Seed biology of *Hopea ponga* (Dennst.) Mabb. (Dipterocarpaceae): An Endemic and Threatened Tree Species of the Western Ghats

Thesis Submitted to
the University of Calicut in partial fulfillment of
the requirement for the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

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Research Supervisors

Dr. N.S. PRADEEP
Dr. P.N. KRISHNAN

KSCSTE-MALABAR BOTANICAL GARDEN & INSTITUTE FOR PLANT SCIENCES
Kozhikode, Kerala, 673014, India

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CERTIFICATE

This is to certify that the thesis entitled "**Seed biology of *Hopea ponga* (Dennst.) Mabb. (Dipterocarpaceae): An endemic and threatened tree species of the Western Ghats**" submitted to the University of Calicut by **Mr. Mithun Venugopal**, in partial fulfillment for the award of the degree of Doctor of Philosophy in Botany is a bonafide record of the research work carried out by him under our supervision and guidance. No part of the present work has previously formed the basis for the award of any other degree or diploma.

Dr. P. N. Krishnan
Co-guide

Dr. N. S. Pradeep
Guide

Kozhikode
Date:

DECLARATION

I hereby declare that the work presented in the thesis entitled "**Seed biology of *Hopea ponga* (Dennst.) Mabb. (Dipterocarpaceae): An endemic and threatened tree species of the Western Ghats**" is based on the original work done by me under the guidance of **Dr. N. S. Pradeep** and **Dr. P.N. Krishnan** and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone a plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

Mithun Venugopal

Dr. N. S. Pradeep

Kozhikode
Date:

Dr. P. N. Krishnan

ACKNOWLEDGEMENTS

I wish to convey my profound gratitude to my Supervisors, Dr. N.S. Pradeep (Principal Scientist & Scientist in Charge, KSCSTE-MBGIPS) and Dr. P.N. Krishnan (Former Director Grade Scientist & Head of Biotechnology and Bioinformatics Division, KSCSTE-JNTBGRI). Their unwavering trust, generous investment of time, and nurturing care were often the sole motivators during the most challenging phases of my work. Having supervisors endowed with remarkable scientific acumen and exceptional human virtues was a true honour. Their dedication to my work, readiness to assist, and prompt, patient responses to my inquiries, always accompanied by invaluable advice, have been deeply appreciated. My heartfelt and abundant thanks go to them.

I gratefully acknowledge Dr. S. Pradeep Kumar, Former Director in Charge of KSCSTE-Malabar Botanical Garden, Kozhikode, for providing the necessary facilities and infrastructure for my research work.

Very special gratitude goes to Dr. P.V. Madhusoodanan (Former Professor & Head of the Botany Department, University of Calicut and Former Emeritus Scientist, KSCSTE-MBGIPS) for his untiring support, encouragement and suggestions to bring this work towards completion. I sincerely acknowledge the help and encouragement of Prof. M. Sabu, CSIR Emeritus Scientist, KSCSTE-MBGIPS, for my study.

I thank Dr. R Jayaraj (Principal Scientist, KSCSTE-KFRI) and Dr. Rajeev K Sukumaran (Senior Principal Scientist, CSIR-NIIST) for their valuable suggestions, constant support, and extended facilities.

Needs a special mention to my friends Dr. Neethu R.S. (Assistant Professor, JDT Islam College of Arts & Science), Dr. Karthik Menon (Senior Research Associate, Bioplus Life Sciences), Dr. Anoop P Balan (Assistant Professor, BAM College), Dr. Jisha Daniel (Assistant Professor, Iqbal College), Dr. Babu K.P, Late Dr. Kamarudheen Kunju (Dept. of Botany, Former Assistant Professor, Kerala University), Mrs. Akhalya Rajendran for the fruitful discussions, valuable suggestions and key points and, unbelievable assistance throughout the period.

I express my sincere thanks to my seniors and colleagues, Dr. Anoop K.P., Dr. Rajilesh V.K., Dr. Prajitha B., Dr. Saranya Babu Jayaprakash, Dr. Swetha Thilak T, Dr. Pavisha P., Dr. Sojan Jose (Assistant Professor, Dept. of Botany, Govt. Victoria College), Mrs. Athulya Sadanand C.P., Mrs. Sinisha E.K, Mr. Bibin Joseph, Mrs. Jaseela V.T., Ms. Pooja Pushkaran and Mr. Harshid P for their enthusiasm and help rendered me during the study. I also acknowledge the help of former field assistant Mr. Lijo C.J.

I thank the field guides, Garden supervisors, and technical Assistants at MBGIPS for their help during my collection trips. I also thank the administrative staff of MBGIPS for their help and support.

I want to express my heartfelt appreciation to the Forest Department and Kerala officials for permitting me to conduct field studies in reserve forests and protected areas.

This would be impossible without my family's constant help, support, love and care. I am cheerfully indebted to my better half Ragina C.T., for the everlasting support and understanding of my goals and aspirations. I find no words to express my loving gratefulness to beloved Mother Remadevi P., Father Venugopalan C., and Sister Mithula Venugopal, who raised me through constant moral support, relieving me from domestic chores, which helped me to concentrate absolutely on the academic chase. I would like to thank all my teachers for their encouragement sincerely.

Mithun Venugopal

LIST OF ABBREVIATIONS

%	Percentage
µg	Microgram
µM	Micro molar
µl	Microlitre
µmol	Micromole
^{0}	Degree
km	Kilometre
^{0}C	Centigrade
A	Absorbance
cm	Centimetre
et al.	et aliorum (and of others)
g	Gram
h	Hour
m	Meter
mg	Milligram
ng	Nanogram
mg/ml	Milligram per millilitre
ml	Millilitre
mM	Millimolar
mm	Millimetre
CBD	Convention on Biological Diversity
N	Normality
NaCl	Sodium chloride
NaOH	Sodium Hydroxide
pH	Hydrogen potential
rpm	Rotations per Minute
UV	Ultraviolet
V	Volts
LEA	Late Embryogenesis Abundant proteins
SMP	Seed maturation protein
UNESCO	United Nations Educational, Scientific, and Cultural Organization
IUCN	International Union for Conservation of Nature
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
MIC	Minimum Inhibitory Concentration
FRAP	Fluorescence recovery after photobleaching
H_2O_2	Hydrogen Peroxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
MnNPs	Manganese Nanoparticles
MTT	3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
MC	Moisture content
EOO	Extension of occurrence
AOO	Area of occupancy
m^2	Meter square
km^2	Kilometre square
DAA	Day after anthesis

ISTA	International Seed Testing Association
RH	Relative humidity
α	Alpha
β	Beta
nm	Nanometer
TCA	Trichloroacetic acid
&	and
v/v	Volume/volume
PCA	Perchloric acid
w/v	Weight/Volume
dr. wt.	Dry weight
fwt.	Fresh weight
Na_2CO_3	Sodium carbonate
H_2SO_4	Sulphuric acid
BSA	Bovine serum albumin
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
MDA	Malondialdehyde
EC	Electrical conductivity
HPLC	High-performance liquid chromatography
EDTA	Ethylenediaminetetraacetic acid
NBT	Nitro blue tetrazolium
POD	Peroxidase
PPO	Polyphenol oxidase
CAT	Catalase
SOD	Superoxide dismutase
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Dipotassium phosphate
FT-IR	Fourier Transform Infrared Spectroscopy
LC-MS	Liquid Chromatography Mass Spectrometry
CE	Collision energy
DP	Declustering potential
D	Days after storage
B-ME	β-mercaptoethanol
SDS	Sodium Dodecyl Sulphate
HCl	Hydrochloric acid
MW	Molecular weight
H_2O	Water
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TEMED	N,N,N′,N′ -Tetramethylethylenediamine
DMRT	Duncan's Multiple Range Test
ANOVA	Analysis of Variance
Lux	Unit of illuminance
sec.	Seconds
hrs	Hours
SE	Standard error
PLU	Plumule
RAD	Radicle
COT	Cotyledon
EMB	Embryonal axes
WS	Whole seed
±	Plus-or-minus sign

HAH	Hours after harvest
PB	Polycarbonate bottles
AF	Aluminium foil
PC	Polythene bags
OT	Open tray
CV	Cryo vials
HPDM	Dried seeds with Methanol
HPDA	Dried seeds in Acetone
HPDH	Dried seeds in Hexane
HPFM	Fresh seeds in Methanol
HPFA	Fresh seeds in Acetone
HPFH	Fresh seeds in Hexane
NaOCl	Sodium hypochlorite
C_2H_5OH	Ethyl Alcohol or Ethanol
PDA	Potato Dextrose Agar
nBLAST	nucleotide-Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
ML	Maximum Likelihood
PCR	Polymerase Chain Reaction
dNTPs	Deoxynucleotide Triphosphates
ITS	Internal transcribed spacer
TE	Tris-EDTA
CTAB	Cetyltrimethylammonium bromide
Lys	Lysine
Arg	Arginine
Thr	Threonine
Gly	Glycine
Cys	Cysteine
Ala	Alanine
Glu	Glutamate
Trp	Tryptophan
ABA	Abscisic acid
THPG	Trihydroxy-phenanthrene glucoside
ROS	Reactive oxygen species
FA	Fatty acid
ATP	Adenosine triphosphate
MSL	Mean Sea Level
Kb	Kilobase
TSS	Total Soluble Sugars
AMY	Alpha-amylase
BAM	Beta-amylase

ABSTRACT

Seeds are a fundamental component of the plant life cycle, as they store the genetic information necessary for the next generation of plants to disperse, establish, develop and eventually reproduce to maintain the species. In tropical rainforests, there is still limited information on the physiology of seeds, phenology data, and ecological perspectives. Most tropical seeds have a short lifespan in soil and become non-viable. The genus Hopea is the largest genus in the Dipterocarpaceae family and is widely distributed throughout the Western Ghats, from lowland evergreen forests on the coastal plains to tropical evergreen forests.

The physiological and biochemical studies provide strong evidence of the hard-to-process nature of the seeds of *Hopea ponga*. This is consistent with their quick growth after falling into damp conditions. The study has also demonstrated that the metabolism of active sugars, proteins, and lipids increased, along with lipid peroxidation, activities of free radical scavenging enzymes such as peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT), electrolyte leakage, phenolic accumulation, and more during the drying out of the seeds. These factors further affected the level of enzyme activities, lipid peroxidation, sugar metabolism, and more. Thus, the active metabolism and related changes during seed desiccation can be signals for identifying seed viability. The study suggests that the optimal seed storage conditions should be 20±2°C&40% RH in closed polycarbonate bottles for *H. ponga*.

The metabolic imbalances that develop during various treatments can provide valuable information about the properties of different biomolecules and their functional behaviour. These imbalances can be considered markers for detecting viability and can help develop proper conservation measures through seed banking.

The equilibrium of species in natural habitats has been destabilized due to human encroachment, endemic nature, over-exploitation, recalcitrancy, habitat specificity, irregular fruiting cycles, and other factors. This study could serve as a model for future research to better understand the seed physiology and biochemistry of other important species. Therefore, the study accomplishes the dual goals of conservation and sustainable utilization, particularly concerning the unique resources found in the Western Ghats.

സാരാംശം

അനുദിനം വികസിച്ചുകൊണ്ടിരിക്കുന്ന ജൈവശാസ്ത്രലോകത്ത് വിത്തുകൾക്കും അവയെ കുറിച്ചുള്ള അറിവുകൾക്കും നിർണായകമായ പങ്കാണുള്ളത്. ഒരു ചെടിയുടെ ജീവിതചക്രത്തിൽ അനിർവചനീയമായ സ്ഥാനമാണ് വിത്തിന്. ഉഷ്ണമേഖലാ മഴക്കാടുകളിൽ കാണപ്പെടുന്ന സസ്യങ്ങളിലെ വിത്തുകളുടെ ജൈവഘടനയെ കുറിച്ചും അവയുടെ സങ്കീർണമായ പാരിസ്ഥിതിക ബന്ധങ്ങളെ കുറിച്ചും വളരെ പരിമിതമായ അറിവുകൾ മാത്രമേ നിലവിലുള്ളൂ, എന്നാൽ നിലനിൽക്കുന്ന അറിവുകൾ പരിമിതവും ക്രോഡീകരിക്കപ്പെടാത്തതുമാണ്. ഭൂരിഭാഗം ഉഷ്ണമേഖലാ വൃക്ഷങ്ങളുടെ വിത്തുകളും മണ്ണിൽ വളരെ കുറച്ചുകാലത്തേക്ക് മാത്രം ജീവനോടെ നിലനിൽക്കുകയും അതിന് ശേഷം അവ നിർജീവമാവുകയും ചെയ്യുന്നു. ഡിപ്റ്റെറോകാർപേസിയെ എന്ന വംശനാശ ഭീഷണി നേരിടുന്ന സസ്യകുടുംബത്തിലെ ഏറ്റവും വലിയ ജനുസാണ് ഹോപ്പിയ. ഈ ജനുസിലെ പ്രധാനപ്പെട്ട അംഗമാണ് ഹോപ്പിയ പോങ്ങ. പശ്ചിമഘട്ടത്തിലുടനീളവും, തീരദേശ സമതലങ്ങളിലെ താഴ്ന്ന പ്രദേശങ്ങളിലും നിത്യഹരിത വനങ്ങൾ മുതൽ ഉഷ്ണമേഖലാ നിത്യഹരിത വനങ്ങൾ വരെയും ഇവയെ കാണപ്പെടുന്നു. ഐ.യു.സി.എന്നിന്റെ വംശനാശ ഭീഷണി നേരിടുന്ന അംഗങ്ങളെ ഉൾപ്പെടുത്തിയിട്ടുള്ള റെഡ് ഡാറ്റ ബുക്കിൽ ഈ സസ്യത്തെ വൾനെറബിൽ എന്ന വിഭാഗത്തിൽ ഉൾപ്പെടുത്തിയിരിക്കുന്നു .

റീകാൾസിട്രെന്റ് (വിത്തുകൾ മണ്ണിൽ എത്തിയാൽ അതിന്റെ ഈർപ്പം ക്രമാതീതമായി കുറയുംമുൻപ് ദ്രുതഗതിയിൽ മുളയ്ക്കുന്നുവ) സ്വഭാവമാണ് ഹോപ്പിയ പോങ്ങയുടെ വിത്തുകൾ പ്രകടിപ്പിക്കുന്നതെന്ന് ഫിസിയോളോജിക്കൽ, ബയോക്കെമിക്കൽ പഠനങ്ങളിലൂടെ സാക്ഷ്യപ്പെടുത്തിയിട്ടുള്ളതാണ്. വിവിധങ്ങളായ എൻസയിമുകൾ വിത്തുകളുടെ ഘടനയെ എങ്ങനെ സ്വാധീനിക്കുന്നു എന്നും അവ വിത്തിന്റെ മറ്റ് ജൈവീക ഘടകങ്ങളുമായി എങ്ങനെ ബന്ധപ്പെട്ടിരിക്കുന്നു എന്നും ഈ പഠനത്തിന്റെ ഭാഗമായി കണ്ടെത്തിയിട്ടുണ്ട്. സ്വാഭാവിക കാലാവസ്ഥയിൽ 7 ദിവസം കൊണ്ടു തന്നെ വിത്തുകളുടെ മുളയ്ക്കുവാനുള്ള ശേഷി നഷ്ടപ്പെടുന്നു, എന്നാൽ 20±2°C ഊഷ്മാവിൽ 40% ആപേക്ഷിക ആർദ്രതയിൽ സൂക്ഷിച്ചാൽ ഈ വിത്തുകളെ 210 ദിവസങ്ങൾ വരെ അവയുടെ മുളയ്ക്കുവാനുള്ള ശേഷി നഷ്ടപ്പെടാതെ നിലനിർത്തുവാനും സാധിക്കും എന്നും കണ്ടെത്തിയിട്ടുണ്ട്.

വ്യത്യസ്തമായ ജൈവ തന്മാത്രകൾ വിത്തിന്റെ ജൈവ ഘടനയെ എങ്ങനെ സ്വാധീനിക്കുന്നു എന്നും അതിൽ വരുന്ന വ്യത്യാനങ്ങൾ മനസിലാക്കി വിത്തിനെ ദീർഘകാല അടിസ്ഥാനത്തിൽ ഏതെല്ലാം രീതിയിൽ

സൂക്ഷിച്ചുവെയ്ക്കാൻ സാധിക്കുമെന്നും കണ്ടെത്താൻ കഴിഞ്ഞിട്ടുണ്ട്. ഭാവിയിൽ സീഡ് ബാങ്കിംഗ് സാങ്കേതികവിദ്യ കൂടി സമന്വയിപ്പിച്ചു വംശനാശ ഭീഷണി നേരിടുന്ന ഈ വൃക്ഷത്തിന്റെ സംരക്ഷണ പ്രക്രിയക്ക് ആക്കം കൂട്ടാൻ ഇത്തരം പഠനങ്ങൾ സഹായകമാകും, എന്നും വിശ്വസിക്കുന്നു.

ഈ പഠനത്തിലൂടെ, പശ്ചിമഘട്ടത്തിലെ ജൈവവൈവിധ്യ സംരക്ഷണത്തിന്റെയും അവയുടെ സുസ്ഥിരമായ ഉപയോഗത്തിന്റെയും ബൃഹത്‌ലക്ഷ്യങ്ങൾ കൈവരിക്കാൻ സാധിച്ചു. മനുഷ്യരുടെ കടന്നുകയറ്റം, ഈ വൃക്ഷത്തിന്റെ വ്യാവസായിക ഉപയോഗത്തിനായി അമിതമായ രീതിയിൽ ചൂഷണം ചെയ്യുന്നതും, ക്രമരഹിതമായ രീതികളിൽ പൂക്കുകയും കായ്ക്കുകയും, സൂക്ഷ്മജീവികളുടെയും പ്രാണികളുടെയും ആക്രമണം തുടങ്ങിയവ പ്രകൃതിദത്ത ആവാസവ്യവസ്ഥയിലെ ഹോപ്പിയ പോങ്ങയുടെ സന്തുലിതാവസ്ഥയെ അസ്ഥിരപ്പെടുത്തുന്നു എന്ന് മനസിലാക്കാൻ സാധിക്കുന്നു. ഹോപ്പിയ പോങ്ങയിൽ നടത്തിയ ഈ പഠനം മറ്റ് ഉഷ്ണമേഖലാ മഴക്കാടുകളിൽ അധിവസിക്കുന്ന ഡിപ്റ്റെറോകാർപേസിയെ വൃക്ഷങ്ങളിലും മറ്റ് വംശനാശ ഭീഷണി നേരിടുന്ന സസ്യങ്ങളിലും മാതൃകയായി അവലംബിക്കാൻ സഹായകമാകും.

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3.	Name of the Supervisor	Dr. N.S. Pradeep (Guide) Dr. P.N. Krishnan (Co-guide)	
4.	Department/Institution	KSCSTE-Malabar Botanical Garden & Institute for Plant Sciences, Kozhikode	
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Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

ABSTRACT

Seeds are a fundamental component of the plant life cycle, as they store the genetic information necessary for the next generation of plants to disperse, establish, develop and eventually reproduce to maintain the species. In tropical rainforests, there is still limited information on the physiology of seeds, phenology data, and ecological perspectives. Most tropical seeds have a short lifespan in soil and become non-viable. The genus Hopea is the largest genus in the Dipterocarpaceae family and is widely distributed throughout the Western Ghats, from lowland evergreen forests on the coastal plains to tropical evergreen forests.

The physiological and biochemical studies provide strong evidence of the hard-to-process nature of the seeds of *Hopea ponga*. This is consistent with their quick growth after falling into damp conditions. The study has also demonstrated that the metabolism of active sugars, proteins, and lipids increased, along with lipid peroxidation, activities of free radical scavenging enzymes such as peroxidase (POD), polyphenol oxidase (PPO), and catalase (CAT), electrolyte leakage, phenolic accumulation, and more during the drying out of the seeds. These factors further affected the level of enzyme activities, lipid peroxidation, sugar metabolism, and more. Thus, the active metabolism and related changes during seed desiccation can be signals for identifying seed viability. The study suggests that the optimal seed storage conditions should be 20±2°C&40% RH in closed polycarbonate bottles for *H. ponga*.

The metabolic imbalances that develop during various treatments can provide valuable information about the properties of different biomolecules and their functional behaviour. These imbalances can be considered markers for detecting viability and can help develop proper conservation measures through seed banking.

The equilibrium of species in natural habitats has been destabilized due to human encroachment, endemic nature, over-exploitation, recalcitrancy, habitat specificity, irregular fruiting cycles, and other factors. This study could serve as a model for future research to better understand the seed physiology and biochemistry of other important species. Therefore, the study accomplishes the dual goals of conservation and sustainable utilization, particularly concerning the unique resources found in the Western Ghats.

Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

സാരാംശം

അനുദിനം വികസിച്ചുകൊണ്ടിരിക്കുന്ന ജൈവശാസ്ത്രലോകത്ത് വിത്തുകൾക്കും അവയെ കുറിച്ചുള്ള അറിവുകൾക്കും നിർണായകമായ പങ്കാണുള്ളത്. ഒരു ചെടിയുടെ ജീവിതചക്രത്തിൽ അനിർവചനീയമായ സ്ഥാനമാണ് വിത്തിന്. ഉഷ്ണമേഖലാ മഴക്കാടുകളിൽ കാണപ്പെടുന്ന സസ്യങ്ങളിലെ വിത്തുകളുടെ ജൈവഘടനയെ കുറിച്ചും അവയുടെ സങ്കീർണമായ പാരിസ്ഥിതിക ബന്ധങ്ങളെ കുറിച്ചും വളരെ പരിമിതമായ അറിവുകൾ മാത്രമേ നിലവിലുള്ളൂ, എന്നാൽ നിലനിൽക്കുന്ന അറിവുകൾ പരിമിതവും ക്രോഡീകരിക്കപ്പെടാത്തതുമാണ്. ഭൂരിഭാഗം ഉഷ്ണമേഖലാ വൃക്ഷങ്ങളുടെ വിത്തുകളും മണ്ണിൽ വളരെ കുറച്ചുകാലത്തേക്ക് മാത്രം ജീവനോടെ നിലനിൽക്കുകയും അതിന് ശേഷം അവ നിർജീവമാവുകയും ചെയ്യുന്നു. ഡിപ്റ്റെറോകാർപേസിയെ എന്ന വംശനാശ ഭീഷണി നേരിടുന്ന സസ്യകുടുംബത്തിലെ ഏറ്റവും വലിയ ജനുസാണ് ഹോപ്പിയ. ഈ ജനുസിലെ പ്രധാനപ്പെട്ട അംഗമാണ് ഹോപ്പിയ പോങ്ങ. പശ്ചിമഘട്ടത്തിലുടനീളവും, തീരദേശ സമതലങ്ങളിലെ താഴ്ന്ന പ്രദേശങ്ങളിലും നിത്യഹരിത വനങ്ങൾ മുതൽ ഉഷ്ണമേഖലാ നിത്യഹരിത വനങ്ങൾ വരെയും ഇവയെ കാണപ്പെടുന്നു. ഐ.യു.സി.എന്നിന്റെ വംശനാശ ഭീഷണി നേരിടുന്ന അംഗങ്ങളെ ഉൾപ്പെടുത്തിയിട്ടുള്ള റെഡ് ഡാറ്റ ബുക്കിൽ ഈ സസ്യത്തെ വൾനെറബിൽ എന്ന വിഭാഗത്തിൽ ഉൾപ്പെടുത്തിയിരിക്കുന്നു .

റീകാൾസിട്രെന്റ് (വിത്തുകൾ മണ്ണിൽ എത്തിയാൽ അതിന്റെ ഈർപ്പം ക്രമാതീതമായി കുറയുംമുൻപ് ദ്രുതഗതിയിൽ മുളയ്ക്കുന്നുവ) സ്വഭാവമാണ് ഹോപ്പിയ പോങ്ങയുടെ വിത്തുകൾ പ്രകടിപ്പിക്കുന്നതെന്ന് ഫിസിയോളോജിക്കൽ, ബയോക്കെമിക്കൽ പഠനങ്ങളിലൂടെ സാക്ഷ്യപ്പെടുത്തിയിട്ടുള്ളതാണ്. വിവിധങ്ങളായ എൻസയിമുകൾ വിത്തുകളുടെ ഘടനയെ എങ്ങനെ സ്വാധീനിക്കുന്നു എന്നും അവ വിത്തിന്റെ മറ്റ് ജൈവീക ഘടകങ്ങളുമായി എങ്ങനെ ബന്ധപ്പെട്ടിരിക്കുന്നു എന്നും ഈ പഠനത്തിന്റെ ഭാഗമായി കണ്ടെത്തിയിട്ടുണ്ട്. സ്വാഭാവിക കാലാവസ്ഥയിൽ 7 ദിവസം കൊണ്ടു തന്നെ വിത്തുകളുടെ മുളയ്ക്കുവാനുള്ള ശേഷി നഷ്ടപ്പെടുന്നു, എന്നാൽ 20±2°C ഊഷ്മാവിൽ 40% ആപേക്ഷിക ആർദ്രതയിൽ സൂക്ഷിച്ചാൽ ഈ വിത്തുകളെ 210 ദിവസങ്ങൾ വരെ അവയുടെ മുളയ്ക്കുവാനുള്ള ശേഷി നഷ്ടപ്പെടാതെ നിലനിർത്തുവാനും സാധിക്കും എന്നും കണ്ടെത്തിയിട്ടുണ്ട്.

വ്യത്യസ്തമായ ജൈവ തന്മാത്രകൾ വിത്തിന്റെ ജൈവ ഘടനയെ എങ്ങനെ സ്വാധീനിക്കുന്നു എന്നും അതിൽ വരുന്ന വ്യത്യാനങ്ങൾ മനസിലാക്കി വിത്തിനെ ദീർഘകാല അടിസ്ഥാനത്തിൽ ഏതെല്ലാം രീതിയിൽ

സൂക്ഷിച്ചുവെയ്ക്കാൻ സാധിക്കുമെന്നും കണ്ടെത്താൻ കഴിഞ്ഞിട്ടുണ്ട്. ഭാവിയിൽ സീഡ് ബാങ്കിംഗ് സാങ്കേതികവിദ്യ കൂടി സമന്വയിപ്പിച്ചു വംശനാശ ഭീഷണി നേരിടുന്ന ഈ വൃക്ഷത്തിന്റെ സംരക്ഷണ പ്രക്രിയക്ക് ആക്കം കൂട്ടാൻ ഇത്തരം പഠനങ്ങൾ സഹായകമാകും, എന്നും വിശ്വസിക്കുന്നു.

ഈ പഠനത്തിലൂടെ, പശ്ചിമഘട്ടത്തിലെ ജൈവവൈവിധ്യ സംരക്ഷണത്തിന്റെയും അവയുടെ സുസ്ഥിരമായ ഉപയോഗത്തിന്റെയും ബൃഹത്‌ലക്ഷ്യങ്ങൾ കൈവരിക്കാൻ സാധിച്ചു. മനുഷ്യരുടെ കടന്നുകയറ്റം, ഈ വൃക്ഷത്തിന്റെ വ്യാവസായിക ഉപയോഗത്തിനായി അമിതമായ രീതിയിൽ ചൂഷണം ചെയ്യുന്നതും, ക്രമരഹിതമായ രീതികളിൽ പൂക്കുകയും കായ്ക്കുകയും, സൂക്ഷ്മജീവികളുടെയും പ്രാണികളുടെയും ആക്രമണം തുടങ്ങിയവ പ്രകൃതിദത്ത ആവാസവ്യവസ്ഥയിലെ ഹോപ്പിയ പോങ്ങയുടെ സന്തുലിതാവസ്ഥയെ അസ്ഥിരപ്പെടുത്തുന്നു എന്ന് മനസിലാക്കാൻ സാധിക്കുന്നു. ഹോപ്പിയ പോങ്ങയിൽ നടത്തിയ ഈ പഠനം മറ്റ് ഉഷ്ണമേഖലാ മഴക്കാടുകളിൽ അധിവസിക്കുന്ന ഡിപ്റ്റെറോകാർപേസിയെ വൃക്ഷങ്ങളിലും മറ്റ് വംശനാശ ഭീഷണി നേരിടുന്ന സസ്യങ്ങളിലും മാതൃകയായി അവലംബിക്കാൻ സഹായകമാകും.

