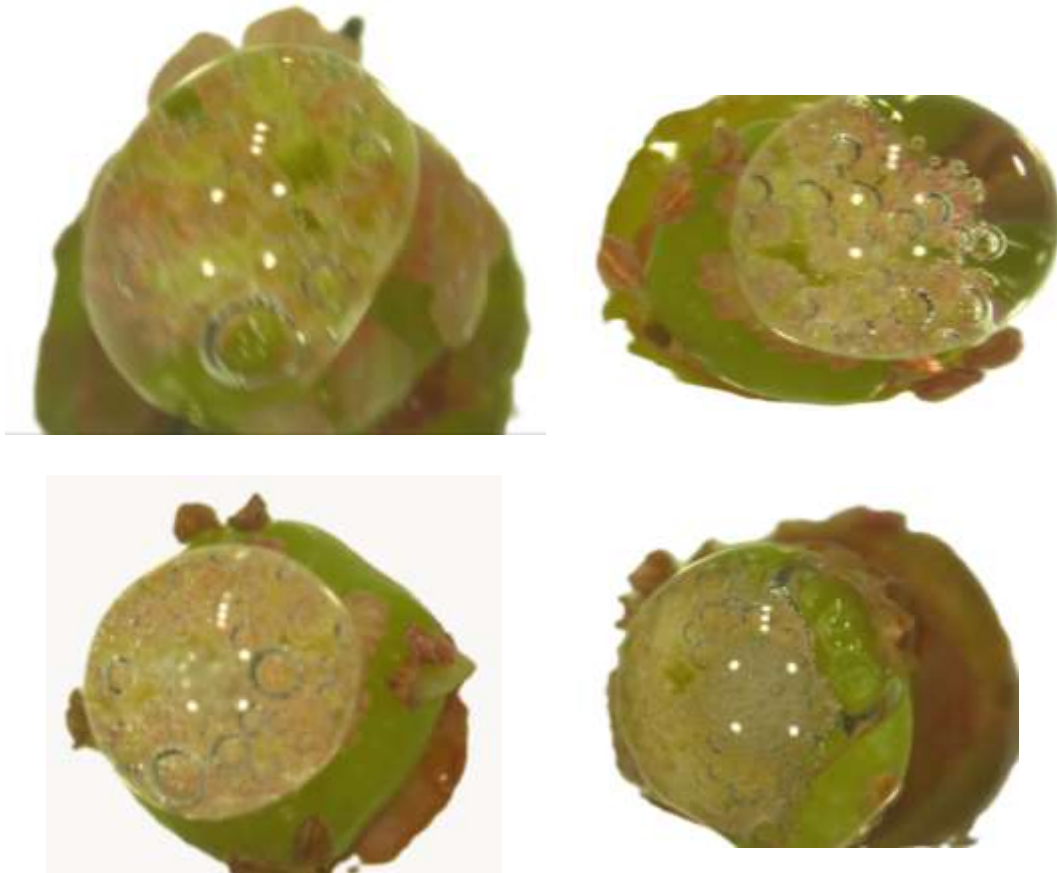


Figure 4.24: Stigma receptivity at different stages (unopen, recently open, half open and fully open)

4.7 Floral and Fruit Productivity between Thingdawl and Thenzawl Hermaphrodite Tree Populations

Comparative analyses were performed to evaluate floral productivity in hermaphrodite trees of *G. lanceifolia* from two different natural populations, namely Thingdawl (Kolasib District) and Thenzawl (Serchhip District). The evaluation was conducted using morphological traits such as trunk diameter and vertical growth, along with flower count data, to assess correlations and predictive power for reproductive yield.

The descriptive statistics show that the vegetative structure and floral output of the two populations were quite different but at the same time similar in degree (Table 4.15). The average girth of the hermaphrodite trees at Thingdawl (32.81 ± 4.89 cm) was just a little more than that at Thenzawl (30.18 ± 7.63 cm), indicating a rough increase of 8.7%. Likewise, mean tree height was considerably much larger in Thingdawl (4.00 ± 0.44 m) than in Thenzawl (3.43 ± 0.82 m), which is notably close to 16.6% more growth upwards ($p < 0.05$). On the contrary, the mean flower production per tree was found to be slightly more in the Thenzawl (1268.9 ± 746.4) than in the Thingdawl (1141.9 ± 447.6), indicating about a 10% increase in the potential yield.

The temporal and spatial variation in fruit productivity was evident across the two sites and years (Table 4.16). At Thenzawl, the total number of flowers per tree was 1268.84 ± 764.65 in 2021 but decreased to 758.44 ± 682.4 in 2022; however, the fruit set percentage remained stable and relatively high ($11.48 \pm 9.6\%$ in 2021 and $11.23 \pm 5.23\%$ in 2022). This consistency in fruit set, despite fluctuating flower numbers, can likely be attributed to Thenzawl's moderate temperature during flowering and fruit formation. Furthermore, the presence of both male and hermaphrodite tree leads to stable and consistence in fruit production percentage (Richards, 1990). In contrast, at Thingdawl, flower production showed little variation (1141.82 ± 454.8 to 1189.96 ± 591.8), but fruit set increased from $6.45 \pm 1.19\%$ to $8.73 \pm 2.47\%$. This improvement in productivity coincides with a cooler flowering and fruit formation period, substantially higher rainfall during the critical early fruit

development phase in 2022, conditions that are known to enhance fruit retention and expansion. High temperatures during sensitive period (flowering and fruit formation) can cause flower bud abortion or lead to poor pollen viability, ultimately reducing the potential fruit set percentage (Vara Prasad et al., 2001). The overall fruit-to-flower ratio (6–12%) aligns with values reported for other *Garcinia* species exhibiting partial androdioecy (Pangsuban et al., 2007; Singh et al., 2019)

The vegetative and reproductive traits of both populations were shown to have strong and significant positive relationships, as revealed by Pearson's correlation coefficients (Figures 4.25 and 4.26). In Thenzawl, the girth-height correlation coefficient was $r = 0.82$ (**), which was almost equal to Thingdawl's $r = 0.80$ (**), indicating that allometric growth patterns have been the same in both populations. The girth-flower number correlation was also slightly better in Thenzawl ($r = 0.76$ (**)) than in Thingdawl ($r = 0.72$ (**)), meaning that tree size is a little more important factor in determining reproductive capacity in Thenzawl. The height-flower correlation was found to be similar among the different populations ($r = 0.69$ and $r = 0.68$, respectively), suggesting that vertical canopy exposure plays an important role in improving flower production through light interception and resource availability.

The correlations were further confirmed through regression analyses. The girth coefficient (β) was 58.7 in Thenzawl and 59.1 in Thingdawl, which means that every centimeter of girth increase causes around 59 more flowers to be produced per tree in both populations. The height coefficient (β) was again slightly higher in Thenzawl (612.3) compared to Thingdawl (598.4), indicating that height has a slightly stronger impact on floral productivity at Thenzawl (+2.3%). The R^2 values of the model were quite high in both populations (0.71 for Thenzawl and 0.74 for Thingdawl), which means that together the size traits accounted for more than 70% of the variance in flower number. The slightly greater R^2 in Thingdawl indicates that the morphological parameters are more reliably correlated with reproductive output in that population.

A one-way ANOVA analysis was performed to check the impact of tree size classes (35 cm girth) on floral productivity. The overall difference in the number of flowers among the size groups was significant ($p < 0.01$) at both sites, with the highest F values of 10.74 and 12.83 at Thenzawl and Thingdawl, respectively. At Thenzawl, the smallest trees (less than 30 cm) were found to generate an average of 755.6 flowers, whereas the medium trees (30–35 cm) produced 1037.8 flowers, and the largest trees (over 35 cm) were responsible for an output of 2240.8 flowers. This stepwise increase in the floral output shows a strong positive relationship between tree size and flowering. On the other hand, Thingdawl's medium-sized trees showed the highest average productivity of 1332.8 flowers, small ones produced 701.7, and large ones 1746.4. In general, Thenzawl's populations were more productive in both the small and large size classes, while Thingdawl's productivity was the highest in the medium size class. The differences in productivity between the sites might be related to habitat heterogeneity, tree age structure, and local microclimatic adaptation, which would affect synchrony of flowering and energy investment in fruiting.

The relationship of flower output to the tree parameters (girth, height, and number of branches) in both populations is shown in Figures 4.25 and 4.26. The graphical pattern suggests a close-to-linear relationship between vegetative growth and the number of flowers, which means that the larger, taller, and more branched trees had a significantly higher floral output. In the populations studied, tree girth (TG), tree height (TH), and number of branch per tree (NoB) turned out to be strong predictors of the total number of flowers per tree (TNoFT) and average number flowers per branch (ANoFB), respectively. The close correspondence of regression lines in both graphs suggests that *G. lanceifolia* had similar structural–reproductive integration over the two locations.

The evident difference in the growth of vegetation, where Thingdawl trees are much taller and thicker, indicates that there are different environmental conditions between the two regions. Soil nutrient profiles, water availability, and light regimes could all be variations (Cramer, 2011; Aczel, 2021). Even though the average tree size is larger at Thingdawl, the higher flower production per tree at

Thenzawl is a reminder of a major rule in plant ecology: resource allocation is a strategic decision (Weiner, 2004). In Thenzawl, trees seem to prefer a reproductive strategy over a vegetative one by committing a lot of resources to the production of flowers. Such a strategy could gain wide acceptance in certain environments as it would be the fittest one. This is in line with the findings about other tropical trees, where the amount of resources allocated to reproduction is influenced by the competition and resource availability in the habitats of different populations (Santos-del-Blanco et al., 2013; Sugiyama and Bazzaz, 1998). The very high and significant positive correlations ($r = 0.80-0.82$) between girth and height in the two populations are a strong indication of a consistent and tightly controlled allometric growth pattern in *G. lanceifolia*. It implies that there is growth architecture particular to the species that is upheld in varied habitats (Bohlman and O'Brien, 2006).

A primary research finding of the current investigation is the remarkable forecast ability of tree shape towards the amount of flowers produced. The high Pearson's correlation coefficients between girth and flower number ($r = 0.76$ and 0.72) and height and flower number ($r = 0.69$ and 0.68) indicate that larger *G. lanceifolia* trees definitely have greater reproductive potential. This connection is probably due to bigger trees having more resources stored and thus their light interception being improved, and thus they photosynthesize more and eventually more of that goes into reproduction (Setsuko and Tomaru, 2011; Sugiyama and Bazzaz, 1998).

The relationship between tree size and flower number has been drawn in quantitative terms by the regression models, indicating that every centimeter increment in girth bears a flower count increase of around 59, which is the case for the two separate populations. The R^2 values, which are notably high (0.71 and 0.74), imply that more than 70% of the variance in flower yield can be accounted for only by tree girth and height. Such a robust allometric rule is advantageous in the identification of high-yielding genotypes for domestic and breeding purposes of *G. lanceifolia*. Just by taking tree size measurements, cultivators can accurately predict the number of flowers produced, thus facilitating the management and conservation of this species (Castilla et al., 2015; Murthy et al., 2018).

Based on the ANOVA findings, it can be stated that the connection between the tree size and flower output is not completely linear and is, to a certain extent, affected by the factors of each site. The gradual increase in the number of flowers produced per size class at Thenzawl is in sharp contrast with the medium-sized trees at Thingdawl, where the lowest productivity was seen. Such a difference indicates that the local conditions, like, for example, light competition, soil quality, or even the age distribution of the tree cohorts, may affect the choice of the size for reproduction. This variability should be taken into account in the conservation plans, as it means that the presence of various sizes within a population might be the valuable to the overall fruit yield (Castilla et al., 2015; Minor and Kobe, 2019).

The interesting research finding that the percentage of fruit set remained stable at Thenzawl (~11%) even though there was a reduction of about 40% in flowers between the years 2021 and 2022. On the other hand, Thingdawl showed a rise in the percentage of fruit set even though the number of flowers stayed the same. This points out that the fruit set in *G. lanceifolia* is not only dependent on flower production but is also a very well-controlled process. The stability at Thenzawl indicates that there is very efficient pollen source due to the presence of male tree, which can withstand the annual fluctuations. The total fruit-to-flower ratio of 6–12% matches the resource-consuming characteristic of fleshy fruits in tropical trees, where a significant portion of the flowers is dropped to produce a small number of high-quality fruits (Cohen and Dukas, 1990; Gross, 1993).

Table 4.15: Comparative Statistical Analysis of Floral Productivity between Thenzawl and Thingdawl Hermaphrodite Tree Populations

Analysis Type	Metric	Thenzawl	Thingdawl	Comparison	Biological Significance
Descriptive Stats	Mean Girth (cm)	30.18 ± 7.63	32.81 ± 4.89	Thingdawl +8.7%	Thingdawl trees are structurally larger
	Mean Height (m)	3.43 ± 0.82	4.00 ± 0.44	Thingdawl +16.6%*	More vertical growth in Thingdawl
	Mean No. Flowers/Tree	1268.9 ± 746.4	1141.9 ± 447.6	Thenzawl +10%	Thenzawl has higher yield potential
Pearson's r	Girth vs Height	0.82**	0.80**	Nearly identical	Strong size integration in both populations
	Girth vs Flowers	0.76**	0.72**	Thenzawl slightly stronger	Size more critical in Thenzawl
	Height vs Flowers	0.69*	0.68*	Comparable	Height equally important in both
Regression	Girth Coefficient (β)	58.7*	59.1*	Nearly identical	1cm girth $\approx$ +59 flowers in both sites
	Height Coefficient (β)	612.3*	598.4*	Thenzawl +2.3%	Height slightly more valuable in Thenzawl
	Model R ²	0.71	0.74	Thingdawl +4%	Better predictability in

					Thingdawl
ANOVA (Size Groups)	Small Trees (<30cm)	755.6 flowers	701.7 flowers	Thenzawl +7.7%	Thenzawl saplings more productive
	Medium Trees (30-35cm)	1037.8 flowers	1332.8 flowers**	Thingdawl +28.4%	Thingdawl excels in mid-size class
	Large Trees (>35cm)	2240.8 flowers	1746.4 flowers	Thenzawl +28.3%***	Thenzawl giants outperform significantly
	F-value (ANOVA)	10.74**	12.83**	Both significant	Size class matters strongly in both sites

Table 4.16: Fruit productivity of *G. lanceifolia*

Location	2021			2022		
	Total no of flower per tree	Total no of fruit per tree	% of Fruit per tree	Total no of flower per tree	Total no of fruit per tree	% of Fruit per tree
Thenzawl	1268.84±764.65	111.88 ± 72.15	11.48 ± 9.6	758.44 ± 682.4	74.54 ± 74.54	11.23 ± 5.23
Thingdawl	1141.82 ± 454.8	70.06 ± 20.3	6.45 ± 1.19	1189.96 ± 591.8	97.02 ± 39.55	8.73 ± 2.47

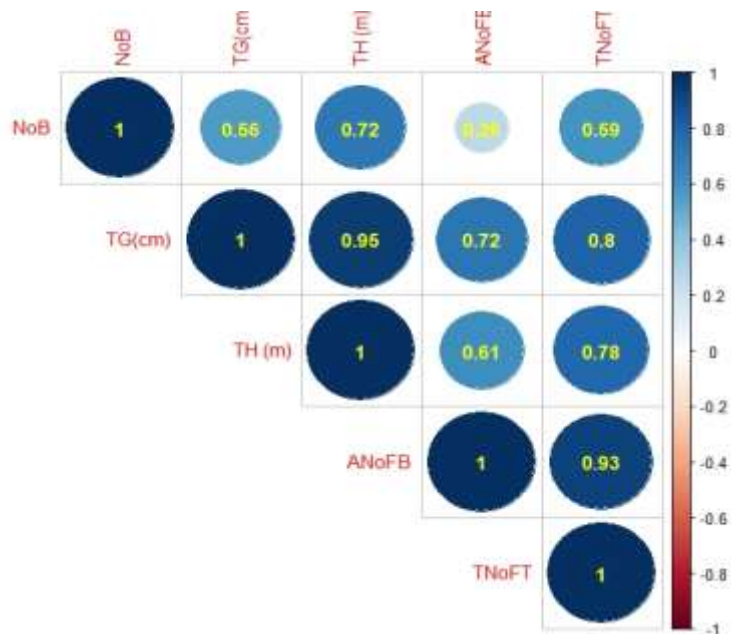


Figure 4.25: Correlation of flower productivity and Tree parameters of Thingdawl

(NoB : No. of branch; TG (cm): Tree girth; TH (m): Tree height; ANoFB : Avg. no of Flower per tree; TNoFT : Total no of flower per tree)

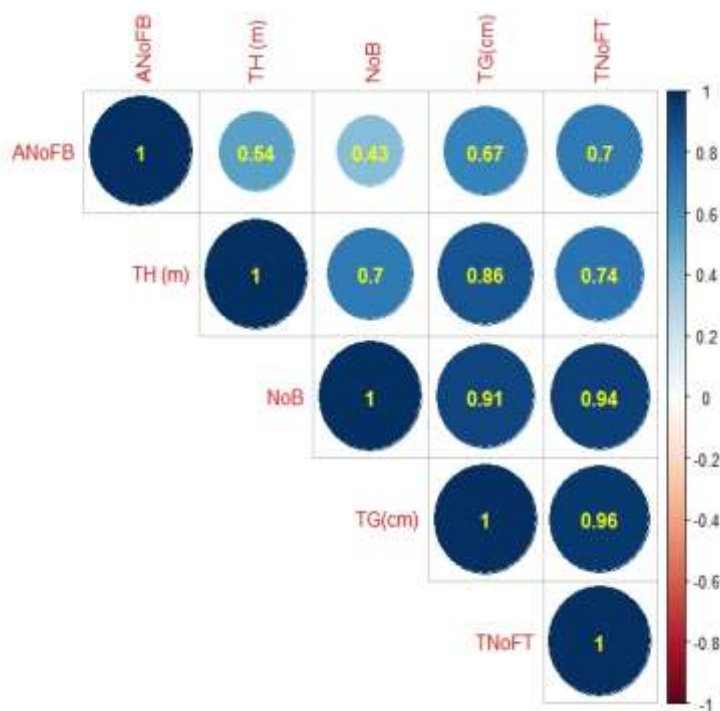


Figure 4.26: Correlation of flower productivity and tree parameters of Thenzawl

4.8 Pollen Production and Viability

4.8.1 Total Pollen Count per Anther

A significant difference was observed in total pollen count per anther of *G. lanceifolia* between male and hermaphrodite flowers (Table 4.17 and figure 4.27). The mean pollen count per anther of male flowers (2208) exceeded that of hermaphrodite flowers (1891.8). The difference in variation among samples was low in male flowers (CV = 14.34%) but high in hermaphrodite flowers (CV = 19.27%), thus pointing out the greater inconsistency in the pollen production of hermaphrodite individuals. The pollen count for male flowers was 1796 to 2782, whereas for hermaphrodite flowers it was 1163 to 2283. These findings imply that the male flowers are not only morphologically but also functionally more adapted to pollen dispersal and, therefore, contribute to the pollen pool to a larger extent than hermaphrodites, thus confirming the same with other androdioecious *Garcinia* species (Baskaware and Deodhar, 2022; Sutthinon et al., 2018).

4.8.2 Pollen Viability per Anther

There were notable variations in pollen viability between the two types of flowers (Table 4.18 and figure 4.28). The hermaphrodite flowers with an average pollen viability of $79.44 \pm 9.96\%$ showed a significant difference compared to male flowers that had only $32.13 \pm 7.32\%$ as the average pollen viability. The viability range of the hermaphrodite pollen was 61.60% to 87.38 % while for the male pollen; it was only 22.34% to 41.61%. The viability of the male pollen is relatively low, and this might be one factor indicating that some possible physiological or developmental differences have a negative impact on the pollen maturation process. The male flowers have a lower viability percentage, but their high total pollen count may act as a compensation for the reduced individual pollen quality, thus ensuring that the reproductive process within the population is not affected by the lack of functional pollen. The pollen's viability characteristics of the different sexual morphs have been documented as similar by other researchers (Delph and Herlihy, 2012; Wang et al., 2021).

4.8.3 Total Anther Production per Flower

The number of anthers per flower was different among flower types and locations (Table 4.19). The hermaphrodite flowers at Thingdawl had the least number of anthers (mean = 9.7 ± 1.69), while the same type of flowers at Thenzawl had more (22.45 ± 3.84). The male flowers at Thenzawl were the ones with the most anthers, which were 42.9 ± 5.6 per flower on average. The results give a clear indication of a pattern in the order of male > hermaphrodite (Thenzawl) > hermaphrodite (Thingdawl). This increase in anther number among male flowers is a common adaptation to facilitate pollen output, thus, promoting crop pollination and keeping the population productive (Baskaware and Deodhar, 2022).

4.8.4 Total Pollen Production per Flower

When the pollen output per flower was calculated (Table 4.20), the male flowers had the highest numbers, with a mean of $94,787 \pm 12,360$ grains per flower and a range of 75,106 to 128,122. On the other hand, Thenzawl hermaphrodite flowers averaged $42,473 \pm 7,600$, while those from Thingdawl showed a lower number at $18,920 \pm 3,380$ grains per flower. The significant disparity between male flowers and the other floral forms underscores the aspect of male flowers' reproduction that is specially adapted to liberate large amounts of pollen. The population differences may also indicate the prevailing ecological factors, such as microclimate and soil nutrition status, that may influence the amount of flowering (Dai and Galloway 2012).

4.8.5 ANOVA for Pollen Count

The analysis of variance (ANOVA) specified that there was a significant difference in pollen production among the different flower types (Table 4.21). The variation between the groups ($SS = 65.2 \times 10^9$) was considerable compared to the variation within the groups ($SS = 5 \times 10^9$), which resulted in a very high F-value of 369.4 ($p < 0.001$). The very low p-value suggests a strong statistical significance that flower type and likely location have a major influence on pollen yield in *G.*

lanceifolia. This supports the idea of a functional differentiation between male and hermaphrodite forms.

4.8.6 Correlation Analysis between Anther Number and Pollen Production

According to Table 4.22, there was a very strong positive correlation between the number of anthers and the total pollen production in all flower types. The correlation coefficients were very high, $r = 0.97$ for Thingdawl hermaphrodites, $r = 0.99$ for Thenzawl hermaphrodites, and $r = 0.99$ for male, all with $p < 0.001$. This implies that thereupon anther number and pollen output exist an absolute similar relationship, and anther count is a trustworthy predictor for the pollen production capacity of this species; moreover, similar patterns of linear association have been reported (Reddi and Reddi, 1986).

4.8.7 Total Pollen Production per Tree

When pollen production was assessed on the basis of individual trees (Table 4.23), it turned out that at Thenzawl, the male trees were the most significant in terms of their average pollen output, which was 156.92×10^6 grains per tree, and even 352.27×10^6 in exceptional cases. As for the hermaphrodite trees, the production was 53.89×10^6 (Thenzawl) and 21.6×10^6 (Thingdawl) respectively. This difference in production among the locations probably indicates that ecological factors affected the trees' investment in flowering, for instance, the microclimate or the nutrients present in the soil (Dai & Galloway, 2012). The male trees, when considered at the tree level, not only managed to produce an overwhelming number of pollen but also were recognized as the most important for pollinators in the ecosystem, thus, their role in the gene flow among populations was confirmed by the theoretical models of androdioecy to be consistent (Ishida and Hiura, 2002; Pannell, 2002).

4.8.8 Pollen–Ovule Ratio and Reproductive Efficiency

The pollen–ovule (P/O) ratio is a definite number measure for the reproductive investment and indicator of the species' breeding system and pollination strategy. In *G. lanceifolia*, a plant with androdioecious sexual system, pollen, and

ovule production were investigated in the hermaphrodite flowers harvested from Thenzawl and Thingdawl, which are the two natural populations.

Results showed a significant distinction in pollen output among the two flower types (Table 4.24). The fruits of the hermaphrodite flowers yielded a large quantity of pollen grains per flower 42,473 at Thenzawl and 18,920 at Thingdawl, and at the same time they had only one functioning ovary with an average of 7–8 ovules.

Therefore, Thenzawl hermaphrodite (approx. 5663:1) and Thingdawl hermaphrodite (approx. 2628:1) showed the highest P/O ratio. The numbers correspond to the values given for obligate outcrossers and androdioecious systems, where high pollen production is a way of compensating for limited chances of successful fertilization (Cruden, 1977; Richards, 1997).