INTRODUCTION

One of the world's eight "hottest hotspots" of biodiversity is the Western Ghats Mountain range, also known as the Sahyadri Range, a UNESCO World Heritage Site. The territory covers over 1,40,000 square kilometres and runs over 1,600 kilometres alongside the western coast of India. The Western Ghats, the unique mountain ranges, spans 6 Indian states, Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra and Gujarat. It runs parallel to the country's western coast with an altitude range from 100- 2695 meters. Due to their high levels of endemism and species richness, the Western Ghats are known as a biodiversity hotspot. Threatened animals like the Lion-tailed Macaque, Nilgiri Tahr, Malabar Civet, and Nilgiri Marten are Vulnerable, endangered and critically endangered animals exclusively habited in the Western Ghats. A wide variety of plant species, including many medicinal plants used in conventional medicine, are found in the forests of the Western Ghats. The Western Ghats are, however, under threat from several factors, including invasive species, climate change, over-exploitation of natural resources, habitat loss, and fragmentation. Habitat damage and biodiversity loss are caused in the area by human activities such as agriculture, mining, and deforestation. To preserve the Western Ghats' biodiversity, conservation activities are required. National parks and animal sanctuaries are only a few protected places that have been developed in the area. These protected areas support sustainable tourism while preserving the area's distinctive flora and fauna. The Western Ghats should be conserved because they are essential for local populations' livelihoods and the region’s climate and biodiversity.

Importance of Western Ghats Forests

The Western Ghats are renowned for their various forest types, which provide a wide diversity of flora and fauna and essential ecological

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functions. Tropical rainforests, evergreen forests, semi-evergreen forests, moist deciduous forests, dry deciduous forests, and shola woods are among the significant kinds of forests in the Western Ghats.

Evergreen, thick woods with considerable annual precipitation are known as tropical rainforests. They are distinguished by their towering trees, dense undergrowth, and lush canopy. There are several plant species present in the Western Ghats' rainforests. Evergreen forests are comparable to tropical rainforests in that they maintain their green foliage all year round. These woods are home to various tree species, including enormous hardwood trees and lower-canopy plants that flourish in the shadow. Semi-evergreen forests are just what their name implies: they combine evergreen and deciduous traits. During specific months, they go through a phase of leaf shedding, yet they still have a sizable amount of green foliage. Between deciduous woods and rainforests, semi-evergreen forests are a transitional stage. The Moist Deciduous Forests are those with a distinct dry season, the bulk of the trees sheds their leaves. They are mainly differentiated by the fusion of both deciduous and evergreen trees and a thick undergrowth of shrubs, herbs, and grasses.

Dry Deciduous Forests are prevalent in areas with lower annual precipitation, and dry deciduous forests experience prolonged droughts. Most trees lose their leaves during the dry season to cope with the lack of water. These woodlands frequently contain prickly and succulent vegetation. Shola woods are distinct and crucial for the environment in the Western Ghats at higher elevations. Evergreen trees stunted and covered in an understory of grasses and shrubs define shola forests. They play a significant role in managing water supplies and are interspersed with grasslands known as "sholas." The largest shola-type forest reserve in the world is near Munnar, in the Mannavan Shola forest region. Mannavan Shola comes under the Marayoor Forest Range of Munnar Forest Division

and is located between 10°10' 00 and 10°12' 18" North latitudes and 77° 9' 50" and 77° 12' 18" East longitudes. The altitudes range between 1600 to 2400 m MSL. Each of these many types of forests has unique ecological importance and is home to a wide range of flora and fauna. The wide variety of vegetation of the Western Ghats considerably contributes to the region's total biodiversity.

Biodiversity Hotspot and Centre of Endemism

The Western Ghats is home to an astonishing variety of plant and animal species, many of which are endemic to the region. More than 7400 species of angiosperms are reported from the Western Ghats, among them 5588 are native or indigenous to the Western Ghats (examples are *Kingiodendron pinnatum* (Roxb. Ex DC.) that are endemic to the southern Western Ghats, *Cynometra travancorica* Bedd. is endemic to Western Ghats in Karnataka, Tamil Nadu, Kerala, *Atuna travancorica* (Bedd.) Kosterm. is endemic to the Western Ghats, Tamil Nadu, Kerala, and *Madhuca bourdillonii* (Gamble) H. J. Lam. is endemic to the southern Western Ghats) and Rest 376 are exotics naturalised and 1438 are cultivated or ornamental species. Further analysis showed that 2253 are endemic to India of them 1273 are exclusively confined to W. Ghats. It is estimated that over 5,000 plant species, 139 mammal species, 508 bird species, 179 amphibian species, and 290 freshwater fish species are endemic to the region (Nayar et al., 2014).

Rare and Endangered Species: The Western Ghats are also home to rare and endangered plant species, such as *Vateria macrocarpa* Gupta (Valiya-vellapayin, Perumpain), *Hopea erosa* (Bedd.) Slooten (Eeyakam, Karakongu), *Aglaia malabarica* Sasidharan (Chuvanna cheeralam, Chuvanna akil), *Cynometra beddomei* Prain (Valliyakoori), *Vateria indica* L., *Garcinia travancorica* Bedd. and *Garcinia imberti* Bourd are some of the species.

Medicinal Plants: Many plants found in the Western Ghats have medicinal properties and are used in traditional folk medicine. The region is rich in plant-based remedies for a wide range of ailments, like "Arogyapacha," the local name for *Trichopus zeylanicus*, a rare and indigenous medicinal plant of India. This plant is famous for its traditional use as an instant energy booster.

Water Catchment Area: The Western Ghats are a crucial water catchment area for India's major rivers, including the Godavari, Krishna, Kaveri, Periyar, Bharathapuzha and many others. The Ghats are the source of many perennial rivers that provide water for drinking, irrigation, and hydropower generation.

Ecological Services: The Western Ghats provide a wide range of ecological services, including soil conservation, carbon sequestration, and climate regulation. Their forests are also important for maintaining regional rainfall patterns and preventing soil erosion.

Cultural Significance: The Western Ghats have a rich cultural and historical heritage. Various indigenous tribal communities inhabit the region, preserving their unique traditions, languages, and customs. The Ghats also have ancient temples, forts, and cave systems, reflecting the area's historical significance.

Economic Importance: The Western Ghats support many local communities by producing timber, non-timber forest products, and medicinal plants. The region is also a significant source of spices, including Pepper, Cardamom, and Cinnamon.

Tourism: The Western Ghats attract millions of tourists annually who come to enjoy the region's natural beauty and biodiversity. The Ghats offer various activities such as trekking, camping, wildlife safaris, bird watching, and water sports.

Conservation Efforts: The Western Ghats face several conservation challenges due to their ecological importance. Deforestation, habitat loss, poaching, and encroachment threaten fragile ecosystems. Efforts are being made to protect and conserve the region, including declaring several wildlife sanctuaries and national parks.

The Western Ghats are a treasure trove of plant diversity, containing thousands of unique and valuable plant species. Protecting and conserving this biodiversity hotspot is essential to ensuring the continued well-being of both humans and the environment. It is also essential to protect the Western Ghats from human activities such as deforestation, mining, and land-use change to ensure their long-term sustainability.

Trees

Trees are woody-stemmed perennial plants with branches and leaves that are the norm. The world is home to various tree species, each with a unique set of traits and adaptations. Due to the year-round retention of their leaves (needles), coniferous trees are frequently called evergreens. They are suited to colder regions and have a distinctive cone-shaped appearance. Coniferous trees include pine, spruce, fir, cedar, and cypress trees, to name a few. During specific seasons, often in the fall, deciduous trees lose their leaves. They are renowned for the fall foliage's vivid colors. Oak, maple, birch, beech, poplar, and ash trees are common deciduous tree species. Tropical climates with high temperatures and rainfall are home to tropical plants. They are frequently distinguished by their broad, big leaves and extensive canopies. Baobab, teak, palm, and mahogany trees are a few examples of tropical trees. Forest trees with conifers: Coniferous woods have chilly temperatures and acidic soils and support species well. Spruce, fir, pine, and hemlock trees are a few examples of trees frequently found in coniferous forests. Many trees, including canopy trees that soar above the forest floor, may be found in rainforests. These trees are adapted to

withstand heavy rainfall and sunlight competition. Brazil nut, mahogany, ebony, and kapok trees are a few examples of rainforest trees. Desert trees have unique characteristics that allow them to live in desert climates with little access to water. Mesquite, acacia, Joshua tree, and Desert willow are a few examples of desert plants.

Tropical Trees

The planet's biodiversity depends heavily on tropical trees, which have various ecological and financial advantages. Tropical trees flourish in the tropics, such as the rainforests of Southeast Asia, rainforests in Congo (Africa), and South America's pride, the Amazon rainforests. Using photosynthesis to take in carbon dioxide from the atmosphere, they play a critical part in controlling the planet's temperature. According to estimates, tropical forests hold around 25% of the carbon globally, making them an essential weapon in preventing climate change. A wide variety of plant and animal species, many peculiar to certain places, may be found in tropical trees, which also serve as their home. Some of the most well-known animals in the world, including orangutans, jaguars, and toucans, may be found in these woods. Tropical trees have considerable commercial worth in addition to their ecological significance. They offer wood for building, fuel, and non-timber forest items, including fruits, nuts, and healing herbs. By supplying food, shelter, and income to local populations, the forest also helps them.

Deforestation, habitat loss, and climate change, however, pose threats to tropical plants. Tropical forests are being lost at an alarming rate due to human activities, including mining, logging, and agriculture. This loss of biodiversity significantly impacts both the ecosystem and human well-being. Conservation activities are crucial to save tropical trees and the biodiversity they sustain. These initiatives comprise the creation of protected areas, the promotion of sustainable forestry methods, and

assistance to regional groups in the preservation of their natural resources. By taking these steps, we can ensure that tropical trees continue to benefit the environment and the economy for many years. These trees are highly significant for several reasons, including:

Biodiversity: Tropical trees are home to a wide variety of animal and plant species, many of which are exclusive to these areas. They provide essential habitats for mammals, birds, reptiles, and insects.

Carbon Storage: As they develop, tropical trees take in carbon dioxide from the atmosphere and store it in their biomass. They are, therefore, essential to preventing climate change since they contribute to a decrease in the atmospheric concentration of greenhouse gases.

Water Cycle: Tropical plants are crucial in controlling the water cycle. They prevent soil erosion and manage water runoff through transpiration (water absorption from the soil surface and releasing it into the earth's atmosphere).

Economic Importance: Many tropical trees are economically significant because they provide lumber, fruits, nuts, and other goods that support local livelihoods and national economies.

Medicinal Properties: Numerous plant species that thrive in tropical climates have therapeutic qualities that are applied to cure various illnesses. Many of these medicinal plants have the potential to lead to brand-new treatments and cures but have not yet been thoroughly explored or utilized.

Tropical trees are vital to the survival of our world because they promote biodiversity, mitigate climate change, provide essential ecological services, boost regional and national economies, and hold the promise of novel medical discoveries. Therefore, it is crucial for the welfare of both people and the environment to preserve and protect these woods.

Seeds

Botanically, a seed is a mature ovule containing the embryo with suitable nutritive tissues enclosed with protective layers of the testa or seed coat. In a broad sense, seeds refer to the whole dispersal unit (diaspore). Seeds

contain a unique genetic composition resulting from mixing parental genetic material. They produce seedlings with genetic variation, which in turn enhances the ecological adaptability. Plants depend on seeds for survival and expansion to produce food, habitat, and oxygen for animals and people. They are crucial for preserving environmental equilibrium and biodiversity. Rare and endangered plant species are maintained in seed banks, ensuring their survival and potential use in future scientific studies or agricultural operations. The genetic variety in seeds may also be harnessed to increase agricultural yields and plant resistance to environmental challenges. Overall, the significance of seeds for sustainable agriculture, ecosystem health, and global food security cannot be emphasized.

Seeds are essential to plant life as the origin of growth and reproduction. In addition to the nutrients needed for germination and early development, they carry all the genetic material required to create a new plant. Seeds are essential to maintaining global food security because they are the primary source of many of the world's major crops, including wheat, rice, and corn. They are also necessary for crops that yield fruits, vegetables, and other edibles rich in vitamins and minerals. Animals commonly disperse them by eating the fleshy fruit that encloses the seed, helping to conserve the species and ensure its survival.

However, seeds are also dangerous from various human activities, like tropical plants. Significant dangers to seed variety and accessibility are posed by habitat loss, climate change, and biodiversity loss. The creation of seed banks and conservation initiatives to preserve and protect seed varieties are examples of seed conservation activities. To ensure that seeds are available for future generations, these activities entail gathering and preserving seeds from various plant species in safe locations. Promoting sustainable agricultural practices can aid in protecting seed variety and

conservation initiatives. Supporting regional farmers and promoting a variety of cropping techniques will assist in keeping a robust and adaptable seed supply that can cope with shifting environmental factors. Tropical trees and seeds serve crucial roles in maintaining ecological and economic systems and are vital to the world's biodiversity. To ensure that these resources continue to serve future generations, conservation measures are required to safeguard them from human activity and climate change dangers.

A seed is a small, dormant object produced by plants that house the embryo of a future plant. Its customary exterior layer of defence is a fruit or a hard shell. Because seeds may be moved by wind, water, animals, or other means to new locations where they can sprout and grow into new plants, seeds are crucial for plant reproduction and dispersal. Many seeds may be used as food by both people and animals since they are rich in nutrients. Additionally, seeds are used in agriculture and horticulture to cultivate crops and grow plants. Seeds are essential in emerging nations. It is necessary to have a basic grasp of seed biology to comprehend community activities such as plant establishment, succession, and natural plant regeneration. Thus, this is an essential method for controlling plant numbers. In the case of tropical rainforests, there are still very few dispersed studies on the physiology of seeds, phenology knowledge, and ecological views. Most tropical seeds can only remain viable in the soil for a brief time before decomposing. The three main classifications of seeds are Recalcitrant, Orthodox, and Intermediate. Recalcitrant seeds can't be stored for long periods and can't endure drying out (desiccation). Contrary to traditional seeds, which can withstand drying and last for years, recalcitrant seeds have a high moisture content and are vulnerable to it. They must be planted or stored in a moist area as soon as they are harvested.

When storing temperate-recalcitrant seeds, the moisture content should be higher as compared to other orthodox seeds, when the proper mature seeds fall from the mother plant. Keeping the seeds wet occasionally during storage may be necessary to maintain this moisture level. Ideally, temperatures should be between 0 and 5°C, while most species won't be harmed by a drop of 1 or 2 degrees below zero. Containers must allow some gas exchange with the environment while impervious to moisture loss. The 0.075 to 1.0 mm wall thickened Polyethylene bags are ideal. While certain Oaknuts may be preserved in this way for three years, some viability will be lost. Like how temperate species are stored, preserving seeds from tropical-resistant plants requires maintaining high temperatures. Although there are variations amongst species, the bottom limits are typically 12 to 20°C. More than a year of successful storage cannot be expected.

Recalcitrant seeds are typically found in humid, tropical, and subtropical climates. Due to their susceptibility to drying, recalcitrant seeds are difficult to transport and store. Tissue culture or vegetative propagation techniques are frequently used to increase their numbers. They are, hence, not as well adapted as traditional seeds for industrial horticulture or agriculture. Recalcitrant seeds do, however, play a crucial role in natural ecosystems by assisting plant regeneration in their native habitats as soon as they fall from the parent tree.

A seed that can tolerate drying and endure for a long time is called an orthodox seed. Because they have a low moisture content, these seeds may be able to survive desiccation. When correctly handled, they may be kept for years without losing vitality. One may usually locate orthodox seeds in temperate regions with drier weather. Typical orthodox seeds include bean, corn, and wheat seeds. Since orthodox seeds can withstand drying, they are easier to handle and store than recalcitrant seeds. They are suited for

use in large-scale agriculture and horticulture because they can be stored and transported long distances without losing their ability to germinate. Orthodox seeds are another key component of plant breeding and genetic preservation. In case of crop failure or natural disasters, they can be stored in seed banks and used to enhance crops in the future.

Both orthodox and recalcitrant seeds have unique properties and roles in plant reproduction and conservation. Understanding these characteristics is essential for effective seed management and conservation activities. Regarding their resistance to drying, seeds classified as intermediates fall between orthodox and recalcitrant seeds. These seeds are not as resistant to drying as traditional seeds, although having sufficient moisture content and may tolerate some drying. Frequently, intermediate seeds may be found in areas with a moderate climate and high humidity. Examples of intermediate seeds are papaya, Ceylon Ironwood, and citrus seeds. Even though intermediate seeds are less resistant to desiccation than orthodox seeds, they may be stored for a shorter time. They are suitable for storage and transportation since they are less resilient than typical seeds. As a backup or for potential crop development, intermediate seeds can also be stored in seed banks for plant breeding and genetic conservation. Their shorter storage life necessitates more frequent monitoring and seed stock renewal.

The Western Ghats are known for their extensive forests in addition to their broad diversity of plants. These woodlands, which serve as a haven for many different animal species, significantly impact the ecological balance of the region. The Western Ghats are home to several distinct types of forests, including montane forests, wet deciduous forests, tropical evergreen forests, and semi-evergreen forests. Each forest type is distinguished from others by its distinctive tree and plant species mix. Tropical evergreen woods may be found in the wettest Western Ghats

locales. The rich flora and enormous trees that form a closed canopy in these woods serve as identifying features. A few significant tree species that may be found in these forests are the Indian mahogany (*Toona ciliata*), Indian ebony (*Diospyros ebenum*), and Malabar tamarind (*Garcinia gummi-gutta*).

In areas with a bit less rainfall, you can find semi-evergreen forests, which are made up of evergreen and deciduous tree species. Some of the common tree species in these forests are wild cinnamon (*Cinnamomum tamala*), rosewood (*Dalbergia latifolia*), and Indian laurel (*Terminalia elliptica*). Moist deciduous forests, found in areas with normal rainfall, may have a mix of deciduous and evergreen tree species. These forests have several significant tree species, including teak (*Tectona grandis*), black dammar (*Canarium strictum*), and Indian gooseberry (*Phyllanthus emblica*). The montane forests of the Western Ghats are characterized by their peculiar flora and cooler temperatures. Some of the tree species that may be found in these woodlands are shola trees, *Grevillea robusta*, *Rhododendron nilgiricum*, and *Syzygium travancoricum*, often known as the Nilgiri plum. These forests are necessary for maintaining the ecosystem, managing the water cycle, preventing soil erosion, and providing a home for various creatures, such as elephants, tigers, and many bird species.

On the other hand, illegal logging, habitat destruction, and deforestation are threats to the Western Ghats and its forests. Initiatives for conservation are being taken to preserve this unique habitat and ensure the survival of its diverse tree composition and wildlife. Recalcitrant seeds may commonly be found in tropical woodlands, especially in the Western Ghats. Unlike conventional seeds, which may be stored for lengthy periods under controlled conditions, recalcitrant seeds have a short shelf life and must be sown as soon as they are collected. As a result, their utility in

conservation and forestry initiatives is limited because of their inability to be stored and disseminated.

Conservation efforts are underway in the Western Ghats to protect resistant seed species and the biodiversity of the woods. By safeguarding these forests, we can ensure the survival of the trees and the various animal species that depend on the woods for their habitat and ecological balance. Knowledge of seed biology is essential in conservation efforts when protecting resistant seed species. These seeds have specific characteristics and needs that must be understood to be stored appropriately, multiplied, and reintroduced into their natural habitats. Conservationists must promptly identify recalcitrant seeds and remove them from parent trees to ensure survival. This comprises planning efforts for harvesting seeds and monitoring the maturation of seeds. After being collected, recalcitrant seeds must be planted straight soon or prepared for storage. Recalcitrant seeds have few storage choices due to their vulnerability to freezing and drying. Therefore, short-term preservation techniques are widely used by conservationists. These methods assist in maintaining the seeds' short-term viability, enabling their use in reforestation projects.

Additionally, research into the biology of seeds can help develop fresh techniques for the long-term storage and preservation of resistant seeds. Researchers are looking at cryopreservation methods, which involve preserving objects at very low temperatures, to lengthen the lifespan of these delicate seeds. The results of this study may help efforts to conserve and reintroduce resistant seed species. Knowledge of the biology of resistant seeds is crucial for their conservation. By knowing about these vital seed species' distinctive characteristics, storage needs, and germination requirements, conservationists can effectively conserve and propagate them, contributing to the overall conservation of tropical forests and their biodiversity.

Tropical forests are one of the planet's most diverse plant flora types. Kerala's Western Ghats Forest contains a range of tropical ecosystems and is home to several native and endangered tree species. Refractory seeds, which have a high moisture content at maturity and become non-viable when they lose around 10% of their moisture content, are produced by most trees in the Western Ghats region. Studies on seed technology have thus far mainly focused on traditional seeds since they can be stored for a longer time. Tropical trees, especially those from ancient families like the Dipterocarpaceae, infamous for their inability to keep their seeds for long periods, are known for having recalcitrant seeds. Since seed is the primary mode of multiplication for many of these tropical tree species, the long-term availability of viable seeds is essential for maintaining a stable, healthy population in the community.

Knowledge of seed physiology is necessary to preserve large volumes of seed, especially for afforestation projects and social forestry efforts. Forest geneticists collect seeds of economically valuable species from diverse geographical places to preserve the genetic variation of the species for use in future genetic development attempts. According to King & Roberts (1979), Chin & Roberts (1980), and Roberts & Ellis (1982), many tropical and subtropical plants produce seeds that cannot tolerate desiccation. Their vulnerability to freezing and desiccation is the fundamental problem that seed technologists and conservationists face in assuring the long-term survival of these kinds of tropical seeds.

According to their fresh weight alone, mature seeds of the species fall off the tree with a higher moisture content under usual climatic circumstances. An in-depth study is necessary to comprehend the seeds' stubborn behaviour because of their great sensitivity to dryness. Orthodox seeds may often be stored at lower temperatures and with little moisture content for several years. According to several sources (Ohga, 1923; Shen-Miller

et al., 1995; Li et al., 2000), lotus seeds can survive 100 to 1300 years. On the other hand, recalcitrant seeds cannot be stored for an extended period since they start to lose viability with only a slight reduction in their initial moisture content level. Refractory species are used in many restoration and reintroduction programs; however, their use is greatly hampered by low seed storage rates, erratic blooming patterns, and seed laying.

Seed banking is a relatively new and underutilized choice. Seed conservation is a critical strategy for ensuring the long-term genetic diversity of rare plant species. It is notable for its efforts to maintain plant diversity globally and make testing and research on plants covered by the "Convention on Biological Diversity (CBD)" quick and straightforward. The best conservation collections, which may replicate up to 50 parent plant diversity in separate packages, will conserve up to 3000 seeds from five populations throughout the species' range. The tropics have vibrant tree variety. However, the pace of extinction of this tree type is extremely rapid because of habitat fragmentation, increased deforestation, overexploitation for use as timber, and consumption of secondary metabolites (Gum, resin, tannins, etc.). Despite the abundance of species, only a few are used in mass planting initiatives, agroforestry systems, and forest restoration initiatives.

History of Seed Biology Studies

When people first started growing plants for food and other uses in ancient times, the history of seed biology began. They had traditional ways to store seeds and retain viability. Nevertheless, the scientific study of seed biology as a separate study area is relatively recent. The major turning points in the development of seed biology are summarized as follows:

Early Observations: Humans have long studied the fundamental characteristics of seeds, such as their capacity to sprout and give rise to new plants. Ancient societies, such as the Egyptians, Greeks, and Romans,

were familiar with seed germination and fundamental agricultural techniques.

Pre-19th Century Developments: In the 16th century, Conrad Gesner, a Swiss physician, botanist, and artist, wrote "Historia Plantarum", which included significant descriptions and pictures of seeds. Scientists like Nehemiah Grew and Stephen Hales performed studies in the 17th and 18th centuries to examine seed germination and structure.

Advent of Microscopy: Improvements in microscopy and the creation of better microscopes in the early 19th century made it possible to examine seeds in more detail. Researchers like Robert Brown (1773-1858) and Matthias Schleiden (1804-1881) contributed to our understanding of cell biology and seed shape.

Mendel's Discoveries: Gregor Mendel's groundbreaking research on pea plants in the 19th century set the groundwork for the field of genetics. His research shed light on how features in plants are inherited, ultimately advancing our knowledge of genetics and seed development.

Seed Dormancy and Germination: In the late 19th and early 20th centuries, there was an increase in interest in seed dormancy and germination. By examining the causes of seed dormancy, breaking seed dormancy, and comprehending the germination process, scientists like Charles Darwin, Francis Darwin, and others made substantial contributions.

Seed Physiology and Biochemistry: The development of biochemical and physiological techniques in the middle of the 20th century enabled researchers to learn more about the internal mechanisms involved in seed germination. Researchers started studying the complex biochemical processes, metabolic pathways, and hormone control that occur during seed germination and development.

Conservation and Seed Banks: The creation of seed banks and conservation initiatives grew in popularity over the 20th century. To preserve plant variety and stop the extinction of endangered species, projects like the Millennium Seed Bank Project started aggressively saving seeds.

Molecular Advances: In the latter half of the 20th century, the development of molecular biology methods such as DNA sequencing and genetic engineering advanced the study of seeds. Thanks to these technologies, it was possible to analyze seed genetics and gene expression and change seed characteristics to achieve various goals for crop improvement, yield, and conservation initiatives.

With an emphasis on seed lifetime, seed banks, seed quality, seed multiplication, and the effect of environmental conditions on seed germination, seed biology is still evolving today in the case of plants that need immediate conservation. Combining classic research methodologies with contemporary technologies is continually improving our knowledge of seeds and their function in plant reproduction, conservation, and agriculture.

Seed Biology in the Current Era

Biochemical and Genetic studies have significantly developed in the field of seed biology, in the last few decades. The molecular mechanisms behind the specific seed storage proteins, in the seed desiccation sensitive proteins and the expression of these subsequent proteins, etc. are studied comprehensively. The analysis of proteins at different stages of seed development, dormancy, and germination has provided invaluable information on the biochemistry of such processes. For example, proteome analyses have shown the importance of protein synthesis during seed germination (Rajjou et al., 2004), as well as factors involved in seed survival and longevity (Rajjou et al., 2008), and metabolome profiling has

shown that metabolic switch a key aspect of seed maturation and germination (Fait et al., 2006).

The key target of advanced seed biology is to explore the specific interactions between the phytohormones and other signalling molecules that control the process of germination and to analyze the cell signalling pathways. In recent years, targeted analysis of plant hormones has revealed new aspects of seed physiology, including the complex metabolic balance between multiple plant hormones during development and germination (Chiwocha et al., 2005, Preston et al., 2009, Kanno et al., 2010).

Dipterocarpaceae Seeds

In tropical regions of the world, particularly in Southeast Asia, tree seeds from the delicate Dipterocarpaceae family of flowering plants are frequently seen. These seeds are well-known for both their unique characteristics and ecological importance. Family Dipterocarpaceae seeds come in a variety of shapes and configurations. They are frequently gigantic and can be as little as a few millimeters or as large as several centimeters. Some species have large, non-winged seeds dispersed by animals or gravity, whereas others have winged seeds dispersed by the wind (Ashton & Hall, 1992). Dipterocarpaceae seeds usually exhibit several dormancy mechanisms that postpone germination when it comes to germination. Examples of these systems include physical barriers, such as hard seed coats, or physiological components that delay germination until specific conditions are met. Diverse species of Dipterocarpaceae may require different conditions for seed germination (Haron et al., 2019). Many dispersion techniques have been created for the seeds of Dipterocarpaceae.

Genus Shorea, one of the largest with more than 200 species, is an essential tree in the Dipterocarpaceae family. It encompasses trees that are native to Southeast Asia and the Indian subcontinent. Because of its valuable timber,

Shorea species are extensively cut and used for the timber industry. In Southeast Asia, the genus Hopea has over 80 species. Hopea trees are well known for their robust wood used in boat building. The Dryobalanops genus has only 7 species, which are restricted to Western Malaysian tropical rain forests (Peninsular Malaysia, Borneo and Sumatra). A resin that is utilized for the preparation of incense sticks and varnishes is yielded from these trees. The resin is commonly called as Damar or Gurjun. These are only a few species that make up the family Dipterocarpaceae.

Every species has a unique set of characteristics and a unique ecological function within the ecosystems of tropical rainforests. The biology of the seeds of Dipterocarpaceae is crucial for their preservation. Every few years, these trees engage in a unique kind of reproduction known as "mast fruiting," during which they simultaneously produce many seeds. This broad production of seeds increases the probability of successful germination and survival. Therefore, understanding the biology of Dipterocarpaceae seeds is essential to ensuring their survival. It helps to identify settings that are best for seed germination and spread and create effective techniques for seed collection, storage, and propagation.

Conservation efforts usually involve gathering seeds from ancient trees and building seed banks or nurseries to ensure the availability of healthy seedlings for future replanting attempts. Research on seed biology helps identify potential threats to seed viability, such as habitat destruction, climate change, or invasive species, and in developing mitigation strategies. Overall, our knowledge of the biology of seeds in these plants is crucial for the conservation and sustainable management of these ecologically vital trees and the several rainforest environments they occupy, including the Dipterocarpaceae.

Seed Biology Studies and its Importance

Seed biology is the study of seeds and their diverse biological characteristics. It covers seed development, maturity, germination, dormancy, and lifespan. Furthermore, the study of seed genetics, physiology, morphology, and ecology are included in seed biology. Seed germination is a significant facet of seed biology. A dormant seed begins to sprout again and transform into a new plant through germination. Successful crop production, reforestation initiatives, and ecological restoration depend on understanding the elements that affect germination, such as temperature, light, water, and hormone control. Another important topic in seed biology research is seed dormancy. When a seed does not germinate even in ideal circumstances, it is said to be in dormancy. Numerous dormancy mechanisms can shield seeds from germination immediately after dispersal, enabling them to endure severe circumstances or timing their germination to coincide with the most favourable climatic conditions.

The biology of seeds is also interested in the longevity of the seeds. Some seeds may survive in a latent state for lengthy periods, even hundreds or millennia. For seed banking, conservation initiatives, and the preservation of genetic variety, knowledge of the elements that affect seed life, such as desiccation tolerance and resistance to oxidative damage, is essential. Research on seed biology has applications in horticulture, forestry, agriculture, and ecological restoration. It offers information on seed treatments, seed storage techniques, agricultural enhancement methods, re-vegetation techniques, and the control of invasive plant species. The study of seeds, including their growth, germination, dormancy, lifespan, physiology, genetics, morphology, and ecological relationships, is the focus of the interdisciplinary area of seed biology. Addressing agricultural issues, protecting biodiversity, and encouraging sustainable land

management techniques all depend on this information. The preservation and propagation of plant species depend heavily on the sciences of seed biology and biodiversity conservation. These are their connections to one another:

Seed as a Conservation Tool: Seeds are fundamental building blocks for maintaining plant species. *Ex-situ* conservation includes gathering, storing, and cultivating seeds outside their original habitat in seed banks, botanical gardens, or nurseries. *In-situ* conservation aims to conserve plant populations in their natural ecosystems. Seed banks serve as repositories for protecting plant genetic variety, particularly that of uncommon, threatened, or agriculturally important species.

Seed Germination: Studying the numerous mechanisms and elements influencing seed germination is the subject of seed biology. Successful seed propagation requires understanding the germination needs of various plant species. Dormancy, moisture, temperature, light, and the properties of the seed coat all impact how well seeds germinate. Scientists and conservationists can develop improved germination techniques for wild and domesticated plant species with the proper knowledge of seed biology.

Genetic Diversity: To preserve and sustain genetic diversity among plant populations, seed biology is essential. Genetic variety is crucial to adapt, be resilient, and evolve in response to shifting environmental conditions. Conservation efforts can protect the possibility for future adaptation and reduce the risks brought on by habitat loss, climate change, and other challenges by keeping a broad variety of seeds, particularly those reflecting various genetic variants within a species.

Ecological Restoration: Ecological restoration initiatives seeking to restore deteriorated ecosystems are centered on seed biology. Local seed sources can restore native plant communities, increase biodiversity, and enhance ecological processes. Conservationists can identify suitable seed

sources, choose the best seeding procedures, and encourage the development of varied and hardy plant populations by having a solid grasp of seed biology.

Crop Improvement: Programs for crop development and agriculture depend heavily on seed biology. Plant breeders choose to produce plant varieties with superior qualities, like higher vigour with good productivity rate, disease resistance and greater nutritional value. Thanks to an understanding of seed biology, breeders can create better seed types, research inheritance patterns, and enhance seed quality, which also increases agricultural yield and sustainability.

Seed biology and biodiversity conservation go hand in hand. Understanding seed germination, protecting genetic variety, aiding in ecological restoration, and supporting agricultural development initiatives are all aspects of seed biology that serve as the basis for effective conservation methods. By safeguarding and using seeds, we can preserve plant biodiversity and assure a healthy future for both natural and agricultural environments.

The development of the embryo and seed maturity, which may be further split into early, medium, and late maturation phases, are the two main sequential steps that make up the process of seed creation. The primary plant organs are established, tissue-specific cell differentiation begins, and the embryo's axial structures are developed (Lau et al., 2012). Storage chemicals are heavily deposited during the early stages of seed development (Bewley et al., 2013). Important occurrences like the start of desiccation tolerance and the switch to dormancy occur during the late stage of seed maturity. According to Lima et al., (2017), the desiccation tolerance of orthodox seeds is based on an accumulation of molecules, including late embryogenesis abundant (LEA) proteins, small heat shock proteins (sHSP), non-reducing oligosaccharides of the raffinose group

(RFO), and low molecular weight antioxidants like glutathione (Cairns et al., 2006), tocopherols (Chen et al., 2016), and carotenoids (Smolikova & Medvedev, 2015).

With abundant (LEA) proteins in the late embryo, seed viability is maintained in large part by LEA proteins (Nagaraju et al., 2019). These polypeptides build up in seeds during the late maturation stage, which means that they are present in dry seeds before germination and disappear after germination. Cis-elements responsive to ABA, drought, and low temperatures were discovered in the promoters of the Arabidopsis genes that code for LEA proteins. In response to drought, salt, and exceptionally high and low temperatures, Chen et al., (2019) found that potato LEA gene expression increased. High glycine (Gly) content, a lack of cysteine (Cys) and tryptophan (Trp) residues, and a preponderance of amino acids like alanine (Ala), glutamate (Glu), lysine/arginine (Lys/Arg), and threonine (Thr) are the characteristics of LEA-proteins. They are highly hydrated and stable across a wide range of temperatures because of their structure.

When a cell dehydrates, LEA-proteins function as chaperons, stabilizing denatured proteins and assisting in their refolding by heavily influencing the structural stability of other proteins and cell membranes. LEA proteins have been found to play a crucial role in cellular stress tolerance, binding to ionic molecules and accumulating in response to dehydration. This accumulation helps safeguard membrane-bound proteins and enzymes from the detrimental effects of excessive salt buildup, thereby preserving cellular function. Despite being found in every cell compartment; LEA proteins are mostly found in the cytoplasm. In aqueous conditions, the majority of LEA-proteins assume randomly coiled, disordered forms. Dehydration, on the other hand, causes them to refold into structures that include a greater proportion of amphiphilic helix. LEA proteins feature adaptable structural elements, including polyproline II helices, which

enhance their ability to interact with nucleic acids like DNA and RNA, as well as other proteins, facilitating versatile binding capabilities. The Arabidopsis genome has 51 LEA protein-coding genes in total.

LEA proteins and a few heat shock proteins are examples of the so-called moonlighting proteins, which have a variety of physiologically significant roles (Chen et al., 2018). Seeds have a significantly greater amount of LEA gene expression than plant vegetative organs. Only seeds express 21 of the 51 Arabidopsis genes that code for LEA proteins, and these genes directly regulate the development of seeds. In *Camellia sinensis*, this trend is even more pronounced: 39 of the 48 LEA genes found in this plant may be crucial for seed development. The LEA-2 family is the biggest subgroup of LEA proteins. These proteins are distinguished by having a larger proportion of hydrophobic amino acids and a traditional three-dimensional structure. Dehydrins are the family of universal protection compounds involved in plant responses to diverse abiotic stresses and are the most researched category of LEA proteins (Jin et al., 2019). Dehydrins' hetero- and homo-complexes, which are frequently multimeric, are what allow them to attach to cellular biopolymers and stabilize their structural integrity. The orthodox, intermediate, and recalcitrant seeds have been revealed to have distinct dehydrin compositions and contents, which is consistent with the idea that a decrease in cellular levels of dehydrins might increase the vulnerability of plant tissues to desiccation (Radwan et al., 2014).

***Hopea ponga* (Dennst.) Mabb.**

The forests of the Western Ghats have been subjected to selective logging and fragmentation, with portions converted for intensive agriculture, including monoculture plantations of Tea, Coffee, Eucalyptus, Rubber, Teak and Oil palm. Additionally, infrastructure development, such as construction, road building, reservoir creation, and railway expansion, has

led to further deforestation. Encroachment within protected areas and grazing activities by cattle and goats have resulted in severe soil erosion on forest slopes, compromising the integrity of these peculiar ecosystems. Tree communities are altered in forests of the tropical region due to habit loss and fragmentation of the population (Laurance et al., 2006). The Western Ghats have been esteemed as a Natural World Heritage site by UNESCO, acknowledging their rich biodiversity and unique ecosystems. Considering this recognition, it is essential to delve into the Indian distribution, phenological patterns, and ecological characteristics of endemic and threatened genera, such as the Hopea genus, to comprehensively understand and conserve these precious natural assets.

With 104 species identified worldwide, including 11 species from India, Hopea is the biggest genus in the Dipterocarpaceae family. It is widely distributed throughout the Western Ghats, from lowland evergreen forests on the coastal plains to tropical evergreen forests. The Western Ghats, mainly Karnataka, Kerala, and Tamil Nadu, are home to 8 of the 11 species that have been documented from India. The species thrives best in Karnataka's lower altitude, warm, and humid regions. The IUCN has classified all 11 species of Hopea that have been documented from India as being in grave danger (Irudhyaraj, 2016). A typical sub-canopy tree of wet evergreen woods, the *Hopea ponga* is unique to the Western Ghats and can reach heights of 900 m. Most of the time, the damp tropical environment is where one can find the tree *Hopea ponga* (Dennst.) Mabb. South and southwest India is the species' native habitat. It's a tall tree from the Western Ghats. On branching inflorescences, bisexual blooms are produced. The ovate-lanceolate petals have exterior hairs on them. There are up to 15 stamens. The fruit's sepals are bigger and more tenacious than wings; they start greenish but become crimson as the fruit ripens.

Hopea ponga, a highly prized timber species, has been subjected to intense logging in recent decades, resulting in a significantly fragmented population. The current situation reveals a dire need for sustainable management practices, as the unchecked exploitation of timber has led to isolated patches of *H. ponga* in evergreen forests and lowland areas, threatening the long-term viability of this species. Studies on the phytosociological vegetation of the Alakyam stream revealed that *H. ponga* was the dominant tree species under severe threat (Irudhyaraj, 2016).

There are only a few studies on the pharmacological and phytochemical aspects of *H ponga*. Six resveratrol oligomers, including E-resveratrol, Viniferin, Trihydroxy-phenanthrene glucoside (THPG), Vaticaphenol A, and Hopeaphenol, as well as four phytochemicals, were identified from the acetone and ethanol extraction of the stem bark of *Hopea ponga* (Sasikumar et al., 2019). Compared to other solvent extracts, the methanol extract of the leaves demonstrated a higher level of anthelmintic activity. To isolate the active compounds and adequately clarify their mechanisms of action, which helps in the development of innovative pharmaceuticals to treat helminthiasis, they concluded that a complete chemical and pharmacological study should be conducted (Shettar & Vedamurthy, 2017).

Species of the Hopea genus are attacked by insects of different orders during flowering and fruiting. Beetles are responsible for the maximum percentage of fruit fall (Irudhyaraj, 2016). It completely arrests differentiation and development of leaf primordia and develops it into multicellular echinate appendages (Raman & Takagi, 1992). An ant species *Oecophylla smaragdina* constructs a nest using leaves and tender twigs thereby affecting the growth of the tree few butterfly species such as *Archopala amantes* (Large Oakblue), *Arhopala centaurus* (Centaur

Oakblue), *Arhopala bazaloides* (Dusted Oakblue), and *Triodes minosact* as pollinators in the adult stage whereas they act as defoliators in the larval stage (Sukesh, 2014; Nitin et al., 2018).

Objectives of the Study

- Seed biology of the selected species, *Hopea ponga* on the line of phenology, seed development, desiccation, and storage behaviour
- To analyze the physiological changes including synthesis and accumulation of metabolites and their role in the development of desiccation sensitivity or tolerance
- To compare the role of specific sugars, Late embryogenesis abundant protein (LEA protein), phenols, lipid peroxidation, and the role of oxidative enzymes and the proteins in desiccation sensitivity or tolerance.

REVIEW OF LITERATURE

With 2.4% of the world's landmass and 7-8% of the world's species, including over 45,500 plant species, recorded in its 10 bio-geographic areas, India is one of the megadiverse nations. Of these, 33% of the plants in India are endemic, meaning they are unique to their native country. India has 10 bio-geographic zones with a rich and diversified history of biodiversity. The nation is also consecrated with a wide range of plant life, including marine, freshwater, desert flora, moist tropical rainforests, alpine forests, and other forest types. In India, forests occupy 64.01 million hectares, or around 19.4% of the nation's total land area (Anonymous, 1993). High endemicity and species richness levels can be seen in tropical forests (Orians & Groom, 2005). Southern India's forests are discovered to be covered with tropical flora, with a wider variety of plants and animals documented at various altitudes, particularly around the Western Ghats.

The Indian Himalayas, Peninsular India, and the Andaman and Nicobar Islands are the three primary phytogeographical zones, accounting for 5752 (29%) of the 20,074 angiosperm species that have been documented from India (Nayar, 1996). Many different fragrant and therapeutic plants may be found throughout the Indian subcontinent. Peninsular India is the most abundant endemic region in the world, home to almost 82% of all endemic taxa. The 1,600 km long Western Ghats Mountain range chain, which runs parallel to Gujarat's and Tamil Nadu's western coasts in a south-to-north orientation, is renowned for its abundance of endemic species. In light of its valuable natural ecosystems, the United Nations Educational, Scientific, and Cultural Organization (UNESCO) designated the Western Ghats as one of the Natural World Heritage sites in 2012 (UNESCO). The variety of latitudinal and altitudinal gradients with varying rainfall and temperatures are to blame for the diversity of the Western Ghats species. These individuals favour a high level of endemism

Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

as well. Nayar (1996) concluded that most endemic species in the peninsular area are paleoendemic and may be found in the Western Ghats' highlands.

A long history of ancient civilizations may be found in the study of seed biology. For instance, it is known that the Egyptians used sealed jars to store and preserve seeds for later use. Science didn't start looking at seed biology, though, until the 17th and 18th centuries. Nehemiah Grew, an English botanist who made significant contributions to the science of seed biology in the late 17th century *via* his studies of plant anatomy and physiology, was one of the forerunners in this discipline. Future study in seed biology was made possible by Grew's observations of seed growth and structure. The binomial system of nomenclature, developed in the 18th century by the Swedish botanist Carl Linnaeus, offered a uniform method for identifying and categorizing plants. According to Taylorson (2000), Linnaeus made important contributions to the study of seed morphology and categorization. With the discovery of plant hormones like auxins and gibberellins, which are essential for seed germination and growth, in the early 20th century, the area of seed biology was greatly enlarged. The comprehension of these hormonal mechanisms has greatly benefited from the work of researchers like Ernst Munch and Frederick Blackman (Bradford & Nonogaki, 2007).

Modern molecular biology and genetics have had a significant impact on seed biology. Scientists can now examine the genetic composition of seeds and comprehend the processes underpinning seed growth and germination thanks to the discovery of DNA and the development of tools like the polymerase chain reaction (PCR). Genetics, physiology, ecology, and conservation are only a few of the areas of plant science that are covered by the interdisciplinary study of seed biology today. The availability of seeds for future agricultural development and environmental restoration is

ensured by seed banks across the world in maintaining and conserving plant genetic variety (Bewley & Black, 2012).

Today's forestry industry is progressively evolving, and using sustainable forest management techniques results in a wider variety of trees being planted. Particularly in vast regions or in environments with high biodiversity, seeds are more affordable than planting seedlings for ecological rejuvenation (Perez et al., 2019). However, fewer than 10% of the seeds planted in the ground can grow into plantlets (James et al., 2011; Merritt & Dixon, 2011; Ceccon et al., 2016). Such a high failure rate is now affordably and severely restricting the success of seed-based restoration in the required quantities due to the difficulties and expenses of procuring and producing indigenous seeds as well as the negative effects of increasing wildlife seed collection rates (Merritt & Dixon, 2011a; Menz et al., 2013; Nevill et al., 2018). The shift away from invasive species like *Eucalyptus sp.*, *Acacia sp.*, *Pinus sp.*, etc., and toward native species is evident throughout time. Through proper seed handling, encouraging endemic tropical tree seed research, and promoting the conservation of threatened tree species, the information on the phenological and physiological parameters will help to further increase the use of local (native) tree species in social forestry programs throughout the tropics thereby conservation and sustainable utilization of these natural resources.

According to Reddy et al., 2020, species like *Hopea ponga, Hopea parviflora*, etc. rely on wind dispersal, in which case their winged seeds are transported away by air currents. Some species produce seeds without wings that fall to the ground, slide down slopes, or land just beneath the parent tree, and some Dipterocarpaceae plants have also been found to be dispersed by animals, mainly mammals and birds. According to Appanah & Turnbull (1998), Dipterocarpaceae seeds are essential for the regeneration and preservation of tropical rainforests. Numerous creatures,

such as rats, primates, and birds, rely on them as a major source of food. Seed predators and dispersers are drawn to the big size and high nutritional content of Dipterocarpaceae seeds (Cannon et al., 1998). Threats to Dipterocarpaceae trees and their seeds include habitat loss brought on by deforestation and irresponsible logging techniques. The dissemination and regeneration of Dipterocarpaceae seeds may be impacted by the fall in seed dispersers like big animals. To maintain the survival of Dipterocarpaceae species, conservation efforts are concentrated on safeguarding remnant forests and advocating for sustainable management techniques (Kettle et al., 2019).

According to Mabberley (2017), the Indo-Malayan area is home to the majority of the 104 species that make up the Dipterocarpaceae genus Hopea Roxb. According to Sanil et al., 2020, 12 species and one variation have been identified in India. The Western Ghats are home to 10 species, eight of which are only found in Kerala and are endangered (Nayar et al., 2014; Sasidharan, 2017; Robi et al., 2020). According to Sanil et al., 2017, the Western Ghats may be one of the foci for the development and diversification of Hopea due to the prevalence of endemism there.

The Dipterocarpaceae family tree *Hopea ponga* (Dennst) Mabberley is an endangered species that is only found in the tropical evergreen forests of the Western Ghats in Tamil Nadu, Kerala, Maharashtra, and Karnataka (Irudhyaraj & Ramasubbu, 2018). The tree is valuable commercially for its timber, and its bark also makes an excellent tanning material and is astringent with a slow diffusion rate (Ashton, 1998). For the manufacture of wooden objects, furniture, and building construction, *H. ponga* wood is employed (Shivaprasad et al., 1999). Researchers looked at how desiccation affected the vigour and germination of seeds from *Hopea parviflora* and *Hopea ponga* (Dayal & Kaveriappa, 2000). *Hopea ponga*

seed survival and the impact of wing loading were studied (Muralikrishnan & Chandrashekar, 1997).

In the Western Ghats, the genus Hopea is one of the economically and environmentally dominating genera (Sanil et al., 2017). According to Pearson & Brown (1932), the genus is frequently used in the plywood and timber industries for a variety of construction tasks, including planking, floors, shipbuilding, masts and spars, dugouts, and heavy packing boxes. In boat construction and handicrafts, a thick solid resin from the Hopea species known as rock dammar is employed as a varnish and anticorrosive covering (Shiva & Jantan, 1998). Although Ashton (1982) supported their placement in the order Malvales as well as in APG IV (Chase et al., 2016, Heckenhauer et al., 2017), the phylogenetic attribution of Dipterocarpaceae among angiosperms has previously been difficult.

Hopea ponga seed wings have been tested for their phytochemical composition, antioxidant capacity, and antibacterial activity (Hidayathulla et al., 2011). To treat piles, the whole plant may be employed (Divakar et al., 2013). The *H. ponga* leaf methanolic extracts show an extraordinary capacity for scavenging certain free radicals. Additionally, the tree produced several active substances with potent antioxidant and antibacterial properties (Rose et al., 2013). According to Agullar et al., 2006, the seeds of Dipterocarpaceae species often have a limited lifespan and are unable to survive desiccation. For the effective use of conservation techniques in the case of endemic and endangered species, knowledge of seed development is crucial.

According to Rose et al., 2013 investigation of antibacterial activity, *Proteus vulgaris* and *Bacillus cereus* were significantly inhibited by the methanol extract of *H. ponga* and a higher concentration of flavonoids and phenols was present in methanol extract as compared to the other studied extracts. An investigation by Sukesh et al., 2011 assessed the antibacterial

properties of water extracts against six bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. circulans*, *Pseudomonas fluorescens*, and *P. putida*. Using the disc diffusion method and Minimum Inhibitory Concentration (MIC) broth dilution, the study revealed notable antibacterial activity, with inhibition zones measuring 18mm (*B. subtilis*), 19mm (*B. circulense*), 20mm (*P. putida*) and 22mm (*S. aureus*).

According to Prashanth (2022), both leaf and blossom extracts, though to varying degrees, were successful at inhibiting all test microorganisms. The extracts demonstrated concentration-dependent antibacterial activity, with leaf extract displaying substantially more antibacterial activity than floral extract. To the greatest extent possible, both extracts suppressed *Shigella flexneri*. The plant looks to be a strong contender for creating brand-new antibacterial compounds. Sukesh et al., 2011 examined the antioxidant activity of this species using in-vitro models like DPPH. The greatest levels of polyphenolic content (176±9.17mgGAE/g), reducing power assay, and DPPH scavenging activity were found in the methanolic extract. Through the use of the FRAP, H_2O_2, DPPH, and PM tests, the antioxidant capacity of plant extracts and MnNPs was assessed. In every test run, the produced MnNPs demonstrated considerable antioxidant activity (Priyadarshini & Ankala, 2019). Numerous researchers, including Hsin (2013), Ching (2013), Shiuan (2013), Kee (2013), and Siju et al., 2017, investigated the antiproliferative activity in various cell lines. The MTT test was used to investigate the anticancer efficacy of *Hopea ponga* leaves on the viability of non-small cell lung cancer (A549) cells in combination with an aqueous extract of *H. ponga* and its generated MnNPs. Strong dose-dependent anticancer activity is reported in *H. ponga* leaf aqueous extract against non-small cell lung cancer cells A549.

Phenology and Seed Development

In a general sense, the seed is genetic material utilized for regeneration. Technically, the seed is a complete ovule that has been fertilized and is

coated with a seed coat, or it is the most basic form of propagation. According to their behaviour during seed storage, Roberts (1973) divided the seeds into two main categories: orthodox and recalcitrant types of seeds. The lifespan of storage is substantially rising as a result of orthodox seed's ability to withstand significant desiccation. Conversely, the storage life of recalcitrant seeds is diminishing (Roberts (1973), Chin (1988), Hanson et al., 1984), as they cannot withstand desiccation and cold temperatures. In addition to two major categories, a third category classification was identified and termed "intermediate" because a group of species has been identified that shows seed storage behaviour intermediate between the orthodox and recalcitrant categories (Ellis et al., 1990), (Ellis et al., 1991a), (Ellis et al., 1991b), (Ellis et al., 1991c), (Hong & Ellis, 1992), (Hong & Ellis, 1995).

The two primary categories (Roberts (1973) and Bonner (1990)) recommended classifying seeds based on storage requirements and adaptations to their native habitats, dividing the species into four groups. They are 1. True-orthodox (seeds can be kept for long periods at temperatures below freezing with a moisture content of less than 10%), 2. Sub-orthodox (seeds can be kept for shorter periods under the same conditions as true orthodox seeds), and 3. Tropical-recalcitrant seeds (seeds are susceptible to damage on desiccation and can't survive in extreme cold temperatures) 4. Temperate-recalcitrant seeds (seeds are those that suffer damage if dried yet can be preserved and stored for a couple of years at temperatures just above the freezing range)

An essential aspect of the forest ecosystem is phenology, which links a species' growth pattern to its physical surroundings. According to Leith (1973), phenological events are useful for defining and interpreting the seasonal characteristics of ecological phenomena. They also aid in the management of the species, the use of bioproducts, and the felling of trees

in a series. Leith (1973) evaluated Harper's (1906) and Keolmeyer's, 1959 research on phenological phenomena. Phenology is the study of the connections between climatic variables and recurring phenomena in plants, according to Daubenmire (1947). Leaf flushing, leaf shedding, vegetative and reproductive development, blooming, fruiting, harvesting of fruits and seeds, insect-pest interactions, pollinators, etc. are typically studied during phenological periods. These are beneficial for maintaining the species' genetic diversity. To account for seasonal fluctuations in individual species as well as emerging community patterns in a given location, observations on phenological events should span several years. To identify irregular blooming and fruiting patterns, a longer time is extremely important (Holttum, 1953).

Phenology is the study of cyclical biological activities in plants, such as blooming, leaf unfolding (or bud burst), seed set, seed dissemination, leaf fall, etc., according to Vilhar (2013). Different fruit and seed maturation processes arise depending on the microclimatic and micrographic conditions at each site within a forest. Due to these variances, determining the planting and ripening periods of each species in various provenances needs a thorough study of forest phenology. New leaves also affect when seedlings are planted in the field, especially for deciduous species (seedlings are often sown before the new leaves appear). Different tree species respond differently to the seasons in terms of their vegetative and reproductive processes. For its practical value regarding many elements, it is becoming more and more crucial to completely comprehend a tree's seasonal behaviour (Rajput, 1992).

There are some detailed phenological studies related to the flowering from the seasonal tropics though many causal observations are fragmented or hidden in floristics or forestry accounts such as those of Croat (1975) in Barro Colorado Island and Boojh & Ramakrishnan (1981) in northeast

India. The majority of trees in the seasonal forest flower annually; flowering occurs either before leaf fall. Flowering mainly occurs at the beginning or towards the end of the dry season (Longman & Jenik, 1974). Climatic conditions affecting phenological events have also been investigated by various workers (Singh & Dixit, 1972). Plant reproductive phenology work conducted by Sakai et al., (1999) in lowland dipterocarp forest reveals that they classified 257 species into flowering types based on timing and frequency of flowering and the predominant flowering pattern was "general flowering" (35%), characterized by blooming exclusively during the General Flowering Period (GFP). In contrast, "supra-annual" (19%), "annual" (13%), and "sub-annual" (5%) flowering types exhibited more varied and less frequent reproductive events. Particularly, the general flowering pattern and synchronized reproductive timing were widespread among diverse species, encompassing various taxonomic categories, life forms, pollination strategies, and fruit types.