Table 4.17: Total Pollen count per anther

Statistic	Male Flowers	Hermaphrodite Flowers
Mean	2208	1891.8
Median	2189.5	1970
Std Dev	316.6	364.5
Variance	1,00,228.40	1,32,868.40
Range	986 (1796–2782)	1120 (1163–2283)
CV (%)	14.34	19.27

Table 4.18: Viability of Pollen per anther

Statistic	Male	Hermaphrodite
Mean (%)	32.13	79.44
SD	7.32	9.96
SE	3.27	4.45
Range (%)	22.34–41.61	61.60–87.38

Table 4.19: Total anther production per flowers

Location	Mean	Std. Dev	Min	Max
Thingdawl Hermaphrodite	9.7	1.69	8	13
Thenzawl Hermaphrodite	22.45	3.84	17	31
Thenzawl Male flower	42.9	5.6	34	58

Table 4.20: Total pollen production per flower

Location	Mean	Std. Dev	Min	Max
Thingdawl Hermaphrodite	18,920	3,380	15,136	24,596
Thenzawl Hermaphrodite	42,473	7,600	32,164	58,652
Thenzawl Male flower	94,787	12,360	75,106	1,28,122

Table 4.21: ANOVA for pollen count

Source	df	SS ($\times 10^9$)	MS ($\times 10^9$)	F-value	p-value
Between	2	65.2	32.6	369.4	<0.001
Within	57	5	0.088		
Total	59	70.2			

Table 4.22: Correlation Analysis (Anthers vs. Pollen)

Location	r (Anthers vs. Pollen)	p-value
Thingdawl	0.97	<0.001
Thenzawl	0.99	<0.001
Male flower	0.99	<0.001

Table 4.23: Total pollen production (in Millions) per Tree

Location	Mean ($\times 10^6$)	SD ($\times 10^6$)	Min ($\times 10^6$)	Max ($\times 10^6$)
Thingdawl Hermaphrodite	21.6	8.61	11.39	35.21
Thenzawl Hermaphrodite	53.89	32.48	11.69	123.37
Thenzawl Male	156.92	117.87	17.63	352.27

Table 4.24: Pollen–Ovule ratio in hermaphrodite flowers

Location / Flower Type	Mean Pollen Grains per Flower	Mean No. of Ovules per Flower	P/O Ratio
Thenzawl	42,473	7.5	5,663:1
Thingdawl	18,920	7.2	2,628:1

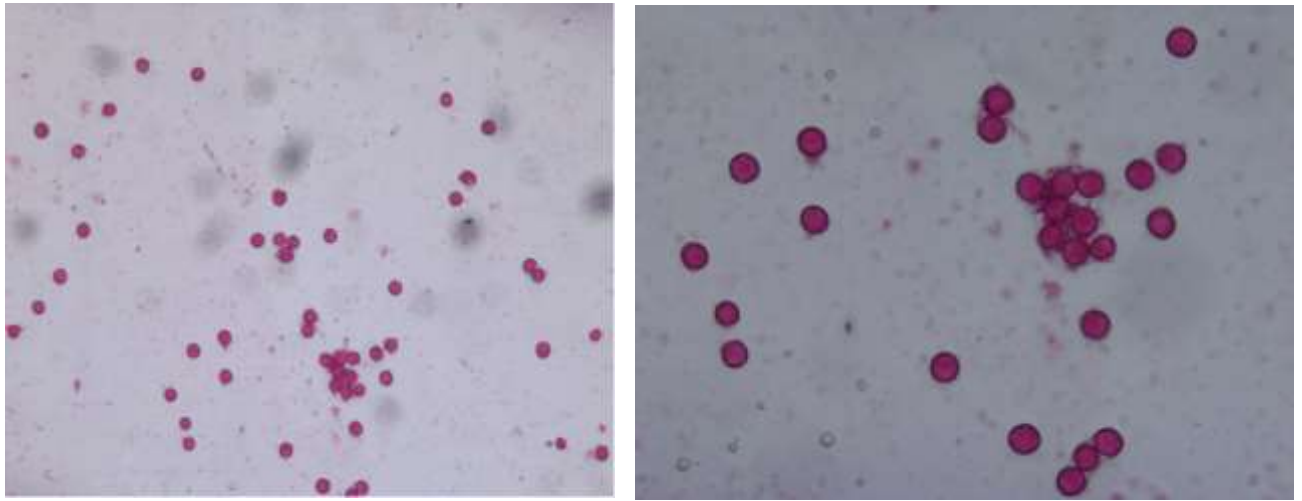


Figure 4.27: Microscopic pollen count following safranin staining at 10x and 20x magnification.

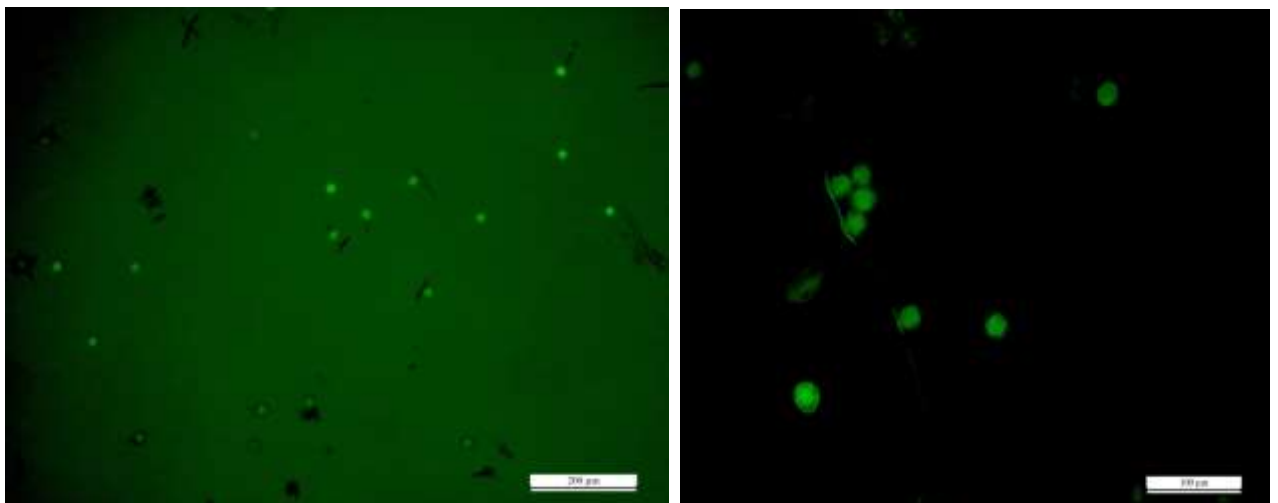


Figure 4.28: Assessment of pollen viability via Fluorescein Diacetate (FDA) staining. Viable pollen grains exhibiting bright green fluorescence under bright-field microscopy at 10x and 20x magnification.

4.9 Mating System in *G. lanceifolia*

The results in Table 4.25 showed different trends between the two hermaphrodite populations. In the case of Thenzawl, geitonogamy had the highest fruit set with a 38.1 % record, while in Thingdawl, a slightly higher geitonogamy rate was recorded at 46.34 %, but this was statistically insignificant ($\chi^2 = 1.01$, $p = 0.315$). The less geitonogamous fruit set would suggest a far more common reproduction path in *G. lanceifolia*: self-pollination within the same individual.

Autogamy, both Thenzawl and Thingdawl had very low fruit set percentages for spontaneous self-pollination within the flower itself (8.33 % and 5.41 % respectively), with no significant difference ($\chi^2=0.45$; $p=0.503$). This shows that few fruits are produced through autogamy, which means that self-pollination is infrequent in this species and, with complete certainty, requires a pollinator or floral mechanism to prevent possible selfing, all mutually exclusive with protogyny or genetic self-incompatibility.

Xenogamous pollination (Crossing between different trees) gave rise to some distinct changes between the two populations. Hermaphrodites in Thenzawl achieved a 27.27 % fruit set as against the 16.67 % in Thingdawl, a significant difference ($\chi^2 = 3.92$, $p = 0.048$). This shows that outcrossing is more efficient in the Thenzawl population, probably as a consequence of greater genetic diversity or more efficient pollen transfer among individuals.

Apomixis, Thingdawl showed — on 27.03% — higher apomictic fruit set than Thenzawl (11.76%); this was done by emasculation and bagging (to exclude pollen), significant at $\chi^2 = 9.12$, $p = 0.003$. Higher apomixis in Thingdawl suggests a stronger tendency towards apomictic seed formation, likely in that population to function as a reproductive assurance mechanism when pollination is limited.

The male-to-hermaphrodite (Hand cross-pollination) treatment done only in Thenzawl gave an intermediate yield of 15.38 % because male flowers can fertilize perfectly hermaphrodite flowers, in other words, female functioning flowers. Results of the study have supported there being a male tree in Thenzawl's population,

which has substantiated the androdioecious breeding pattern in *G. lanceifolia*. No action like this could be introduced in Thingdawl due to the non-availability of male flowers or trees in the village.

The reproduction data of *G. lanceifolia* suggest a facultative mixed mating system, with the two extremes being sexual outcrossing and asexual apomixis; this balance shifts notably between the Thenzawl and Thingdawl populations. It is an example of how local habitats can influence the choice of reproductive strategy (Suijkerbuijk, 2025).

In cases of self-pollination (autogamy), a minimum fruit set was observed, indicating a low rate of self-fertilization in the population. This is true for tropical trees, such as possible enforced outcrossing by way of self-incompatibility or protogyny (Bawa, 1974). The occurrence of a very low, non-zero seed production in these bagged flowers, supported by the positive outcome in the apomixis test done separately (emasculated, no pollination), has led to the conclusion that this fruit set is actually caused by facultative apomixis rather than by self-fertilization (Pangsuban et al., 2009).

Alternatively, facilitated geitonogamy (hand-pollination with pollen of another plant on the same tree) with a high fruit set (40–46%) shows that *G. lanceifolia* is at least partially self-compatible. Moderate yields suggest that while physical incompatibility barriers are bypassed, there is still some degree of self-incompatibility, as found in related species like *G. atroviridis*, where the fruit remains seedless or aborted when self-pollinated (Pangsuban et al., 2007). Also, in facultative apomicts, pollination itself, even with self-pollen, directly triggers the development of clonal seeds known as pseudogamy (Hörandl, 2010). Thus, it is likely that geitonogamous fruit set is more likely to result from limited self-fertilized seeds and more (or all) apomictic seeds initiated by pollen stimulus.

The most vivid manifestations of inter-population variation were in xenogamy and apomixis tests. Notably higher xenogamous fruit set at Thenzawl (27.3%) in comparison with Thingdawl (16.7%) pointed out more effective sexual reproduction with respect to the former locality. The phenomenon could be due to

the possibly better availability of compatible pollen or greater genetic diversity among trees that would result in more successful crosses (Aabd et al., 2021). Conversely, lower xenogamy success in Thingdawl would clearly support a case of high genetic uniformity of this population, originating possibly from very few clonal lineages. Thus, cross-pollination between different related neighboring trees would be conceptually identical to inbreeding, which results in in-hybrid depression (Tambarussi et al., 2017; Charlesworth and Willis, 2009).

The most notable variance presented was based on the rate of apomixis. The rate of production of fruits apomictically by the population of Thingdawl is more than double that of Thenzawl (27 vs. 12%). This unique reliance on apomixis at Thingdawl is well expressed in the term "reproductive assurance," where the formation of seeds through asexual means would assure that one had progeny when sexual reproduction was not satisfactory (Mráz et al., 2019; Hojsgaard and Hörandl, 2019). It is reported that apomictic fruits likely may be inferior in their characteristics. Results from a study on *G. atroviridis* suggested that apomictic fruits are much smaller with lower vigor of seed germination and that the clonal mode of reproduction tends to lose genetic diversity gradually, having the potential of limiting the adaptive potential (Pangsuban et al., 2009).

Thenzawl population mating further clarifies the breeding system by the introduction of a male-to-hermaphrodite (M→F) cross-pollination test. Indeed, the proof of successful fruit set (15%) indicates that males produce functional pollen capable of fertilizing hermaphrodites. The non-existence of male flowers in Thingdawl population will be a significant difference between their sexual expressions. In comparison, Thenzawl would be considered androdioecious, where both hermaphrodites and male trees occur in a mixture, and the result may usually encourage outcrossing (Vassiliadis et al., 2002). In contradiction, Thingdawl typically consists entirely of hermaphrodites, hence restricting pollen diversity and rather tending toward such observed changes as apomixis (Huang et al., 2009; Maurice and Fleming, 1995). The variation study between two habitats provides an excellent example of a dynamic trade-off in *G. lanceifolia*. In this primary outcrossing species, ecological constraints such as pollinator limitation or low mate

availability, as inferred for Thingdawl, can shift towards heavy options with respect to asexual seed production.

Table 4.25: Comparative analysis of mating systems between Thenzawl and Thingdawl hermaphrodite populations.

Mating System	Thenzawl (%)	Thingdawl (%)	χ^2 Value	p-value	Significance
Autogamy	8.33	5.41	0.45	0.503	NS
Geitonogamy	38.1	46.34	1.01	0.315	NS
Xenogamy	27.27	16.67	3.92	0.048	*
Apomixis	11.76	27.03	9.12	0.003	**
Male tree to tree hermaphrodite (M→F)	15.38	NA	—	—	—

4.10 Evidence of Androdioecy in *G. lanceifolia* Roxb. (Clusiaceae) in Mizoram

Androdioecy is the rarest and evolutionarily interesting reproductive system in plants, characterized by the presence of hermaphrodite and male individuals in a single population. This is an intermediate condition recorded between hermaphroditism and dioecy, thus becoming a good model to understand the evolution of reproductive differentiation and sexual system (Charlesworth, 1984; Pannell, 2002; Duan et al., 2019). Recorded cases of androdioecy are very few in number, and confirmed examples are from conserved families such as Apiaceae, Cucurbitaceae, Clusiaceae, Datiscaceae, Euphorbiaceae (Liston et al., 1990; Spigler & Ashman, 2012).

The present study provided the first detailed confirmation of androdioecy in *G. lanceifolia* Roxb. based on field observations, floral morphology, and sex ratio analysis across seven natural populations in Mizoram, whereby duplication of pistils is dealt with. The geographical coordinates and elevations of the sites investigated are presented in Table 4.26 and Figure 4.29 covering a broad altitude range which, in from 780 to 990 m above mean sea level.

4.10.1 Sexual Morph Identification

Field and floral examinations confirmed the presence of two distinct sexual morphs within natural populations of *G.lanceifolia*:

1. Male trees, which bear exclusively staminate (male) flowers, and
2. Hermaphrodite trees bear only bisexual (perfect) flowers.

Most inflorescences were terminal though axillary clusters were occasionally present. Flowers were solitary, or paired, or sometimes occurred in small fascicles. Each flower is enclosed by four concave sepals and four petals which are initially green but turn yellowish-red or pinkish-red as they mature (Fig. 4.30).

Hermaphroditic flowers have the superior ovary placed centrally, which was convoluted by the stamens in four bundles consisting of 2-7 stamens (Figure 4.31). The stigma had 5-8 lobes corresponding to the number of locules of the ovary, with

each typically having 5-8 ovules (Figure 4.32). In contrast, male flowers lacked any trace of an ovary and exhibited approximately 40 stamens, arranged compactly in a dense, hairless androecial mass. The anthers were narrow, slightly angled, and nearly sessile (stalkless), with four pollen sacs per anther. They were introrse, opening via longitudinal slits to release copious pollen. These morphological distinctions unambiguously delineate the two sexual morphs, confirming the presence of functionally male and hermaphrodite individuals—a hallmark of androdioecy.

4.10.2 Morphological Differences

The data showed significant differences in most floral traits between the male and hermaphrodite flowers (Table 4.27). In general, the hermaphrodite flowers are bigger, heavier, and have a greater peduncle length compared to male flowers and also exhibit greater flower weight, diameter, stalk length, and overall flower length than their male counterparts. The difference in the size of the floral opening between the two types was not significant. Conversely, with male flowers, anthers were clearly higher in number than those observed in hermaphrodite flowers, estimated at almost double.

4.10.3 Sex Ratio Variation

The male-to-hermaphrodite ratio of trees was significantly different between the sites (Table 4.28). At most of the locations, the hermaphrodite trees were very much in excess and were dominant in at least certain of the sites. One was more balanced between both sexes, with all others showing the prevalence of hermaphrodite plants.

The present research intends to bring out comprehensive knowledge on the reproductive morphology and sexual distribution of *G. lanceifolia* in its natural habitat in Mizoram. For correct identification, floral assessment is essential, as no difference is found vegetatively between male and hermaphrodite trees. It is clear that the majority of *Garcinia* species exhibit their sexual dimorphism through the floral characteristics rather than vegetatively, similar to what was recorded in previous studies (Baskaware and Deodhar, 2022).

The morphological differentiation between the male and hermaphrodite flower concurs with functional specialization in the androdioecious species. Compared to male flowers, hermaphrodite flowers tend to be larger and heavier, thus probably putting into male and female reproductive structures a greater amount of resource investment. On the other hand, male flowers, comprising several anthers, are specialized for pollen production (Niu et al., 2017; Lyu et al., 2024). The presence of multiple anthers in male flowers is a typical feature of pollen specialization to allow for an enhancement of male reproductive fitness, especially in outcrossing systems (Barrett, 2010).

Most sites were dominated by hermaphrodite trees, especially in Tanhril and Nisapui, because they acquired the capacity for autonomous reproduction to ensure reproductive success in the absence of males (Ramirez & Brito 1990). The prevalence of hermaphrodite trees may reflect reproductive assurance, particularly in a fragmented or isolated habitat (Pannell, 2002). This involves a dual system of enabling both selfing and outcrossing strategies and is crucial to the minimization of the loss of population-level genetic diversity (Barret 2002).

The consistent maintenance of floral aperture size makes a point for pollination strategies shared or ecological constraints across morphs (Galen et al., 1987; Lamenager et al., 2024). The variation in sex ratios across localities seems to indicate either local environmental influences, pollinator behavior, or effects of demographic processes such as genetic drift (Devlin and Stephenson, 1987; Pickup and Barrett, 2013).

The discovery of androdioecy in *G. lanceifolia* widened our horizons on plant sexual diversity in the tropics. Quite significant ecological and morphological differences prevail between male and perfect flowers, through which species get adapted. This leads to opportunities for research, like understanding how genetic aspects of sex determination operate and qualifications to multiply opportunities under changing environmental conditions. Eventually, testing for assigning pollinator function in maintaining this rare form of sexuality will be conducted.

Table 4.26: Coordinates and elevation of the sites.

Sites	Coordinates	Elevation (AMSL)
Bukpui	24°04'55"N 92°47'35"E	952.41 m
Lungmuat	23°57'57"N 92°46'44"E	867.07 m
North Chaltlang	24°01'04"N 92°46'13"E	783.34 m
Nisapui	23°55'55"N 92°45'09"E	989.62 m
Thenzawl Site 1	23°17'08"N 92°46'51"E	785.92 m
Thenzawl site 2	23°17'11"N 92°47'01"E	796.8 m
Tanhril	23°43'46"N 92°39'29"E	822.53 m

Table 4.27: Morphological comparisons between male and hermaphrodite flowers.

Parameter	Male (Mean $\pm$ SD)	Hermaphrodite (Mean $\pm$ SD)	<i>p</i> -value
Weight (g)	0.25 $\pm$ 0.03	0.43 $\pm$ 0.07	< 0.001
Diameter (mm)	8.81 $\pm$ 0.38	10.24 $\pm$ 0.68	< 0.001
Stalk length (mm)	4.82 $\pm$ 0.58	6.83 $\pm$ 0.81	< 0.001
Flower opening (mm)	4.88 $\pm$ 0.41	4.96 $\pm$ 0.66	0.59
Flower length (mm)	6.24 $\pm$ 0.28	6.57 $\pm$ 0.62	0.023
No. of anthers	43.35 $\pm$ 5.21	22.85 $\pm$ 0.62	< 0.001
Significant at $p \leq 0.05$			

Table 4.28: Male to hermaphrodite tree ratios.

Site	Male:Hermaphrodite Ratio
Bukpui	1:1.83
Lungmuat	1:3
North Chaltlang	1:3.2
Nisapui	1:5
Thenzawl 1	1:4.5
Thenzawl 2	1:3.6
Tanhril	1:6

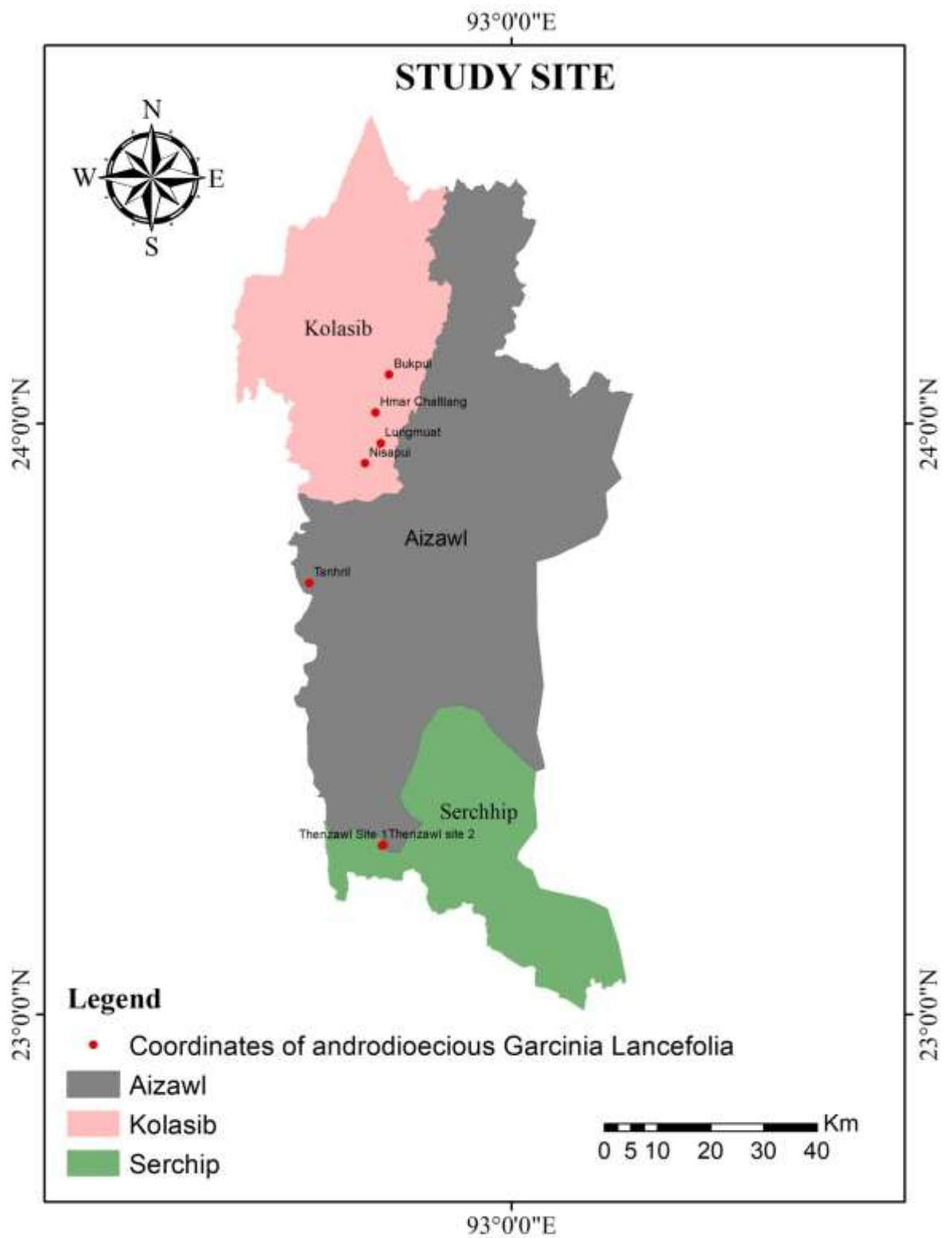


Figure 4.29: Geographical distribution of documented androdioecious populations



Figure 4.30: Changes of colors as the flower mature in both and male and hermaphrodite flower

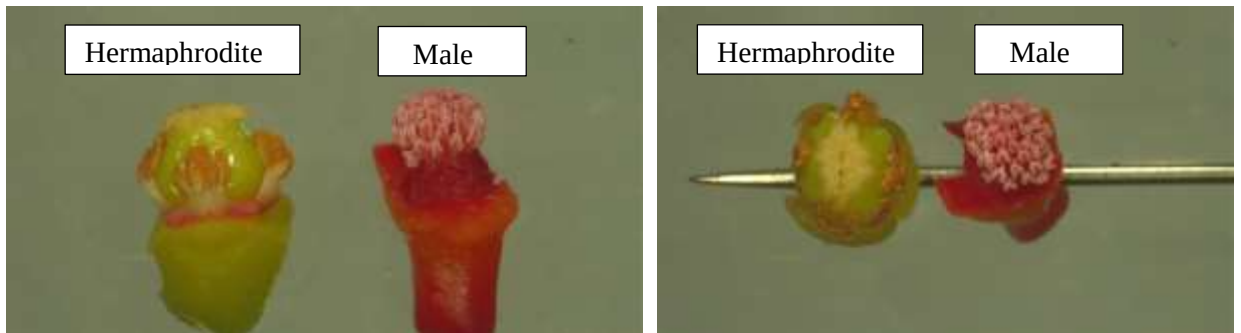


Figure 4.31: Side view and top view of both hermaphrodite and male flower



Figure 4.32: Figure 4.24: LS, TS view of hermaphrodite flower, ovary and stigma

4.11 Transcriptome Assembly, Annotation, and Analysis of Sex Differentiation in *G. lanceifolia*

4.11.1 De Novo Transcriptome Assembly and Functional Annotation

The floral transcriptome of *G. lanceifolia* was assembled de novo into a resource that is of high quality, ideal for studying its reproductive system and biology. The assembly resulted in 154,734 transcripts with a total length of approximately 121.4 Mb in size and an N50 statistic of 1,071 bp (Table 4.29). N50 whereby half of the assembled transcripts are equal to or longer than this length, along with an average transcript length of ~784 bp, indicates how contiguous and comprehensive the assembly. This is a level of assembly quality that is on par with other excellent plant flower transcriptomes, like the result established for *Chrysanthemum dichrum*, having N50 of around 1.4 kb and a mean length of ~0.84 kb (Matra et al., 2016; Rowley et al., 2012). These statistics are indicative of high confidence that most mRNA sequences' capture, most of which are within the range of full-length capturing, are expressed, which could serve as a foundation for downstream gene discovery and differential expression analyses. The result was precipitated by good read coverage and articulate optimization of assembly parameters such that highly expressed and rarer transcripts are comprehensively represented in the input data.