A study by Anderson et al., (2005) recorded that phenology patterns of tropical trees are associated with fluctuations in typical temperature, rainfall, and solar radiations, along with variations in phenological patterns were primarily driven by transient changes in rainfall and temperature, indicating a strong sensitivity of life cycles to short-term environmental fluctuations. Selwyn & Parthasarathy, (2006) found a significant correlation between floral characteristics and pollination modes, with bee pollination playing a crucial role in pollination systems. Phenological observations of 22 woody species revealed that trees and lianas primarily reproduced during the dry season (January to June), exhibiting a seasonal and uni-modal flowering pattern. However, fruiting activity displayed a bimodal pattern, with one peak during the dry season and another during the wet season. Notably, many species showed synchronized flowering

and fruiting periods, indicating temporal aggregation in their reproductive cycles.

Nanda et al., (2017) conducted a tree phenological study on 47 trees of the tropical evergreen forest of Southern India, revealing that rainfall had a significant negative influence on both vegetative and reproductive phenologies. Aside from leaf senescence, various phenophases of reproductive and vegetative phenologies exhibited pronounced seasonality. Consequently, phenology studies serve as a vital indicator of terrestrial ecosystem dynamics and a sensitive gauge of climate change, providing valuable insights into the intricate relationships between biological processes and environmental factors. Bhat (1992) observed Noticeable seasonal shifts in the reproductive and vegetative phenology of tree species in the tropical moist forest of Uttara Kannada district of Karnataka. The increase in daylight hours and temperature during the pre-monsoon dry period likely triggers leaf flushing and maturation, whereas the shorter daylight hours and cooling temperatures in the post-monsoon period may induce leaf senescence. The synchronized flowering in the early pre-monsoon dry period may serve to attract pollinators, while the concurrent fruit ripening by most species during this period may provide a post-dispersal advantage for seed germination, enhancing their chances of successful establishment.

A study by Bach (2002) reveals that leafing, flowering, and fruiting events are closely linked to the wet season phenological patterns exhibit a high degree of annual recurrence and predictability, mirroring the consistent alternation of wet and dry seasons. This cyclical nature of phenological events allows for forecasting and anticipation of biological responses to environmental cues, enabling a deeper understanding of ecosystem dynamics and the intricate relationships between species and their environment. The diverse fruiting patterns create a dynamic tapestry of

food resources for frugivores, ensuring a constant and varied supply of nutrients throughout the ecosystem. This is important in conserving and maintaining the frugivore rainforest system in northern Australia. Sundarapandian et al., (2005) observed a broad spectrum of variations in phenological events, including leaf flushing, senescence, flowering, and fruiting, which were influenced by abiotic factors to a certain extent. The peak periods of leaf fall and emergence coincided with the onset of the dry season, allowing for optimal timing of vegetative and reproductive development to capitalize on the subsequent rainy season. The peak flowering period overlapped with leaf fall or flushing, potentially serving to attract pollinators. Furthermore, the initial rainy season marked the commencement of fruit ripening and dispersal, reaching its peak to leverage available soil moisture for seed germination and seedling establishment.

Weber et al., (2005) studied the Molecular physiology of legume seed development, showing that seed development in legume seeds is done by progressive differentiation of organs and tissue resulting in development gradients. In the case of maturing cotyledons photo heterotrophic metabolism improves initial oxygen supply and biosynthetic fluxes and assimilates partitioning. Woldearegay (2020) reviewed the impacts of climate change on plant distribution and phenology, the far-reaching consequences of climate change encompass significant alterations to phenological traits, such as shifts in flowering schedules, modifications to species ranges and diversity, and transformations in community composition, among other critical impacts.

Wright & van Schaik (1994) study reveals that seasonal variations in irradiance (changes in cloud cover, daylight, solar angle, etc.) have a role in phenology. New leaves and new flower formations occurred at the maximal irradiance period. The drier season is characterized by a

convergence of low insect dynamics and high irradiance levels, leading to an investigation of the alternative hypothesis that the reduced pest pressure during this period drives the selection of dry season flowering and fruiting strategies in certain plant species. Nagireddy et al., 2013 studied the fruit set of the endemic tree *Croton scabiosus* and observed 46% higher under natural conditions compared to artificial pollination. The results of geitonogamy were compared with those of xenogamy, indicating that plants are self-compatible. The average fruit set was observed in this plant. In their studies, the seed germination rate due to seed attack and predation was recorded as 10%. A large number of seedlings were found clustered near the mother plant and the threatening factors for this species were attributed to nutrient-poor habitat, frequent fires, rocky habitat, and habitat destruction.

Daskalakou et al., 2015 stated that *Cedrus brevifolia* seeds remaining in the soil undergo cold stratification (during winter), which is said to be manifested in early spring germination and seedling emergence and establishment in spring. The records showed that a longer period of cold stratification promotes seed germination in spring when climatic conditions are favourable for seedling survival, development, and growth. Of course, seedlings appear in nature in early spring after the snow melts. Experiments conducted in growth chambers during the winter months, involving seeds subjected to various light conditions, corroborated these findings. The comparable germination rates observed in seeds exposed to darkness, white light, and red light suggested that seed germination was unaffected by light exposure, implying that seeds sown, lying on the soil surface, or situated in forest openings or under vegetation would exhibit similar germination patterns. The data presented in this study confirmed that Cyprian cedar seeds maintain high viability for at least 12 months

when dried at a relatively low (11%) moisture content, provided the storage temperature is also low (5°C).

Seed Collection and Seed Characterization

The time between seed maturity and dispersal is frequently brief, however, the timing of seeding can vary by several weeks depending on the environment in a particular year. Early springs and dry summers in the temperate zone can hasten the maturity of seeds, while strong, dry winds hasten the dissemination of the mature seeds. On the other side, cool, rainy weather can cause ripening and dispersal to be postponed by weeks or even months (Stein et al., 1974). Similar yearly fluctuations in the beginning dates of the rainy and dry seasons may be found in the dry tropics. Therefore, it is vital to examine the crop itself each year to verify the proper timing of gathering. Abscission and fruit shedding are common indicators of fruit maturity, and it is reasonable to suppose that they also point to a high number of developed seeds. The opposite is not always true. The earliest *Tectona grandis* fruits in Thailand begin to shed in March, however, observations have revealed that the most viable fruits are the last to shed, therefore it is advised to pick fruits in April and suggested that the initial seeds or fruits that naturally fall are frequently of low quality; as a result, it is advised to discard them and defer gathering until the height and second half of the season (Hedegart, 1975). According to Sceber & Aejpaoa (1976), the fruit of Dipterocarp species that fall as they mature are often faulty and should not be collected until the majority of the fruit has dropped. White (1994) has also studied the phenological pattern of fruit fall.

The only method to gather tree seeds in India, especially dipterocarp seeds, is to wait until the majority of the fruit is mature and to gather the seeds before they have completely dispersed. On the other side, early collection leads to the gathering of immature, poorly germinal seeds. Because not all

seeds ripen at once, collecting seeds can be challenging. According to Harrington (1973), when the seed reaches its maximum dry weight and no longer receives nutrients from the mother plant, it reaches physiological maturity. Studies on the growth and maturity of seeds are crucial because they can be collected before they start to lose viability and vigour and perform poorly in the field. Many forest trees are fairly erratic in their seed production from year to year. One or more years with a weak seed harvest, or perhaps none at all, may come after a year with a big yield (Morandini, 1962). In a good year, there are several benefits to collecting seeds. Due to the crop's concentration, a high level of good seed bearers may be selected, and the cost of collecting is also reduced. *Tectona grandis* typically produces good blooming every year, but on a three- or four-year cycle, unusually strong seed years have been seen in some locales (Murthy & Krishna, 1973). The number of seeds that may be readily gathered by a man depends on the proportional size and quantity of natural dispersion units.

The techniques for figuring out how to gather seeds rely on the fruit's features, such as size, number, location, distribution of fruits, peduncles' resistance to shaking, tugging, breaking, or cutting, and the time between ripening and opening. It must be emphasized that a good seed has high viability and vigour as well as genetic suitability for the location and the intended use (Sharma & Jain, 1981). The effectiveness and success of growing plants in nurseries and then establishing them in forest plantations are highly dependent on the quality of the seeds utilized. The fit and quality of the seeds have a significant impact on the success of the plantations that are grown from them in tropical locations where trees are reproduced from seed. The greatest way to ensure fast-growing, healthy plantations that can produce high-quality wood is generally agreed upon to be the adoption of sound seed from stands of high intrinsic quality (Aldhous, 1972).

Characters from seeds result from the interplay of different environmental factors. According to Mayer & Poljakoff, (1975), the structure and form of each environmental circumstance in which plants develop throughout seed germination affect the seeds' size and shape. In the case of *Theobroma cacao,* variation in seed shape, length, width, thickness, and weight has been associated with genetic origin (Clement et al., 2003).

Seed Desiccation

Seeds that can be safely dried to moisture contents between 6 and 10% and stored successfully at low temperatures have been described as ‘orthodox’ in storage behaviour, while seeds that cannot be dried to these levels without losing viability have been described as ‘recalcitrant’ (Roberts, 1973). While these terms have been characterized as less than ideal choices to describe the physiological behaviour of seeds (Berjak et al., 1990), they have been widely accepted and are commonly used by all seed scientists today. Within commercially valuable trees, experienced seed handling and processing experts can easily differentiate the orthodox and recalcitrant seeds. Examples of temperate tree species exhibiting recalcitrant seed behaviour include Castanea (Pritchard & Manger, 1990), Aesculus, Quercus, and certain species within the Acer genus (Bonner, 1990), which are characterized by their sensitivity to desiccation and specialized storage requirements.

Tropical recalcitrant tree species include some Hopea, Shorea, and Dipterocarpus (Tang & Tamari, 1973; Yap, 1986; Tompsett, 1987), and several economically important fruits, such as Cocoa, Mango, Jackfruit, and Durian (Chin & Roberts, 1980). Despite progress in understanding seed storage behaviour, many lesser-known species remain unclassified, and the underlying physiological mechanisms driving recalcitrance are still not fully elucidated. Although various hypotheses have been put forward to explain recalcitrant behaviour, further research is needed to

unravel the complex physiological processes involved. One proposal suggests that the storage problems are associated with changes in lipid composition or with physical disruption of the seed membranes which can occur as the seeds age or during drying and/or chilling (Flood & Sinclair, 1981; Seewaldt et al., 1981; Priestly & Leopold, 1983). Seed moisture content (MC) is also closely linked to seed decay and death; the critical MC below which recalcitrant seeds should not be dried reportedly varies from 12-31%, depending on the species being studied (Roberts, 1973; Pammenter et al., 1991; Wesley-Smith et al., 1992). Farrant et al., (1985, 1988) first proposed the hypothesis that a heightened requirement for water bound to cellular structures increases the sensitivity of seeds to desiccation, thereby rendering them more susceptible to damage from water loss. It is now recognized that recalcitrant seed behaviour is based on an increasingly aberrant metabolism during hydrated storage (Pammenter et al., 1994) and as water is lost (Berjak & Pammenter, 1997). A systematic approach to investigating seed desiccation tolerance involves uniformly drying the seeds and conducting regular, intermittent assessments of their moisture content and germination capacity, thereby enabling a comprehensive understanding of the relationships between water content, viability, and desiccation sensitivity.

The classification of seeds as orthodox or recalcitrant can be determined by assessing their viability at moisture contents of 10% or lower. If seeds retain viability at this level, they are deemed orthodox. Conversely, if viability is compromised before reaching this threshold, they are considered recalcitrant. However, the testing protocol for recalcitrance can be complex, as it depends on factors such as drying rate and temperature. The test conditions are often described in terms of relative humidity and temperature, adding nuance to the evaluation process. A range of temperatures has been employed in seed desiccation studies, 2 °C, as reported by Gosling (1989), 15 and 20 °C, utilized by Tompsett (1984) and

Tompsett & Pritchard (1993), 25 °C, chosen by Farrant et al., (1993) and 35 °C, selected by Becwar (1982). Relative humidities are not always specified, but those reported have ranged from 15% (Tompsett, 1984) to 50% (Farrant et al., 1993). Tompsett (1984) concluded that viability loss was independent of the drying method for nine species of Araucaria. Pritchard et al., (1995) reported similar results for excised embryos of seven species of Inga. Berjak et al., (1990) took the opposite position, based on studies with excised embryos and intact seeds of *Avicennia marina* at different stages of differentiation. Additional complications may be seed size (Tompsett & Pritchard, 1993), stage of seed development or maturity (Finch-Savage, 1992; Farrant et al., 1993; Tompsett & Pritchard, 1993), and chemical nature of the principal food reserves (Tompsett, 1984).

Chandrashekar (2013) studied the influence of temperature on the viability of *Vatica chinensis* L., a critically endangered tree species. The seed will not germinate when the moisture content level becomes 61%. Concerning the lapse of time, seed vigour was decreased, and electrolyte leakage was increased. The pivotal factor governing germination and seed viability is moisture content. A synergistic interplay between decreased moisture levels, elevated electrolyte leakage, and alterations in protein and sugar composition likely contribute to the diminished viability of *Vatica chinensis* seeds. Some recognized seed biology studies of the plant species were short-listed in Table 1.

Sl No.	Species	Studies	References
1	*Bauhinia variegata, Careya arborea, Dillenia pentagyna, Sterculia colorata, Sterculia villosa* and *Terminalia belerica*	Phenology and population structure	Dutta & Devi, 2015
2	*Lysiloma divaricatum, Lysiloma acapulcense, Acacia bilimekii* and *Haematoxylum brasiletto*	Germination	Cervantes et al., 2014
3	*Cassia fistula*	Flowering Phenology	Okusanya et al., 2016
4	*Bauhinia variegata, Caesalpinia pulcherrima, Cassia fistula, Cassia glauca, Cassia siamea, Delonix regia, Parkinsonia aculeata, Acacia auriculiformis, Acacia nilotica, Albizia lebbeck, Calliandra tweedii, Prosopis juliflora, Abrus precatorius, Butea monosperma* and *Dalbergia sissoo*	Phenology	Kaur et al., 2013
5	*Aglaia formosana, Bischofia javanica, Dendrocnide meyeniana, Diospyros maritima, Fraxinus griffithii, Koelreuteria henryi, Lagerstroemia subcostal, Macaranga tanarius, Pisonia umbellifera, Radermachia sinica* and *Sapindus mukorossii*	Seed distribution	Lin et al., 2011
6	*Lilium polyphyllum, Polygonatum verticillatum* and *Polygonatum cirrhifolium*	Seed biology, dormancy, and phenophase of seed germination	Sharma et al., 2015
7	Maize, Soyabean, and Wheat	Water Relations in Seed Biology	Villela, 1998
8	*Quercus robur*	Desiccation stress	Finch-Savage et al., 1996
9	*Hevea brasiliensis*	Desiccation and Cryopreservation	Normah et al., 1986
10	*Azadirachta indica*	Desiccation	Varghese & Naithani, 2001
11	*Fagus sylvatica* and *Fagus crenata*	Seed storage behaviour	Lobos & Ellis, 2002
12	*Buchanania lanzan, Diospyros melanoxylon, Gmelina arborea* and *Madhuca indica*	Seed storage physiology	Naithani et al., 2004
13	*Swietenia macrophylla*	Imbibition	Paiva et al., 2006
14	*Elaeis guineensis*	Seed storage behaviour	Ellis et al., 1991a

15	*Shorea robusta*	Analyze the role of lipid superoxide dismutase and peroxidation during the germination	Chaitanya & Naithani, 1994
16	*Madhuca indica*	Seed storage behaviour	Varghese et al., 2002
17	*Ornithopus compressus*	Seed imbibition	Taylor & Revell, 2002
18	*Enterolobium contortisiliquum*	Dormancy breaking and germination	Malavasi & Malavasi, 2004
19	*Acacia catechu, A. nilotica, Albizia lebbek, Dalbergia sissoo* and *Tectona grandis*	Germination	Khera & Singh, 2005
20	*Annona glabra* and *Pachira aquatica*	Germination	Mata & Moreno-Casasola, 2005
21	*Gmelina arborea*	Germination and storability	Naithani et al., 2006
22	*Glycine max*	Biochemical composition and physical characteristics	Kumar et al., 2006
23	*Angelica glauca* and *Pleurospermum angelicoides*	Seed germination	Kandari et al., 2007
24	*Peltophorum pterocarpum, Albizzia lebbek, A. procera, Leucaena leucocephala,* and *Acacia auriculiformis*	Seed germination	Sharma et al., 2008
25	*Glycine max*	Biochemistry and Molecular Biology	Krishnan, 2000
26	*Aesculus hippocastanum*	Physiological and biochemical properties of dormant seeds	Gumilevskaya & Azarkovich, 2007
27	*Dipcadi saxorum*	Seed storage behaviour	Rao et al., 2009
28	*Pinus gerardiana*	Storage and biochemical changes	Malik & Shamet, 2009
29	*Capsicum annuum*	Physiological and biochemical changes in seed quality	Bhanuprakash et al., 2010
30	*Oryza sativa*	Physiological and biochemical changes during seed deterioration	Kapoor et al., 2011
31	*Sterculia urens*	Biochemical changes	Satyanarayana et al., 2011
32	*Elaeis guineensis*	Biochemical characterisation during seed development	Kok et al., 2013
33	*Shorea robusta*	Protein metabolism during desiccation	Parkhey et al., 2014
34	*Trigonella foenum-graecum*	Physiological and biochemical properties	Zandi et al., 2015
35	*Adenanthera pavonina, Alibertia edulis, Aniba rosaeodora, Astrocaryum aculeatum, Bactris gasipaes, Bertholletia*	Seed storage behaviour	Lima et al., 2014

	excelsa, Bixa orellana, Calophyllum brasiliense, Calycophyllum spruceanum, Carapa guianensis, Carapa procera, Cariniana micrantha, Casearia sylvestris, Cassia ferruginea, Cedrela fissilis, Cedrela odorata, Ceiba pentandra, Cenostigma tocantinum, Copaifera multijuga, Cordia goeldiana, Couepia longipendula, Couratari atrovinosa, Couratari guianensis, Couratari longipedicellata, Couratari stellata, Cynometra bauhiniifolia, Dinizia excelsa, Dipteryx odorata, Enterolobium schomburgkii, Eugenia stipitata, Euterpe oleracea, Euterpe precatoria, Genipa americana, Goupia glabra, Handroanthus serratifolius, Helicostylis tomentosa, Hevea brasiliensis, Hymenaea courbaril, Lecythis pisonis, Mauritia flexuosa, Minquartia guianensis, Myrciaria dubia, Ochroma pyramidale,Oenocarpus bacaba, Oenocarpus bataua, Oenocarpus mapora, Oenocarpus minor, Ormosia excelsa, Parkia discolor, Parkia multijuga, Parkia nitida, Parkia pendula, Pseudobombax munguba, Psidium acutangulum, Schefflera morototoni, Schizolobium amazonicum, Spondias mombin, Stryphnodendron guianense, Stryphnodendron microstachyum, Swietenia macrophylla, Tapirira guianensis, Tetragastris panamensis, Theobroma grandiflorum and *Virola surinamensis*		
36	*Bursera bipinnata, Bursera copallifera, Bursera fagaroides var. elongate, Bursera glabrifolia, Eysenhardtia polystachya Leucaena esculenta, Leucaena macrophylla, Guazuma ulmifolia*	Reproductive Phenology and Seed germination	Nunez-Cruz et al., 2018
37	*Oryza sativa*	Seed Development and Stress Response	Naithani et al., 2017
38	*Carica papaya*	Effect of Bioregulators and Chemicals on Seed Germination and Seedling Growth	Sharma et al., 2018
39	*Radermarchera xylocarpa* and *Dolicandrone falcata*	Seed germination	Trivedi et al., 2016

40	*Stevia rebaudiana*	Seed germination, seedling growth and selected biochemical properties	Simlat et al., 2016
41	*Silybum marianum*	Seed germination	Khan et al., 2016
42	*Peltophorum dubium* and *Mimosa bimucronata*	Seed structures in water uptake, dormancy release, and germination	Geisler et al., 2017
43	*Acacia karroo* and *Gleditsia triacanthos*	Seed germination	Kheloufi, 2017
44	*Mesua ferrea*	Seed storage	Mithun et al., 2021
45	*Hopea ponga*	Physiology and biochemical changes during seed development	Mithun et al., 2020
46	*Mesua ferrea*	Phenology and seed development	Mithun et al., 2020
47	*Mesua ferrea*	Biochemical changes during seed storage	Mithun et al., 2023
48	*Hopea parviflora*	Phenology and seed development	Kamarudeen et al., 2017a
49	*Hydnocarpus alpina*	Desiccation on germination and biochemistry	Kamarudeen et al., 2015
50	*Vateria indica*	Phenology and seed development	Kamarudeen et al., 2017
51	*Cucurbita maxima, Cucurbita moschata*	Biochemical changes during various stages	Silva et al., 2017
52	*Euterpe precatoria*	Effects of Temperature, Light and Seed Moisture Content on Germination	Costa et al., 2018
53	*Glycine max* and *Zea mays*	Imbibition	Yang et al., 2018
54	*Lactuca sativa*	Physiological and biochemical changes	Catao et al., 2018
55	*Carthamus tinctorius*	Biochemical changes	Onder et al., 2020
56	*Medicago polymorpha*	Physiological and Biochemical Changes	Li et al., 2023
57	*Tilia miqueiana*	Biochemical Changes in Fruit and Seed Development	Wu et al., 2023
58	*Linum usitatissimum*	Biochemical changes	Paliwal et al., 2024
59	*Lupinus albus*	Biochemical Characterization	Spina et al., 2024

Table 1: Relevant seed biological studies on some plant species

MATERIALS AND METHODS

Hopea ponga (Dennst.) Mabb., a huge tree up to 20 m in height and in the family Dipterocarpaceae, is endangered. In Malayalam, it is also known as "Kambakam" or "Irumbakam." This species is only found in the Western Ghats and South-West Sahyadris; most populations have relatively few. It may be found in the lowlands and evergreen and semi-evergreen woods. There are presently 65-70 locations where the species may be found. This species has experienced habitat fragmentation due to the species' current distribution and the extent of land conversion to holy groves. This species is relatively prevalent in this habitat in the southern Western Ghats and even in the plains. This species has an area of occupancy (AOO) of 256 km^2 and an extension of occurrence (EOO) of 76,972.194 km^2. The occupancy region was determined only via collections; therefore, this species may be under-collected. However, given what we know about this species, the occupied area is unlikely to be more than 500 km^2. Since there has been a population drop of more than 30% in the past and a significant degree of habitat degradation within this species' range of occurrence, the species is classified as Vulnerable (IUCN, 2021).

Our current study, a beacon of hope for the Mabb tree, has gathered seeds and fruits from various locations in Kerala. The process was meticulous, involving gently shaking branches to collect ripe seeds on plastic sheets. The KSCSTE- MBGIPS, Olavanna, Kozhikode district, is home to a thriving population of this tree taxon. Other significant sites include the Sreenarayanpuram (S.N) Sacred Grove in the Keralan, Thrissur district, Kayyath Nagam Kavu, Kannur district, Iringole Sacred Grove, Ernakulam district, Calicut University Campus and Vazhayoor in the of Malappuram district, and the Parappa forest range of Kasargod district. These findings are crucial for understanding the species' survival and can guide future conservation efforts. This plant usually reproduces via seed; however, the

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Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

seeds are refractory, and when they separate from the mother plant, they quickly lose viability in typical environmental circumstances.

Population Studies

To analyze different life forms of the tree species in natural habitats, a detailed analysis of seed dispersal, seed development, and adult individuals was calculated and noted. To analyze the proper dispersal mechanism of seeds, approximately 1x1 meter plots were randomly created in selected natural habitats, and the average number of seeds per square meter plot was calculated. Also, the number of seedlings germinated per m^2 was calculated randomly at selected study sites. The number of medium-sized (10-12 years) trees grown per km^2 and several mature individuals available per square km was also assessed for about 4 years from 2019-2023.

Phenology and Seed Development

Phenological studies deal with reproductive processes, including leaf flushing, flower initiation, anthesis, pollination, and identification of pollinators. The quantity of pollen grains has been determined using a hemocytometer to determine the pollen-ovule ratio. Frequent trips have been made to examine flower commencement, anthesis, seed development, etc. By extracting a slice of the ovary at an early stage, the number of ovules in an ovary has been determined. To ascertain the total floral abundance of a tree, a two-step approach was employed: first, the number of flowers within each inflorescence was tallied, followed by a count of the total number of inflorescences on each branch, thereby enabling a comprehensive calculation of the tree's overall floral yield. Counting the branches on each tree has determined an average number of blooms. By looking at the stigma during blossoming, the maturity of the stigma has been identified. The phenophase events were recorded as per the method suggested by Dafni et al., (2005).

Pollen counts were obtained by crushing a certain number of anthers in 1 ml of distilled water. After that, it was attached to a hemocytometer. The average number of pollen grains in each anther was calculated using ten repetitions. The ovules were exposed by being pressed with a needle after incubating in 0.1N NaOH. They were then examined using a dissection microscope to determine the number of ovules in each ovary. Calculating the average number of buds and flowers per inflorescence, as well as the average number of branches and fruits produced, can gain valuable insights into the plant's reproductive efficiency and seed yield potential, ultimately shedding light on its ability to propagate and disseminate. For the experimental studies, equally aged flower buds were tagged, and the developing fruits were collected periodically at an interval of initially at 4 days till 48DAA and then 60DAA and 80DAA at 14 developmental stages.

A comprehensive approach was employed for the pollination study, combining both *ex-situ* and *in-situ* observations. To facilitate laboratory analysis, blooming twigs were carefully collected and preserved in a humid environment, allowing for detailed examinations and observations to be made later.

The gathered growing fruits were placed in plastic bags and sealed to prevent moisture loss. Vernier callipers were used to measure the fruits' length and diameter, and an electronic balance (Sartorius, Model-BSA 224S-CW) was used to determine their weight. To determine the approximate age of the growing fruits and seeds, external fruit characteristics such as texture, colour, and hairiness were also examined. According to ISTA (1985), the Low Constant Air Owen technique (103°C for 17 hours) was used to determine the moisture content of the gathered seeds.

In *Hopea ponga*, 14 developmental stages, starting from flowering to seed maturity, were identified. From all the stages, one gram of seed tissue was

used for biochemical analysis. Up to 40 DAA, whole seeds were used for biochemical analysis because differentiation of cotyledon and embryo started only after 44 DAA. On 44 DAA (Day after anthesis), one gram of whole seed, cotyledon, and embryo were taken for biochemical analysis.

Moisture Content Determination

According to ISTA regulations from 1985, the High Constant Air Owen technique, which involves drying the seeds at 130°C for an hour, was used to evaluate the moisture content of the seeds at each interval. The moisture content of the developing and desiccated seeds was analyzed following this method. The seeds were finely chopped before being put into weighing bottles with labels and pre-added weights. The loss of moisture throughout the procedure was carefully controlled. The bottles are maintained in sealed desiccators with activated silica gel right away after drying so they can reach room temperature before being weighed. A fresh weight basis was used to compute the moisture content, which was then represented as a percentage of moisture.

Collection of Seeds

A total of 35 locations are analyzed as part of this work. When some ripe fruits begin to separate from the mother plant, mature fruits are gathered from the chosen plants. Fruit-bearing branches were gently shaken to harvest the fruits. The fruits weren't allowed to fall to the ground to prevent harm. The fruit wall was gently removed, and the seeds were carefully gathered in little bags. The seeds were collected only after the seeds were fully mature because the viability of the seeds relies on the timing of harvest. The colour change of the fruit wall after the maturity phase has been used to determine the seed harvesting ripeness. Due to the plant's irregular annual blossoming, care was taken to pick as many fruits as possible. Polyethylene bags were used to transport the collected seeds to the laboratory. Before being de-husked, fruits were spread out on

absorbent paper in the laboratory to be inspected for fungal and other microbial assaults.

Isolation of Seed-Borne Fungi

Plant Surface Sterilization

The most employed technique for detecting and quantifying seed-borne fungi involves isolating them from surface-sterilized plant (seed) tissues. To achieve this, collected plant material undergoes surface sterilization following established protocols with minor changes (Santos et al., 2003), allowing for the effective isolation of fungi from the seed surface. Prior to fungal isolation, plant material underwent a thorough cleaning and surface sterilization regimen. Initially, the material was washed repeatedly under running tap water to remove loose debris. Subsequently, surface sterilization was conducted through a sequential rinse protocol involving:

- 70% ethyl alcohol (C_2H_5OH) for 3 minutes
- 5% sodium hypochlorite (NaOCl) for 7 minutes
- 70% ethanol for 1 minute
- Sterile distilled water, repeated four times

Following sterilization, the plant material was gently dried between sterile filter paper folds to remove excess moisture. This meticulous preparation enabled the successful isolation of seed-borne fungi.

Isolation of Fungal Isolates

Following thorough drying, the surface-sterilized seed materials were segmented into smaller fragments to reveal the inner tissue. Each fragment, now referred to as an explant, was then carefully placed onto a Potato Dextrose Agar (PDA) medium that had been enriched with chloramphenicol (0.5 mg/ml) to inhibit bacterial growth. This precise preparation enabled the explants to be cultured in a controlled environment, facilitating the isolation and growth of seed-borne fungi. Seven explants were put on each agar plate and boundaries were sealed with parafilm to avoid contamination. A total of

35 healthy and surface sterilized plant materials were inoculated on five agar-poured Petri plates. All the plates were incubated at a temperature of 28±2°C (room temperature) to facilitate fungal growth. The plates were regularly inspected for any signs of mycelial development. Upon observing mycelial growth, a subsequent subculturing procedure was performed to further isolate and purify the fungal cultures.

Management of Pathogens

The isolated endophytic fungi were purified and individually transferred to Potato Dextrose Agar (PDA) slants for storage. At last, all the purified fungal endophytes were preserved at a refrigerated temperature of 4°C for future use.

Identification of Seed-Borne Fungi with Molecular Technique

Genomic DNA Isolation

Genomic DNA extraction from selected endophytic fungal isolates was accomplished by adapting the protocol of Moller et al., (1992). Initially, freshly cultured, purified fungal samples were introduced into potato dextrose broth and incubated at 28±2°C for 6-7 days to achieve optimal maturation.

Subsequently, the resulting mycelial biomass was harvested through filtration using autoclaved muslin cloth and desiccated to yield approximately 50 mg of material per sample. The dried mycelial mass was then pulverized into a fine powder using liquid nitrogen and an autoclaved mortar, followed by transfer to a sterilized microcentrifuge tube.

The powdered sample was mixed with 500 µl of pre-warmed TES lysis buffer and incubated at 60°C for 60 minutes, facilitating cellular disruption. Proteinase K was added, and the mixture was further incubated for 60 minutes to ensure complete protein degradation.

The resulting suspension was then combined with 140 µl of 5 M NaCl and 64 µl of 10% (w/v) CTAB, followed by incubation at 65°C for 10 minutes. DNA extraction was performed using an equal volume of

phenol:chloroform:isoamyl alcohol, followed by centrifugation at 14,000 g for 10 minutes. This step was repeated twice to ensure optimal DNA purity.

Further purification was achieved through additional chloroform washes and RNA digestion using RNAase. The DNA was then precipitated using 3 M sodium acetate (pH 5.2) and maintained at -20°C overnight. The DNA pellet was collected by centrifugation, washed twice with 70% ethanol, and air-dried to remove residual ethanol. Finally, the pellet was resuspended in 50 µl of TE buffer.

Estimation of DNA

The concentration of DNA was assessed by electrophoresis on 0.8% agarose gel by comparing the intensities of the band of fungal DNA isolates with standard DNA (lambda DNA marker) of known concentration (300ng/µl). DNA quantification was cross-checked spectrophotometrically using Nanodrop. A standard of known DNA concentration was prepared, and the readings were measured through a UV spectrophotometer at 260nm. The absorbance ratio at 260 nm and 280 nm (A260/A280) was utilized to evaluate the purity and concentration of the extracted DNA. A ratio of 1.8 is commonly regarded as indicative of high-purity DNA, suggesting minimal contamination with proteins or other impurities.

PCR Amplification of ITS Region

To attain homogeneity, DNA samples of disparate concentrations were normalized to a uniform concentration of 50 ng/µl through dilution with TE buffer, a crucial prerequisite for PCR amplification. Subsequently, specific genomic loci of the isolated fungal DNA were magnified using ITS-specific oligonucleotides, namely ITS1 and ITS4 (White et al., 1990), which target eukaryotic ITS sequences. The amplification process was executed in a Bio-Rad Thermal Cycler, facilitating precise thermoregulation throughout the PCR reaction. For each DNA sample, a PCR reaction was run for a 25µl reaction mixture. The PCR reaction mixture for specific amplification of ITS-1 region

of ribosomal genes of endophytic fungal isolates was prepared according to the following recipe after proper standardization. PCR reaction involves the following main steps: Denaturation of DNA strands, annealing of primers to complementary sequences of the template DNA, and finally extension or elongation which is facilitated by polymerases and complementary dNTPs to synthesize new strands. The PCR program was run according to the optimized conditions after proper standardization as tabulated below.

Sequencing of Amplified PCR Product for Fungal Identification

PCR products after amplification were analysed electrophoretically on 1% agarose gel in 1X-TAE buffer and detected by ethidium bromide. The amplicon size was determined by comparing it with the 1kb molecular weight DNA ladder. The amplified DNA fragments were subsequently submitted for sequencing, yielding results in FASTA format. These data were then subjected to in-depth analysis using the nBLAST (nucleotide-basic Local Alignment Search Tool) algorithm from NCBI (National Center for Biotechnology Information). The resulting information was cross-referenced against the comprehensive GenBank database to determine the taxonomic identity of the isolated fungal endophytes. All the sequences were submitted to NCBI GenBank to get the NCBI GenBank accession numbers.

Phylogenetic Analysis

Sequence alignment and nucleotide similarity calculations were performed using the CLUSTAL MUSCLE algorithm (Edger, 2004). Phylogenetic reconstruction was achieved through the Maximum Likelihood (ML) approach (Saitou & Nei, 1987). Evolutionary distances were estimated employing the Maximum Composite Likelihood method (Tamura et al., 2004). Tree topologies were validated by conducting bootstrap resampling with 1000 replications (Felsentein, 1985). All evolutionary analyses were executed within the MEGA 11 framework (Kumar et al., 2018).

Desiccation Studies

In a scientific setting, seeds for desiccation tests were dispersed in a try to ascertain how the moisture content changed during air drying at laboratory conditions. For the analysis of moisture content, germination percentage and biochemical analysis entire seeds were taken at regular intervals in triplicate. The seeds were moved to a humidity chamber before planting for germination to lessen imbibitional damage when moving immediately into the water.

During germination, the quantitative estimation of starch, sugar, protein, amino acids, phenols, lipids, etc., was carried out.

Seed Germination Studies

The viability of seeds was determined based on the percentage of germination. When the pericarp of the seed opens, and the radicle extends to a length of 5mm, the seed is considered to have germinated (ISTA 1993). Studies on seed germination were carried out in Petri plates and acid-free (pH value 7.0) germinating papers (12×18 inch in dimension) from Alkarty Company in New Delhi, India. Three replicates of 100 seeds each were used for the germination test, conducted under standard laboratory conditions (28±2°C and 65%RH).

Biochemical Estimation During Seed Germination

During the germination process, starch, sugar, protein, amino acids, phenols, lipids, etc., were quantitatively estimated. Enzyme assays of α-amylase, β -amylase, and protease were also conducted.

Amylase Enzyme Extraction

A one-gram seed sample was pulverised and mixed with 0.1 M CH_3COONa (Sodium Acetate) Buffer (pH 4.7) to create a uniform suspension. The resulting mixture was then subjected to centrifugation at 5000 rpm for 30 minutes at a temperature of 4°C. Following the removal of the supernatant, the extraction process was repeated twice using the same homogenizing buffer. The collective supernatant obtained from these steps was the source of α-amylase and β-amylase enzymes.

Total Enzyme Assay (α-amylase and β -amylase)

The reaction mixture consisted of 1 ml of 1% starch solution in sodium acetate buffer (0.1 M, pH 4.7) and 1 ml of appropriately diluted enzyme source, which was then incubated for 15 minutes. The enzymatic reaction was terminated by adding 2 ml of 1% dinitro salicylic acid (prepared with 0.2% crystalline phenol, and 0.05% sodium sulphite in 1% NaOH). The mixture was subsequently heated in a boiling water bath for 5 minutes, followed by the addition of 1 ml of 40% sodium potassium tartrate solution. After cooling, the mixture was diluted to 10 ml with distilled water. The resulting orange-red color (nitro aminosalicylic acid) was measured spectrophotometrically at 560 nm. A control sample was prepared by adding dinitro salicylic acid before incubation. Amylase activity was expressed as the amount of maltose released 15 min^{-1}mg $protein^{-1}$.

α-Amylase & β -Amylase

Before adding the enzyme to the incubation mixture, the activity of α-amylase was eliminated by heating the enzyme extract at 70°C for 5 minutes, resulting in only a-amylase. From the total amylase activity, α-amylase activity was subtracted, and the β-amylase activity was calculated.

Protease Enzyme Extraction

Tissue specimens were finely ground in a pre-cooled mortar and pestle with 5.0 ml of ice-chilled 0.1 M sodium phosphate buffer (pH 7.0). The resulting homogenate was subjected to centrifugation at 3000 rpm for 10 minutes (REMI, Model- C24 Plus), and the supernatant fraction was retrieved. The remaining residue was subsequently washed three times with a defined volume of extraction buffer, and the combined supernatant fractions were utilized as the enzyme source for the protease assay. All enzyme extraction procedures were conducted at a temperature of 4 °C.

Protease Assay

Protease activity was assessed by quantifying the amount of protein hydrolysed into amino acids, relative to a control, using the method described

by Snell & Snell (1971). The reaction mixture consisted of 1 ml of 1% casein in sodium phosphate buffer (0.1 M, pH 7) and 1 ml of appropriately diluted enzyme source, which was incubated at 37°C for 1 hour in a test tube. Subsequently, 1 ml of 12% trichloroacetic acid (TCA) was added to precipitate proteins. The mixture was then cooled, and centrifuged at 3000 rpm for 5 minutes, and the supernatant was carefully decanted. The protein content in the supernatant was determined using the Lowry et al., method (1951). A control sample was prepared by adding 1 ml of 12% TCA before incubation.

Seed Storage Studies

The fruits were gathered, de-husked, cleaned, and washed in running water for 30 minutes. Then, they were spread over absorbent paper to dry the seeds. They were kept in eight distinct environments. The storage conditions studied are detailed below.

1. Seeds stored at 0°C in closed polycarbonate bottles and polythene bags.
2. Seeds stored at 5°C closed polycarbonate bottles and polythene bags.
3. Seeds stored at 10°C closed polycarbonate bottles and polythene bags.
4. Seeds stored at 20±2°C and 40% RH in closed polycarbonate bottles and open plastic trays.
5. Seeds are stored in room conditions (laboratory conditions 28±2°C and 65% RH) in closed polycarbonate bottles, polythene bags, and open plastic trays.
6. Seeds stored at -20°C in aluminium foil packets.
7. Seeds stored at -80 °C in aluminium foil packets.
8. Seeds stored at -196 °C (liquid nitrogen) in plastic cryo vials.

The germination percentage was used to determine the viability of seed samples collected at intervals of 1 to 5 days. The durability of the seeds

spread out in open plastic trays at laboratory temperatures (28±2°C & 65% RH) was compared to the longevity of the seeds kept in various settings.

Fast drying was accomplished using the modified Yao et al., (2014) approach. The seeds were dried in closed jars with an active silica gel-to-seed ratio of 18:1 by volume. The conditions were 28±2°C, 4% RH, and activated silica gel. Once the blue tint fades, the silica is once again active.

Biochemical Estimation During Seed Storage

During seed storage, the quantitative estimation of starch, sugar, protein, amino acid, phenols, lipids, etc. was carried out. In each storage condition, each protein fraction (Albumin, Globulin, Prolamin, Glutelin) and total protein content was analyzed. Enzyme assays of α-amylase, β -amylase, and protease were also conducted during germination.

Biochemical Analysis

During seed development, desiccation, and storage, metabolites like total sugars, individual sugars, phenolics, amino acids, protein, lipids, and starch were examined at different intervals. To analyze the stress-induced alterations happening during the desiccation of the seeds, the activity of antioxidant enzymes such as peroxidase and polyphenol oxidase was analyzed at different intervals. After moisture analysis, every procedure was completed except for the enzyme assay. The de-coated seeds were steeped in water before the enzyme test. The measurement of MDA produced to determine the level of lipid peroxidation was used to assess the membrane disintegration related to desiccation. Measurements of conductivity and tetrazolium treatment were also used to evaluate the viability loss of the seed.

Extraction of Metabolites

Seed samples (1gm) from various experiments at the time points were homogenized with a measured volume of 80% ethanol (v/v) in distilled water. The mixture was then subjected to centrifugation at 4000 rpm for

ten minutes. Following centrifugation, the supernatant was collected and adjusted to a volume of 10 ml. The remaining residue was rewashed twice with 10.0 ml of 80% ethanol (v/v) in distilled water, and the combined supernatant was utilized as the source for analyzing Total sugars, phenolics, and amino acids. A 5 ml aliquot of the extract was transferred to a Chinese dish and dried in a hot air oven at 60 °C. After drying, 5 ml of distilled water was added, and the mixture was thoroughly mixed. The resulting mixture was then centrifuged at 3000 rpm for ten minutes to obtain the supernatant, which was used to quantify the amount of total soluble sugar (TSS). The residue was washed two times with 4 ml of PCA (15%) centrifuged at 3000 rpm for ten minutes each, and the combined extract was used to estimate starch.

Estimation of Total Soluble Sugars

The technique suggested by Montgomery (1957) was used to estimate the total sugar content. The 1 ml aliquot from the sample used to calculate the amount of sugar was combined with 0.1 ml and 80% (w/v) phenol and well blended. A burette was rapidly used to add 5 ml of strong sulfuric acid to the tube. The optical density of the resulting solution was subsequently measured at 540 nm using a spectrophotometer (JASCO, V-730) after cooling. D-glucose served as the reference standard. In place of the sample, 1 ml of distilled water was used as the blank solution. By utilizing a glucose standard curve, the total sugar content was calculated and expressed as milligrams per gram of dry weight (mg g^{-1} dr. wt.).

Quantification of Phenol

The phenol content of the tissues was quantified using the Swain & Hillis (1959) method. Following vigorous agitation of 1 ml of the aliquot with 0.5 ml of Folin-Ciacoltaue reagent for 3 minutes, 2 ml of 20% Na_2CO_3 was added. The tubes were thoroughly mixed before being immersed in a hot water bath for three minutes. After cooling, the resulting color was measured at 650 nm. Utilizing catechol as the reference standard, the total

phenolic content was calculated from a standard curve and expressed as milligrams per gram of dry weight (mg g^{-1} dr. wt.)

Estimation of Total Amino Acid

Sadasivam & Manickam (1996) assessed the amino acid content in diverse tissues. One millilitre of the alcoholic supernatant and two millilitres of the Ninhydrin reagent (0.2% Ninhydrin in 80% ethanol) made up the reaction mixture. For 15 minutes, the mixture was brought to a boil in a water bath. It is then chilled and diluted with 50% ethanol to make 5ml. The tissue's total amino acid content was determined using a standard graph plotted against L-Leucine and represented as mg of amino acid per mg^{-1}dr.wt. The optical density was measured at 620 nm.

Extraction and Estimation of Starch

The supernatants extracted using PCA were pooled and utilized as a source for quantifying starch content according to the method of McCready et al., (1950). The assay mixture consisted of 4.0 ml of chilled Anthrone reagent (0.2% in 96% H_2SO_4) and 1.0 ml of appropriately diluted aliquot. The mixture was heated for 8 minutes in a boiling water bath, and after cooling in the (Julabo Corio CO) water bath, the resulting green color was measured at 630 nm. D-glucose served as the reference standard. The starch content was calculated as milligrams per gram of dry weight (mg g^{-1} dr. wt.) by multiplying the measured value by a conversion factor of 0.9.

Extraction and Quantification of Protein

The method described by Lowry et al., (1951) was employed to quantify the total protein content. A 100 mg sample was homogenized in a medium containing 50 mM phosphate buffer (pH 7.5) and 50 mM 2-mercaptoethanol using a mortar and pestle. The homogenate was then centrifuged at 4000 rpm (REMI, Model- C24 Plus) for 10 minutes. The resulting supernatant was collected and adjusted to a volume of 10 ml. A 2.0 ml portion of the supernatant was transferred to a centrifuge tube,

combined with an equal volume of 10% (w/v) trichloroacetic acid (TCA), and thoroughly mixed. For an hour, the mixture was placed in an ice bath to help the protein flocculate. After 10 minutes of centrifugation of the mixture, the supernatant was discarded. The residue was centrifuged again after being washed with 2.0% (w/v) TCA. To remove starch and lipids, the precipitate was washed twice with 15% (v/v) PCA and centrifuged thrice with diethyl ether. To get rid of any pigments, the precipitate was washed with 80% (v/v) acetone. The centrifuged pellet was immersed in a water bath for 10 minutes to solubilize it in 5 ml of 0.1N sodium hydroxide. The resulting supernatant was then collected following centrifugation. Aliquots of known volume were pipette out in triplicate from the supernatant and diluted with distilled water to 1.0 ml. This was then combined thoroughly with 5 ml of alkaline copper reagent. Following a 10-minute interval, 0.5 ml of 1N Folin-Ciocalteu phenol reagent was added, and the mixture was vigorously agitated. The tubes were then allowed to stand for 30 minutes to facilitate color development. The optical density was subsequently measured at 700 nm using a JASCO, V-730 spectrophotometer. Bovine serum albumin (BSA) served as the reference standard. The total protein content was expressed as milligrams per gram of dry weight (mg g^{-1} dr. wt.)

Lipid Extraction and Estimation

The technique developed by Bligh & Dyer (1959) was used to assess the total extractable lipids. One gram of tissue was mashed in a glass mortar and pestle with a pinch of acid-washed sand and 10 ml of distilled water. The fine paste was put into a conical flask filled with chloroform and methanol (2:1 v/v) and well stirred. The flasks were left to extract the lipids completely while remaining at room temperature and in the dark all night. The flask was once more filled with 20 ml of water. After incubation, the slurry was subjected to centrifugation at 5000 rpm for 15 minutes. This

resulted in the formation of a distinct chloroform layer at the bottom, which was carefully decanted and removed. A pre-weighed, oven-dried China dish was transferred to the chloroform layer, which was then slowly evaporated to dryness. Chloroform was completely evaporated, and the weight was once more determined with an electronic balance. The total weight of lipids in the tissues was calculated as the difference between these two weights and given as mg g^{-1}dr.wt.

Cellular Membrane Damage Assay

Malondialdehyde (MDA) can be detected using the thiobarbituric acid (TBA) reaction test (Yusuf et al., 2016). In 5 mL of 5% trichloroacetic acid (TCA), finely powdered samples (0.5 g) were homogenized before being centrifuged at 10,000 g for 10 min. The mixture of two mL of pure supernatant and two mL of TBA in TCA was incubated at 95°C for 60 minutes. Using a spectrophotometer, the absorbance at 450, 532, and 600 nm was used to determine the MDA concentration.

Electrolyte Estimation

Based on Lutts et al., (1996), electrolyte leakage was assessed. After 24 hours of incubation on a rotary shaker, the samples' electrical conductivity (EC1) was measured. The samples were inserted into tightly sealed vials containing 20 mL of highly purified water, achieved through a double distillation process. After autoclaving the samples for 20 minutes at 120 °C, the electrical conductivity (EC2) was again determined. It had the following definition and percentage representation: EC1/EC2 × 100.

Extraction and Estimation of Sugars through HPLC

Sample Preparation

A one-gram seed sample was soaked in 95 % ethanol and ground into a fine paste in a mortar. After that, the mixture was refluxed in a water bath for two hours (Harborne, 1973). To get rid of the alcohol, the clear reflux filtrate was concentrated. Double-distilled water is introduced to the mixture to augment its volume to a final volume of 5 ml. Any insoluble

elements were removed using filtration. Petroleum ether was added to extract the lipids, which were then separated in a funnel. The aqueous extract was subsequently filtered through the Whatman No. 1 paper, and the resulting filtrate was then employed for high-performance liquid chromatography (HPLC) analysis.

HPLC Analysis

The aqueous solutions produced during sample preparation were filtered using a 0.45-m Nylon filter before running through an HPLC system (Shimadzu LC 2010A HT) with a guard column. The mobile phase of the LC-NH2 column was an acetonitrile-water (70:30) combination flowing at a 1 ml/min rate. To find the sugars, 20: l µl of the sample was put into the column. Sugars were found using a detector for refractive index. The peak areas were determined by the automated computer integrator using standard solutions of sugars such as glucose, galactose, arabinose, fructose, sucrose, and ethylene glycol at a concentration of 1 mg/5 ml. Calculated sugar concentrations were compared to standard solution concentrations. Retention durations for glucose, galactose, arabinose, fructose, and sucrose were also recorded.

Enzyme Assay

Extraction of Enzymes

The 1-gram samples of seed tissue, collected at various stages, were homogenized with a small amount of pure sand, 0.1M cold sodium phosphate buffer (pH 7.0), in a pre-chilled mortar and pestle. The resulting homogenate was then centrifuged at 5000 rpm for 20 minutes in a chilled centrifuge, and the supernatant was carefully collected. The proteins present in the supernatant were precipitated twice using cold acetone and subsequently resuspended in the extraction buffer. The supernatant from the centrifuged extract served as the enzyme source for the peroxidase and polyphenol oxidase assays. The entire extraction procedure was carried out at a temperature of 4°C.

Determination of Lipid Peroxidation

The extent of lipid peroxidation was assessed by quantifying the amount of malondialdehyde (MDA) produced in the thiobarbituric acid (TBA) reaction, as described by Heath & Packer (1968). Seed samples (0.5 g) were pulverized in 10 mL of 5% trichloroacetic acid (TCA) solution. The resulting homogenate was centrifuged at 15000 × g for 10 minutes, and the supernatant was collected. A 2-millilitre aliquot of the supernatant was then mixed with 4 millilitres of 0.5% thiobarbituric acid in 20% TCA. The mixture was heated at 95°C for 30 minutes, rapidly cooled in an ice bath, and centrifuged at 10,000 × g for 10 minutes. The absorbance of the supernatant was measured at 532 and 600 nm. The MDA concentration was calculated using its molar extinction coefficient (155 mM^{-1} cm^{-1}), corrected for non-specific absorbance at 600 nm, and expressed as mol MDA g^{-1} fresh weight.

Antioxidant Enzyme Activities

Assay of SOD Activity

The activity of superoxide dismutase (SOD) was evaluated using the method described by Gianopolitis & Ries (1977). The reaction mixture comprised 13 mM methionine, 0.025 mM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate, and 0.5 mL of enzyme extract in a total volume of 3.0 mL. The reaction was initiated by adding 0.002 mM riboflavin, and the tubes were then agitated and placed under two 15 W fluorescent lights. The reaction was started by illuminating the mixture at 30°C. After a 15-minute incubation, the lights were turned off, and the tubes were covered with black cloth. The non-irradiated reaction mixture served as a control, while the enzyme-free reaction medium produced the maximum color and absorbance at 560 nm. One unit of SOD activity was defined as the amount

of enzyme required to achieve a 50% inhibition of the photochemical reduction of NBT and was expressed as Units/min/mg protein.

Assay of POD Activity

Based on the method described by Maehly & Chance (1954), the activity of peroxidase (POD) was evaluated by monitoring the oxidation of pyrogallol in the presence of hydrogen peroxide (H_2O_2). The reaction mixture (3 ml) consisted of 10 mM phosphate buffer (KH_2PO_4/K_2HPO_4) at pH 7.0, 10 mM H_2O_2, 20 mM pyrogallol, and 0.5 mL of enzyme extract. The formation of purpurogallin increased absorbance, which was quantified at 470 nm (Klapheck et al., 1990), and the enzyme activity was expressed as A470/min/mg protein.

Assay of CAT Activity

A 3 mL reaction solution comprising 50 mM phosphate buffer (pH 7.0), 30% (w/v) hydrogen peroxide (H_2O_2), and 0.5 mL enzyme extract was employed to determine the catalase (CAT) activity, following the method described by Aebi (1984). The initiation of the reaction was triggered by the addition of the enzyme extract. The catalytic activity was quantified by measuring the decrease in absorbance at 240 nm over 1 minute, after H_2O_2 consumption (Havir & McHale, 1987), and expressed as Units/min/mg protein.

Extraction of Seed Samples for FT-IR and LC-MS Analysis

Sample Preparation

Fresh Seed Samples

Fresh seed samples are collected, de-husked, and cleaned in running water to remove unwanted materials. They are then dried to remove the surface water content after washing. Then, they are chopped or ground for Soxhlet extraction. In this study, mostly grinding and milling methods are employed for size reduction.

Drying of Seed Samples for Dry Samples

Drying is a vital step in the extraction of plant materials, as fresh plant materials contain active enzymes that can alter the composition of the extracts through ongoing metabolic reactions. Therefore, drying is essential for preparing plant materials before extraction. To prevent the loss of volatile compounds and light-sensitive constituents, many researchers dry plant materials under shade in a darkroom using air-drying. However, various drying methods such as microwave drying, oven drying, silica gel drying, salt drying, and freeze-drying are also employed. In this study, oven drying was chosen as the preferred method due to the high moisture content of the seeds. Air drying was not feasible, as fungal growth occurs within 2-3 days under these conditions

Grinding

Particle size reduction through grinding and milling is a critical step in the Soxhlet extraction process, as it significantly enhances the surface area of the powdered particles. A larger surface area facilitates optimal interaction between the powdered particles and the extraction solvent, leading to efficient extraction. Various grinding and milling techniques are commonly utilized for size reduction, and this approach was adopted in the present study.

Sieving

Particle size uniformity is essential for efficient Soxhlet extraction, as it enables the solvent to permeate the particles in the thimble consistently. Both excessively fine and coarse powders are suboptimal for effective extraction; the former may cause clogging during the process, while the latter can extend the extraction duration. Size separation is typically achieved through sieving, and particle size can be determined by methods such as sieving and microscopy. Researchers employ different particle size ranges for extracting various plant materials using the Soxhlet method.

Selection of Solvent for Soxhlet Extraction

The choice of solvent for Soxhlet extraction depends on the process of isolating phytoconstituents. The solvent must be easily removable and inert. Typically, solvent selection follows the order of increasing polarity, such as hexane, acetone, and methanol.

Defatting is crucial for certain plant materials because waxy substances can cause emulsification with the solvent, disrupting the extraction process. Methanol, being a semi-polar solvent, can extract a wide range of phytoconstituents.