The discovery of the assembled transcript is that the floral genetic toolkit of *G. lanceifolia* is highly conserved from the predicted 3,072 coding sequences. Of this, 88.4% (46,918) find significant homology with NCBI NR proteins (Table 4.30). This is an excellent annotation rate, compared with other non-model plant transcriptomes: like annotation of unigenes against NR in *C. dichrum* is about 56.9%, and for most species, it falls in the 50-80% range of successfully annotated genes (Liu et al., 2021). Therefore, perhaps *G. lanceifolia* flowers have conserved orthologous genes expressed across angiosperms, which are significant for flowering and reproduction. And in this way, also the ~ 11.6% unigenes without ORFs in the present databases could be considered novel gene resources contributing to the very particular biology of *Garcinia*.

A comparative documentation across diverse databases, including NR, Uniprot, KOG, and Pfam, reflects a broad functional coverage (Table 4.31). NR database resulted in the highest number of hits, which stands at 46,918; UniProt is the second with 38200 hits. They also show considerable overlap in their annotations across the databases noted by the Venn diagram (Figure 4.33). Thus, there can be a kernel, a central set of transcripts, consistently detected by different algorithms, most likely housekeeping and developmental, that is highly conserved. Transcripts with single- or no-hit hits in a database may be considered specific to specialization-function classes or newly ordered elements (Segata and Huttenhower, 2011). Combining such a window on the annotated profile will show whether there is quite a vast functional repertoire of the floral transcriptome of *G. lanceifolia*, much of which is similar to that found with other plant species, but unique components will be found in it.

4.11.1.1. Gene Ontology (GO) and Pathway Enrichment Analysis

The more in-depth functional insights come from Gene Ontology (GO) classification, with about 10,378 transcripts assigned with GO terms in three major domains--Biological Process, Cellular Component, and Molecular Function (Table 3.32). In Biological Process, the vast preponderance of terms issued concerned "the metabolic process" and "cellular process," reflecting major biochemical processes for flower development. The Molecular Function category is largely filled with terms related to "catalytic activity," "binding," and accounts for thousands of gene annotations. This pattern, in fact, mirrors floral transcriptomes by encoding a significant fraction of pelleted genes, from enzymes to regulatory proteins, like kinases and transcription factors, needed in the processes of organogenesis and development (Liu et al., 2021; Wang et al., 2021). It has been plentiful from "metabolic" to "catalytic at," indicating the tremendous demand for energy and the molecular precursors necessary to make pigments, fragrances, and hormones essential to carrying on its reproductive function.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of functional landscape was further contextualized through transcriptomic work (Table 4.33). A large number of these transcripts are mapped against metabolic pathways, with the biggest set in carbohydrate metabolism (945 transcripts), amino acid metabolism (562 transcripts), lipid metabolism, and energy metabolism. This enrichment underlines the huge energetic and biosynthetic needs of growing floral organs and gametes (Mühlemann et al. 2012). The signaling mechanisms of these processes are best explained as actively dividing cells in the floral meristems and gametophytes, using abundant resources for the number of transcripts directly affecting glycolysis, TCA cycle, amino acids, and other metabolites (Wuest et al., 2010).

In contrast, out of the "Environmental Information Processing" category, signal transduction pathways appeared as one of the major groups, consisting of 1,473 transcripts in total. Hypothetically, these must represent all the possible cell-cell communications and hormonal signalling cascades that act during the course of flower development. Auxin, ethylene, cytokinin, and gibberellin signaling cascades at the level of hormone signals must be relatively well covered. Consequently, hormonal signals are known to overlap and interact with each other during sex expression and differentiation of organs in plants (Dhall et al., 2023; Patil and Pawar, 2019). Cucurbits serve as good examples, in which ethylene is a plant hormone involved in the development of female flowers, and the essential enzymes in this process are the biosynthesis enzymes for ethylene (Zhang et al., 2023). This dominance of signal transduction in the *G. lanceifolia* transcriptome shares the same states as its counterparts and specifies that floral development is a close and metabolically coordinated affair of regulation with intricate pathways.

4.11.2 Discovery of Microsatellite and its Potential as a Molecular Marker

A re-examination of the repeat sequences in the cDNA library revealed 3,356 SSR loci, which can be used as important tools in genetics and breeding studies (Katrin et al., 2008). The most surprising fact is: 5.7% of all sequences (in total, 53,072) contain at least one SSR, which also represents the general threshold of

discovered EST, when analyzed in plants. Distribution of repeated motifs is heavily skewed; trinucleotide repeats are overwhelmingly the most abundant (2, 984 SSRs, ~80.9%), followed by dimeric repeats (547); and tetra-, penta-, and hexa-nucleotide repeats can just be counted by handfuls (Table 4.34 and Table 4.35).

This prevalence of trinucleotide SSRs occurs quite frequently among plant coding sequences because insertions or deletions of triplets do not cause frameshift mutations, making trinucleotide step size gaps more tolerable in open reading frames (Metzgar et al., 2000). In all the EST-SSR studies examined in this review, this framework is observed regardless of plant species. These genic SSRs, given their direct association with expressed genes, are particularly attractive as molecular markers and hold great promise for the identification of sex-linked tags for early sex identification in plants case that was efficiently demonstrated in other dioecious species such as *Actinidia arguta* (kiwiberry) and *Pistacia chinensis* (pistachio), where sex-specific markers were developed for breeding programs (Guo et al., 2023; Cheng et al., 2022). At least 3300 SSR loci uncovered in this analysis may lend the genetic diversity studies organized on the lowland genetic diversity of *G. lanceifolia*, together with leading in designing the linkage maps- besides being a helpful resource in the field of research.

4.11.3 Differential Gene Expression Underlying Sexual Dimorphism

The main goal of the research is to decipher the cellular basis of sexual dimorphism in *G. lanceifolia*, with a transcriptomic analysis of male and hermaphrodite floral transcriptomes. The variance in gene fall-out response evaluation analyses between the two morphs of flower was well resolved. We found 30,594 transcripts with differences in expression, of which 4,099 were found very significantly different at a false discovery rate (FDR) below 0.05 (Table 4.36). This large number of regulated genes (DEGs), approximately 7.7% of total sequenced transcripts, suggests that sexual differentiation in this species is an extensive developmental program affecting a variety of essential cellular functions. Of note is the scope of sex- biased gene expression in *G. lanceifolia*, which is consistent with other plant systems. For example, sex-biased gene expression in *G. lanceifolia* is

similar to that in other plant systems, with an example being a recent study on the transcriptomics of unisexual and bisexual flowers of an orchid (*Cymbidium tortisepalum*), which identified ~4,291 sex-biased DEGs among floral organs (Ma et al., 2023), compared to the order of such magnitude found here.

Indeed, the distribution of differential expression of genes in male and hermaphroditic sex was balanced: 1,577 differentially expressed genes were found up-regulated in male flowers, while 2,522 in hermaphroditic flowers. A slightly higher number of hermaphrodite genes is anticipated biologically since they should have expression programming for the development of functional carpels and male stamens. On the other hand, the male flowers only develop stamens. This kind of observation is similar to similar observations related to sexual dimorphism in many species, such as papaya (*Carica papaya*), where in female and hermaphrodite flowers, increased expression of genes are required for carpel and ovule development, which are repressed in male flowers (Zerpa-Catanho et al., 2019).

4.11.3.1 Key Regulatory Genes and Pathways

- i. **MADS-Box Transcription Factors:** Genes displaying significant sex-bias are transcripts of MADS-box family transcription factors representing a seminal part of the floral organ identity regulatory network (ABCDE) model). Several MADS-box transcripts were found to be differentially expressed. The study is further affirmed by the presence of over 327 differentially expressed MADS-box genes identified during transcriptomic analysis in *Garcinia indica*-analogues active in different sex types, which include core regulators like APETALA1 (AP1), AGAMOUS (AG), and SEPALLATA (SEP) (Patil and Pawar, 2019; Jabbour et al., 2022). Notably, the genes involved in ovule identity, such as *AGAMOUS-LIKE 11 (AGL11/SEEDSTICK)*, showed highly spatiotemporally localized expression within female functions in the flower. The male flowers of *G. lanceifolia* likely achieve such a program of development because the carpel-initiating genes would fail to function and thus fail to form proper functioning female organs.

- ii. Hormone Synthesis and Signaling:** Phytohormones are the key elements in plant sex determination. In transcriptome data, genes involved in the biosynthesis and signaling pathway of hormones have shown high differential expression distinctly in relation to auxin biosynthesis, ethylene, gibberellin, and jasmonic acid. Then, it follows results seen in other species: in papaya, ABA and auxin signaling-sex characterized genes such as ABA 8-hydroxylases, AUX/IAA and ARF factors, and reactive oxygen species (ROS) regulation (Zerpa-Catanho et al., 2019). In *Garcinia indica*, many of the auxin response factors (ARFs) and ethylene response factors (ERFs) are expressed differently in male, female, and bisexual flowers, pinpointing auxin and ethylene as significant players in sexual differentiation in the species (Patil and Pawar, 2019). In *G. lanceifolia*, hermaphrodite flowers up-regulate auxin and cytokinin pathways to promote ovary formation in hermaphrodite flowers. It can be speculated that gibberellin or jasmonate activity may be altered in male flowers. Excitingly, Tasselseed2 (TS2) in maize is an example of a gene that encodes a specific jasmonate biosynthesis that induces programmed cell death in female primordia in male florets; the mutation results in sex changes. The homologs of such key regulatory genes could form the category of male-up modulated DEGs in our data set and may be among the candidates for suppressing carpel growth.
- iii. Other Key Regulators:** Besides the MADS-box and hormone genes, other transcription factor families have been reported such as the YABBY type, which has been found as a molecular switch for carpel development and sex determination. In dioecious species like the grapevine, a YABBY gene on the Y chromosome will repress hermaphroditism, but a simple overexpression of the gene in a hermaphroditic individual can change its gender expression. Identification of YABBY homologs within our DEG set hints at their possible incorporation into *G. lanceifolia* complex in the reproductive/differentiation network.(please add a reference)

To visualize the major expression shifts, a heatmap of the top DEGs (Figure 4.34) shows clear clustering by flower type: hermaphrodite samples cluster together, expressing a set of genes (shown in red on the heatmap) that are minimally expressed in males, and vice versa for the male-upregulated genes (blue in hermaphrodites). Many of these top DEGs likely belong to the categories discussed above (floral regulators, hormone-related genes, and possibly secondary metabolism enzymes that differ between the sexes). The distinct expression modules we observe echo what has been seen in other sex-differentiating transcriptomes.

Table 4.29: Master assembly transcript statistics of *G. lanceifolia* flower transcriptome.

Description	Statistics
Total no. of transcripts	1,54,734
Total transcriptome size (bp)	12,13,76,262
Average transcript length (bp)	784
Maximum transcript length (bp)	26,733
Minimum transcript length (bp)	300
Transcript N50 (bp)	1071

Table 4.30: BLAST statistics against NR database.

Sample Name	Total CDS	CDS with Hits	CDS with No Hits
Master CDS	53,072	46,918	6,154

Table 4.31: BLAST statistics against protein databases.

Blast Database	Hits against database
Total	53,072
NR	46,918
Uniprot	38,200
KOG	23,377
Pfam	23,186

Table 4.32: GO distribution for annotated transcripts of *G.lanceifolia*.

Description	CDS with GO (GO terms)
Total GO	10,378
Biological Process	7,159
Cellular Component	5,729
Molecular Function	8,038

Table 4.33: KEGG pathway statistics for *G. lanceifolia* flower CDS.

# Level 1	Level 2	Count
09100 Metabolism	09101 Carbohydrate metabolism	945
	09102 Energy metabolism	481
	09103 Lipid metabolism	407
	09104 Nucleotide metabolism	142
	09105 Amino acid metabolism	562
	09106 Metabolism of other amino acids	186
	09107 Glycan biosynthesis and metabolism	216
	09108 Metabolism of cofactors and vitamins	308
	09109 Metabolism of terpenoids and polyketides	125
	09110 Biosynthesis of other secondary metabolites	198
	09111 Xenobiotics biodegradation and metabolism	155
09120 Genetic Information Processing	09121 Transcription	329
	09122 Translation	703
	09123 Folding, sorting and degradation	582
	09124 Replication and repair	276
	09125 Information processing in viruses	36
09130 Environmental Information Processing	09131 Membrane transport	35
	09132 Signal transduction	1473

	09133 Signaling molecules and interaction	5
09140 Cellular Processes	09141 Transport and catabolism	717
	09142 Cell motility	112
	09143 Cell growth and death	665
	09144 Cellular community - eukaryotes	139

Table 4.34: SSR statistics for *G. lanceifolia* flower transcriptome.

Sample Name	Total CDS
Total number of sequences examined	53,072
Total size of examined sequences (bp)	38828112
Total number of identified SSRs	3,356
Number of SSR-containing sequences	2,852
Number of sequences containing >1 SSR	407
Number of SSRs present in compound formation	292

Table 4.35: SSR pattern distribution in *G. lanceifolia* transcriptome.

Sample Name	Total CDS
Dinucleotide	547
Trinucleotide	2,747
Tetranucleotide	32
Pentanucleotide	17
Hexanucleotide	13

Table 4.36: Differential gene expression statistics for *G. lanceifolia* male vs. hermaphrodite flowers.

DGE combinations	Total DGEs	Down regulated	Up regulated	Significant Down regulated	Significant Up regulated	Total Significant DGE
Male Flower vsHermaphrodite Flower	30,594	15,047	15,547	2,522	1,577	4,099

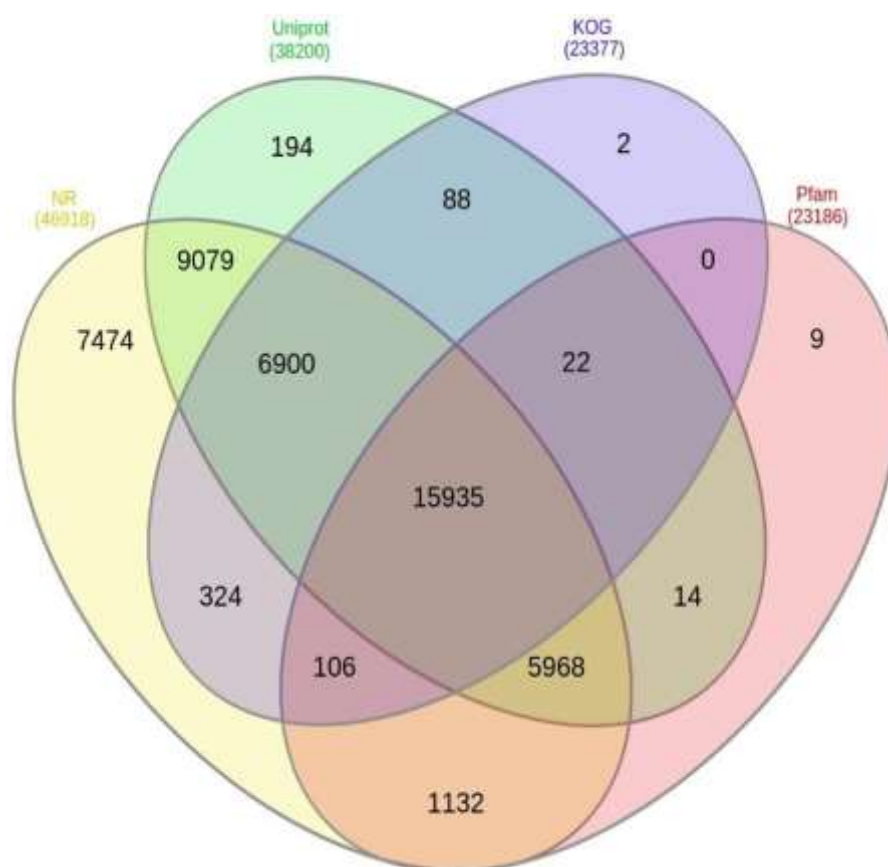


Figure 4.33: Venn diagram for annotated proteins in different database

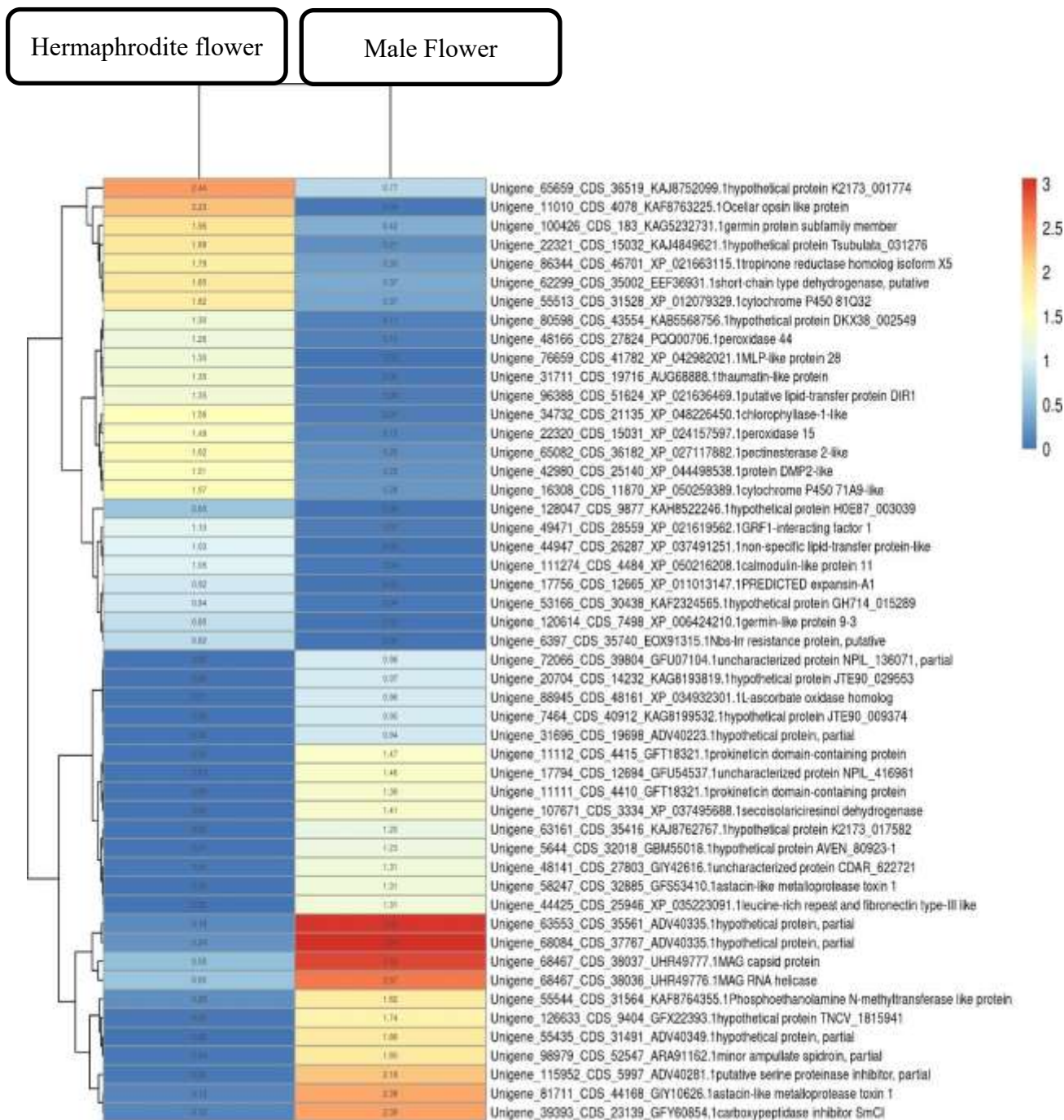


Figure 4.34: Heat map representing most significant genes expressed in both the samples was plotted using log10 of normalized read count values (CPM) for *G. lanceifolia* Male Flower vs *G. lanceifolia* Hermaphrodite Flower, where shades of blue represent down regulated genes and shades of red represent highly expressed genes

4.12. Germination and Seedling Growth Response of *G. lanceifolia*

4.12. 1. Germination Response to Chemical and Hormonal Pre-treatments

In the pretreatment comparison, the germination behavior of *G. lanceifolia*'s seeds clearly indicated physiological aspects is important for this endemic species' seed biology. The influence of the applied pretreatment was showed in in Table 4.37 (Figure 35). The seeds that underwent treatment with potassium nitrate (KNO_3) showed the highest germination percentage of 91.1%, which was also significantly higher than the control group's germination percentage (85.6%, $p < 0.01$). This superior performance of KNO_3 across key germination metrics is visually summarized in Figure 37, which illustrates the significantly higher germination percentage and energy compared to all other treatments. Furthermore, Figure 38 confirms that KNO_3 priming also resulted in the highest germination value and index, underscoring its role in enhancing both the speed and robustness of the germination process. This is consistent with other species' studies showing that KNO_3 priming resulted in an increase in the rate of germination and its uniformity as well. The probable reason for this is the metabolic activation and oxygen absorption to a higher degree during the soaking process (priming "wakes up internal metabolism") (Shim et al., 2008). It is also noted that KNO_3 contributed to the highest germination energy, value, and index, which is in line with the metabolic enhancement.

Gibberellic acid (GA_3) pretreatment was also found to be effective in increasing germination compared to the control treatment. At 300 ppm GA_3 , germination was 88.9% (higher than the control, $p < 0.5$), but there was no increase in germination at 500 ppm. This indicates the possibility of an optimal dose as reported in other crops (e.g. GA_3 priming significantly increases germination speed in cereals). In general, the application of exogenous GA_3 is associated with the breaking of dormancy and the obtaining of vigor, thus leading to better germination metrics (Mohammed and Baldwin, 2023).

On the other hand, the priming with thiourea and kinetin gave rise to germination rates (84.4% and 80.0%) which were almost the same as that of the

control (statistical significance $p > 0.5$). Thus, the conclusion was made that the treatments did not provide a substantial increase in germination. In addition, the earlier studies pointed out that kinetin priming did not have much impact on the final germination percentage of seeds (for example, in the case of aged eggplant seeds) (Demir et al., 1994). The finding indicated that, even though cytokinins like kinetin might jack up the metabolism, they did not raise overall germination significantly under our conditions.

The harsh treatments caused strong inhibition. The use of sulphuric acid scarification (H_2SO_4) led to a dramatic reduction in germination (11.1%, $p < 0.001$), indicating damage to both the seed coat and embryo. In fact, other species have shown that even very short contact with concentrated H_2SO_4 seriously destroyed seed viability (Stoian-Dod et al., 2023). Likewise, NAA (500 ppm) led to a reduction in germination (24.4%, $p < 0.001$), which was posited to be a result of hormonal imbalance: the high auxin levels increase the ABA effects and keep the seed dormant, hence germination is inhibited. In the end, KNO_3 priming and GA_3 (300 ppm) were found to be the best pretreatments, which supported the priming theory (metabolic activation) and were in accordance with the previous studies in related *Garcinia* species.