Post Extraction Process

After completing the Soxhlet extraction process, the extracted materials are subjected to concentration, the solvent is removed through evaporation, and the resulting extract is stored for later analysis or application. Distillation or various evaporators can be used to separate the extract from the solvent. Once collected, the extract is kept in a tightly sealed container, wrapped in aluminium foil, and refrigerated. The raw material weighed beforehand, is placed in a capsule made of filter paper within the Soxhlet apparatus. The solvent, in a 1:3 ratio to the mass of the raw material, is added to a round-bottom flask and heated in a water bath with a temperature control. The water bath's temperature is set according to the solvent's boiling point. The duration of extraction varies with the solvent used. After the Soxhlet extraction, the solvent in the flask becomes enriched with the bioactive components dissolved from the plant material.

FT-IR Analysis

Infrared spectroscopy investigates the interaction between infrared electromagnetic radiation and matter, enabling the measurement of molecular vibrations. This technique facilitates both qualitative and quantitative analyses of samples. A Fourier transform infrared (FTIR)

spectrometer is an optical instrument for identifying unknown organic and inorganic samples in various physical states.

When infrared radiation traverses a material, a portion of the intensity remains unabsorbed, while the remaining intensity interacts with molecules and is absorbed. The ratio of absorbed intensity to total incident intensity is directly proportional to the concentration of absorbing molecules, as described by Beer-Lambert's law. The methodology employed was based on the description by Sacithraa et al., (2013). The sample was analyzed using FT-IR spectroscopy, with an infrared light source generating a wavelength range of 4000 to 400 cm^{-1}, scanned 32 times per sample, at a resolution of 4. The infrared spectrum was Fourier transformed and recorded in absorption mode. The resulting FT-IR Interferogram displayed the relationship between wave number and absorption. IR solution software was utilized to generate the spectrum.

LC-MS Analysis

Chromatographic analyses were conducted using an Agilent 1100 liquid chromatograph (Agilent Technologies, USA), comprising a binary pump and an automated sampler. Before injection (50 µL), the extracts were filtered through a 0.45 µm Millex HA filter (Millipore Corp.). Chromatographic separation was achieved on a Phermerex C18 column (25 cm × 4.6 mm, 5 µm particle size) coupled with a 5 µm C18 guard column, maintained at 25 °C. The mobile phases consisted of (A) formic acid/water (1:99, v/v) and (B) methanol, with a gradient elution program: 0-43 min, 10-90% B; 43-45 min, 90-10% B; 45-50 min, 10% B. The solvent flow rate was 0.9 mL/min. Mass spectrometric detection was performed in positive ion mode using a Q Trap mass spectrometer (Applied Biosystems/MDS Sciex, USA) equipped with a Turbo ion spray source at 480 °C. The ion spray voltage was set to 5000 V, with a declustering potential (DP) of 35 V and collision energy (CE) of 21 V. Nitrogen served as the nebulizing and collision gas. Data acquisition and

processing were performed using Analyst 1.6 software (Applied Biosystems/MDS Sciex, USA).

Late Embryogenesis Abundant (LEA) Protein

Frozen embryos weighing 50 milligrams extracted from the seeds that were stored at 20±2°C and 40% RH in closed polycarbonate bottles 30 days after storage (30 D), 60 D, 90 D, 150 D, 210 D, and 240 D are used for analysis. Embryos were homogenized in 1 mL of high-salt extraction buffer (50 mM Tris-HCl, pH 8.5, 15 mM Beta-Mercaptoethanol, 1 mM Dithiothreitol) using chilled mortars and pestles. The resulting extracts were transferred to 1.5 mL Eppendorf tubes and centrifuged at 16,000 × g for 15 minutes at 4°C. The supernatants were collected, and the precipitate was discarded. Before electrophoresis, protein quantification was performed using the Bradford (1976) method, and volumes were adjusted to contain 40 µg of soluble protein per sample. A 10 µL "Magic Mix" loading buffer solution (SDS 1%, B-ME 1%, EDTA 0.074%, glycerol 10%, bromophenol blue 0.05%, in Tris-HCl 50 mM, pH 6.8) was added to each sample (Table2). The soluble protein samples were denatured in a boiling water bath for 5 minutes and loaded onto a polyacrylamide-sodium dodecyl sulfate (SDS) gel (10 × 10 cm). A 12% acrylamide gel (Laemmli, 1970) was used, and electrophoresis was performed at 120 V for 2 hours using a Tris-glycine run buffer (Tris-HCl 0.1% pH 8.0, glycine 1.15%, SDS 0.8%) (Table 3). The gel was stained with Coomassie blue for 2 hours and destained in a solution of acetic acid (10%), methanol (40%), and water (50%) (Laemmli, 1970). The molecular weight of the separated proteins was determined using a calibration curve generated from a kit of protein markers with known molecular weights (Prestained SDS-PAGE Standards, Low range, Bio-Rad, Hercules, CA, USA).

Sl No.	Solutions	pH	Components	Required Quantity
1	Solution A Tris Buffer 1.5M	8.8	Tris HCL H_2O	18.17gm 100ml
2	Solution B Tris Buffer 1.5M	6.8	Tris HCL H_2O	3.03gm 50ml
3	Solution C Acrylamide: Bis-Acrylamide Solution (30%)	-	Acrylamide Bis Acrylamide H_2O	29gm 1gm 100ml
4	Solution D Ammonium persulphate (10%)	-	Ammonium persulphate H_2O	1gm 10ml
5	Solution E Sodium dodecyl sulfate (10%)	-	Sodium dodecyl sulphate H_2O	10gm 100ml
6	Running Buffer	8.3	Tris base Glycene SDS H_2O	7.25gm 34.56gm 24gm 2.4l
7	Fixative TCA (10%)	-	TCA Methanol H_2O	20gm 80ml 200ml
8	Staining Solution Commassie Blue (0.25%)	-	Commassie Blue Methanol Acetic Acid H_2O	0.25gm 45ml 10ml 45ml
9	Destaining Solution	-	Methanol Acetic Acid H_2O	200ml 70ml 230ml

Table 2: Preparation of stock solutions for SDS-PAGE

Sl No.	Solution	Separating gel (15%) – 5ml	Stacking gel (4%) – 3ml
1	1.5M Tris Buffer (pH 8.8)	1.25ml	-
2	0.5M Tris Buffer (pH 6.8)	-	0.375ml
3	Acrylamide-Bis acrylamide solution (30%)	2ml	0.5ml
4	Sodium dodecyl sulfate 10%	0.05ml	0.03ml
5	Distilled Water	1.65ml	2.065ml
6	Ammonium persulfate (10%)	0.05ml	0.03ml
7	TEMED	0.002ml	0.003ml

Table 3: Preparation of SDS-PAGE Gel

Statistical Analysis

The experimental data were subjected to Analysis of Variance (ANOVA) at a 1% significance level to identify significant differences. Post-hoc tests,

including the Least Significant Difference (LSD) test and Duncan's Multiple Range Test (DMRT), were then employed at the same 1% significance level to compare and distinguish the mean values among treatments.

RESULTS

Study Area

The population study was conducted in thirty-five locations across Kerala's political boundary, from Kasargod to Thiruvananthapuram districts (Fig.1 & Table: 4). Kozhikode district comprises a significant cluster for *Hopea ponga*, featuring ten populations with the highest individual counts. The temperature range within these populations is 25-33°C, humidity varies from 60-80%, and light intensity spans 2000-20,000 lux. The mean soil temperature of the study areas is 20-22°C, soil moisture content ranges from 14-27%, and the annual mean precipitation lies between 2500-3100 mm. *Hopea ponga*, a typical sub-canopy tree of the wet evergreen forests, is endemic to the Western Ghats and can grow to an altitude of 900 m. The KSCSTE-Malabar Botanical Garden & Institute for Plant Sciences hosts one of the largest natural populations of *Hopea ponga* with 107 individuals. Other thriving populations include those in Silent Valley and the Melpalani Murugan temple. Some populations are in protected areas like Botanical Gardens, National Parks, and Sacred Groves. Some populations are fragmented in nature and some crucial anthropogenic interferences are reasons leading to the decline of populations in the form of road widening, Conversion of land into agriculture and other plantations, Expansion of railway lines, and other developmental activities. Generally, regeneration of this species is only through seeds. Still, the recalcitrant behaviour of seeds with loss of their viability within a short period in natural conditions is leading to the decline of the population without further regeneration.

Sl No.	Locations	GPS coordinates
1	Muzhakkunne, Kannur	11°55'57"N 75°44'23"E
2	Iringole kavu, Rayamangalam, Ernakulam	10°06'54.9"N 76°29'47.8"E
3	Adoor, Kasaragod, Near Pazhaswini River	12°34'13.8"N 75°17'04.4"E
4	Vazhayur, Malappuram	11°12'12.6"N 75°53'19.0"E
5	Bellipady, Kasargod, Kerala	12°34'55"N 75°19'28"E
6	Calicut University Campus, Malappuram	11°08'01.9"N 75°53'26.3"E

MITHUN VENUGOPAL, Ph.D. Thesis
Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

7	Kuruwadweep, Wayanad	11°49'18.0"N 76°05'32.0"E
8	Vallikattukavu, Kozhikode	11°23'18.2"N 75°47'11.4"E
9	Parapanangadi, Malappuram	11°02'51.0"N 75°51'36.4"E
10	Peringave, Malappuram	11°11'52.7"N 75°53'16.5"E
11	Melpalani Murugan temple, Kozhikode	11°16'24.4"N 75°51'33.8"E
12	Mukkali, Palakkad	11°02'53.5"N 76°32'23.6"E
13	Tusharagiri, Kozhikode	11°28'22.4"N 76°03'15.0"E
14	KSCSTE- Malabar Botanical Garden & Institute for Plant Sciences	11°14'31.6"N 75°49'43.7"E
15	Vazhayur- Peringave route	11°13'44.8"N 75°54'17.3"E
16	Mavoor, Kozhikode	11°15'46.8"N 75°56'28.7"E
17	Poolacode, Kozhikode	11°17'50.6"N 75°56'22.6"E
18	Ramanattukara, Kozhikode	11°11'05.5"N 75°53'04.0"E
19	CMS College, Kottayam	9°35'51.59" N 76º31'19.88" E
20	Sri Shangukulangara Bhagavathi Kavu at Sree Narayana Puram, Thrissur	10°31'50.73"N 76°11'54.40"E
21	NagaruKavu, Puliyoor Konam, Thiruvananthapuram	8°31'6.83"N 76°58'27.16"E
22	Siva temple Kavu, Ambalathara, Thiruvananthapuram	8°27'11.90"N 76°56'57.89"E
23	Chalakkudy, Thrissur	10°18'15.20"N 76°35'47.00"E
24	Karimpuzha, Malappuram	10°56'22.98"N 76°24'13.57"E
25	Rosemala, Kollam	8°54'54.00"N 77°10'12.23"E
26	Attapady, Palakkad	11° 4'15.57"N 76°34'3.97"E
27	Poochipara, Palakkad	11° 6'44.25"N 76°25'9.35"E
28	Vaniyampuzha, Malappuram	11°27'13.09"N 76°14'7.00"E
29	Nedumkayam Forest, Malappuram	11°17'34.75"N 76°20'28.39"E
30	Thenmala, Kollam	8°57'59.85"N 77° 3'51.75"E
31	Syrendhri, Silent Valley National Park, Palakkad	11° 5'57.26"N 76°27'5.76"E
32	Inchathotty, Neriamangalam, Idukki	10° 5'15.29"N 76°44'44.12"E
33	Shendurney Wildlife Sanctuary	8°54'39.72"N 77°10'44.40"E
34	Ponnamveli, Alapuzha	9°44'20.18"N 76°18'43.90"E
35	Janakikadu, Kuttiady, Kozhikode	11°37'32.78"N 75°47'36.69"E

Table: 4 Population assessed during the study period

Phenology and Seed Development

Hopea ponga is an evergreen tree 18 meters in height with grey or brown colored bark. In mature individuals, white resin is exudated from the bark. Leaves are simple, alternate, and narrow oblong-lanceolate to oblanceolate-elliptic, base is obtuse to oblique. Margins are entire, apex acute to acuminate. In *Hopea ponga,* leaf flushing started from September onwards and the process extended up to December. Young leaves are yellowish green and change to dark green on maturity. Though the leaf flushing occurred every

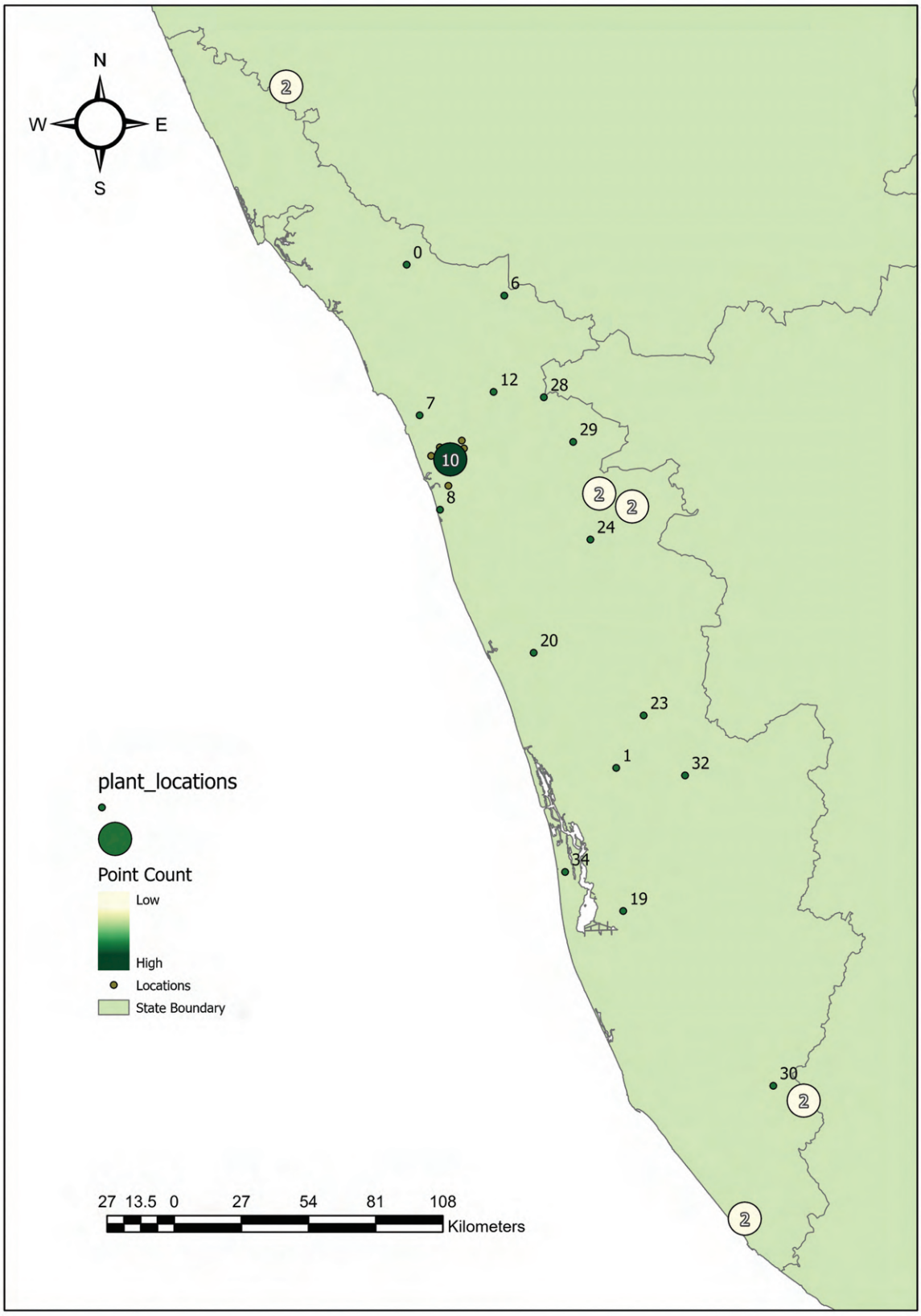


Fig.1 Study area - Political boundary of Kerala State, India

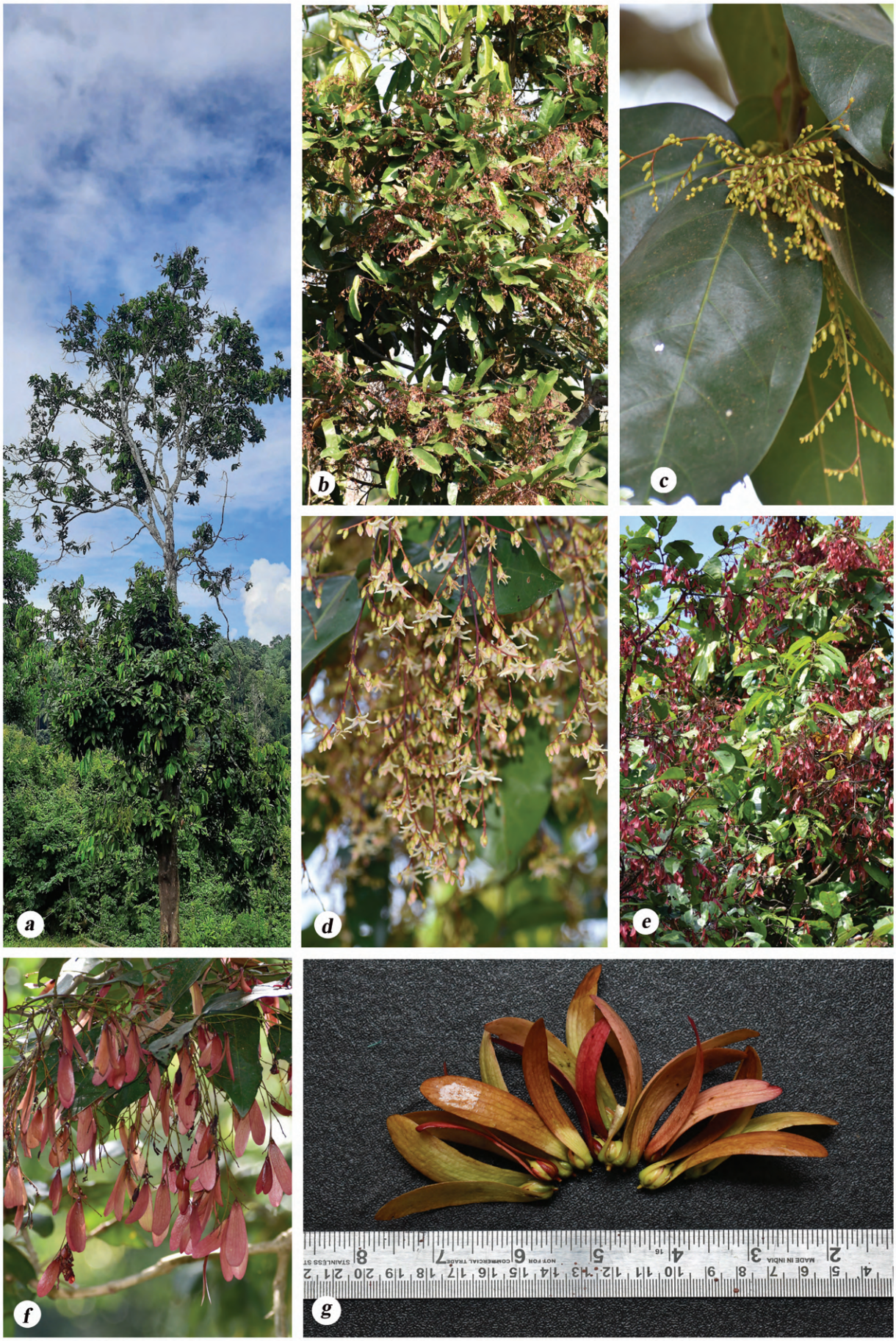


Fig.2 a. Habit, b. Mast Flowering, c. Inflorescence with buds, d. Inflorescence, e. Mast Fruiting, f. Immature fruits, g. Mature Fruits

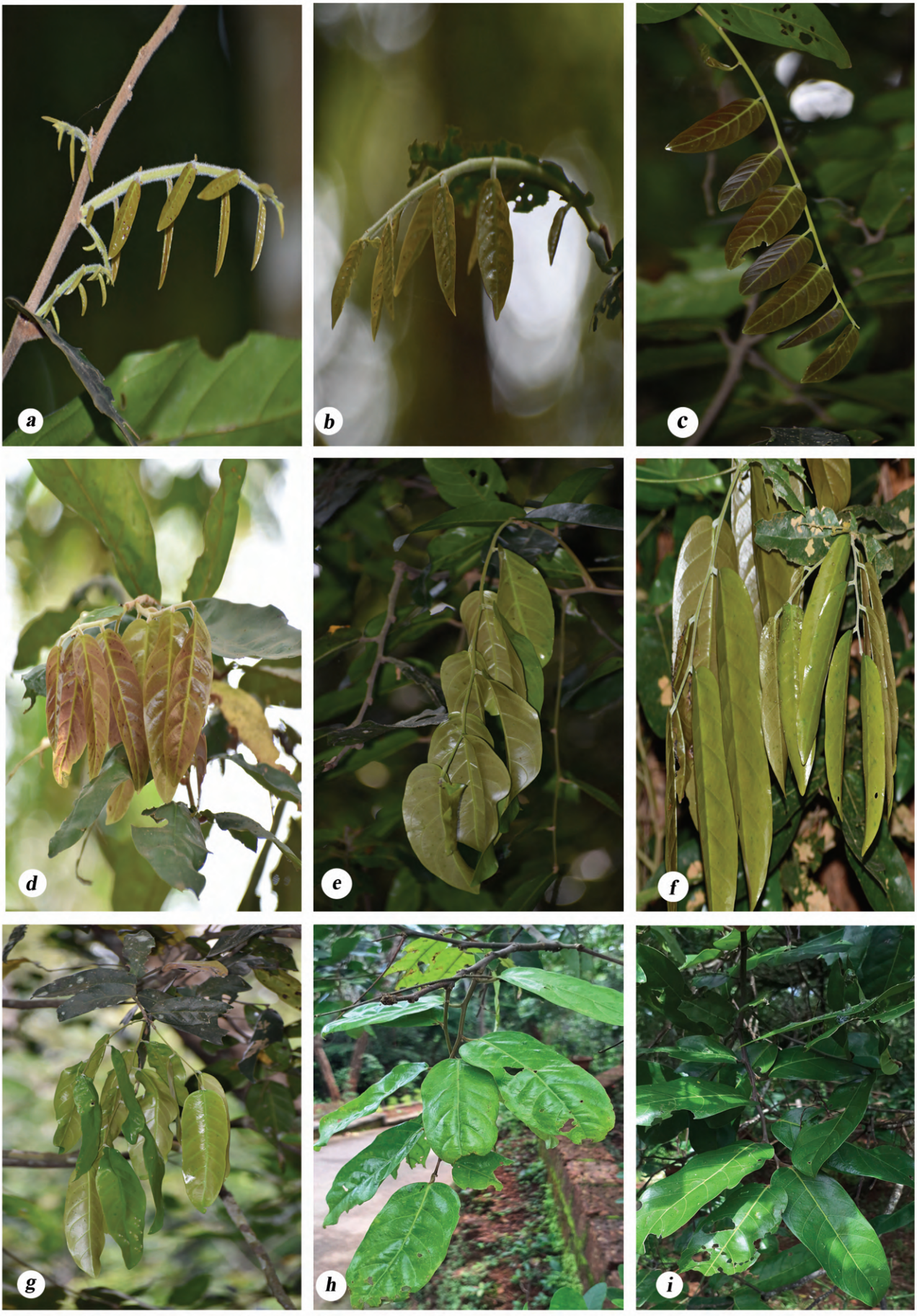


Fig.3 Different stages of leaf flushing - a. Initial stage, b. After 5 days, c. After 10 days, d. After 15 days, e. After 20 days, f. After 25 days, g. After 30 days, h. After 35 days, i. Final stage.

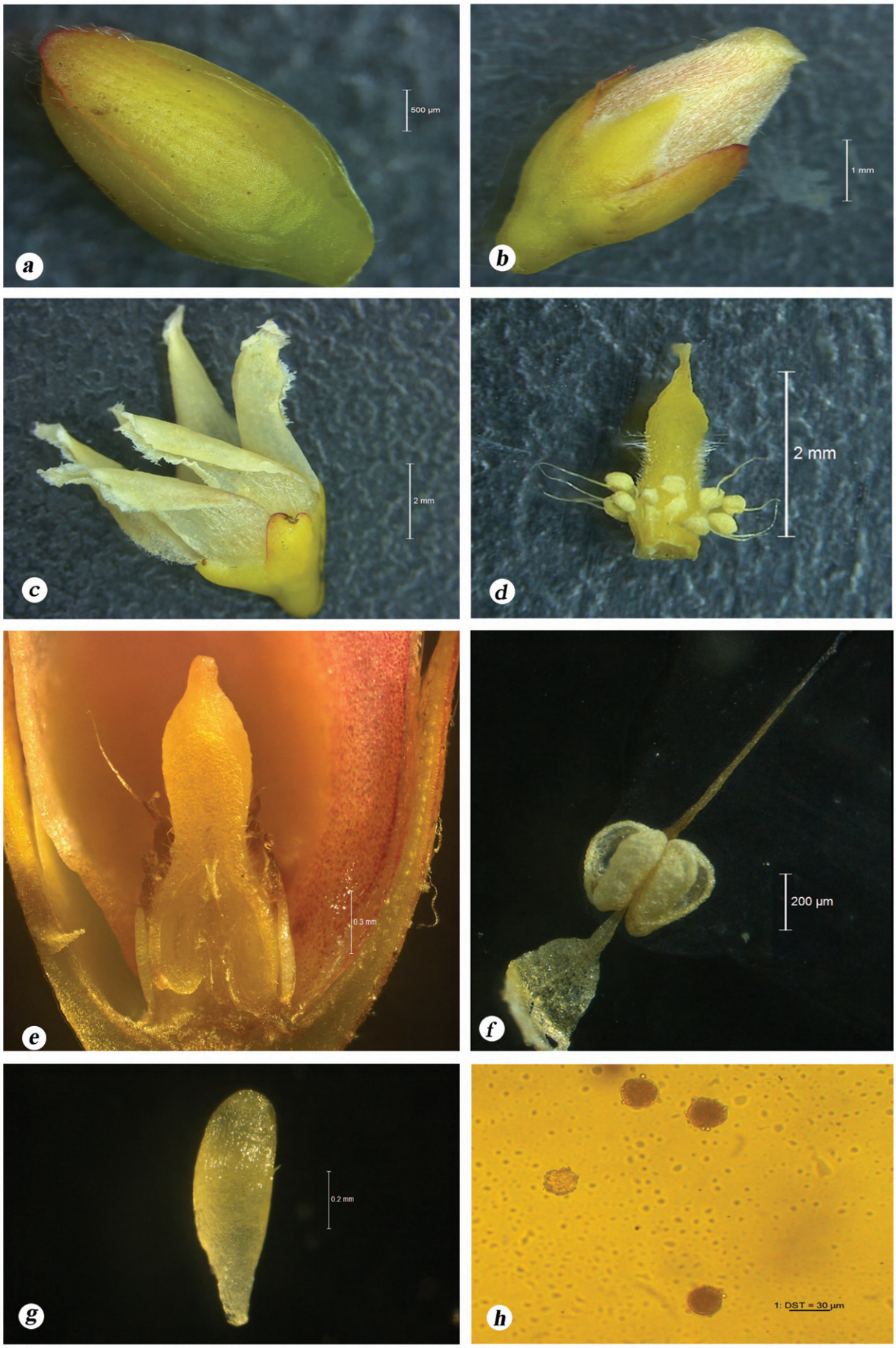


Fig.4 a. Immature bud, b. Mature bud, c. A single flower, d. Gynoecium & Androecium, e. Ovary, f. Anther, g. Ovule, h. Pollen grains (Acetocarmine staining)

year, flowering was observed only at an interval of 2-4 years. The newly developed leaves appeared along with mature leaves and the colour changed to yellowish green and then to dark green on maturity (Fig.3). The flower initiation starts at the light green stages of leaves. Observations revealed that most flowers appeared at the tip region of the branches (Fig.2). The flowering and fruiting behaviour of *Hopea ponga* was studied and the results showed that flowering is infrequent, with gregarious flowering occurring once in 2-4 years followed by sporadic, irregular flowering activities. The disparity in the timing (period) of flower initiation was also noted. The flower initiation started from the last week of January and extended up to March. 27-36 days are required for full blooming of a flower from the bud initiation.