4.12.2. Early Seedling Growth Performance under Pre-treatments

Pre-treatment effects also influenced seedling growth at the early stage (Table 4.38). GA_3 applied seedlings gave rise to the tallest and most robust seedlings. The maximum shoot height (12.56 cm) and root length (9.62 cm) were measured with a 500 ppm GA_3 application, which was very close to the results obtained with 300 ppm GA_3 . This is somewhat predictable since it is known that GA_3 enhances both cell elongation and shoot elongation in other plants (Chaudhary et al., 2020). In addition to that, GA_3 seedlings had the highest biomass accumulation: total dry weight was 1.32 g (shoot dry 0.97 g) at 500 ppm, which is significantly higher than the control ($p < 0.001$). The results are consistent with other reports stating that application of gibberellin has positive influence on vegetative growth and dry mass (e.g., barley seedlings) (Mohammed and Baldwin, 2023). On the other hand, the root–shoot ratio

slightly decreased with the increase in GA₃, indicating that the preferential elongation of the shoot occurred.

The seedlings that got Potassium nitrate as a priming agent exhibited moderate vigor: the heights of the plants (9.91 cm) and the biomass were greater than those of the control but smaller than those of the treatments with GA₃. The collar diameter was almost the same for most treatments (1.52–1.68 mm, $p = 0.013$). Pre-treatments with thiourea and kinetin produced seedlings that were indistinguishable from the control in all aspects. In conclusion, GA₃ (both at 300 and 500 ppm) gave rise to the most vigorous seedlings, followed by KNO₃; the treatments with kinetin and thiourea had minimal impact on growth (mostly similar to untreated seedlings).

4.12.3. Influence of Potting Mixtures on Seedling Development

The use of different substrates for potting has a significant impact on the seedlings' growth (Table 4.39) (Figure 36). The mixed substrate of forest soil, sand, and vermicompost (P2, 3:2:1) gave the largest seedlings with all measurements taken. For P2, the average height of the plants was 32.22 cm and 4.32 mm for the collar diameter—these were both significantly higher than others ($p < 0.001$). The pronounced effect of the potting mixtures on key morphological traits is vividly captured in Figure 39, which demonstrates the superior seedling growth parameters achieved with the P2 substrate. P2 produced the highest total biomass (5.67 g) and the most number of leaves (35.09), thus giving the largest Dickson quality index (0.50) to this mixture. This is further corroborated by Figure 40, which clearly shows that the P2 mixture supported the highest total seedling biomass and the superior Dickson Quality Index, reflecting the production of well-balanced, high-quality seedlings. On the other hand, the mix P3 (forest soil: sand: FYM: vermicompost, 3:2:1:1) produced the weakest growth over all traits. Its high organic matter content likely led to poor aeration and nutrient imbalance, creating very unfavorable conditions for the root system to grow. P0 (pure forest soil) and P1 (soil: sand: FYM) were in between. These findings are in line with other studies reporting that moderate levels of vermicompost can enhance seedling growth, while too much compost can lower quality (Frata et al., 2025).

- 1) P2 (Soil: Sand: Vermicompost 3:2:1): The height, diameter, leaf number, and dry weight, all aspects of performance, were at their highest. The greatest biomass was obtained with the highest vertical growth (32.22 cm) seedlings, which is in line with results that substrates enriched with vermicompost improve the availability of nutrients and vigor (Tejada and Benítez, 2015).
- 2) P1 (Soil: Sand: FYM 3:2:1) and P0 (Soil alone): Moderate growth; better than P3 but below P2. Organic amendment (FYM) improved over soil alone, but not as much as vermicompost.
- 3) P3 (Soil: Sand: FYM:Vermicompost): Poorest growth. Its high organic fraction is probably the main reason for poor aeration and potential salt/nutrient stress. Previous studies indicate that although the addition of compost can enhance DQI up to a certain level, an excessive vermicomposting ratio (more than ~75:25) would decrease seedling quality (Erşahin et al., 2017). The outcome indicated that P2 was able to find the right compromise.

Consequently, seedlings flourished in the P2 medium, which is characterized by good aeration and is rich in nutrients. The results suggest that vermicompost is an important ingredient in the substrate for the growth and development of *Garcinia* seedlings (Frata et al. 2025; Tejada and Benítez, 2015). On the other hand, excessive organic amendment could lead to reduced Dickson Quality Index and lesser vigor among the plant. The seeds of *Garcinia* species are known to show recalcitrant seed characteristics, which means that they lose their viability quickly during storage. The seeds are very much prone to water deficiency and temperature extremes; this is the reason why the conditions required for long-term storage are difficult to establish. The critical moisture content of *G. gummi-gutta* seeds is around 34%, at less than which the viability decreases rapidly, and becomes nil at 24% and 17% moisture content at 25°C and 35°C respectively (Joshi and Phartyal, 2017). Larger *Garcinia* seeds maintain moisture content longer than smaller ones, resulting in higher germination percentages (Noor et al., 2016).

4.12.4. Statistical Significance and Post-hoc Analysis

One-way ANOVA showed that there were highly significant differences ($p < 0.001$) between all treatments for each parameter measured, thus supporting the view that both pretreatments and potting mixtures have a big impact on germination and seedling growth of *G. lanceifolia* (Table 4.40). The F-values which are very high for total dry weight (71.56), shoot weight (62.34), and number of leaves (78.91) point out that the sensitivity of these biomass-related parameters to practices. This support of the statistical robustness makes the practical significance of the differences observed stronger and thus, the experimental approach for finding the best propagation protocols for this species is validated.

The post hoc analysis of Tukey's HSD (Table 4.40) provided accurate grouping of treatments, where P2 was always the best (a group) in all parameters, and P0 and P1 followed next ('b' and 'c' groups), whereas P3 was always the worst performer (d group). This distinct statistical division is a very helpful and practical tool for nursery managers as it clearly indicates the vermicompost-enriched substrate (P2) as the best choice for seedling production. The statistical proof of P2's superiority over others in terms of multiple growth parameters further enhances the recommendation for its use in propagation protocols, being the standard one.

Table 4.37: Germination parameters of *G. lanceifolia* seeds under different treatments

Treatment	Germination (%)	Germination Energy (%)	Germination Value (GV)	Germination Index (GI)	Statistical Significance (p < 0.05)
Distilled Water (Control)	85.6 ± 2.0 ^b	15.6 ± 1.2 ^c	28.50 ± 1.5 ^c	2.20 ± 0.08 ^c	–
KNO ₃	91.1 ± 1.7 ^a	25.6 ± 1.8 ^a	45.20 ± 2.2 ^a	2.75 ± 0.10 ^a	Superior to control (p < 0.01)
Sulphuric acid	11.1 ± 1.2 ^d	2.2 ± 0.5 ^d	0.44 ± 0.05 ^d	0.22 ± 0.03 ^d	Strong inhibition (p < 0.001)
GA ₃ 300 ppm	88.9 ± 1.9 ^{ab}	22.2 ± 1.5 ^b	38.90 ± 1.8 ^b	2.65 ± 0.09 ^{ab}	Better than control (p < 0.05)
GA ₃ 500 ppm	86.7 ± 2.0 ^b	20.0 ± 1.4 ^{bc}	35.80 ± 1.7 ^{bc}	2.55 ± 0.08 ^b	No significant difference (p > 0.05)
NAA 500 ppm	24.4 ± 1.5 ^c	6.7 ± 0.8 ^d	1.61 ± 0.12 ^d	0.49 ± 0.04 ^d	Severe inhibition (p < 0.001)
Thiourea	84.4 ± 1.8 ^b	18.9 ± 1.3 ^c	32.10 ± 1.6 ^c	2.45 ± 0.07 ^{bc}	No significant difference (p > 0.05)
Kinetin 500 ppm	80.0 ± 1.6 ^c	16.7 ± 1.1 ^c	28.90 ± 1.4 ^c	2.30 ± 0.06 ^c	No significant difference (p > 0.05)

Table 4.38: Growth parameters across treatments

Parameter	Dist. Water	H ₂ O ₂	KNO ₃	Kinetin	GA ₃ 300 ppm	GA ₃ 500 ppm	Thiourea	p-value
Collar D (mm)	1.65 ± 0.07	1.61 ± 0.18	1.67 ± 0.09	1.59 ± 0.10	1.68 ± 0.12	1.64 ± 0.10	1.52 ± 0.14	0.013
Plant height (cm)	9.75 ± 0.71	9.59 ± 1.13	9.91 ± 0.74	9.67 ± 1.31	12.11 ± 1.51	12.56 ± 1.43	9.72 ± 1.10	<0.001
Root length (cm)	8.62 ± 0.61	8.19 ± 1.00	7.87 ± 0.52	7.66 ± 0.73	8.93 ± 1.21	9.62 ± 0.93	7.91 ± 1.12	<0.001
No. of leaves	5.38 ± 0.65	5.63 ± 0.49	5.38 ± 0.49	5.13 ± 1.13	6.50 ± 0.76	6.29 ± 0.87	5.29 ± 0.64	<0.001
Root weight (g)	0.32 ± 0.02	0.30 ± 0.03	0.30 ± 0.02	0.29 ± 0.02	0.33 ± 0.05	0.35 ± 0.04	0.28 ± 0.04	<0.001
Shoot weight (g)	0.80 ± 0.07	0.81 ± 0.07	0.80 ± 0.04	0.77 ± 0.13	0.97 ± 0.11	0.97 ± 0.09	0.79 ± 0.07	<0.001
Total dry wt (g)	1.12 ± 0.08	1.12 ± 0.09	1.10 ± 0.05	1.06 ± 0.14	1.30 ± 0.13	1.32 ± 0.11	1.07 ± 0.10	<0.001
Root-shoot ratio	0.42 ± 0.06	0.37 ± 0.05	0.37 ± 0.03	0.39 ± 0.07	0.35 ± 0.05	0.37 ± 0.05	0.36 ± 0.04	0.038
DQI	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.003

Table 4.39: Growth parameters of *G. lanceifolia* seedlings across potting mixtures

Parameter	P0	P1	P2	P3
Collar D (mm)	3.66 ± 0.28	3.50 ± 0.30	4.32 ± 0.25	2.72 ± 0.49
Plant Height (cm)	23.36 ± 2.31	25.38 ± 2.48	32.22 ± 2.27	19.06 ± 4.45
Root Length (cm)	26.49 ± 2.37	25.11 ± 5.32	26.68 ± 3.72	22.13 ± 2.45
No. of Leaves	20.18 ± 2.37	11.97 ± 2.58	35.09 ± 2.82	11.15 ± 5.42
Root Weight (g)	0.92 ± 0.09	0.64 ± 0.17	1.17 ± 0.09	0.34 ± 0.14
Shoot Weight (g)	2.07 ± 0.52	1.73 ± 0.38	4.50 ± 0.56	1.13 ± 0.45
Total Dry Weight (g)	2.99 ± 0.62	2.36 ± 0.54	5.67 ± 0.57	1.47 ± 0.63
Root–Shoot Ratio	0.47 ± 0.10	0.37 ± 0.03	0.27 ± 0.04	0.30 ± 0.03
DQI	0.34 ± 0.04	0.24 ± 0.07	0.50 ± 0.05	0.14 ± 0.06

P0 = Forest soil (control); P1 = Forest soil : Sand : FYM (3:2:1); P2 = Forest soil : Sand : Vermicompost (3:2:1); P3 = Forest soil : Sand : FYM : Vermicompost (3:2:1:1).

Table 4.40: One-way ANOVA and Tukey's HSD for seedling growth parameters

Parameter	F-value	p-value	Tukey's HSD
Collar Diameter (mm)	22.45	< 0.001	P2 ^a > P0 ^b , P1 ^b > P3 ^c
Plant Height (cm)	35.67	< 0.001	P2 ^a > P1 ^b , P0 ^b > P3 ^c
Root Length (cm)	3.12	0.042	P2 ^a , P0 ^a > P3 ^b ; P1 ^{ab}
No. of Leaves	78.91	< 0.001	P2 ^a > P0 ^b > P1 ^c , P3 ^c
Root Weight (g)	45.23	< 0.001	P2 ^a > P0 ^b > P1 ^c > P3 ^d
Shoot Weight (g)	62.34	< 0.001	P2 ^a > P0 ^b > P1 ^c > P3 ^d
Total Dry Weight (g)	71.56	< 0.001	P2 ^a > P0 ^b > P1 ^c > P3 ^d
Root-Shoot Ratio	12.89	< 0.001	P0 ^a > P1 ^b , P3 ^b > P2 ^c
Dickson Quality Index	48.76	< 0.001	P2 ^a > P0 ^b > P1 ^c > P3 ^d



Figure 4.35: Germination experiment of seeds to pre-treatment with various chemicals and plant growth regulators in a mist chamber environment.



Figure 4.36: Potting media (different proportion) experiment on plant growth and yield under mist chamber environment.

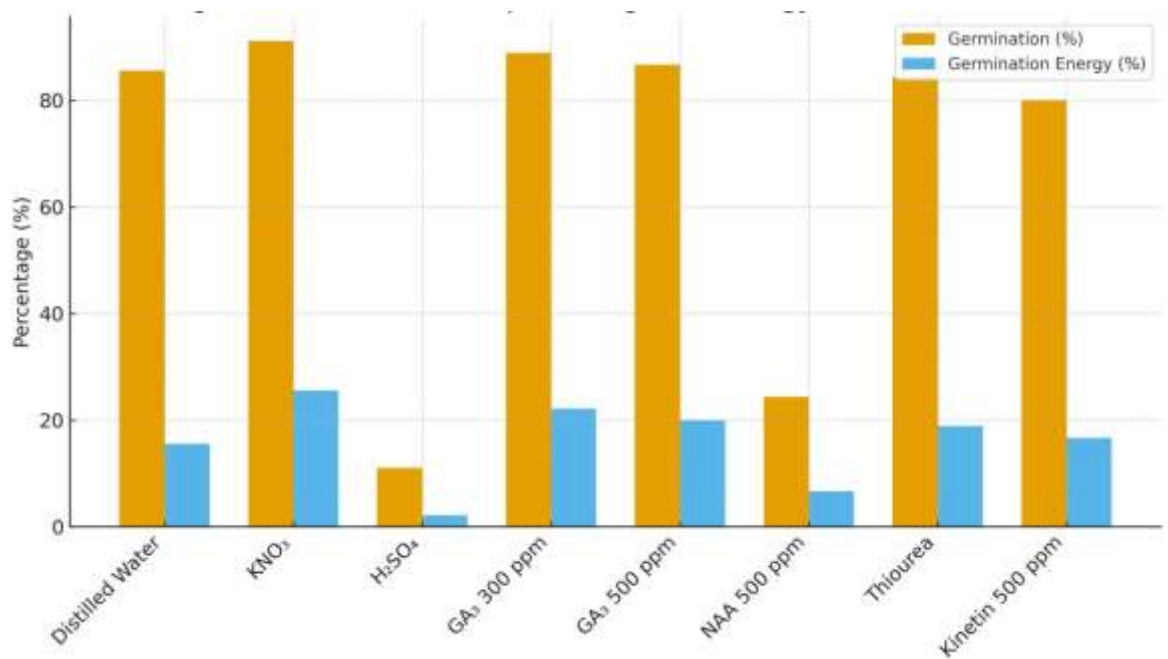


Figure 4.37: Germination percentage and energy across treatments

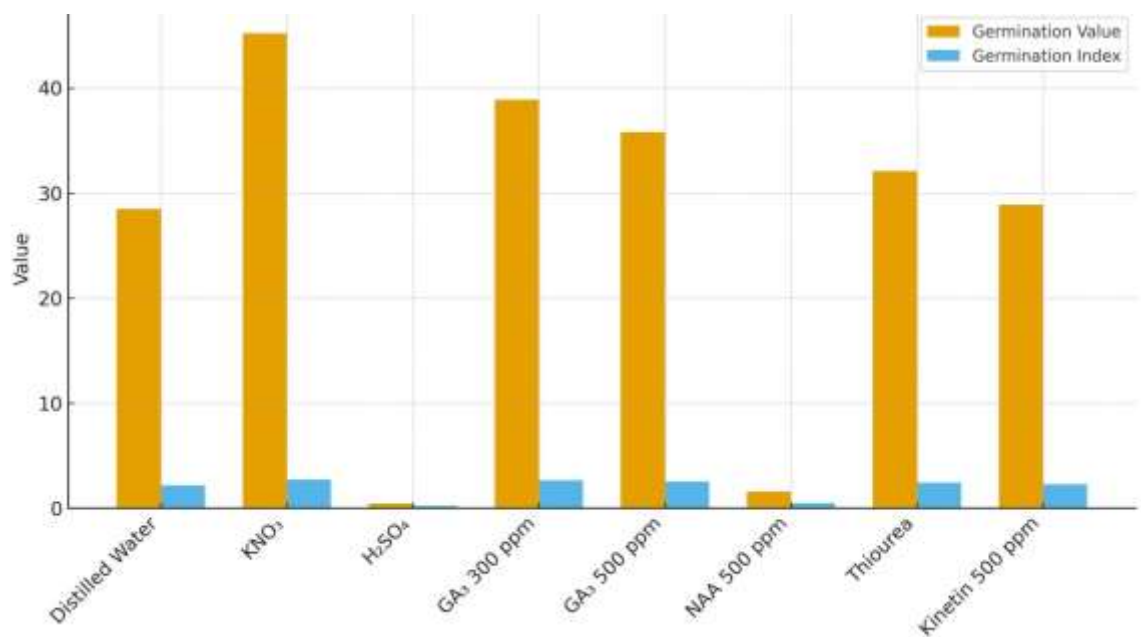


Figure 4.38: Germination value and index under different treatments

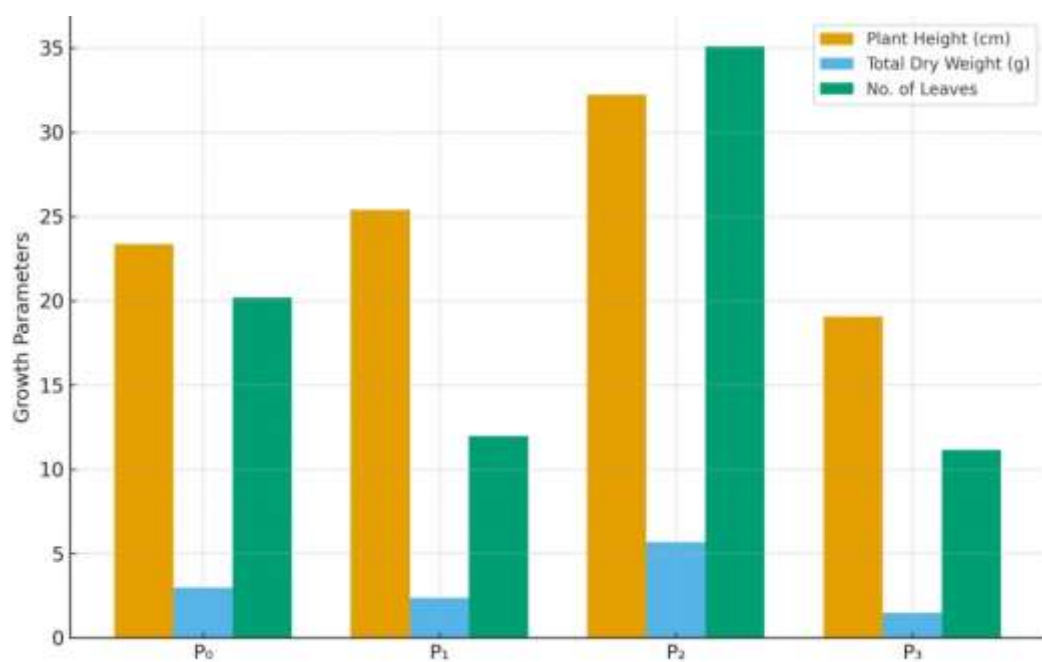


Figure 4.39: Effect of different potting mixtures on seedling growth parameters

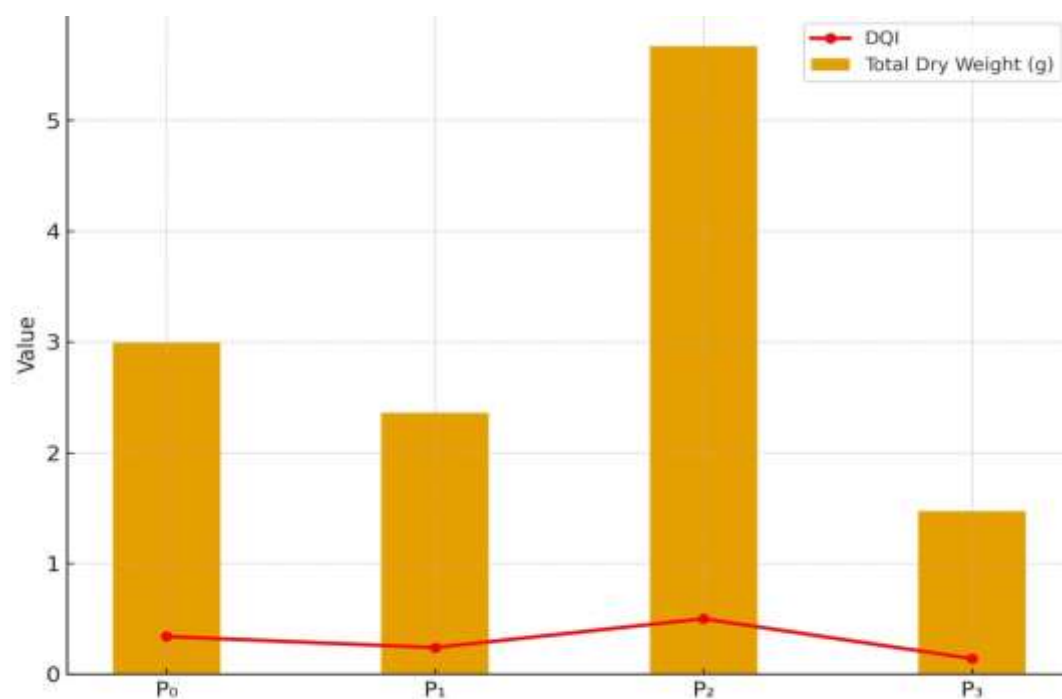


Figure 4.40: Total biomass and Dickson Quality Index (DQI) across potting mixtures

4.13 Spectro-chemical characterization of seed oil of *G. lanceifolia* Roxb

4.13.1 Physico-chemical properties of *G. lanceifolia* seed oil extract.

The seed count per fruit of *G. lanceifolia* ranged from 0 to 8, with an average of 3.85 ± 1.8 seeds per fruit. The mean dry weight of individual seeds was 0.347 ± 0.167 g, with a mean seed length of 12.78 ± 3.659 mm and breadth of 8.023 ± 1.69 mm. The seed oil extract exhibits a solid state at room temperature, liquefying at elevated temperatures (Figure 41). The oil yield from *G. lanceifolia* seeds was recorded at $43.36 \pm 2.035\%$ (w/w), which is noticeably higher than the yields from other species like *G. gummi-gutta* (33.53%) or *G. kola* (34.00%) (Rani et al., 2022; Ogemdi et al., 2006). This indicates the importance that *G. lanceifolia* has the potential to become a significant oilseed species, in comparison to other *Garcinia* species.

Table 4.41 presents several physicochemical properties of the *G. lanceifolia* seed oil extract including the acid value. The acid value of *G. lanceifolia* (4.43 ± 0.33 mg KOH/g) is higher than the one in peanut oil (2.7 mg KOH/g) (Ajayi et al., 2007) and sunflower oil (3.09 mg KOH/g) but lower than olive oil (6.6 mg KOH/g). This acid value comparing is close to that of some other species of *Garcinia* like *G. cambogia* (5.04 mg KOH/g) (Choppa et al., 2015), *G. gummi-gutta* (3.7 mg KOH/g), *G. mangostana* (4.5 mg KOH/g), *G. xanthochymus* (4.8 mg KOH/g), *G. indica* (4.9 mg KOH/g) (Parthasarathy et al., 2014). This species was also found to have an equivalent acid value to that of *G. cambogia* (5.04 mg KOH/g). The presented acid value is in the range of the other species of *Garcinia* such as *G. cambogia*. A moderate acid value like this indicates good oil stability, which makes the *G. lanceifolia* seed oil still a preferred choice despite the need for refining by reducing more free fatty acids for improving shelf life and reducing rancidity (Payamalle et al., 2016).

The peroxide value (2 ± 0.349 meq/kg) of *G. lanceifolia* seed oil is lower than the cold-pressed mustard (3.66 meq/kg), sunflower (4.19 meq/kg), olive (6.39 meq/kg), peanut (8.39 meq/kg), and rapeseed oils (9.46 meq/kg) (Konuskan et al., 2019). Having

a lower peroxide value is a great advantage because it suggests the oil has better oxidative stability, and its shelf life is accordingly longer, thus, it becomes a feasible option for long-term storage and industrial applications where stability is of paramount importance (Moigradean et al., 2012).