Flowers are small, bisexual, pale pink/ creamy white-coloured, and slightly fragrant in tomentose panicles (Fig.4). Each flower consists of 13±2 epipetalous anthers. Anthesis was noted between 1:30- 5:30 pm with a peak at 3 pm. The ovary is trilocular and ovoid. Ovules are arranged in two rows with axile placentation. Style is filiform, short, and cylindric and the stigma is thick. Stigma receptivity extended for 5-10 hours. The calculated flower-fruit and pollen-ovule ratios were 6:1 and 31,776:1 respectively.

Fruits are nut-like with globose-ovoid and enclosed in calyx tubes with two accrescent lobes changed as wings. Fruit that only bears a single seed is adnate at the basal portion with unequal cotyledons. The fruit-setting percentage in *H. ponga* is only 21.83%. The phenological and reproductive efficiency is depicted in Table: 5.

Sl No.	Floral Characters	Observations
1	Inflorescence type	Racemose
2	Flower type	Bisexual, Actinomorphic
3	Colour of the flower	Pale pink /Creamy white
4	Odour	Mild fragrance
5	No. of flowers per inflorescence	84.60 ± 18.29
6	Average of flowers in a tree (At luxurious flowering peak)	4,92,100 ± 1,12,400
7	Flower opening time	6:45 – 11:30 hrs
8	Anther type	Epipetalous anthers

9	No. of anthers/ flower	13 ± 2
10	Anther dehiscence time	One day before the anthesis
11	Stigma type	Dry and Papillate
12	Duration of Stigma receptivity	5-10 hrs
13	Flower - fruit ratio	5: 1
14	Fruit setting percentage	21.83%
15	Ovary type	Superior Ovary with puberulous tip
16	Ovules/flower	6±1
17	Ovule - Seed ratio	6:1
18	No. of flowers per inflorescence	88.50±27.30
19	Flower opening time	1:30-5:30 pm
20	Flower-Fruit ratio	6:1
21	Pollen- ovule ratio	31,776: 1

Table: 5 Phenology and Reproductive Efficiency of *H. ponga*

Pollinators

An ant (*Oecophylla smaragdina*) was also recorded as a floral visitor and spent 12±2 sec. per flower and contributed to the transfer of pollen grains within and between the flowers. They were found as colonies on the floral branches of *Hopea ponga* and visited all flowers of the inflorescence. *O. smaragdina* was targeted to collect nectar and nectarines from the basal part of the flower without entering the flower. *Apis dorsata*, *Apis cerana*, and *Tetragonula iridipennis* were found to be active pollinators of *H. ponga* and visited the flowers from the morning to evening (Table: 6). Butterflies, *Troides minos* and *Euploea core* visit the inflorescences' fresh flowers. *Lymantria sp.* of moth and *Rathinda amora* (monkey puzzle butterfly) are frequent visitors at nighttime and daytime. Some pollinators and their interactions are depicted in Fig.5.

Floral Visitors	**Visiting Phase** (Day or night)	**Foraging Period**	**Time Interval**	**Frequency of Pollinator visit /Inflorescence**	**Reward for Pollination**
Troides minos	Day	08:00 – 12:15 hrs.	09 ± 2sec.	Visit 1 or 2 flowers	Nectar
Euploea core	Day	08:00- 12:00 hrs.	13± 2sec.	Visit 8 or 9 flowers	Nectar
Apis dorsata	Day	07:30 – 15:30 hrs.	30 ± 1 sec.	>16 flowers	Nectar and pollen

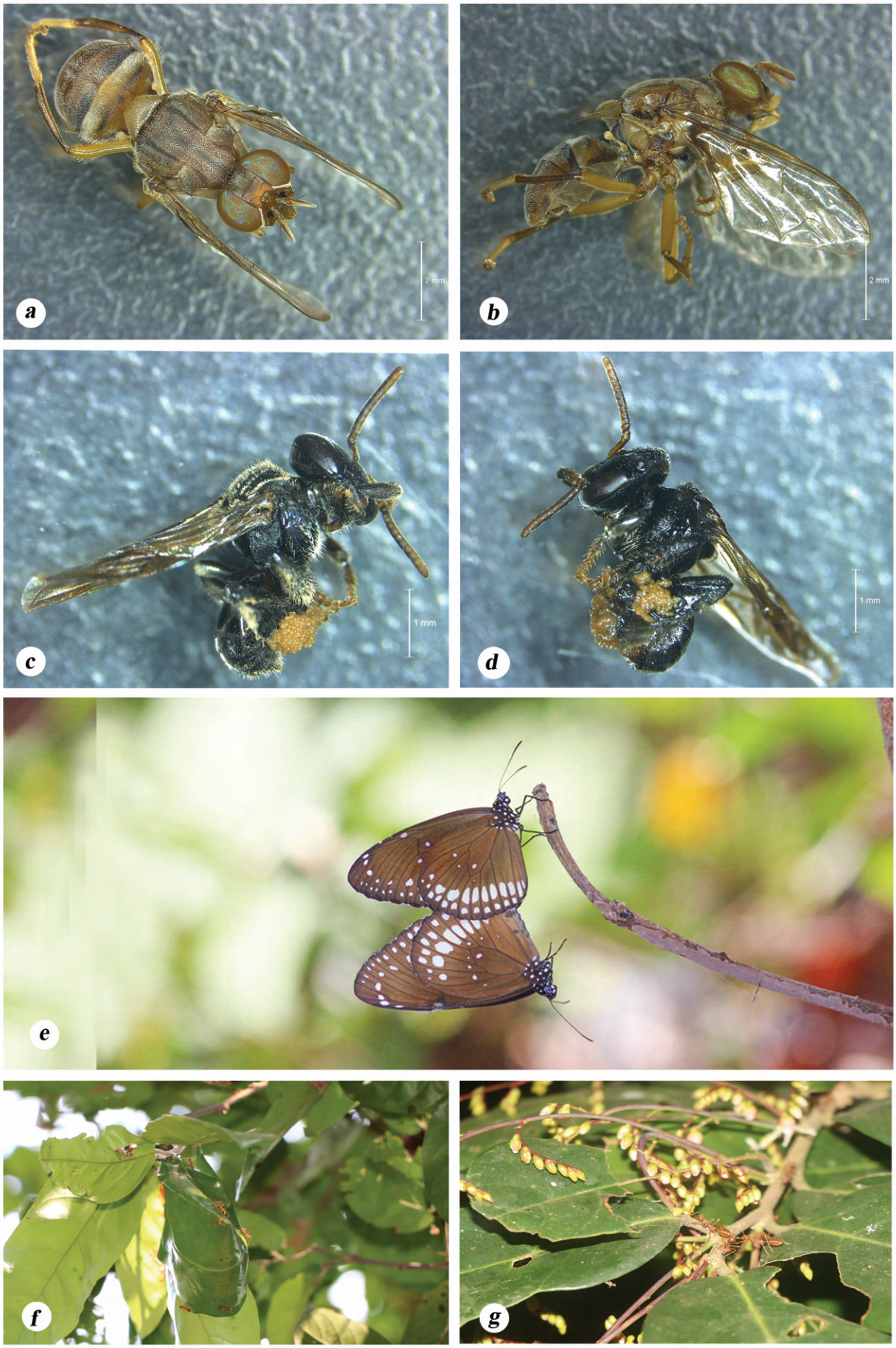


Fig.5 a. *Bactrocera sp*.-top view, b. *Bactrocera sp*.-side view, c. *Tetragonula iridipennis*-side view, d. *T. iridipennis*-lower view, e. *Eupolea core*, f. *Oecophylla smaragdina* ant colony on the leaves of *Hopea ponga*

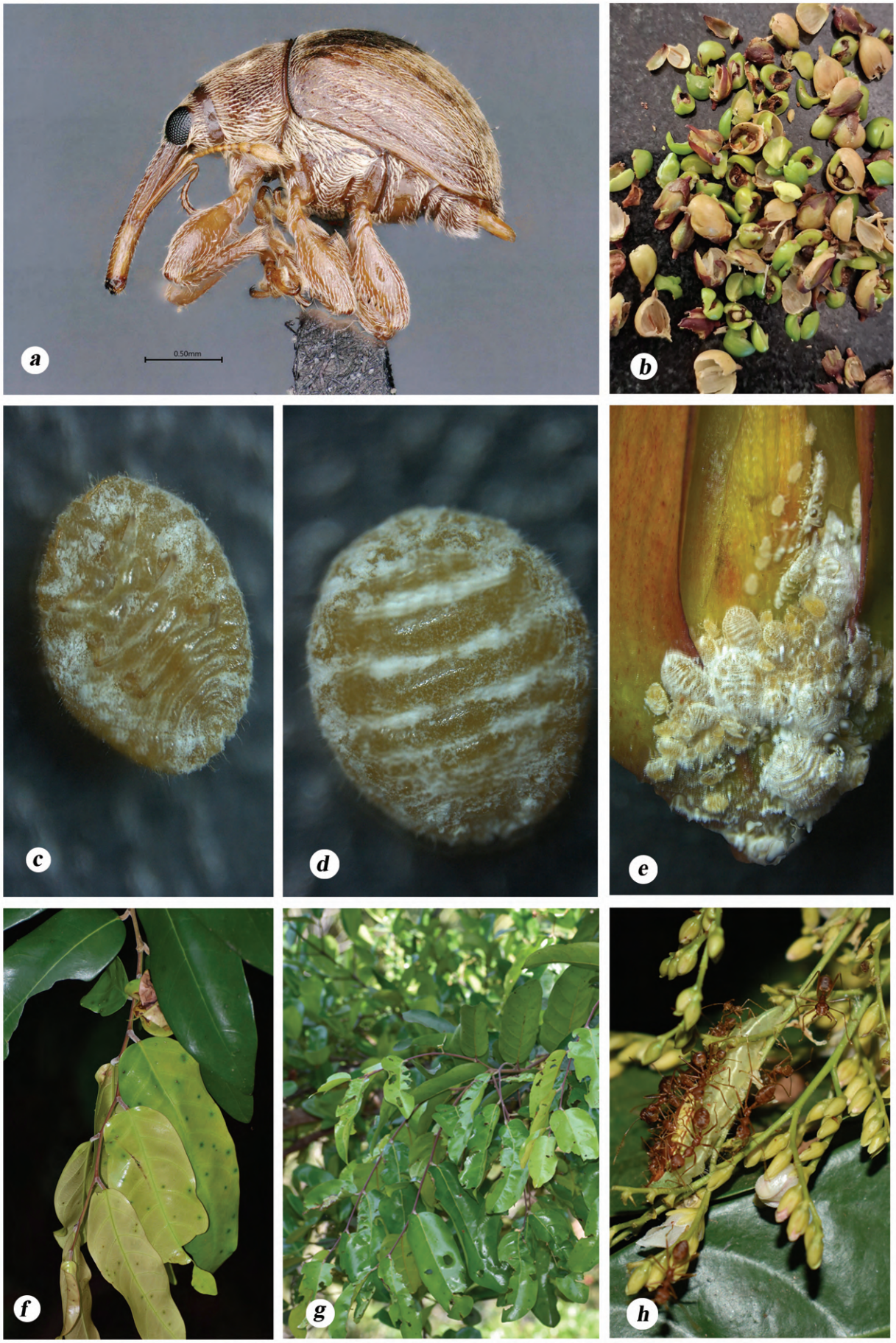


Fig.6 a. *Anthonomous sp.*, b. Beetle infestation at the time of seed storage, c. Mealy bug- Lower surface, d. Mealy bug- Upper surface, e. Mealy bug infestation, f. Spider mite Infestation in newly flushed leaves, g. Hopper attack in mature leaves, h. Caterpillar attack in inflorescence.

Apis cerana	Day	07:30-15:00 hrs.	25±2 sec.	>24 flowers	Nectar and pollen
Tetragonula iridipennis	Day	07:30 – 15:30 hrs.	27±2 sec.	>18 flowers	Nectar and pollen
Oecophylla smaragdina	Day & Night	Always on the inflorescence due to nesting over there	12± 2 sec.	<10 flowers	Nectar and pollen
Bactrocera sp.	Day	0800 – 1600 hrs.	07 ± 3 sec.	Visit selected flowers	Nectar/Floral parts
Funambulus palmarum	Day	0600 – 16:00 hrs.	20 ± 2 sec.	Random visits	Nectar / Floral parts

Table: 6 Pollinator behavior in *H. ponga*

Biological Threats

Small *Anthonomous* sp. weevil severely attacks the fruits (young as well as mature). Around 65% dry matter of the developing seeds is damaged by the potential activity of the weevil. They also lay eggs inside the fruits. Mealy bug infestation is also common in certain populations at the maturation stages of seed development. However, only 2-3 % of flowers finally develop into fruits. Caterpillars of the Common crow butterfly eat young leaves. A severe attack of treehoppers was also seen in young leaves. Beetles were found to be illegitimate floral visitors that caused damage to the developing ovary and contributed to the immature fruit fall. During the early phenology stages, plant hoppers and spider mites often severely attack tender foliage. Spider mite attacks cause blister-like appearances on the tender foliage, while hopper infestations result in patches on the leaf surface. The major biological threats are shown in Fig.6. Fungal attacks are common during the desiccation and storage period of *Hopea ponga* seeds. In the investigation, six fungal species were identified such as *Aspergillus aculeatus*, *Rhizopus arrhizus*, *Aspergillus terreus*, *Pencillium oxalicum*, *Pencillium sp*. and *Curvularia geniculata* (Fig.7). Approximately 35% of the seeds are affected by fungal pathogens when stored at room temperature (28±2°C).

Molecular taxonomic characterization of the seed-borne pathogens was done by extracting genomic DNA followed by PCR amplification of ITS region,

sequencing and sequence analysis. The amplified product was sequenced and compared with sequences in the BLAST alignment program of the Genbank database. The sequence of HP1 revealed 97.37% similarity to the sequence of *Aspergillus aculeatus*, HP2 showed 99.71% similarity to *Rhizopus arrhizus*, HP3 to *Aspergillus terreus* (94.10%), HP4 revealed similarity to *Pencillium oxalicum* (95.33%), HP5 to *Pencillium sp*. (99.16%) and HP6 to *Curvularia geniculata* (99.33%). To confirm the relationships between 30 other strains and the isolates, the maximum likelihood method was conducted with 1000 bootstrap replications and a phylogenetic tree was constructed using MEGA11. Each isolate in this tree is clustered together in a strongly supported clade with a reference strain. The dendrograms of corresponding seed-borne fungi are depicted in Fig.8 to Fig.13

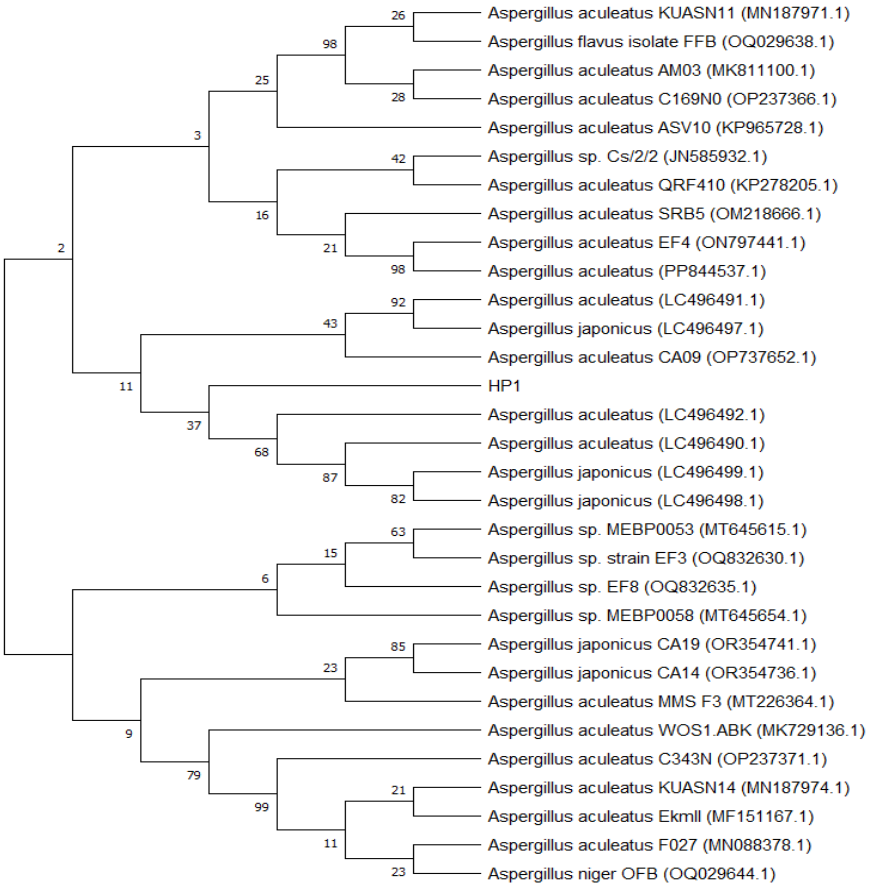


Fig.8. Phylogenetic tree showing evolutionary relationships of HP1 with 30 taxa based on the similarities of ITS1&ITS4

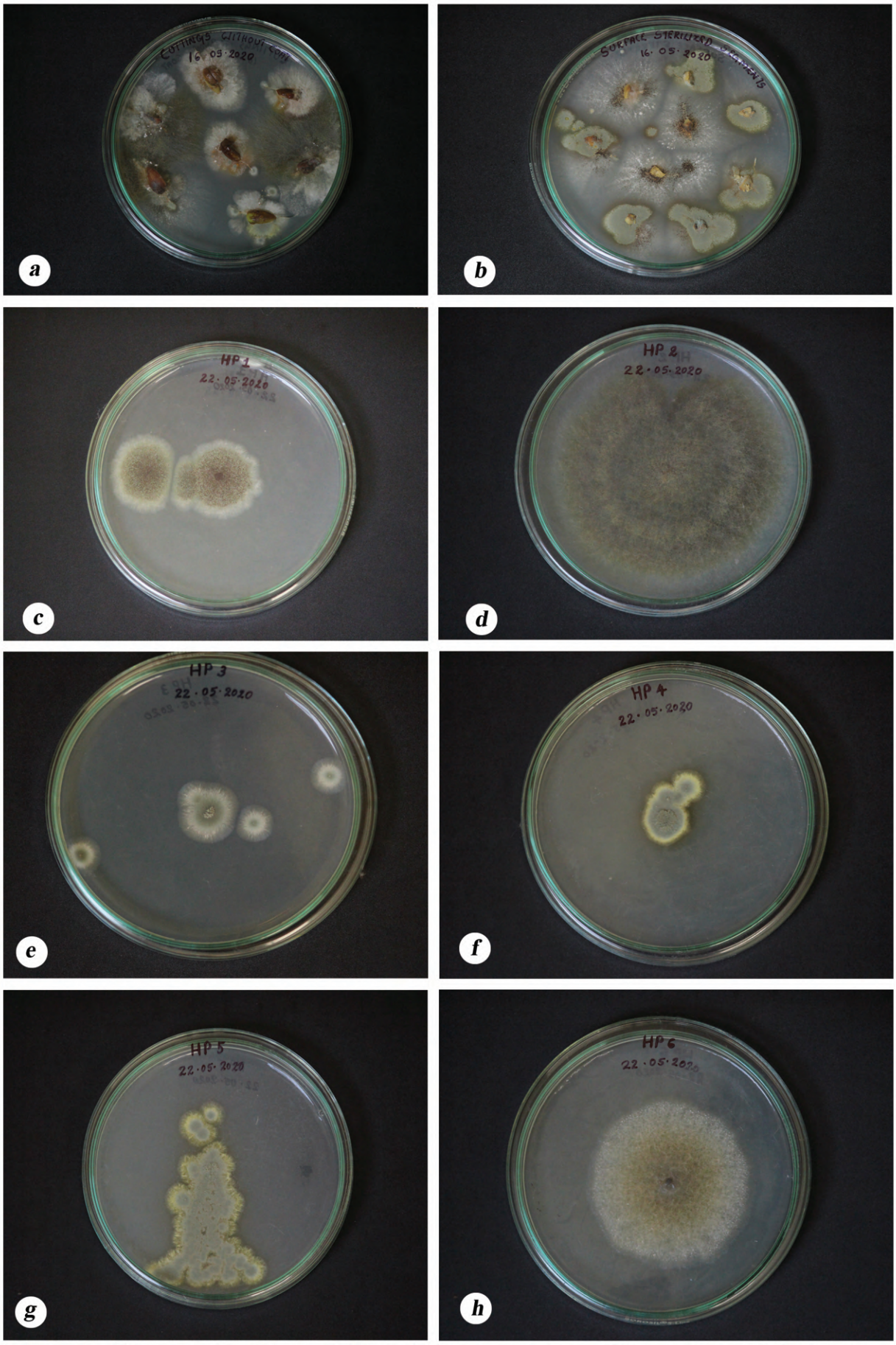


Fig.7 Seed-borne pathogens - a. & b.Isolation plates, c. *Aspergillus aculeatus*, d. *Rhizopus arrhizus*, e. *Aspergillus terreus*, f. *Pencillium oxalicum*, g.*Pencillium sp.*, h. *Curvularia geniculata*

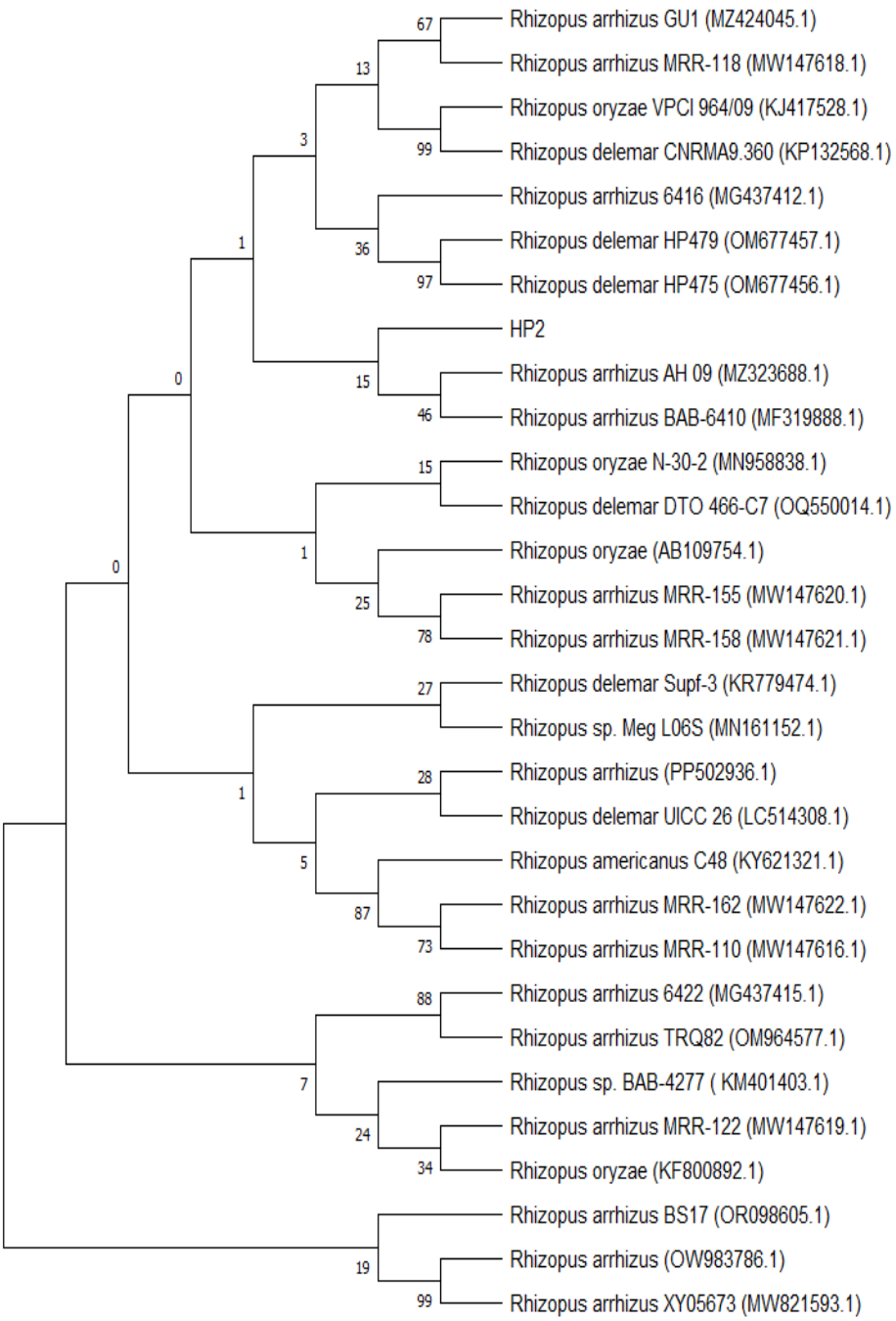


Fig.9. Phylogenetic tree showing evolutionary relationships of HP2 with 30 taxa based on the similarities of ITS1&ITS4

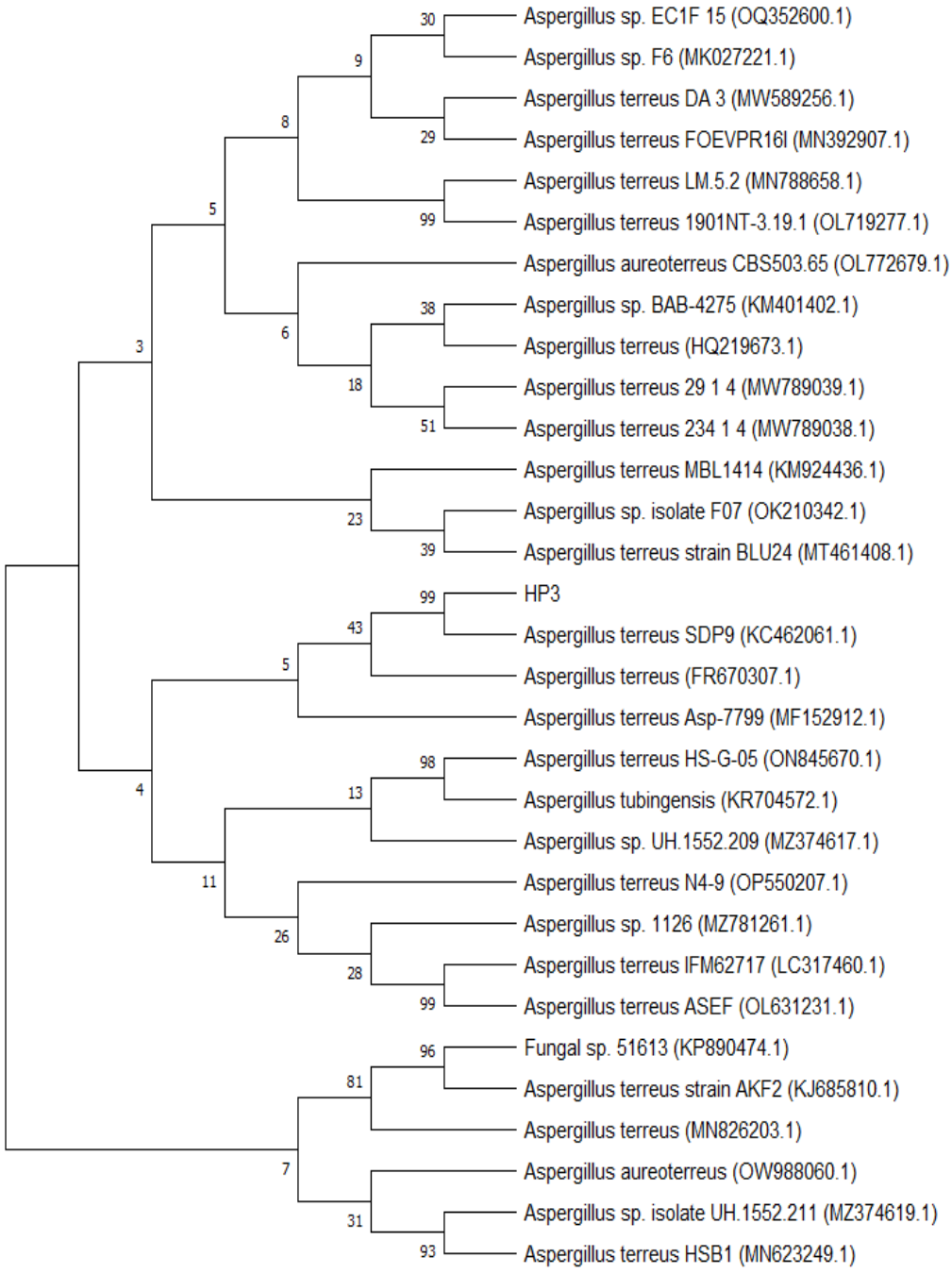


Fig.10. Phylogenetic tree showing evolutionary relationships of HP3 with 30 taxa based on the similarities of ITS1&ITS4