The iodine value of *G. lanceifolia* seed oil, being the measure of unsaturation, is 113 ± 1.29 g/100 g oil. Such a figure puts it among the sunflower oil range (27-135 g/100 g) (Muttagi and Joshi, 2020), close to soybean oil (109 g/100 g), and higher than olive oil (94 g/100 g) (Choppa et al., 2015). When it comes to iodine value, *G. lanceifolia* is one of the *Garcinia* species with the highest value, for instance the oil of *G. morella* has the value of 39.8 g/100 g (Payamalle et al., 2016), also *G. xanthochymus* (37.4 g/100 g), *G. indica* (39.4 g/100 g), *G. gummi-gutta* (50.2 g/100 g), and *G. mangostana* (51.8 g/100 g), while it has lower iodine value than *G. cambogia* (131 g/100 g) (Parthasarathy et al., 2014). The increased iodine value that characterizes the oil indicates that the oil is very unsaturated and it thus would be more suitable for applications requiring oils with a higher degree of unsaturation, as the ones found in the food and cosmetics products (Dyminska et al., 2017)

The saponification value of *G. lanceifolia* seed oil (189.3 ± 2.8 mg KOH/g) is higher than that of *G. cambogia* (145.36 mg KOH/g) and *G. mangostana* (140.5 mg KOH/g), but lower than that of *G. indica* (200.2 mg KOH/g) and about the same as *G. gummi-gutta* (187.9 mg KOH/g) and *G. xanthochymus* (190.3 mg KOH/g) (Choppa et al., 2015; Parthasarathy et al., 2014). The saponification value being higher of the two shows that the substance in the oil consists of a higher percentage of lower molecular weight triglycerides. This makes it good for soap and cosmetic industrial uses (Hassan and Wawata, 2019).

The specific gravity, which is 0.879, and the viscosity, which is 29.18, of the seed oil extracted with hexane, one can say that *G. lanceifolia* oil has a low viscosity and is not as occlusive as hydrocarbon-based oils. Given this first characteristic plus the moderate specific gravity, the *G. lanceifolia* oil can be compared to oleic acid. Such oil

would not only be fit for applying as a general cosmetic compound, but also for use in a new segment of industrial applications (Noureddini et al. 1992).

4.13. 2 Fatty acid Methyl ester (FAME) analysis of *G. lanceifolia* seed oil

The analysis was done to find out the oil's fatty form occurring naturally as in the seed oil extract of *G. lanceifolia*. The detected compounds by GC-MS and NIST library are given in the form of a table in 4.42 and of a figure 4.42, with their chemical structure in Figure 4.47. These four different fatty acids detected are either saturated (Stearic acid, Palmitic acid) or having two double bonds (Linoleic acid) and only one double bond (Oleic acid). As it can be seen, Stearic acid, Palmitic acid, Oleic acid and Linoleic acid and other than the cited *G. lanceifolia* species, the bean-oil of *G. gummi-gutta*, *G. indica*, *G. xanthochymus*, *G. mangostana* and *G. morella* is a good source of the same fatty acids (Parthasarathy et al., 2014; Payamalle et al., 2016). The benefits for human health and the use of these constituents in industry are listed in the form of Table 4.43. The major compound among the four is found to be a saturated one, methyl stearate, which adds up to 56.93 % of the total contribution, followed by 29.91% of oleate, 10.75 % of Linoleate and finally 2.41 % of palmitate. According to the report, it is determined that the agrochemicals methyl stearate and methyl palmitate are eco-friendly in the aspect of banana plants by reducing the damage of root-knot nematodes (Lu et al., 2020). Vegetable and seed oils are some of the sources of fatty acid methyl esters (FAMEs) that are beneficial with antioxidant and antifungal properties (Pinto et al 2017). The *G. lanceifolia* seed oil is highly fatty, and although methyl stearate, the largest component, is beneficial and also can affect high blood pressure through the heart condition, a situation where drugs are required, and also reduce the risk of cancer (Rani et al., 2021).

4.13.3 UV-Visible spectrum analysis

The UV-visible spectrum of *G. lanceifolia* seed oil extracts showed a blue/hypsochromic shift. The UV-visible scan of seed oil showed the following: 228 nm (11.2635 abs), 258 nm (10.5748 abs), 274 nm (1.076 abs), 292 nm (1.0474 abs), 344 nm (0.8543 abs), and 668 nm (0.8662 abs). The values of absorbance decreased as the wavelength increased (Figure 4.43). The main peaks were located in the UV-C region (100-280 nm), and the sub-peaks were in UV-B (280-315 nm), UV-A (315-400), and the visible region (400 to 700). Soybean oil, olive oil, and *G. cambogia* share the absorption spectra of maximum absorbance at 228 and 258 nm due to the existence of high diene and triene conjugation systems (Oomah et al., 2006; Choppa et al., 2015). The UV-visible range between 200 and 300 nm is often used for characterizing the quality of the oil. The maximum absorbance of the seed oil in the UV-C region is significantly lower as compared to soybean oil, olive oil, and *G. Cambogia* (Choppa et al., 2015). The minor absorption peaks in the UV-A and UV-B regions are of upmost value to humans as they are lenses fatal UV-B rays unlike in the UV-B regions (Choppa et al., 2015). The UV-B support of the oil can be highly important in the skincare industry especially in the development of sunscreen creams with sun protection factor (SPF) 50 and above (Nehdi, 2011).

4.13.4 FTIR spectrum analysis

Ten peaks could be clearly identified in the IR spectrum of seed oil. The peaks recorded were 2916.37, 2846.93, 1735.93, 1465.90, 1249.87, 1219.01, 1172.72, 1111.0, 771.53, and 717.52 cm^{-1} , which can be associated with different functional groups (Table 4.44 and Figure 4.44). The features at 2916.37 and 2846.93 cm^{-1} stand for the presence of sp^3 hybridized carbons in saturated aliphatic methylene CH asymmetric and symmetric stretch, respectively. The stretching vibration at 1735 cm^{-1} showed that a carbonyl compound exists with the six-member ring lactone/carbonyl ester. The peak at 1465 cm^{-1} clearly indicated the saturated aliphatic methylene $>\text{CH}_2$ bend. The peak at 1219 cm^{-1} indicates the presence of C-O stretch (Coates, 2000). A comparison was

made between the spectrum of extracted seed oil and the spectrum of *G. cambogia*, and seed oil of corn, olive and sunflower. The same source of similarity among the spectrum was resolved to be the *G. lanceifolia* seed oil (Chippa et al., 2015).

4.13.4 ¹³C Nuclear Magnetic Resonance (NMR) Analysis

The peak at 14.088 ppm corresponds to the last carbon of the methyl groups; the peak between δ 22.557 and δ 34.173 ppm is a signal of the presence of methylene carbons in the fatty acids' long carbon chains. The peak at 62.070 ppm corresponds to the triglyceride moiety's C-1 and C-3 terminal carbons. The peak at 68.867 ppm corresponds, and is the most obvious signal for, the C-2 carbons in the triglyceride moiety, the peak at δ 127.866 and the peak at δ 129.654 are most likely indicative of hydroxyl fatty acids or the unsaturation of the compound (Tariq et al., 2011; Choppa et al., 2015). (Table 4.45 and Figure 4.45)

4.13.5 ¹H NMR analysis: ¹H NMR (300 MHz, CDCl₃) (δ , ppm)

The terminal CH₃ was differentiated from the signatures of the main non-polar hydrocarbon chain of lipids, the δ 0.857 ppm peak being the terminal methyl, and the δ 1.253 and δ 1.302 ppm signals being the -CH₂ of long acyl chains of fatty acids. The doublet δ 1.607 & 1.630 ppm signal may indicate the presence of the beta-methyl group, the δ 2.000 to 2.059 signals may represent the α methylene group to one double bond of unsaturated acyl chains, the peak at δ 2.205 – 2.373 may be indices of α -methylene group to acyl chains of fatty acid, and the crowning singlet was seen at δ 2.771 was the methylene group multiply bound to respective linoleyl chains. The methylenes on C2 and C3 of the glycerol backbone were also identified by a quartet at δ 4.114 and 4.323 ppm. A δ 5.252 – 5.394 ppm couple, benzyl protons at C2 of the -O-CO-R- moiety of triacylglycerides and diene/cis-unsaturated FAs, was also identified (Table 4.46 and Figure 4.46) (Nehdi, 2011; Choppa et al., 2015)

Seed oil extracted from *G. lanceifolia* has been found to hold a promising potential for utilization in various sectors, including the food, cosmetics, and

pharmaceutical industries due to the composition of stearic, oleic, linoleic, and palmitic acids. The dominance of the esters of saturated fatty acids led by methyl stearate (56.93%) and methyl oleate (29.91%) as well as a low peroxide value (2 ± 0.349 meq/kg) shows that the oil has got good oxidative stability and an extended shelf life, thus being suited for storing over longer periods of time or to be employed for industrial purposes. Also, the detected iodine value of 113 ± 1.29 g/100 g oil placed it at a moderately unsaturated level, this plus the density of the oil further indicates its possible application in cases demanding purity of the oils such as in the cosmetic and food industries. The characterization and identification of the long-chain fatty acids were done by matching them with the NIST library and they were also strongly backed up by the UV-visible spectrum, FTIR spectrum, ^1H NMR to their respective rigorous and ^{13}C NMR characteristic peaks.

Table 4.41: Specific properties of the extracted seed oil sample of *G. lanceifolia*

Parameter	Value
Acid value (mg KOH/g)	4.43 ± 0.33
Saponification value (mg KOH/g)	189.3 ± 2.80
Peroxide Value (meq/kg)	2.0 ± 0.349
Iodine value (g/100 g oil)	113.32 ± 1.29
Specific gravity	0.879
Viscosity	29.18
Seed oil content (%)	$43.36 \pm 2.035 \%$

Table 4.42: Detected Fatty acid methyl esters profile of *G. lanceifolia* seed oil by the GCMS technique

Sl. No.	Compound Name	Class of compound	RT	Scan	MW	Molecular formula	Annotation Source	Area %
1	Methyl Palmitate	Saturated fatty acid	23.882	4175	270.5	C ₁₇ H ₃₄ O ₂	NIST library	2.41
2	Methyl linoleate	Triunsaturated fatty acid	24.687	4336	294.5	C ₁₉ H ₃₄ O ₂	NIST library	10.75
3	Methyl oleate	Monounsaturated	24.722	4343	296.5	C ₁₉ H ₃₆ O ₂	NIST library	29.91
4	Methyl stearate	Saturated fatty acid	24.852	4369	298.5	C ₁₉ H ₃₈ O ₂	NIST library	56.93

Table 4.43: Biological and commercial importance of the detected fatty acids, their derivatives and other compounds in *G. lanceifolia* seed oil

Sl. No	Compounds	C-Number	Biological and commercial importance	Reference
1.	Stearic acid	18:0	It is mainly found in animal and plant fats (Shea and Cocoa butter). Highly abundant in the Western diet. Potential component for soy-lecithin green plastics.	van Rooijen and Mensink (2020)
2.	Oleic acid	18:1	Most common monounsaturated fatty acids found in animal and vegetable fats and oil. Major constituents of Olive oil attributed to anti-inflammatory effect in autoimmune disease, anti-breast cancer, immune system improvement, reduction in low-density lipoprotein, and atherogenesis risk. Major component of soap as emulsifying and moisturizer agent.	Sales-Campos et al. (2013)
3.	Linoleic acid	18:2	It is an essential polyunsaturated omega-6 fatty acid mainly found in plant oils. Used in cosmetic products.	Ando et al. (1998)
4.	Palmitic acid	16:1	Most common fatty acid found in human body with 20-30% total fatty acids in membrane phospholipids. Major constituents of Palm oil, dairy products and meat.	Carta et al. (2017)

Table 4.44: Characteristic peaks in FTIR spectrum of extracted seed oil sample of *G. lanceifolia*

Sl. No.	Wave Number (cm ⁻¹) (Test Sample)	Wave Number (cm ⁻¹) (Reference article)	Functional group/ assignment
1.	2916.37	2935-2915	Saturated aliphatic Methylene C-H (>CH ₂) asymmetric stretch
2.	2846.93	2865-2845	Saturated aliphatic Methylene C-H (>CH ₂) symmetric stretch
3.	1735.93	1735	Six-membered ring lactone/Carbonyl ester
4.	1465.90	1485-1445	Saturated aliphatic Methylene C-H (>CH ₂) bend
5.	1249.87	1270-1230	Aromatic ethers, aryl-O-stretch
6.	1219.01	1210-1320	C-O stretch
7.	1172.72	1190-1130	Secondary amine, CN stretch
8.	1111.0	1150-1050	Alkyl-substituted ether, C-O stretch
9.	771.53	770-735	Aromatic ring C-H 1,2-distribution(ortho)
10.	717.52	720-590	Alcohol, -OH out of plane bend

[Peak assigned as per (Coates, 2000)]

Table 4.45: Functional groups of ¹³C NMR

Sl.No.	Peak(s)	Functional group/ assignment
1.	14.088	Terminal carbon of methyl groups
2.	22-34	Methylene carbons of long carbon chain in fatty acids
3.	62.070	The C-1 and C-3 terminal carbons of the triglyceride moiety
4.	68.867	C-2 carbons of triglyceride moiety/methoxy group (O-Me) attached to the ester keto group
5.	127-130	Aromatic regions signal indicates for hydroxyl fatty acids/may correspond to the unsaturation in the compound
6.	172.825 and 173.273	Esters group in fatty acids
7.	179.131	Carbonyl group in fatty acids

Table 4.46: Functional groups of ^1H NMR

Sl. No.	Peak(s)	Functional group/ assignment
1.	0.857- 0.900	Terminal methyl group
2.	1.253 & 1.302	Backbone $-\text{CH}_2$ of long chain fatty acids
3.	1.607 & 1.630	beta-methylene proton
4.	2.000 -2.059	alpha-methylene group to one double bond
5.	2.311	alpha-methylene group to ester
6.	2.342 & 2.347	alpha-methylene group to acid
7.	2.771	alpha-methylene group to two double bond
8.	4.114-4.323	Methylene group (C1 and C3) of glyceride
9.	5.252	Methine proton at C2 of glyceride
10.	5.3-03-5.394	Olefinic protons



Figure 4.41: *G. lanceifolia* seeds, extracted seed oil at ambient temperature and heated seed oil

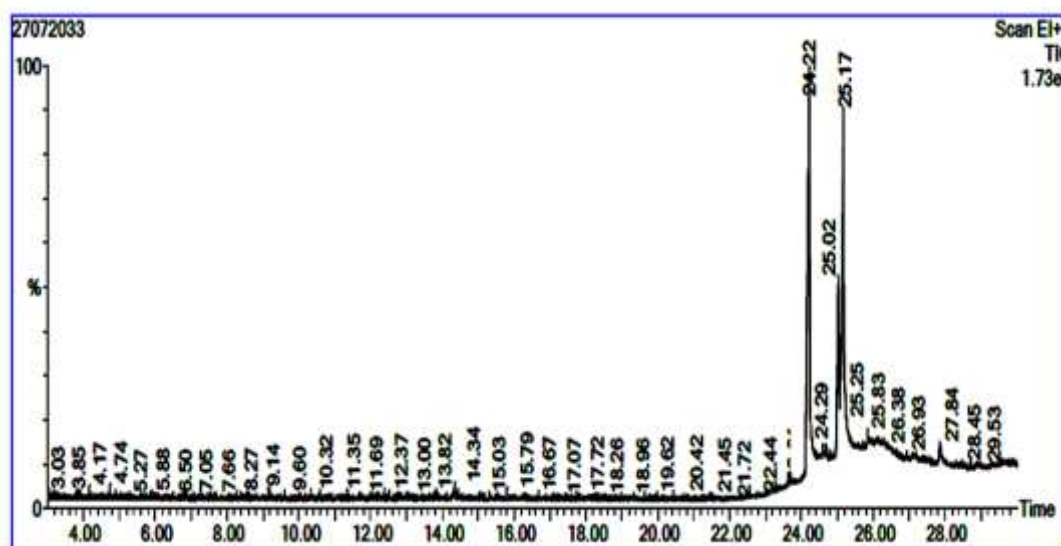


Figure 4.42: FAME-GCMS chromatogram of *G. lanceifolia* seed oil

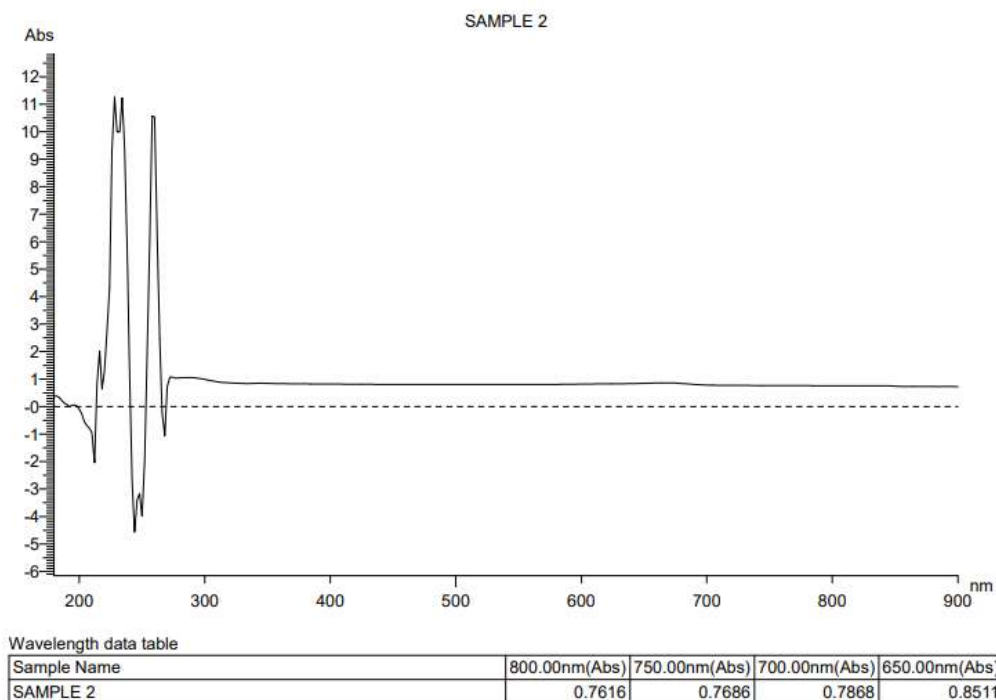


Figure 4.43: UV-Visible spectrum of the extracted seed oil dissolved in DMSO of *G. lanceifolia*

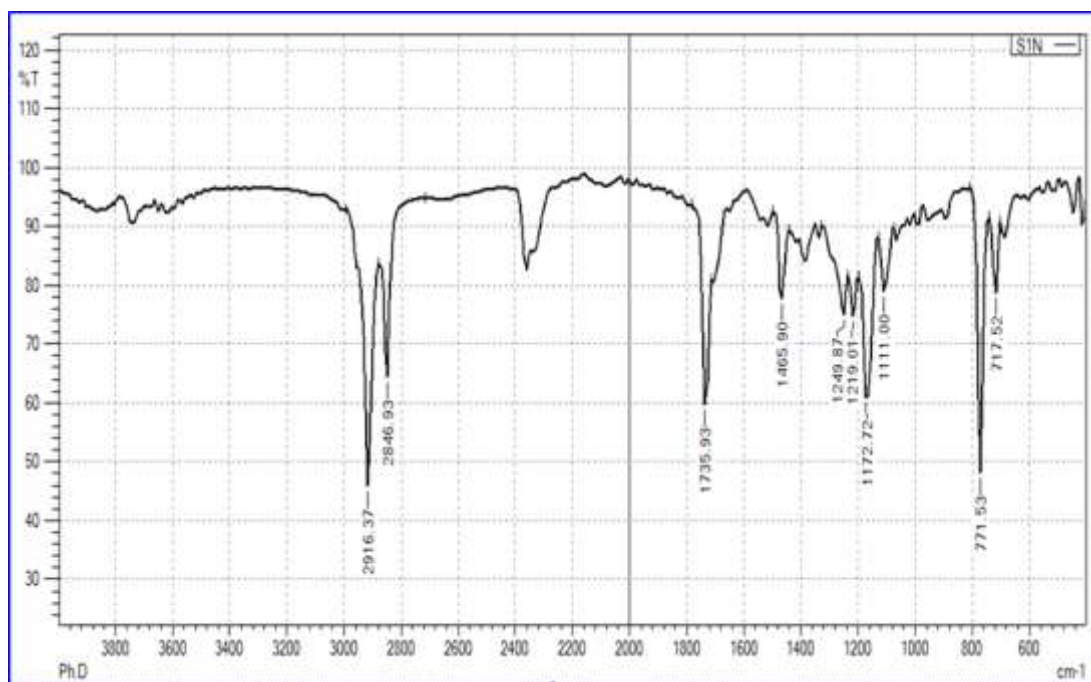


Figure 4.44: FTIR spectrum of the extracted seed oil of *G. lanceifolia*

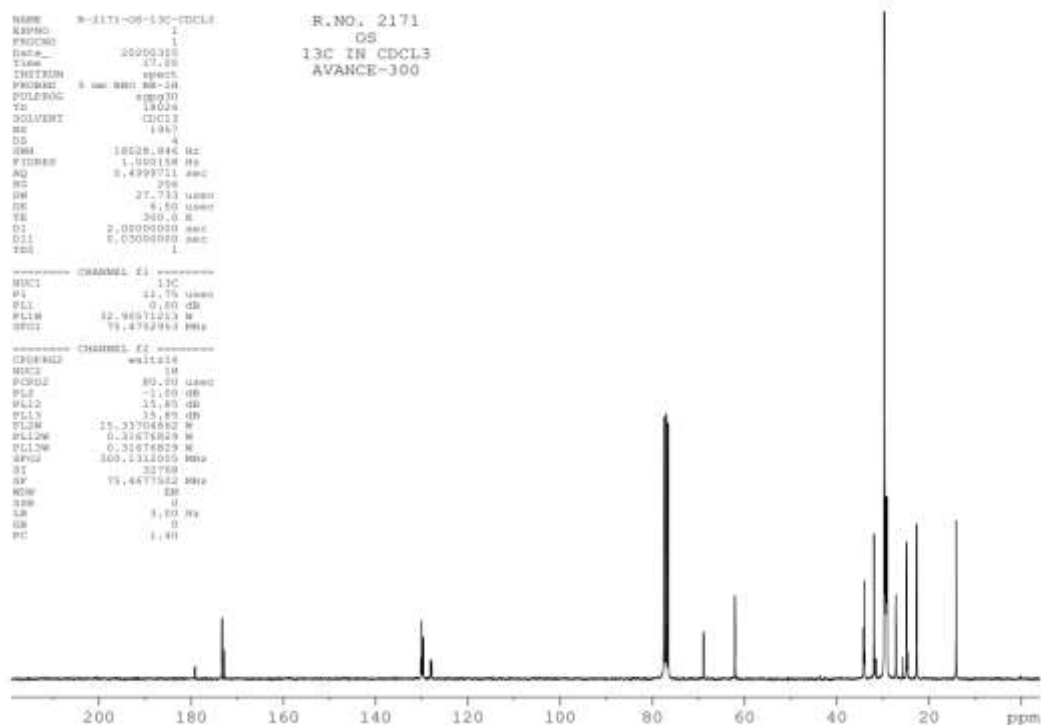


Figure 4.45: ^{13}C NMR spectrum of *G. lanceifolia* seed oil dissolved in CDCl_3

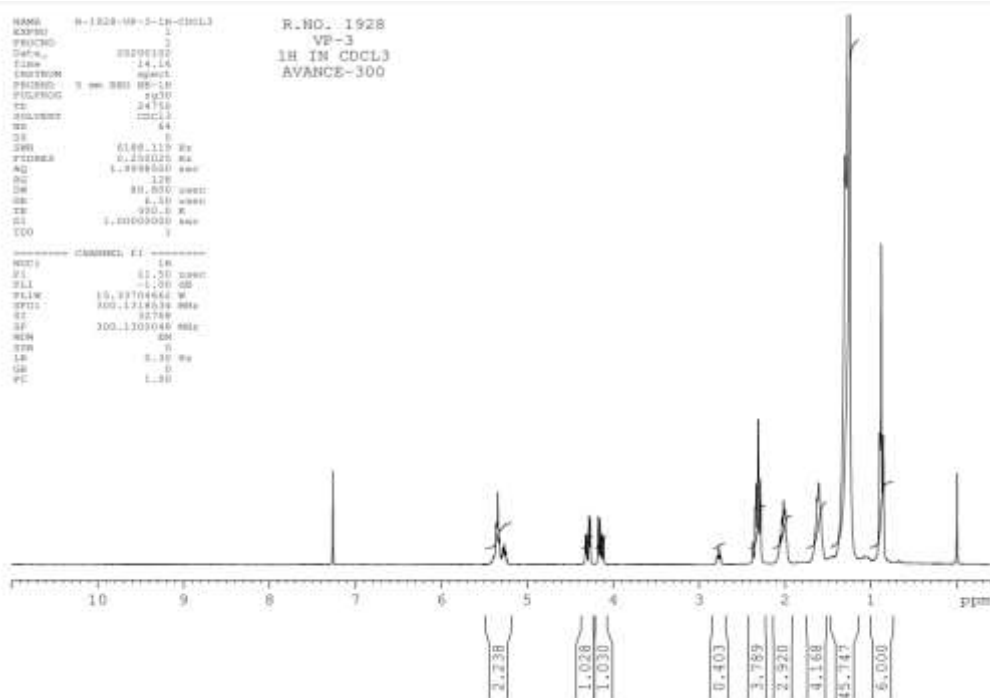


Figure 4.46: ^1H NMR spectrum of *G. lanceifolia* seed oil dissolved in CDCl_3

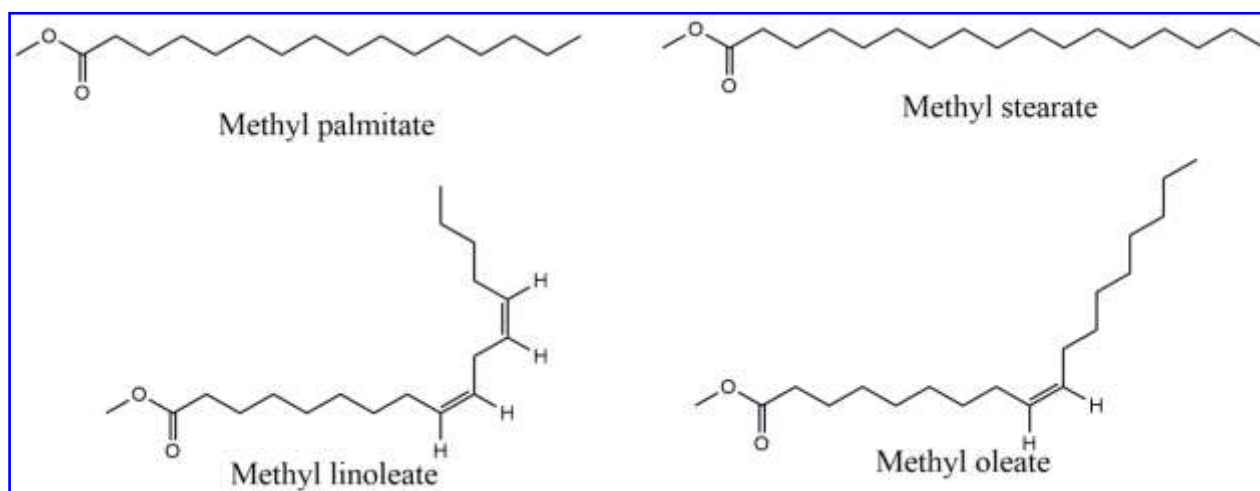
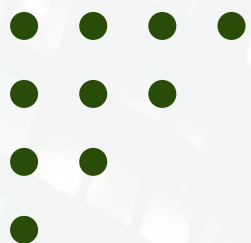


Figure 4.47: Structures of the fatty acid methyl esters identified by GC-MS with reference to NIST library



CHAPTER - V

SUMMARY AND CONCLUSION



CHAPTER 5

SUMMARY AND CONCLUSION

5.1 Overview

The research conducted on "Reproductive and Seed Biology of *Garcinia lanceifolia* Roxb. in Mizoram" aimed to uncover morphological variations, flower development, mating systems, and the whole gamut of biological factors, including germination rates of this rare eastern Indian *Garcinia* species. The researchers intended to adopt an integrated approach to revealing its natural variation, reproductive ecology, and propagation, which are the areas of utmost importance for its conservation and sustainable use in the future. The study involved systematic field surveys, morphometric analyses, controlled pollination experiments, and molecular studies, primarily in Thingdawl (Kolasib District) and Thenzawl (Serchhip District). The investigation showed that *G. lanceifolia* exhibits high variability in vegetative and flowering characteristics, as well as reproductive stages, both among and within populations, thus indicating strong ecological adaptability. This was the first verification of *G. lanceifolia* as an androdioecious sexual system, with differential pollen biology and transcriptomic profiles between male and hermaphrodite flowers. The species further produces high-quality seed oil with potential biochemical properties and promising ethnobotanical and economic prospects.

5.2 Summary of Major Findings

5.2.1 Morphological and Phenotypical Variation

Morphological assessment affirmed the taxonomical identification of the species being examined as *Garcinia lanceifolia* Roxb. The small tree, based on its characteristics, showed opposite, decussate branching, and its leaves were shiny and lanceolate. The flowering and vegetative traits matched the descriptions in the literature; therefore, the plants were recognized with very high certainty. The analysis of phenotypic coefficient of variation showed moderate to high variability for the major architectural characters, especially: tree girth, height, and branching. Analysis of phenotypic coefficient of variation reveal moderate to high variability in

important architectural characters, particularly tree girth, height, and branching. The Thenzawl population thus showed greater plasticity than that from Thingdawl, indicating greater heterogeneity at the microclimatic level and greater adaptability. Sexual dimorphism was apparent in leaf morphometry, with male trees producing heavier and broader leaves. In contrast, both petiole and leaf blade length were greater for hermaphrodites, suggesting a differential allocation of energy associated with reproductive roles.

5.2.2 Floral Morphometry and Sexual Dimorphism

High interpopulation and intersexual variation in floral characters was noticed. Hermaphrodite flowers were significantly larger, heavier, and had longer stalks than males, whereas males bore almost twice the number of anthers. This feature showed functional specialization: male flowers maximize pollen output, while hermaphrodites invest more in structures that ensure successful pollination and fruit set. For morphs, weight, diameter, and length (all parameters considered) showed strong allometry ($r > 0.8$), indicating very tight regulation of ontogenetic scaling. Regression models confirmed that floral size traits are strong predictors of reproductive investment. Such morphological dimorphism coincides with theoretical models of resource partitioning in androdioecious species, in which males maximize pollen dispersal efficiency while hermaphrodites balance both male and female functions.

5.2.3 Fruit Morphology

Characteristics of fruits collected from nature and the market give high morphological diversity. Thingdawl and Thenzawl fruits collected from the natural sites were comparable in weight and size but differed largely as to rind proportion, the rind being thicker in Thenzawl fruits (67%) as compared to the rinds of Thingdawl fruits (55%). There was considerable diversity in size, rind thickness, and moisture content among the fruits gathered from the markets, suggesting environmental and genetic factors as the reasons. Bukpui fruits were the biggest (85.9 g; 59 mm diameter), while Zanlawn fruits were the smallest. The first two components of PCA accounted for 84.8% of the total variation, with PC1

representing overall fruit size and PC2 representing trade-offs between rind thickness and seed count. These variations may reflect phenotypic plasticity in *G. lanceifolia*, enabling local adaptation to ecological conditions.

5.2.4 Flowering Phenology

During phenological observation, it was noted that the reproductive cycle was long and sequential, lasting 125 to 150 days, with flowering starting late in October and peak flowering between December and January. Fruits mature in March–April in the time preceding pre-monsoon rain, which is possibly an adaptive measure in facilitating seed dispersal and germination during the monsoon. Long flowering duration (120–145 days per population) with partial synchrony between sites is an adaptive trait that ensures reproductive assurance under variable ecological and climatic conditions.

5.2.5: Floral and Fruit Productivity

Tree size (girth and height) was found to be a strong predictor of floral production. An increase of 1 cm in girth corresponded to an average increase of ~59 flowers per tree, with high model predictability ($R^2 > 0.7$). The flower production of the Thenzawl populations was lower than that of Thingdawl, yet they had higher fruit-to-flower ratios. Moreover, reproductive efficiency was consistent over the years. Consequently, large, strong trees are a far better source of gene pools for collection and propagation and thus are to be given priority.

5.2.6 Pollen Biology and Reproductive Efficiency

In general, male flowers produced significantly higher pollen counts (~94,800 grains/flower) than hermaphrodites (18,900–42,400 grains/flower), affirming their primary role as pollen producers. However, pollen from hermaphrodites showed a visibly higher viability (~79%) than that of males (~32%), suggesting a balance between quantity and quality of pollen within populations.

5.2.7 Mating System and Androdioecy

Pollination experiments under controlled conditions indicated that the mating system was facultative mixed, allowing for xenogamy, geitonogamy, and apomixis. Xenogamy resulted in higher fruit set in Thenzawl (27.3%) than in Thingdawl (16.7%), suggesting pollen transfer was more effective in the former and that genetic diversity was higher there. Also, apomixis was more prevalent in Thingdawl (27%) than in Thenzawl (12%), providing reproductive assurance under pollen limitation. The successful male•female pollination (15%) indicated that males produce pollen functional for fertilizing hermaphrodite flowers and confirmed the androdioecious reproductive system.

5.2.8 Transcriptomic Insights into Sex Differentiation

The de novo transcriptome assembly of floral material yielded >154,000 transcripts (N50 length = 1,071 bp), 88% of which were functionally annotated. The male and hermaphrodite flower expression profiling revealed a total of 4,099 DEGs, among which important regulators of floral identity and sex differentiation were also included, such as MADS-box and YABBY genes and hormone signaling pathways. The profiles reveal the molecular pathways underlying sexual dimorphism, thereby confirming that the genes preferentially expressed in males are associated with pollen formation, whereas those preferentially expressed in hermaphrodites are implicated in carpel and ovule development.

5.2.9 Germination and Seedling Development

The Seed Germination work showed that *G. lanceifolia* seeds are recalcitrant but very sensitive to pretreatment. The highest germination percentages of 91.1% and 88.9%, respectively, were achieved in potassium nitrate (KNO₃) and gibberellic acid (GA₃ 300 ppm) as the pretreatment agents. The mixture of forest soil, sand, and vermicompost in a ratio of 3:2:1 created ideal conditions for the seedlings to grow, and they reached the highest height (32.2 cm), collar diameter (4.3 mm), and total dry weight (5.67 g), thus proving the excellent balance between aeration and the nutrients' availability.

5.2.10 Spectrochemical and Physicochemical Characterization of Seed Oil

The seed oil yield from *G. lanceifolia* ($43.36 \pm 2.03\%$) is significantly more than that obtained from other *Garcinia* species. The analysis of the physicochemical properties, namely peroxide, iodine, and saponification values, gave the following results: (4.43 mg KOH/g, 2 meq/kg, 113 g /100 g, and 189 mg KOH/g, respectively). The results indicated that the oil was quite stable, highly unsaturated, and thus, suitable for the food and cosmetic industries. The GC-MS technique employed for determining the fatty acid profile revealed the presence of four main methyl esters: methyl stearate (56.93%), methyl oleate (29.91%), methyl linoleate (10.75%), and methyl palmitate (2.41%), which are indicative of the lipid composition being a perfect blend of both saturated and unsaturated types.

Conclusion

The core of the present investigation was the explanation of the reproductive and seed biology of *Garcinia lanceifolia* Roxb. in Mizoram through the complex interplay of morphology, physiology, and molecular factors that assured its reproductive and ecological survival. The species shows phenotypic and genotypic variability, enabling it to exist under various environmental conditions prevalent in the subtropical forests of Mizoram. This heterogeneity in vegetative and reproductive traits confers a strong selective advantage, thereby contributing to the species' resilience during climatic and habitat shifts.

Another significant observation made in this study was the confirmation of androdioecy-the coexistence of male and hermaphrodite individuals within populations-which is rare and truly interesting from an evolutionary standpoint within the Clusiaceae family. The coexistence of these two morphs reflects a balanced reproductive strategy, in which male trees maximize pollen production and dispersal efficiency, while hermaphrodite individuals ensure seed set and population perpetuation. The fact that the morphs differ functionally and phenotypically and at the molecular level underscores the adaptive evolution of reproductive strategies within the genus *Garcinia*.

Studies and experiments on pollen biology and the mating system showed that *G. lanceifolia* is mainly cross-pollinated, but some apomixis also occurs. The presence of a mixed mating system ensures reproduction in the absence or limited availability of pollinators, thereby facilitating population stability. The existence of viable apomictic seeds at some locations, especially Thingdawl, further signifies the sexual and asexual reproductive options available to the species for survival under different ecological constraints.

At the subcellular level, transcriptomic investigations have greatly shed light on the genetic regulation of floral development and sex differentiation. The identification of differentially expressed genes related to hormone signaling and MADS-box transcription factors provides new insights into the molecular mechanisms underlying male and hermaphrodite flower development. These

discoveries serve as a first step toward unraveling the genetic basis of androdioecy and provide a more robust molecular resource, including SSR markers, for future population genetic and breeding investigations.

Seed germinating and seedling growth experiments prove that seeds of *G. lanceifolia* have high viability and respond positively to chemical pretreatments such as potassium nitrate (KNO₃) and gibberellic acid (GA₃). The application of KNO₃ and GA₃ resulted in a remarkable increase in germination percentage, germination rate, and seedling vigor, and therefore could be implemented as a seedling technique in conserving and reforesting activities. Besides, seedlings grown in a vermicompost substrate mixture exhibited higher growth performance, indicating that the application of organic matter could enhance nursery success rates.

G. lanceifolia represents a promising opportunity for the commercial development of high-value seed oil, capitalizing on its ecological and reproductive importance. Physicochemical studies and spectral characterization showed that the seed oil has a fairly stable composition, with high levels of stearic and oleic acids, with implications for food and cosmetics. Thus, viable options may be considered for sustainable bioprospecting and for community cultivation of *G. lanceifolia* in northeastern India.

The study provided insight into *G. lanceifolia* Roxb. as a resilient, adaptive, and economically critical endemic species with distinct reproductive mechanisms. The inclusion of field ecology, reproductive biology, and molecular analysis gave a robust framework for studying life history and conservation requirements. By clearly establishing morphological associations with the pollination system, gene expression, and germination biology, this study has significantly contributed to broader knowledge of the reproductive ecology of tropical trees and has laid a solid scientific basis for the conservation, sustainable utilization, and domestication needs of this lesser-studied species inhabiting the Indo-Burma biodiversity hotspot.



APPENDICES



PHOTO PLATES



Plate 1: *G. lanceifolia* tree bearing mature red fruit in natural habitate (Forest stand)



Plate 2: *G. lanceifolia* fruit in a local village market

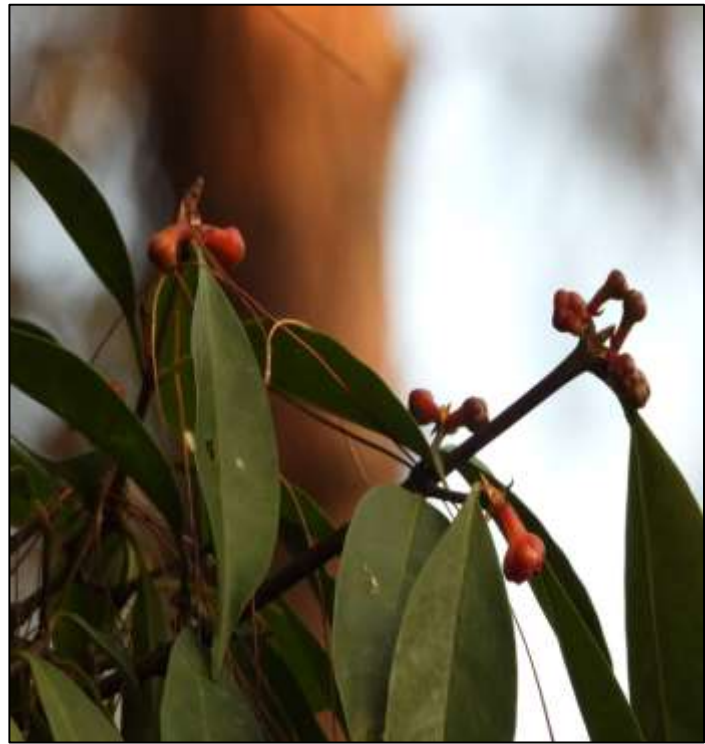


Plate 3: Harmaphrodite and male flower of *G. lanceifolia*

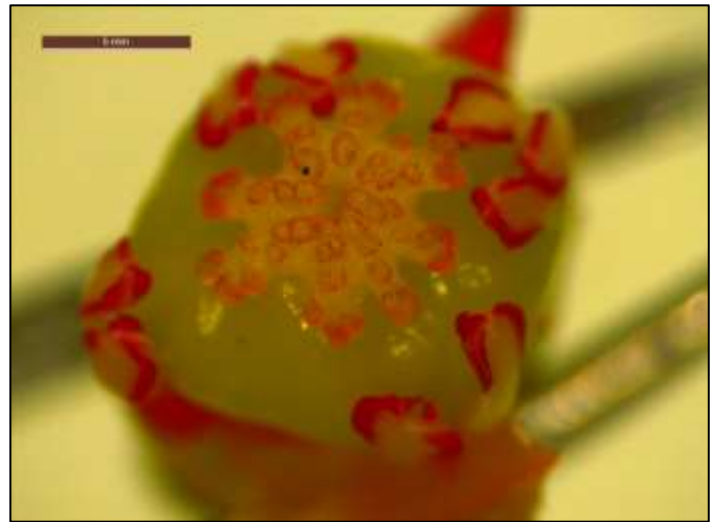


Plate 4: Flower of *G. lanceifolia* through Stereo zoom microscope



Plate 5: Ovary (ovules) and stigma of *G. lanceifolia* through stereo zoom microscope

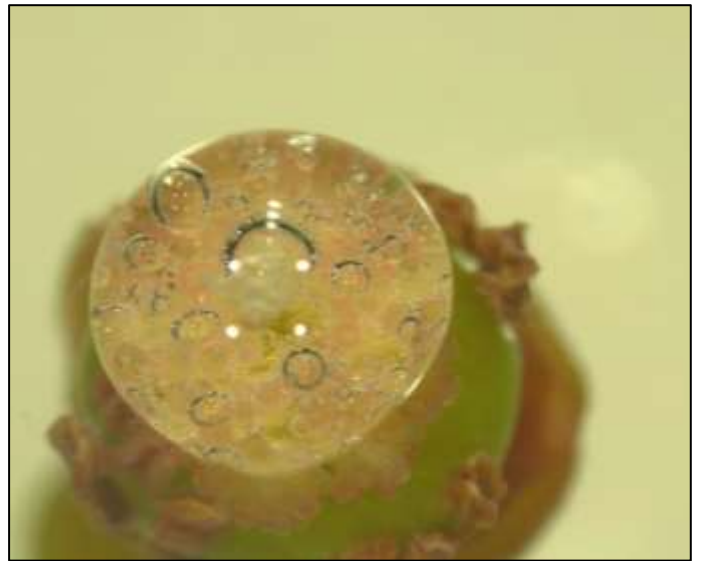
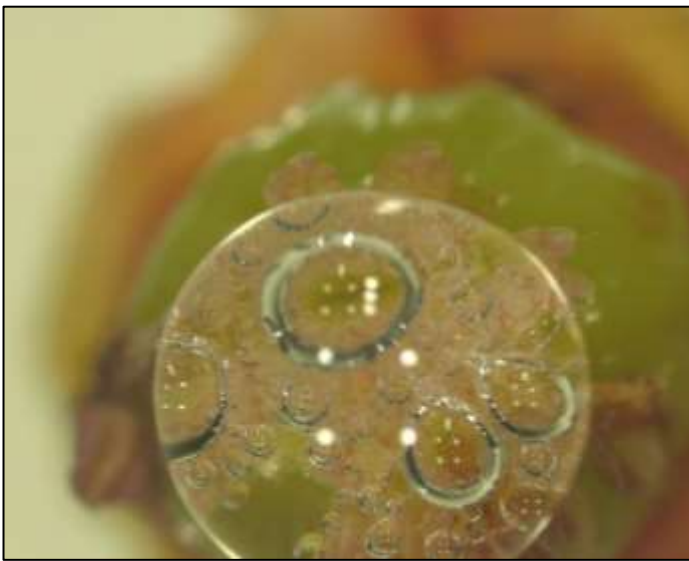
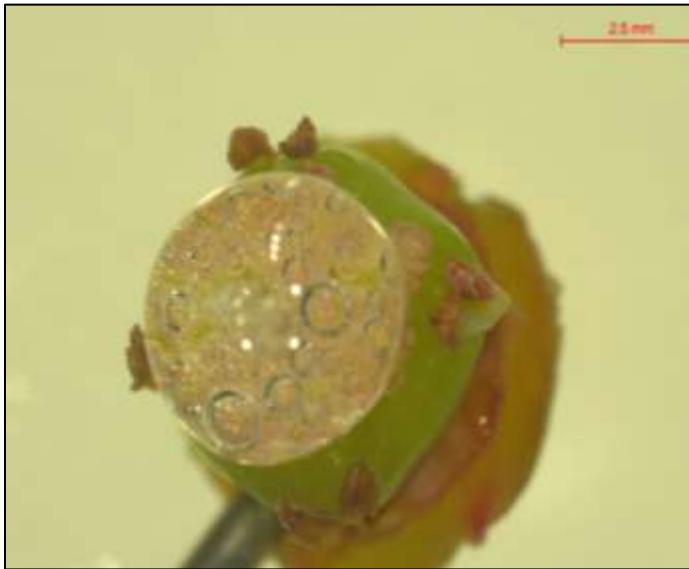


Plate 6: Stigma receptivity of *G. lanceifolia* using a drop of Hydrogen peroxide



Plate 7: Germination experiment of *G. lanceifolia* seeds in mist chamber, Dept. of Forestry, MZU



Plate 8: Potting mixture experiment on *G. lanceifolia* seedlings growth and biomass production



Plate 9: Estimation of plant growth parameters and biomass production in *G. lanceifolia* seedlings



Plate 10: Seeds of *G. lanceifolia*



Plate 11: Seed (in powdered form) and seed oil extracts (at room temperature and heated) of *G. lanceifolia*



Plate 12: Out-planting of *G. lanceifolia* seedlings for ex situ genetic conservation in the Horticulture Garden, Mizoram University.



Plate 13: Out-planting of a *G. lanceifolia* seedling at a roadside location within the Mizoram University campus



Plate 14: Promoting the domestication of *G. lanceifolia* through the dissemination of seedlings to home gardens.



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B.Sc (Agriculture)	Punjabi University, Patiala	2016	First	67.17
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List of publications

- **Vanlalmalsawmropuia, H.**, Kumar, K. S., Singh, A. B., & Reddy, M. S. (2025). Spectro-chemical characterization of seed oil of *Garcinia lanceifolia* Roxb. (Clusiaceae): An endemic medicinal fruit tree of Indo-Burma region of North-East India. *National Academy Science Letters*. Advance online publication. <https://doi.org/10.1007/s40009-025-01815-y>
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Presentation (Conference/ Seminar/ Workshop)

1. “Evaluation of important chemical properties of *Garcinia lanceifolia* Roxb. Seed oil extract”. Presented at National Conference on Recent Advances in Plant Biology with Special References to North-East, India, organized by Department of Botany, School of Life Sciences, Mizoram University, Aizawl from April 20-21, 2023.
2. “High Resolution Chromatography / Mass Spectroscopy Profile of *Garcinia lanceifolia* Roxb. seed oil extract. Presented at International Conference on Biodiversity, Biogeochemistry and Ecosystem Sustainability in Changing Environment, jointly organised by Department of Forestry, Mizoram University and Indian Ecological Society from 14 – 16 June 2023

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE : H. VANLALMALSAWMROUPUA
DEGREE : Ph.D.
DEPARTMENT : Forestry
TITLE OF THESIS : Reproductive and Seed biology of
Garcinia lanceifolia Roxb. in Mizoram.
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ABSTRACT

**REPRODUCTIVE AND SEED BIOLOGY OF *GARCINIA*
LANCEIFOLIA ROXB. IN MIZORAM**

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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**DEPARTMENT OF FORESTRY
SCHOOL OF EARTH SCIENCES AND NATURAL RESOURCES
MANAGEMENT
NOVEMBER, 2025**

**REPRODUCTIVE AND SEED BIOLOGY OF *GARCINIA LANCEIFOLIA*
ROXB. IN MIZORAM**

BY

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Submitted

**In partial fulfillment of the requirement of the Degree of Doctor of Philosophy
in Forestry of Mizoram University, Aizawl.**

Introduction

The Indo-Burma region is one of the world's biodiversity hotspots and is considered to be exceptional for its species richness and endemism. In the meantime, the northeastern state of Mizoram is known for the great variety of its plants, especially those with many flowering plants. In Mizoram, over 2,500 varieties of flowering plants in different hues adorn the site, offering beauty that could very well be turned into food or medicine. Habitat loss, land alteration, and increased climate change are the major threats to this unique, biodiversity-rich region. Nonetheless, many trees, including *Garcinia*, which significantly contribute to the forest niche, human diet, and the local healthcare system, are still not adequately researched, documented, and conserved.

The preservation of the region's many species and cultural elements has been the main reason Northeast India's nature and culture has remained rich. Villagers in the region have been practicing slash-and-burn agriculture for a long time, along with planting trees in the fields. Although the latter practice is not as widespread today as it was in the past, and even though there are debates about the sustainability of these activities, the management of wild edibles and medicinal plants, both in home gardens and forests, has been an ancient practice. In Mizoram, there are still large numbers of unknown edibles and herbs that are indeed consumed by several ethnic groups of the Mizo, including neighboring ethnic groups such as the Garo and the Khasi. These plants are found to be tangential rather than integral parts of the agricultural and medical sectors. Changes in the environment make livelihoods and conservation issues overlap. This is where solutions based on the combined efforts of ecological research and the local population's know-how and production channels come in.

Garcinia (Clusiaceae; formerly Guttiferae) is notable for its taxonomic diversity, distribution, and species richness, and for its great economic importance. The worldwide total of documented species is about 250, and the region with the largest number of species is tropical Southeast Asia. The culinary and medicinal uses of several species are rooted in the far past. *G. mangostana* (mangosteen), with its

sweet arils and pigment-rich pericarp, is one of the most famous and valuable of the fruit species. *G. indica* (kokum) has a sour rind rich in garcinol, hydroxycitric acid (HCA), and anthocyanins, and the fruit is also used as a source of medicine. *G. cambogia* (syn. *G. gummi-gutta*) has a widespread reputation in the food supplement industry as a source of HCA. In the entire Genus, the principal classes of compounds of interest to biomedical researchers are xanthenes, benzophenones, flavonoids, and organic acids. Because modern pharmacological screening methods are yielding results supportive of traditional uses, these compounds are receiving scientific validation. The sour fruits that can be consumed and the special chemical composition of *Garcinia* make it a primary "bridge genus" that connects various aspects of ecology, such as forests, household cooking, health care, and maybe bioprospecting opportunities.

India is the natural habitat of about 43 species of the Genus *Garcinia*, which are mainly distributed across three distinct phytogeographic regions: the Andaman & Nicobar Islands, the Western Ghats, and Northeast India. The evergreen forests in these humid, shaded areas, which experience similar temperature changes, meet the habitat requirements of many of the species in the Genus. In the case of Northeast, *G. pedunculata*, *G. cowa*, *G. morella*, and *G. lanceifolia* are common in the Genus, with each species living in somewhat different ecological settings and playing a distinct role in the culture. As for *G. lanceifolia*, it has been reported from Assam, Nagaland, and Meghalaya, and it also ranges into Bangladesh (Chittagong Hill Tracts), Myanmar, Thailand, Laos, and Vietnam, demonstrating that it belongs to the Indo–Burma affinity zone.

In botanical terms, *G. lanceifolia* Roxb. is an evergreen tree or shrub, very small to medium in size, typically reaching 1-6 m in height, with dark green, shiny, narrow leaves and bright orange to yellow latex, which emphasize the characteristics of the Clusiaceae family. The species is predominantly dioecious, with male and female flowers borne on separate individuals, though occasional polygamous-dioecious forms have been noted. Flowers are small, about 5–8 mm across, orange to reddish, and appear either solitary or in small fascicles on woody stems. The fruits

have a circular shape, a shallowly lobed surface, are 2.2–4.5 cm long and wide, and turn bright orange or red when they are mature. Inside, there are a few medium-sized seeds inside the sweet-sour flesh of the pulp. The flowering season usually falls between October and April, while ripening occurs from January to June. The plant can be found in the wet forest at low and mountainous sites at an elevation of about 300–700 m, mainly under the shelters of dense canopies—a situation typical of trees that make up the second layer of a forest and are also adapted to stable microclimates.

The species is deeply embedded in local food cultures. In Mizoram, it is widely known as "chengkek" (also Pelh or Pelhte), while Assamese communities call it "Rupohi Thekera." Ripe fruits fall into the category of perishable food and are acidic. However, they can be stored and consumed as well as used as a raw material for the preparation of preserved food. One common way is to spread slices of ripe fruit on a tray and sun-dry them with salt to make a tangy-flavored curry and chutney agent, which is stable at room temperature and a common kitchen ingredient in South India, as well as the culinary hub of India. Another method is to juice, pickle, or use the raw material in stews to give them a sharp and varied taste. Even the young leaves of the fruit plant are edible; they are prepared and served as cooked dishes and kept as pickles. The use of *G. lanceifolia* in these ways places it in the same category as other sour *Garcinia* species, as a unique seasonal flavor and a source of micronutrients for household diets. Significant reflection of both subsistence utilization and market utilization when drying, packaging, or other methods that make the products fit and available.

Ethnomedical records list various beneficial uses of the species. In Assam and Mizoram, traditional medicine practitioners use bark and leaves for various gastrointestinal problems such as dyspepsia, ulcers, diarrhoea, and dysentery, and also for metabolic disorders, including diabetes. The fruit is a remedy for diarrhea and dysentery, and is said to have a protective effect on the liver and kidneys, and the leaves are referred to as stomachic and diuretic agents. Based on laboratory experiments, the hydroalcoholic extracts of the tree's bark displayed prominent anti-

ulcer and anti-diabetic properties in Wistar rats, while antibacterial studies indicated the presence of bioactive compounds resembling those of xanthone- and benzophenone-bearing Clusiaceae. In this respect, *G. lanceifolia* is a potential candidate for future bioprospecting, even though it is a much-ignored fruit tree in the locality where it grows.

Beyond medicinal promise, this plant species is nutritional and may be used as a food ingredient. Research on the fruits of other *Garcinia* species indicates that the processing methods used in their manufacture are responsible for the retention of antioxidants and organic acids; with respect to kokum, the garcinol- and HCA-standardized extracts serve as the basis for nutraceutical applications. Preliminary work on *G. lanceifolia* reveals that the nutrient profile and antioxidant activity can be maintained using the freeze-drying method, suggesting potential for value addition if postharvest handling conditions are optimally set. The adoption of such processing rules would then pave the way for the plant to shift its use from household to artisanal and, finally, to commercial production, provided that harvests are sustainable and cultivation areas are enlarged to lessen the burden on the surviving wild populations.

When the *G. lanceifolia* is observed in terms of the hotspot Indo–Burma, all the work done in this regard contributes considerably to a larger conservation obligation. This plant sums up the very essence of the biodiversity-rich, resource-scarce area: in pure ecological terms, it is vital as a shade-tolerant, fruit-bearing understory tree; culturally, it is interconnected through food and medicine; economically, it shows promise via small-scale processing; and biologically, it is difficult through its dioecious nature, recalcitrant seeds, and storage sensitivity. The knowledge and lessons gained from the reproductive and seed biology of *G. lanceifolia* will be relevant to a broader group of species beyond it, including related *Garcinia* species such as *G. pedunculata*, *G. morella*, and *G. cowa*, and, more generally, to tropical trees with ethnobotanical value and complex life-history traits. Concretely, to achieve all of these, one would unite field ecology, pollination biology, and seed science so they complement and enrich community and market

aspects. The outcome would be propagation protocols that are not only scientifically sound but also feasible for local nurseries and farmer groups to implement; value-added products that promote remarkable well-being; and conservation methods that safeguard genetic diversity while sustaining use.

Objectives of the study

1. To survey potential areas of spatial distribution and natural variability (morphological variation in leaf, flower, and fruit).
2. To study the reproductive biology with respect to pollen production, female flower production, and reproductive success.
3. To study the effect of various pre-sowing treatments on germination and seedling growth of *Garcinia lanceifolia*.

Review of Literature

The *Garcinia* group is part of the family Clusiaceae (Guttiferae) and comprises about 400 species of flowering plants found across tropical regions of Asia, the Americas, Australia, Africa, and Polynesia. *G. lanceifolia* Roxb. is a lesser-known plant of the Genus and is native to the northeastern India, particularly in Assam and the neighboring areas like Mizoram. The local community highly values the plant because it is used in various traditional practices, from food preparation to medical use. Today, it is still consumed raw or pickled and is regarded for its ability to cure dysentery, dyspepsia, and biliousness. Bark from the plant also demonstrates good antibacterial activity, hence steering or justifying its use in some diseases caused by gastrointestinal disorders. *G. lanceifolia* is, therefore, an understudied plant with potential as a promising drug, bridging traditional knowledge and modern scientific research.

The ethnobotanical significance of the species was extended beyond the Indian landscape to numerous other regions of China, where the Genus *Garcinia* has been used historically for food, health, building, and cultural purposes. In Assam, the plant is commonly known as "Rupohitheker." Pharmacological tests have shown

that this plant possesses analgesic and anti-inflammatory properties. In Assam, many *Garcinia* species contain bioactive compounds such as hydroxycitric acid, xanthenes, and flavonoids, which are potent antioxidants, antibacterials, hypolipidemic, antimalarials, and cardioprotective. One more *Garcinia* species is available in Assam, *G. pedunculata*, which is also considered an excellent source of antioxidants, with the ability to prevent free radical diseases and human pathogenic diseases. It is a versatile plant with medicinal properties, including antibacterial, anthelmintic, hypolipidemic, anti-diabetic, hepatoprotective, neuroprotective, and nephroprotective, making it very beneficial to human health. Due to both ecological and economic relevance, *G. lanceifolia* has not been utilized significantly, and further studies are needed on its reproductive biology and seed ecology.

The reproductive biology of the *Garcinia* species exhibits a wide range of mechanisms, including hermaphroditism and dioecy, polygamodioecy, apomixis, and agamospermy. These strategies also affect the reproductive success, ecological adaptation, genetic variability, and conservation potential of the species. The majority of the species in the Genus exhibit apomixis, some of which are obligate apomicts, while others are facultative. *G. mangostana* var. *mangostana* (mangosteen) exemplifies a classic case of obligatory apomixis, characterized by male sterility, with the result that reproduction takes place solely through asexual seed formation. The majority of mangosteen trees is female and reproduces clonally via seeds. This genotype has maintained the limitation of extreme phenotypic and genetic diversity, while being characterized by dioecious ancestry. It is hypothesized that mangosteen originated as an allopolyploid, developed from *G. hombroniana* and *G. malaccensis* through single hybridization events. Neither of the parent species resembles the mangosteen, which is clearly an obligate agamosperm because it can reproduce both sexually and asexually. The Genus also affords a certain degree of obligate and facultative apomixis in its other members; *G. celebica* and *G. prainiana* are facultative apomicts, while *G. atroviridis* has a mixed system whereby both sexual and asexual reproduction types are achievable. Unpollinated flowers of *G. atroviridis* can set fruit, but it should be noted that better fruit setting and seed viability are

generally obtained via cross-pollination. This species exhibits gynodioecy, in which female trees produce larger fruit and seeds than hermaphrodites.

The study of seed biology reveals one of the basic elements of plant life and, in addition, governs population oscillations, species distribution, and regrowth success. Besides, seeds have become an essential source of spermatophyte colonization and human food. It is not only temperature but also water that could affect seed dormancy and germination. Climate change is therefore an important factor in the modification process, which will result in alterations in regenerating patterns and changes in germination timing; in the end, this will affect population and community dynamics. Progress in seed biology has been to view the seed not as a single individual but as an ecological individual. Thus, it has been recognized that molecular genetics and ecology can provide insight into how environmental signals influence development, dormancy, and germination.

These species produce seeds within fleshy, edible fruits that are unique due to their biological characteristics. The seeds have a rather recalcitrant nature; they are not very resistant to desiccation, and their size and moisture content greatly determine their germination ability. The seeds' recalcitrant nature, and therefore their high desiccation and cold sensitivity, are the reason behind the problem of long-term storage. One of the notable features of *Garcinia* seeds is that they lack a distinct embryo, endosperm, or embryonic axis, and therefore, are not seeds in botanical terms. A major part of *the Garcinia* species is known to be apomictic, especially through agamospermic, or asexual, reproduction, which may be facultative or obligate depending on the species. Most *Garcinia* species exhibit typical *Garcinia*-type germination, characterized by the emergence of radicles and shoots from opposite ends during germination.

Materials and Methods

Study Area

Mizoram, the study site of this research, is located in the northeast of India and is considered part of the Indo-Myanmar biodiversity hotspot, one of the richest areas with distinct endemic flora and fauna. It has been observed that the local community's efforts to conserve plants have led to greater preservation of plant species diversity in community-conserved areas than in formal protected areas; however, they are challenged by the loss of endemic and threatened species. The Eastern Himalayan mountain range spans a variety of forest types that support populations and Mizoram stands out, registering a 1.4% increase in forest cover over 30 years, despite the country's deforestation. Despite its small size, Mizoram's total area is around 21,081 sq. km, accounting for only 0.64% of India's total land area. In the northeastern part, Mizoram lies between the latitudes of 21°56' and 24°35' N, and longitudes of 92°16' and 93°26' E. The Tropic of Cancer crosses the state at 23°30'N, which has a great effect on its climate and ecology. It has Myanmar to the east, and Bangladesh to the west and south. It also borders Assam, Manipur, and Tripura. The mainly mountainous region is home to evergreen rainforests, which are a key source of livelihoods for the local community, mainly through jhum cultivation, hunting, and gathering.

Mizoram has characteristics of both a tropical monsoon climate and a subtropical moist climate; it receives high annual rainfall, with noticeable seasonal changes. Precipitation is heaviest during the four months from May to September, totaling 2,000 to 3,500 mm per year. The normal high and low summer temperatures in each place are typically 14°C to 30°C. On the other hand, winter has mild temperatures of 6°C to 24°C. The lands are typically lower in pH, high in humus, and mostly have a sandy loam to loam texture, which is favorable for the growth of a variety of vegetation.

Among Indian states, Mizoram has a particularly high percentage of forest cover. According to the India State of Forest Report (ISFR 2021), India has a forest

cover of 84.53% of its total geographical area, amounting to 17,820 km². The forest cover percentage has decreased substantially from 88.93% (18,748 km²) reported in ISFR 2015 to a similar figure in ISFR 2019. Such a substantial reduction is a clear case of human-caused harm, largely attributable to shifting cultivation and frequent habitat conversion. Traditional cultivation practices, such as jhum cultivation and home gardening, remain major aspects of agrarian life and reflect the deep-rooted power in the socio-economic fabric of the people of Mizoram.

Ecologically, Mizoram has diverse forests that span a wide range of altitudes, rainfall regimes, and species composition. The contributing factors have been acknowledged and segmented into six main categories. The tropical wet evergreen forests, the montane subtropical forests, the temperate forests, the bamboo forests, the *Quercus* forests, and the Jhum fallow. The climate and flora of Mizoram are clearly different from those of other places. Besides, it is part of a global biodiversity hotspot, making Mizoram a significant place for the research of plant taxa such as *G. lanceifolia* Roxb. The hills and valleys in the region, and its mild but well-circulated air, are among the best characteristics for studying the reproductive biology, seed ecology, and conservation strategies of this species, which is not widely known but is very significant in botany and ethnology.

Study Sites

For this research, two locations were selected in Mizoram, which include Thenzawl in Serchhip District and Thingdawl in Kolasib District. The two differ in their ecological features, making them a good and diverse source of habitats for the investigation of *G. lanceifolia* Roxb.

Thenzawl(23°17'08" N, 92°46'51" E) lies in the heart of Mizoram at an elevation of about 786 m above sea level. The area lies within the subtropical evergreen forest zone, with dense natural vegetation thriving under limited human interference. A humid climate has an average rainfall of 2299 mm, with the western or southwest monsoon accounting for most of it. Seasonal temperatures range from as low as 11°C in winter to about 28 °C in summer.

Thingdawl (24°11'04" N, 92°41'39" E) is located in northern Mizoram at an altitude of about 714 m above sea level. The area falls within a semi-evergreen forest zone where the vegetation is a mixture of natural forest patches and landscapes altered by human activities. With an average annual rainfall of nearly 2,540 mm, the region's temperatures usually range from 13°C in winter to 30°C in summer.

Morphological Characteristics of *G. lanceifolia*

All morphological characters were assessed following the procedure described by Kumari (2020) with slight modifications. The morphological traits of *Garcinia lanceifolia* were described by documenting natural variability in different attributes. Observations were conducted on different plants using standardized procedures and visual methods. Measurements of tree height and leaf size, and assessments of leaf and tree form, along with qualitative categories of traits such as foliage density, leaf shape, type of apex, surface texture, and trunk color, were also conducted during the investigation.

Floral biology, flowering Phenology, and Pollen Production

A study was carried out following the methodologies for floral biology, flowering phenology, and pollen production, with necessary modifications from Kumari (2020), Mujadadi et al. (2024), and Shivanna & Rangaswamy (2012). Systematic field observations documenting flowering patterns, floral traits, and pollen output are critical to understanding reproductive biology and, by extension, reproductive success in *G. lanceifolia*. Standardized procedures ensure consistency in data recording for the determination of flowering time, peak flowering, inflorescences, and flower size, as well as anthesis timing, pollen production, and female flower production.

The Duration of Flowering was determined by recording the dates for the first and last appearance of flowers, with the opening of 50 fresh flowers per tree on a given day kept as the criterion for the beginning of the flowering season. The Time of Peak Flowering was recorded when 50% of the flowers on the tree had opened. The Length of the Inflorescence was measured in centimeters from the base of the

flower to the tip of the inflorescence. The Size of the Flower was measured in centimeters using a measuring scale. For anthesis, about 50 flower buds expected to open the next day were tagged and observed the following day for the time of anthesis, with counts taken at half-hour intervals. The time of anthesis was recorded over 5 days during peak flowering.

Pollen Production per anther

Pollen Production per anther was calculated using the formula

$$.P= TpA/N$$

Where,

TpA = Total number of pollen grains counted in all anthers

N Total number of anthers (slides) used for counting

Pollen per Flower (PF)

Pollen production per flower was estimated by multiplying the number of pollen grains per anther by the total number of anthers per flower.

$$PF=PA \times AF$$

Where:

PA = Mean pollens per anther

AF = Average number of anthers per flower

Pollen Production per Tree (TP)

The total pollen production per tree was estimated using the formula:

$$TP= PA \times AF \times FB \times BT$$

Where:

PA = Average number of pollens per anther

AF = Average number of anthers per flower

FB = Average number of flowers per branch

BT = Average number of branches per tree

Female flower production

Total flower production per tree was estimated by multiplying the average number of branches per tree by the average number of flowers per branch.

Pollen viability

Pollen viability was determined using the Fluorochromatic reaction (FCR) based on the method given by Heslop-Harrison and Heslop-Harrison (1970), and Heslop-Harrison et al. (1984), with required modifications. FDA stock ($2 \text{ mg}\cdot\text{mL}^{-1}$ in acetone) was prepared and stored at -20°C . A working solution ($20 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$) was made by diluting stock 1:100 into 10% (w/v) sucrose. Fresh pollen was mounted in a drop of the working FDA solution, incubated in the dark for 5–15 min, and observed with a fluorescence microscope (FITC filter). Viable pollen grains exhibited bright green fluorescence and were counted; viability was expressed as a percentage.

Seed Germination Study

Seed germination experiments were conducted to investigate the effectiveness of various pre-sowing treatment methods on improving seed germination performance in *G. lanceifolia*. Since the species itself bears recalcitrant seeds characterized by a relatively limited storability, a germination boost is declared imperative in this species of plant to propagate and conserve. Seeds collected from various sites of Mizoram were combined and used to obtain several replications under controlled polyhouse conditions. Various pre-sowing treatments used are as follows: Dist. Water (18 hrs), Sulphuric acid 30% (30 min), KNO_3 (Potassium

Nitrate) (0.2% 18 hrs), kinetin (500 ppm 18 hrs), Gibberellic acid (300 and 500ppm 18hrs), and Thiourea (2% 18hrs).

Germination and Seedling Emergence Parameters

Seeds of *G.lanceifolia* with different pre-sowing treatments were sown in a mist chamber seed bed, which provided suitable humidity and temperature. Thus, the rate and degree of germination are optimally assessed after measuring most germination parameters and seedling emergence.

Germination Percentage (GP) was calculated by dividing the number of seeds germinated by the total number of seeds sown and expressed as a percentage:

$$GP = (\text{Germinated seeds} / \text{Total seeds sown}) \times 100.$$

Germination Capacity (GC%) was determined as the cumulative number of seeds that germinated during the 28-day test period, plus the number of seeds that remained viable but ungerminated at the end of the test:

$$GC (\%) = ((\text{Cumulative germinated seeds} + \text{Viable un-germinated seeds}) / \text{Seed sown}) \times 100.$$

Germination Energy (GE% %) was expressed as the percentage of seeds that germinated up to the time of peak germination, usually the highest number of germinations recorded in 24 hours:

$$GE (\%) = \times 100 (\text{Seeds germinated during peak 24-hour period} / \text{Total Seeds}).$$

Germination Value (GV) was calculated as:

Germination value = PV×MDG, where PV = Peak value of germination and MDG = Mean daily germination.

Germination Index (GI) was calculated as:

$$GI = \text{Total germinated seeds} / \text{Time to 50\% germination (days)}.$$

Potting Mixtures

The effect of different substrate compositions on the establishment of seedlings was investigated using various potting mixtures of materials available locally. Forest soil was considered the base medium since it closely resembles the natural habitat of the species and provides inherent fertility. Sand was incorporated for the absorbent purposes to increase drainage, aeration, and prevent waterlogging, which is detrimental to seedling survival. Most importantly, organic amendments such as farmyard manure (FYM) and vermicompost are introduced to supply essential nutrients to the overall substrate, enhance microbial activity, and improve soil structure. The ratio of treatments (various potting mixtures) is as follows: Control (Forest soil) – P0; Forest soil: Sand: FYM (3:2:1) – P1; Forest soil: Sand: Vermicompost (3:2:1) – P2; Forest soil: Sand: FYM: Vermicompost (3:2:1:1) – P3.

Seed oil extraction

Extraction of seed oil from collected *G. lanceifolia* seeds was performed in the laboratory using the following procedure. The collected fruits were cut, and the seeds were removed and air-dried. Seeds were cleaned and ground into a fine powder by a mixer grinder. The seed oil was extracted through a cold percolation method; the fine-powdered seed material was soaked in hexane solvent for four days with intermittent flask shaking. Further, the seed oil was separated from hexane using a rotary vacuum evaporator (Büchi Rotavapor R-300), dried at 60 °C, and weighed.

Physicochemical properties of seed oil

Acid value, saponification value, peroxide value, and iodine value of extracted seed oil of *G. lanceifolia* were determined according to "Fats and Oils Handbook" AOCS (American Oil Chemists' Society) Press, referencing to the method standard number for acid value (AOCS: Cd 3a-63); saponification value (AOCS: Cd 3-25, Cd 3b-76); peroxide value (AOCS: Cd 8-53 and Cd 8b-90) and iodine value (AOCS: Cd 1-25, Cd 1b-87 and Cd 1d-92). The specific gravity of the seed oil was determined using a dry pycnometer, and the viscosity of the seed oil sample was estimated as per Zahir et al. (2017).

Results and Discussion

Morphological Description of *G.lanceifolia*

The species under consideration was established as *Garcinia lanceifolia* Roxb. following the uniform suite of distinctive and consistent morphological characters observed across all sampled populations. The tree appears straight and pyramidal in habit. The branching is opposite-decussate, with emerging nodes being right-angled symmetrical pairs above and below. The leaves are placed diametrically opposite each other and are oblong-elliptic or elliptic-lanceolate, with an apiculate acute point at the apex, with a coherent margin. The leaf sheet is glabrous, smooth, dark green, and lustrous, and the flowers are noticeable because they are deep red, with a fleshy, hard perianth; in most cases, there are greenish hints. The above characteristics are not just valuable for determination, but also manifestations of adaptations in *G. lanceifolia*. Opposite-decussate leaf arrangement and a dense, pyramidal crown may serve a useful function by maximizing light capture in the forest's understorey habitat. Such thick, leathery leaves with entire margins suggest increased tolerance to humid tropical environments, thus reducing water loss and herbivorous damages. This is a common feature in many *Garcinia species* (opposite, coriaceous, shiny leaves), indicating a high level of conservation within the Genus; possibly due to the evergreen nature and resource-conservation strategies. The presence of multiple stigmatic rays on the ovary is one of the characteristics of such a section of *Garcinia*, to which *G. lanceifolia* belongs. Altogether, these morphological details are well-aligned with published botanical revisions. *G. lanceifolia* is described as a dioecious shrub or small tree 1-6 m tall with opposite, elliptic-lanceolate, shiny leaves (4.5-10 × 1.5-3 cm) and small reddish-orange flowers about 5-8 mm in diameter, consistent with our observation in most cases. To this extent, our observations are consistent with the literature and provide an accurate determination of our specimens, adding confidence that any such differences are interpreted as biological variability rather than a case of misidentification.

Phenotypic Variability in Tree Architecture

Phenotypic coefficient of variation (PCV) quantified the extent of variability in key architectural traits across populations, revealing a gradient that was usually distinguishable by both site and the sexual form of the trees.

In Thingdawl, a hermaphrodite population showed moderate phenotypic variability of 14.23% in girth and 11.28% in height. Much higher variability was observed in the total number of branches (16.26%). On the other hand, the Thenzawl populations showed a very different pattern: both hermaphrodite and male trees had a high to very high level of variability across all traits recorded. For example, tree girth and height showed high variability ($PCV > 22\%$) while the number of branches ranged from 6.47% in hermaphrodite trees up to 39.61% in male trees; thus, the mean overall phenotypic differentiation between the two populations was ordered by a higher plasticity in respect to growth form of the population from both hermaphrodite and male trees as to the lesser population from Thingdawl.

Quartile analysis clearly reveals wider interquartile ranges in the Thenzawl population, especially for branch number, highlighting morphological heterogeneity. Thenzawl males exhibit extremely high fourth-quartile and maximum branch numbers, indicating higher phenotypic plasticity and reproductive potential, which tallies with the elevated PCV values.

The variation in branching architecture and, in particular, branching pattern is indicative of tropical tree phenotypic plasticity in variable light and moisture conditions, and is greater in Thenzawl because of more heterogeneous terrain and microclimatic gradients. Other than variation in stem diameter and branch number, male trees differ from females in their amount of branching flexibility. Staminate trees, which do not have to invest resources in producing reproductive biomass, can thus allocate more resources to the vegetative part of the tree, which is an excellent help in colonizing forest fragments. The results of this study indicated that the *G. lanceifolia* population remains highly variable with respect to intra-specific differences. Consequently, this provides a basis for local adaptation and resilience.

Floral Morphometry and Dimorphism

1. Inter-Population Variation in Floral Traits

The differences in floral characteristics across all three groups were evident in the results. One-way ANOVA showed that differences in floral traits among the groups were significant ($p < 0.001$). Post-hoc Tukey's tests placed the populations into the following categories: Thenzawl hermaphrodite flowers were always the largest, being considerably heavier and having a larger diameter than both Thenzawl male and Thingdawl hermaphrodite (TDH) flowers. Thenzawl male flowers were generally of intermediate size but were statistically distinct, thereby forming separate groups for weight and diameter. Thingdawl hermaphrodite flowers were the smallest in all respects. For instance, the size of flower openings was equal in Thenzawl hermaphrodite and male flowers, but much smaller in the Thingdawl population.

The comprehensive study of floral traits in *G. lanceifolia* has revealed a complex diversity of patterns, the result of the combined effects of sexual dimorphism and site-specific environmental factors. The major differences in flower size and weight between the Thenzawl and Thingdawl groups, along with the conspicuous sexual dimorphism in the Thenzawl group, provide important indications of the species' reproductive method and adaptive capabilities.

The evident inter-population variation, in which Thenzawl hermaphrodites had the largest and heaviest flowers. In contrast, Thingdawl hermaphrodites showed the opposite, highlighting that the phenotype is not solely determined by genetic sex. Such geographical variation in flower traits is a normal occurrence and may result from different selection pressures acting across environments. Thenzawl's diverse landscape and microclimatic gradients, cited as factors shaping architectural traits, are likely to exert a variety of selective pressures. One example is the climate, which can directly affect resources such as light and water, which in turn determine the amount of energy a plant can devote to its reproductive structures. Consequently, the larger flowers of Thenzawl indicate a more conducive environment or that the large, attractive flowers are subject to stronger selection in such an ecological context.

1. Comparison of Sexual Dimorphism in Flowers

Independent-samples t-tests on Thenzawl hermaphrodite and male flowers yielded a significant difference, confirming sexual dimorphism. Hermaphrodite flowers were much larger than male flowers in a few dimensions, such as weight (70% heavier, $p < 0.001$), diameter (17% larger, $p < 0.001$), and stalk length (42% longer, $p < 0.001$). On the other hand, flower opening size ($p = 0.804$) and overall flower length ($p = 0.241$) showed no significant differences, indicating that these traits have been retained across the sexual morphs.

The powerful allometric links (such as those between weight, diameter, and length) across the two flower types indicate simultaneous growth and provide reliable models for estimating flower investment. The greater phenotypic variability (CV) found in hermaphrodite flowers, especially in stalk height and flower opening, implies more phenotypic plasticity in this flower type. Studies show that phenotypic plasticity in floral traits has critical adaptive roles in various plant species. In *Mercurialis annua*, hermaphrodites from populations with the presence of males exhibited higher plasticity in sex allocation, decreasing pollen production in low resources, and on the other hand, increasing seed production in high resources, as opposed to hermaphrodites from populations with no males. In the same way, *Solanum hirtum* exhibits developmental plasticity in sex expression, with differences among genotypes and an influence of the individual's resource status. This plasticity can be a benefit in changing environments, allowing plants to modify their floral displays in response to environmental factors, such as the presence of pollinators or resource availability.

From a conservation perspective, the significant intraspecific variation documented in this study is an important source of resilience. The Thenzawl population, which possesses high variability in both architectural and floral traits, is a potential source of adaptive genetic diversity. In the case of the Genus *Paphiopedilum*, knowledge of the genetic structure and morphological variation among populations has been shown to be fundamental in setting conservation priorities. The results of this study indicate that conservation planning for *G.*

lanceifolia should give primary importance to protecting different populations, such as in Thenzawl and Thingdawl, to maintain the full range of the species' adaptive capacity, particularly in the context of environmental change.

Pollen Production and Viability

1. Total Pollen Count per Anther

A significant difference was observed in total pollen count per anther of *G. lanceifolia* between male and hermaphrodite flowers. The mean pollen count per anther of male flowers (2208) exceeded that of hermaphrodite flowers (1891.8). The difference in variation among samples was low in male flowers (CV = 14.34%) but high in hermaphrodite flowers (CV = 19.27%), thus pointing out the greater inconsistency in the pollen production of hermaphrodite individuals. The pollen count for male flowers was 1796 to 2782, whereas for hermaphrodite flowers it was 1163 to 2283. These findings imply that male flowers are not only morphologically but also functionally better adapted to pollen dispersal and, therefore, contribute to the pollen pool to a greater extent than hermaphrodites, thus confirming this in other androdioecious *Garcinia* species.

1. Pollen Viability per Anther

There were notable variations in pollen viability between the two types of flowers. The hermaphrodite flowers with an average pollen viability of $79.44 \pm 9.96\%$ showed a significant difference compared to male flowers that had only $32.13 \pm 7.32\%$ as the average pollen viability. The viability range of the hermaphrodite pollen was 61.60% to 87.38 % while for the male pollen it was only 22.34% to 41.61%. The viability of male pollen is relatively low, suggesting physiological or developmental factors that negatively affect pollen maturation. The male flowers have lower viability, but their high total pollen count may compensate for reduced individual pollen quality, ensuring that the reproductive process within the population is not affected by the lack of functional pollen. The pollen viability characteristics of the different sexual morphs have been documented as similar by other researchers.

1. Total Pollen Production per Flower

When the pollen output per flower was calculated, the male flowers had the highest numbers, with a mean of $94,787 \pm 12,360$ grains per flower and a range of 75,106 to 128,122. On the other hand, Thenzawl hermaphrodite flowers averaged $42,473 \pm 7,600$, while those from Thingdawl showed a lower number at $18,920 \pm 3,380$ grains per flower. The large disparity between male flowers and the other floral forms underscores the male flowers' reproductive strategy, which is specifically adapted to liberate large amounts of pollen. The population differences may also indicate the prevailing ecological factors, such as microclimate and soil nutrition status, that influence the amount of flowering.

Mating System in *Garcinialanceifolia*

The results showed different trends between the two hermaphrodite populations. In the case of Thenzawl, geitonogamy had the highest fruit set with a 38.1 % record, while in Thingdawl, a slightly higher geitonogamy rate was recorded at 46.34 %, but this was statistically insignificant ($\chi^2 = 1.01$, $p = 0.315$). The lower geitonogamous fruit set suggests a far more common reproductive path in *G. lanceifolia*: self-pollination within the same individual.

Autogamy, both Thenzawl and Thingdawl had very low fruit set percentages for spontaneous self-pollination within the flower itself (8.33 % and 5.41 % respectively), with no significant difference ($\chi^2=0.45$; $p=0.503$). This shows that few fruits are produced through autogamy, meaning that self-pollination is very infrequent in this species and, with complete certainty, requires a pollinator or a floral mechanism to prevent possible selfing, which is mutually exclusive with protogyny or genetic self-incompatibility.

Xenogamous pollination (Crossing between different trees) gave rise to some distinct changes between the two populations. Hermaphrodites in Thenzawl achieved a 27.27 % fruit set as against the 16.67 % in Thingdawl, a significant difference ($\chi^2 = 3.92$, $p = 0.048$). This shows that outcrossing is more efficient in the Thenzawl

population, probably as a consequence of greater genetic diversity or more efficient pollen transfer among individuals.

Thingdawl showed 27.03% higher apomictic fruit set than Thenzawl (11.76%); this was achieved through emasculation and bagging (to exclude pollen), significant at $\chi^2 = 9.12$, $p = 0.003$. Higher apomixis in Thingdawl suggests a stronger tendency towards apomictic seed formation, likely functioning as a reproductive assurance mechanism when pollination is limited.

The male-to-female pollination treatment, performed only in Thenzawl, yielded an intermediate 15.38% due to the fact that male flowers can fertilize perfectly hermaphrodite flowers, i.e., female functioning flowers. The study's results have supported the presence of a few male units or branches in Thenzawl's population, substantiating the androdioecious breeding pattern in *G. lanceifolia*. No action like this could be introduced in Thingdawl due to the nonavailability of male flowers or trees in the village.

The reproduction data of *G. lanceifolia* suggests a facultative mixed mating system, with the two extremes being sexual outcrossing and asexual apomixis; this balance shifts notably between the Thenzawl and Thingdawl populations. It is an example of how local habitats can influence the choice of reproductive strategy.

Evidence of Androdioecy in *G.lanceifolia* Roxb.(Clusiaceae) in Mizoram

Androdioecy is the rarest and evolutionarily interesting reproductive system in plants, characterized by the presence of hermaphrodite and male individuals in a single population. This is an intermediate condition recorded between hermaphroditism and dioecy, thus making it a good model for understanding the evolution of reproductive differentiation and sexual systems. Recorded cases of androdioecy are very few in number, and confirmed examples are from conserved families such as Apiaceae, Cucurbitaceae, Clusiaceae, Datisceae, and Euphorbiaceae.

The present study provided the first detailed confirmation of androdioecy in *G. lanceifolia* based on field observations, floral morphology, and sex-ratio analysis across seven natural populations in Mizoram, and addressed the issue of pistil duplication.

1. Sexual Morph Identification

Field and floral examinations confirmed the presence of two distinct sexual morphs within natural populations of *G. lanceifolia*: Male trees, which bear exclusively staminate (male) flowers, and Hermaphrodite trees, which bear only bisexual (perfect) flowers.

Most inflorescences were terminal, though axillary clusters were occasionally present. Flowers were solitary or paired or sometimes occurred in small fascicles. Each flower is enclosed by four concave sepals and four petals, which are initially green but turn yellowish-red or pinkish-red as they mature.

Hermaphroditic flowers have the superior ovary centrally placed, which is convoluted by the stamens in four bundles of 2-7 stamens. The stigma had 5-8 lobes, corresponding to the number of locules in the ovary, with each typically bearing 5-8 ovules. The ovary is smooth, glabrous, and distinctly five-locular.

In contrast, male flowers lacked any trace of an ovary and exhibited approximately 40 stamens, arranged compactly in a dense, hairless androecial mass. The anthers were narrow, slightly angled, and nearly sessile (stalkless), with four pollen sacs per anther. They were introrse, opening via longitudinal slits to release copious pollen. These morphological distinctions unambiguously delineate the two sexual morphs, confirming the presence of functionally male and hermaphrodite individuals - a hallmark of androdioecy.

Germination and Seedling Growth Response of *G.lanceifolia*

1. Germination Response to Chemical and Hormonal Pre-treatments

In the pre-treatment comparison, the germination behavior of *G. lanceifolia*'s seeds clearly indicated physiological aspects important for this endemic species' seed biology. The influence of the applied pre-treatment was so dramatic that it was reflected. The seeds that underwent treatment with potassium nitrate (KNO_3) showed the highest germination percentage of 91.1%, which was also significantly higher than the control group's germination percentage (85.6%, $p < 0.01$). This is consistent with other studies on other species, which show that KNO_3 priming increased germination rate and uniformity. The probable reason for this is the metabolic activation and oxygen absorption to a higher degree during the soaking process (priming "wakes up internal metabolism"). It is also noted that KNO_3 contributed to the highest germination energy, value, and index, which is in line with the metabolic enhancement.

Gibberellic acid (GA_3) pre-treatment was also found to be effective in increasing germination compared to the control treatment. At 300 ppm GA_3 , germination was 88.9% (higher than the control, $p < 0.5$), but there was no increase in germination at 500 ppm. This indicates the possibility of an optimal dose as reported in other crops (e.g., GA_3 priming significantly increases germination speed in cereals). In general, the application of exogenous GA_3 is associated with the breaking of dormancy and the obtaining of vigor, thus leading to better germination metrics.

On the other hand, the priming with thiourea and kinetin gave rise to germination rates (84.4% and 80.0%) which were almost the same as that of the control (statistical significance $p > 0.5$). Thus, the conclusion was made that the treatments did not provide a substantial increase in germination. In addition, the earlier studies pointed out that kinetin priming did not have much impact on the final germination percentage of seeds (for example, in the case of aged eggplant seeds). The finding indicated that, even though cytokinins like kinetin might jack up the metabolism, they did not raise overall germination significantly under our conditions.

The harsh treatments caused strong inhibition. The use of sulphuric acid scarification (H_2SO_4) led to a dramatic reduction in germination (11.1%, $p < 0.001$), indicating damage to both the seed coat and embryo. In fact, other species have shown that even very short contact with concentrated H_2SO_4 seriously destroyed seed viability. Likewise, NAA (500 ppm) led to a reduction in germination (24.4%, $p < 0.001$), which was posited to result from hormonal imbalance: the high auxin levels increase ABA effects and keep the seed dormant, thereby inhibiting germination. In the end, KNO_3 priming and GA_3 (300 ppm) were found to be the best pre-treatments, supporting the priming theory (metabolic activation) and consistent with previous studies on related *Garcinia* species.

1. Influence of Potting Mixtures on Seedling Development

The use of different substrates for potting has a significant impact on seedling growth. The mixed substrate of forest soil, sand, and vermicompost (P2, 3:2:1) produced the largest seedlings, as measured across all parameters. For P2, the average height of the plants was 32.22 cm and 4.32 mm for the collar diameter—these were both significantly higher than others ($p < 0.001$). P2 produced the highest total biomass (5.67 g) and the largest number of leaves (35.09), thus giving the largest Dickson quality index (0.50) to this mixture. On the other hand, the mix P3 (forest soil: sand: FYM: vermicompost, 3:2:1:1) produced the weakest growth over all traits. Its high organic matter content likely led to poor aeration and nutrient imbalance, creating very unfavorable conditions for the root system to grow. P0 (pure forest soil) and P1 (soil: sand: FYM) were in between. These findings are in line with other studies reporting that moderate levels of vermicompost can enhance seedling growth, while too much compost can lower quality.

P2 (Soil:Sand: Vermicompost 3:2:1): The height, diameter, leaf number, and dry weight, all aspects of performance, were at their highest. The greatest biomass was obtained with the tallest seedlings (32.22 cm), which is in line with results showing that substrates enriched with vermicompost improve nutrient availability and vigor.

P1 (Soil:Sand: FYM 3:2:1) and P0 (Soil alone): Moderate growth; better than P3 but below P2. Organic amendment (FYM) improved over soil alone, but not as much as vermicompost.

P3 (Soil:Sand:FYM: Vermicompost): Poorest growth. Its high organic fraction is probably the main reason for poor aeration and potential salt/nutrient stress. Previous studies indicate that although adding compost can enhance DQI (Dickson Quality Index) to a certain extent, an excessive vermicomposting ratio (e.g., > 75:25) can decrease seedling quality. The outcome indicated that P2 found the right compromise.

Consequently, seedlings flourished in the P2 medium, which provided good aeration and ample nutrients. The results suggest that vermicompost is an important ingredient in the substrate for the growth and development of *Garcinia* seedlings. On the other hand, if organic amendment is excessive, it could lead to a lower Dickson Quality Index and reduced vigor among the plants.

Spectro-chemical characterization of seed oil of *Garcinia lanceifolia* Roxb

1. Physico-chemical properties of *G. lanceifolia* seed oil extract.

The acid value of *G. lanceifolia* (4.43 ± 0.33 mg KOH/g) is higher than that in peanut oil (2.7 mg KOH/g) and sunflower oil (3.09 mg KOH/g) but lower than olive oil (6.6 mg KOH/g). This acid value comparison is close to that of some other species of *Garcinia*, like *G. cambogia* (5.04 mg KOH/g), *G. gummi-gutta* (3.7 mg KOH/g), *G. mangostana* (4.5 mg KOH/g), *G. xanthochymus* (4.8 mg KOH/g), and *G. indica* (4.9 mg KOH/g). This indicates the importance of *G. lanceifolia* as a significant oilseed species in comparison to other *Garcinia* species.

G. lanceifolia demonstrates a higher acid value of 4.43 ± 0.33 mg KOH/g than peanut oil, with a 2.7 mg KOH/g acid value. This acid value is also higher than that of sunflower oil (3.09 mg KOH/g) but is still lower than that of olive oil (6.6 mg KOH/g). This comparison of acid values is closely related to other species of *Garcinia*, such as the following: *G. cambogia* (5.04 mg KOH/g), *G. gummi-gutta*

(3.7 mg KOH/g), *G. mangostana* (4.5 mg KOH/g), *G. xanthochymus* (4.8 mg KOH/g), *G. indica* (4.9 mg KOH/g). The presented acid value is in the range of the other species of *Garcinia*, such as *G. cambogia*. A moderate acid value like this indicates good oil stability, which makes the *G. lanceifolia* seed oil still a preferred choice despite the need for refining by reducing more free fatty acids for improving shelf life and reducing rancidity.

The peroxide value (2 ± 0.349 meq/kg) of *G. lanceifolia* seed oil is lower than that of cold-pressed mustard (3.66 meq/kg), sunflower (4.19 meq/kg), olive (6.39 meq/kg), peanut (8.39 meq/kg), and rapeseed oils (9.46 meq/kg). Having a lower peroxide value is a significant advantage because it suggests the oil has better oxidative stability and a longer shelf life; thus, it becomes a feasible option for long-term storage and industrial applications where stability is of paramount importance.

The iodine value of *G. lanceifolia* seed oil, being the measure of unsaturation, is 113 ± 1.29 g/100 g oil. Such a figure puts it among the sunflower oil range (27-135 g/100 g), close to soybean oil (109 g/100 g), and higher than olive oil (94 g/100 g). In terms of iodine value, *G. lanceifolia* is among the *Garcinia* species with the highest value. For instance, the oil of *G. morella* has the value of 39.8 g/100 g, also *G. xanthochymus* (37.4 g/100 g), *G. indica* (39.4 g/100 g), *G. gummi-gutta* (50.2 g/100 g), and *G. mangostana* (51.8 g/100 g). In contrast, it has a lower iodine value than *G. cambogia* (131 g/100 g). The increased iodine value, which characterizes the oil, indicates that it is highly unsaturated and thus more suitable for applications that require oils with a higher degree of unsaturation, such as food and cosmetic products.

The saponification value of *G. lanceifolia* seed oil (189.3 ± 2.8 mg KOH/g) is higher than that of *G. cambogia* (145.36 mg KOH/g) and *G. mangostana* (140.5 mg KOH/g), but lower than that of *G. indica* (200.2 mg KOH/g) and about the same as *G. gummi-gutta* (187.9 mg KOH/g) and *G. xanthochymus* (190.3 mg KOH/g). The higher saponification value shows that the oil contains a higher percentage of lower-molecular-weight triglycerides. This makes it well-suited for industrial soap and cosmetic applications.

The specific gravity, which is 0.879, and the viscosity, which is 29.18, of the seed oil extracted with hexane, indicate that *G. lanceifolia* oil has a low viscosity and is not as occlusive as hydrocarbon-based oils. Given this first characteristic plus the moderate specific gravity, the *G. lanceifolia* oil can be compared to oleic acid. Such oil would not only be suitable for use as a general cosmetic compound, but also for a new segment of industrial applications.

1. Fatty acid Methyl ester (FAME) analysis of *G. lanceifolia* seed oil

The analysis was conducted to determine the oil's fatty form, as observed in the seed oil extract of *G. lanceifolia*. The four fatty acids detected are either saturated (Stearic acid, Palmitic acid) or have two double bonds (Linoleic acid) or only one double bond (Oleic acid). As can be seen, Stearic acid, Palmitic acid, Oleic acid, Linoleic acid, and the bean oil of the cited *G. lanceifolia* species are not the only sources of these fatty acids; the bean oils of *G. gummi-gutta*, *G. indica*, *G. xanthochymus*, *G. mangostana*, and *G. morella* are also good sources of these fatty acids. The major compound among the four is a saturated one, methyl stearate, which accounts for 56.93% of the total contribution, followed by 29.91% of oleate, 10.75% of Linoleate, and finally 2.41% of palmitate. According to the report, the agrochemicals methyl stearate and methyl palmitate are eco-friendly for banana plants, reducing damage from root-knot nematodes. Vegetable and seed oils are among the sources of fatty acid methyl esters (FAMES), which have antioxidant and antifungal properties. The *G. lanceifolia* seed oil is highly fatty. Although methyl stearate, the most significant component, is beneficial and can also affect high blood pressure through the heart condition, a situation where drugs are required, and in the prevention of cancer, it would also be then.

Seed oil extracted from *G. lanceifolia* has been found to have promising potential for use in various sectors, including the food, cosmetics, and pharmaceutical industries, due to its composition of stearic, oleic, linoleic, and palmitic acids. The dominance of the esters of saturated fatty acids led by methyl stearate (56.93%) and methyl oleate (29.91%) as well as a low peroxide value (2 ± 0.349 meq/kg) shows that the oil has got good oxidative stability and an extended shelf life, thus being

suited for storing over longer periods of time or to be employed for industrial purposes. Also, the detected iodine value of 113 ± 1.29 g/100 g oil placed it at a moderately unsaturated level; this, along with the oil's density, further indicates its possible application in cases requiring oil purity, such as in the cosmetic and food industries. The characterization and identification of the long-chain fatty acids were performed by matching them to the NIST library and were further supported by UV-visible, FTIR, and ^1H NMR spectra, as well as their characteristic ^{13}C NMR peaks.

Synthesis

This research provides a comprehensive scientific assessment of *G. lanceifolia* Roxb., an endemic and underutilized fruit tree of the Indo-Burma hotspot. It moves beyond basic botanical description to deliver profound insights into the species' reproductive ecology, functional biology, and economic potential. The confirmation of androdioecy places *G. lanceifolia* within a select group of plants with a rare and evolutionarily significant sexual system. The documentation of high phenotypic plasticity and site-specific variation in morphological and reproductive traits underscores the species' capacity for local adaptation, a critical trait in the face of environmental change. The elucidation of its facultative mixed mating system, combining sexual outcrossing with apomixis, reveals a resilient reproductive strategy that balances genetic diversity with reproductive assurance. From a practical standpoint, the research delivers ready-to-use protocols for breaking seed dormancy (KNO_3 priming), promoting seedling vigor (GA_3 application), and optimizing nursery production (vermicompost-enriched substrate). These protocols demystify the propagation of this previously recalcitrant species. Furthermore, the characterization of the high-yield, high-quality seed oil opens a tangible pathway for economic valorization, linking conservation directly to livelihood security. In conclusion, the findings of this study provide a robust scientific foundation for the conservation and sustainable use of *G. lanceifolia*, offering a clear roadmap for its domestication and value addition, and serving as a model for the integrated conservation of neglected yet valuable species in the Indo-Burma hotspot.