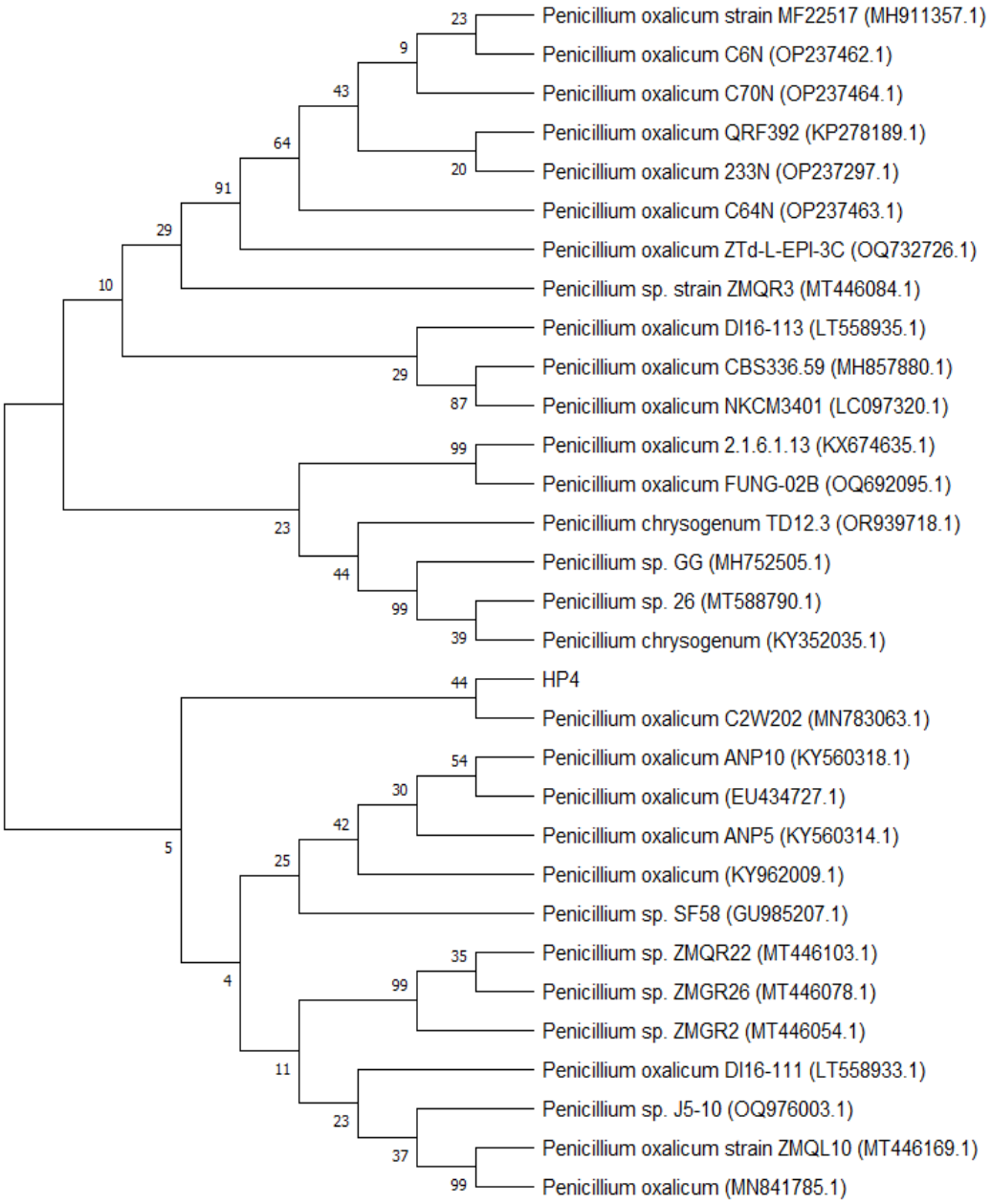


Fig.11. Phylogenetic tree showing evolutionary relationships of HP4 with 30 taxa based on the similarities of ITS1&ITS4

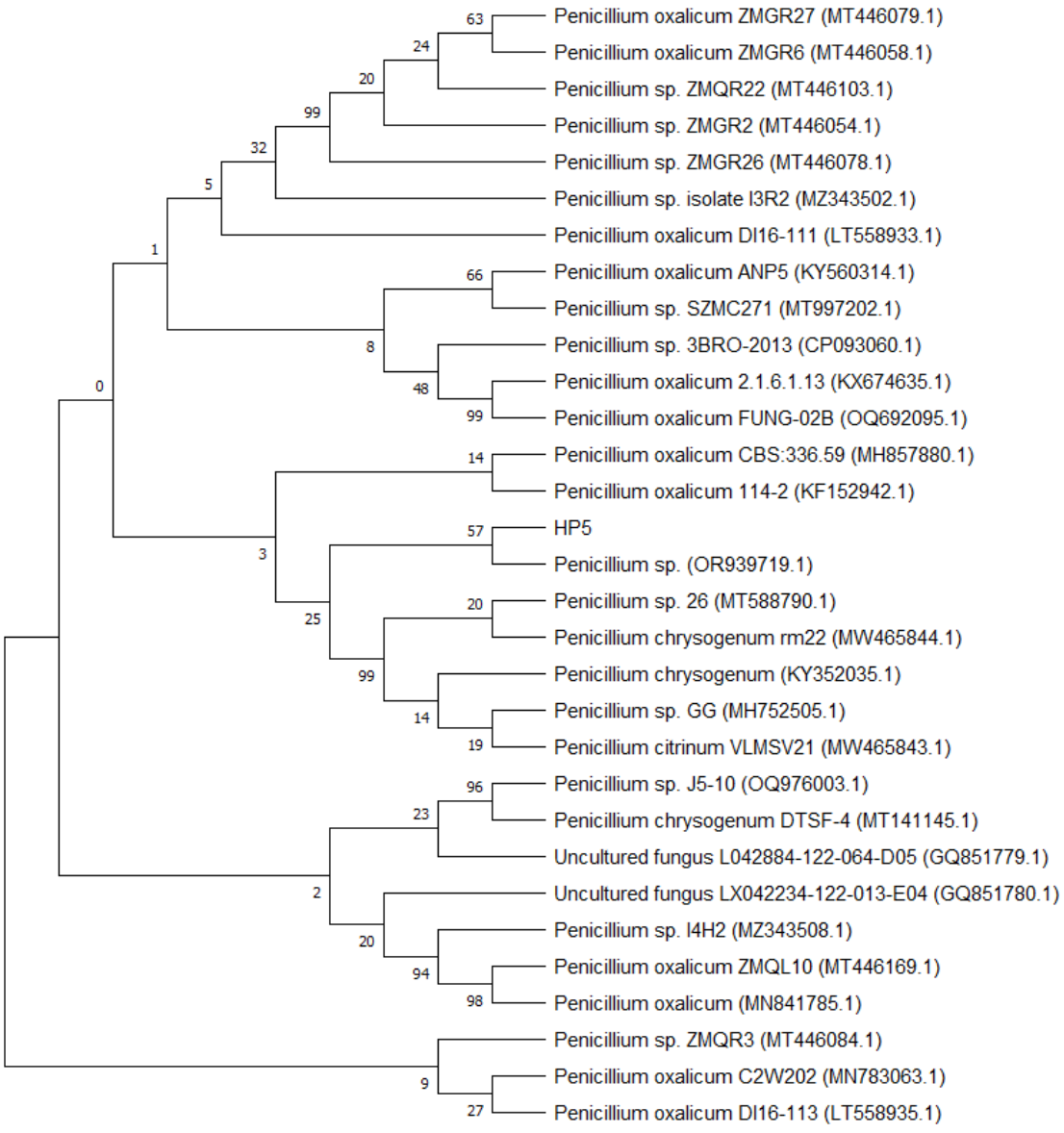


Fig.12. Phylogenetic tree showing evolutionary relationships of HP5 with 30 taxa based on the similarities of ITS1&ITS4

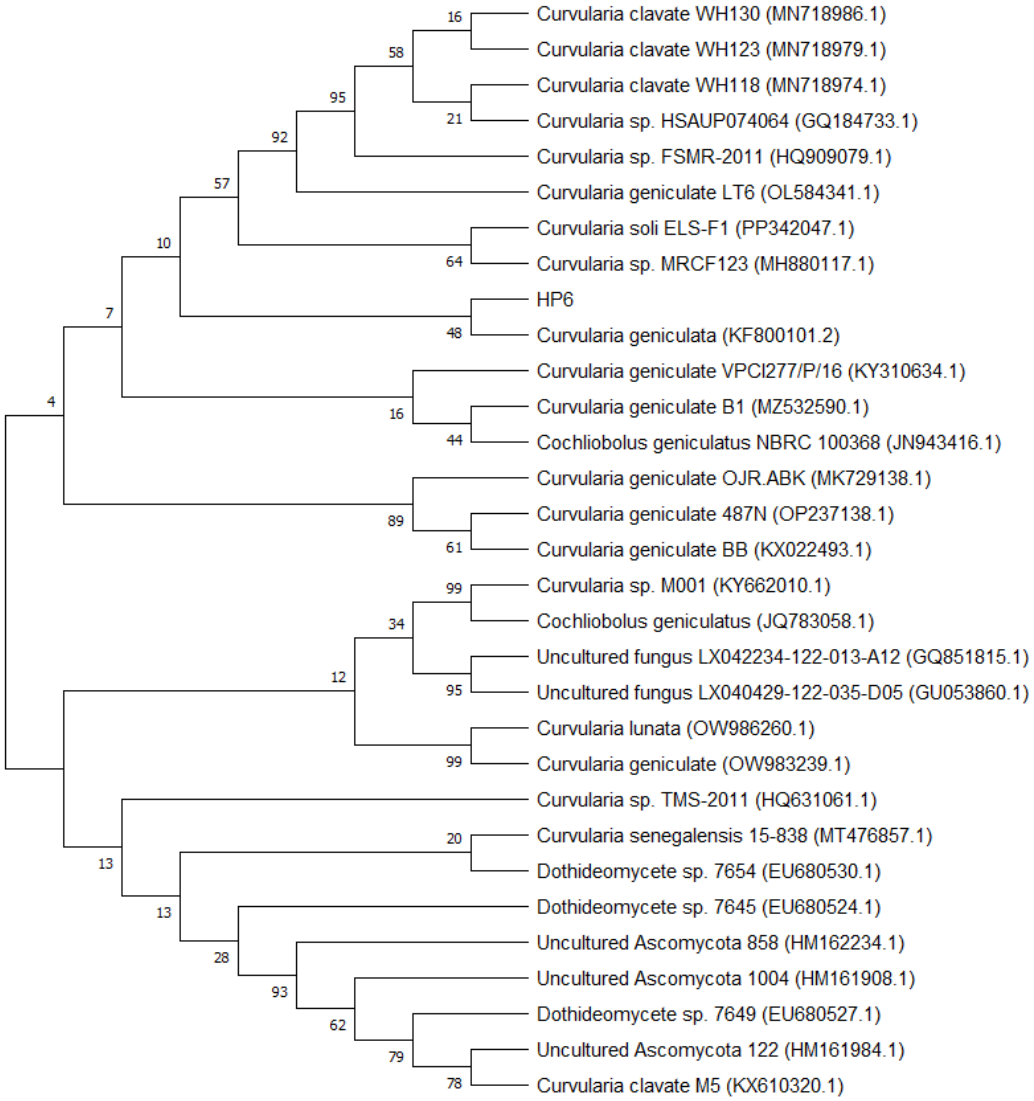


Fig.13. Phylogenetic tree showing evolutionary relationships of HP6 with 30 taxa based on the similarities of ITS1&ITS4

Anthropogenic Threats

The major threats to this species were deforestation and habitat destruction through developmental activities and conversion to agriculture and plantations. The habitat of *H. ponga* is frequently impacted by road construction, selective logging, infrastructure growth, and highway development, posing significant threats to the species' survival in its native habitat. According to the present investigation, habitat fragmentation is visible

in the Karad region, the expansion of road infrastructure and the construction of a concrete drainage system have led to the felling of twenty-four *H. ponga* trees over the past two years (Fig.14). This species, listed as Vulnerable on the IUCN Red List, now faces further risk as the remaining trees are situated along the periphery of the newly built drainage system.

The transformation of sacred groves into traditional temple complexes poses a significant risk to the habitats of various species. For instance, at Vallikattu Kavu, a sacred grove, developmental initiatives funded by the Malabar Devaswom Board have led to the felling of four *H. ponga* trees along the forest pathways. Similarly, in Iringole Kavu, eight trees have been cut down as a part of the development work within the grove.

The expansion and electrification of railway lines are adversely affecting the *H. ponga* population in the regions stretching from Parappanangadi to Vallikunnu. Numerous felled trees remain in these areas. Furthermore, widening railway boundaries poses a significant threat to this population.

Formation of Stem Galls

After the start of the southwest monsoon, the newly hatched female nymphs of *Mangalorea hopea* spread across the entire vegetative branches of *H. ponga*, particularly on the tender axillary vegetative buds. The vegetative buds of axillary branches are densely covered with single-celled hairs (Fig.15). The female nymphs settled on the axillary vegetative branches and fed on the tissues inside. Internally, the tissues are normal, and the structure of the shoot meristem has been identified as having tunica and corpus layers with epidermis combined with several unicellular hairs. Previously, researchers mistook the galls for fruit and categorized the species *Artocarpus ponga* under the family Moraceae. The misidentification of the species has been corrected with a valid description.

The galls that formed on the branches resembled sea urchins and were mostly found on the leaf axis instead of the shoot tips. These galls were spherical, approximately 3.13 cm in diameter, and green in colour, with numerous thick

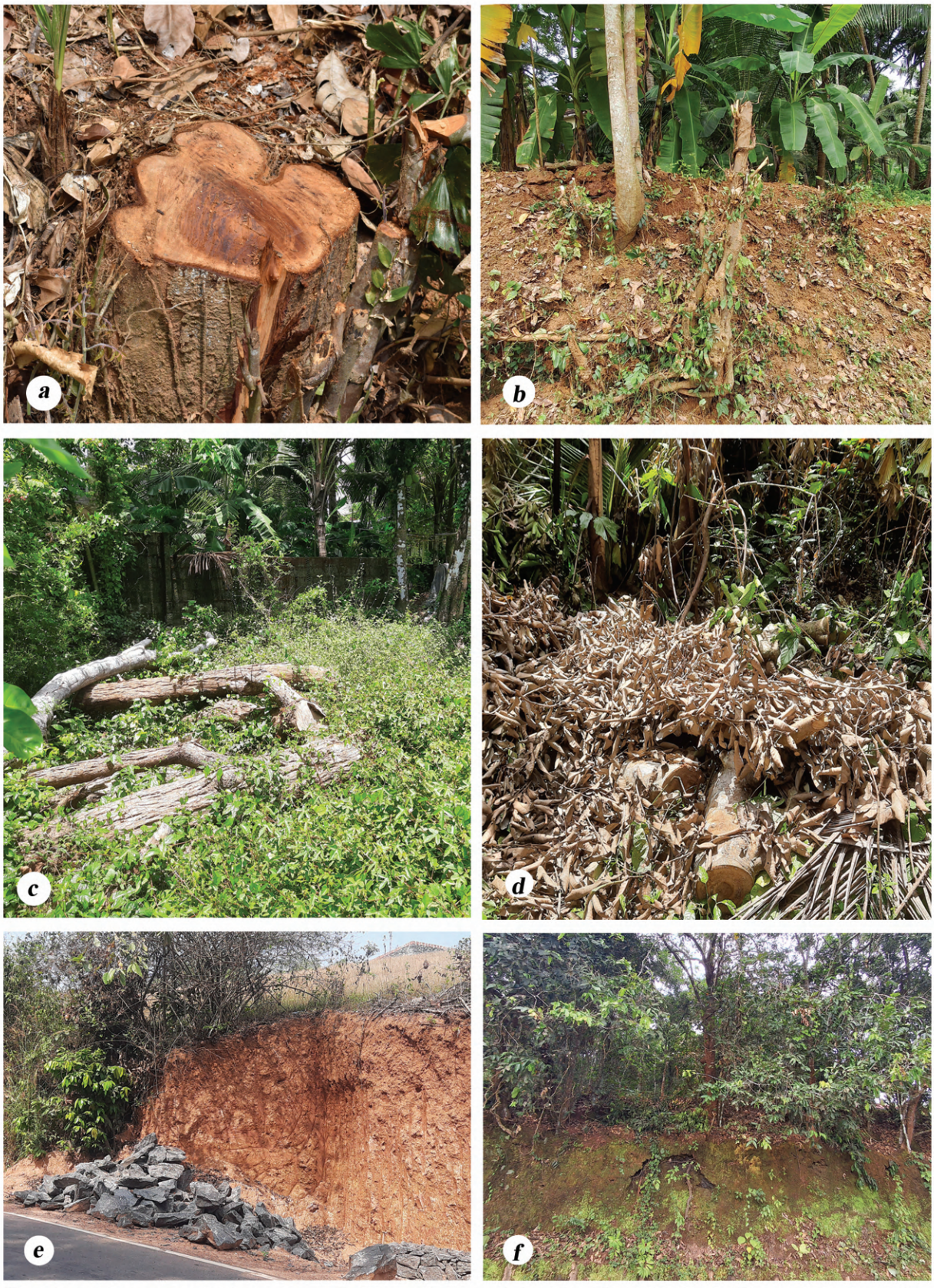


Fig.14 Anthropogenic threats - a. Felling of tree at Mavoor, Kozhikode, b. Pruned the branches of the tree at Thengilakadavu, Kozhikode, c. Trees were felled as part of the railway line expansion in Parappanangadi, Malappuram, d. Trees have been felled for the construction of a villa project in Iringaloor, Kozhikode, e. Habitat destruction in the Kakkove region of Malappuram district, Kerala, is a consequence of road expansion., f. A susceptible population of *Hopea ponga* exists along the roadside boundary in Peringave, Malappuram.

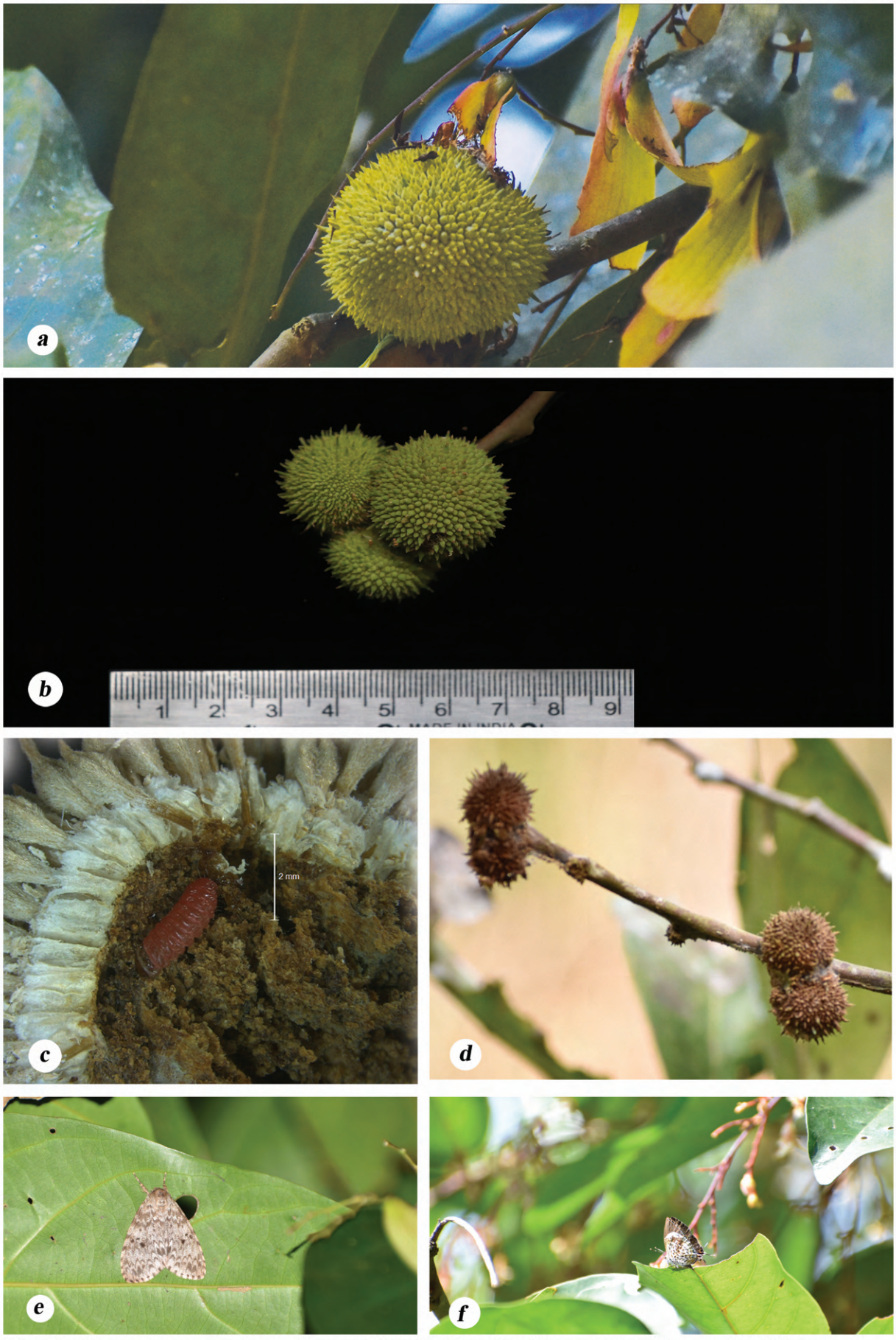


Fig.15 *Hopea ponga* - gall - a. Stem gall, b. Tri-lobed galls, c. C.S of stem gall with insect caterpillar,d. Old galls previous season, e. *Lymantria sp*., f. *Rathinda amor* (Monkey puzzle butterfly)

and pointed bristles. They initially appeared pale green and eventually darkened. There were 2-4 galls developing on each axil. The development of floral buds on the trees was significantly affected by the galls on the axillary branches. This damage led to a reduction in the initiation of floral buds, impacting the frequency of buds and the flowering season.

Dry Matter Accumulation in Seeds of *Hopea ponga* During Development

The length and breadth of the *Hopea ponga* seeds gradually increased during their development (i.e. from 3.14 x 0.94 mm to 10.09 x 6.58 mm). Dry matter accumulation of *H. ponga* seeds during development showed a definite lag in the initial period i.e. up to 36 DAA followed by a gradual increase up to the mature stage. The dry weight of cotyledon continued to increase greatly throughout their development compared to the embryonal axis (Table: 7).

The moisture content of the developing seeds showed a gradual increase up to 36 DAA (peak moisture content 79.85%), followed by a gradual reduction in their moisture content up to the maturity stage. Concomitant with the decrease in moisture content, an increase in dry weight was observed on maturity. The increase was significant at all stages in whole seeds, cotyledon, and embryonal axis. The embryo can easily be separated from the developed seeds after 36 DAA. The cotyledon and the embryonal axis are green in color throughout the developmental period of the seeds in *H. ponga*. The embryonal axis showed some variations in its color during the final stages of development as the embryonal axis became pinkish yellow with a light green color.

Biochemical Changes During Seed Development

Total Soluble Sugar Accumulation (TSS)

The level of TSS in the developing *Hopea ponga* seeds increased steadily up to 60 DAA. After that, the level of TSS was reduced slightly during the maturity stage, (Table: 8).

Starch Accumulation

The starch content of *Hopea ponga* seeds increased rapidly throughout the seed development. Around 55% of starch accumulation happened after 40

DAA. Starch accumulation attained a maximum (286.000±0.796 mg/g dwt.) at the 60 DAA of their development, followed by a gradual decline at the maturity stage. A high level of starch accumulation was noted in cotyledons than in the embryonal axis. At the final stage of seed development, the starch level of the embryonal axis showed a rapid decline (Table: 8).

Phenols Accumulation

Total phenol content in *Hopea ponga* increased gradually from 117.802±1.396 mg/g dwt. at 0 DAA to 180.746±0.329 mg/g dwt. at maturity. However, a slight reduction was noticed at 40 and 44 DAA followed by a gradual increase (Table: 8). Phenol content was maximum in the embryonal axis than cotyledon (186.853±0.459 mg/g dwt.) at the maturity stage.

Lipid Accumulation

Total lipid accumulation was maximum at 80 DAA (115.843±.531 mg/g dwt.) during the mature stages of seed development in *Hopea ponga* (Table: 8). Around 60% of lipid accumulation occurred between the 4 DAA and 60 DAA.

Amino Acid Accumulation

The data in Table 8 show that *Hopea ponga*'s amino acid accumulation reaches its maximum level in the embryonal axis of the mature seed (4.183±.014 mg/g dwt.) rather than in cotyledons (2.126±0.032 mg/g dwt.).

Protein Content During Seed Development

Albumin

The water-soluble protein (Albumin) content of *Hopea ponga* seeds gradually increased during development from 54.276±1.196 mg/g dwt. at 0 DAA to 133.803±0.118 mg/g dwt. at maturity (Table: 9). During the last growth phase, there was a maximum level of albumin accumulation in the embryonal axis (165.751±0.341 mg/g dwt.) of the *H. ponga* seeds at 80 DAA.

Globulin

The sodium chloride-soluble protein fraction (globulin) in *Hopea ponga* seeds during development increased from 4 days after anthesis (DAA) and peaked at 28 DAA, rising from 2.896±0.023 mg/g dwt. to 5.186±0.214 mg/g dwt.

Subsequently, a slight decrease was observed at 32 DAA, followed by a steady increase until the seeds reached maturity at 80 DAA. The highest concentration of globulins, 13.663±0.088 mg/g dwt., was detected in the embryonal axis, surpassing the levels in the cotyledons, which were 6.810±0.083 mg/g dwt., at the mature stage (Table 9).

Prolamin : The alcohol-soluble protein fraction (prolamin) significantly increased from 4 days after anthesis (DAA) to 40 DAA, followed by a minor decrease up to 48 DAA. Subsequently, it gradually rose as development continued towards maturity. The highest level of prolamin content was observed in the embryonic axis, reaching 38.223±0.237 mg/g dry weight, during the advanced stages of development (Table: 9).

Glutelins : Glutelins are divided into two fractions based on the solvent used for extraction: 0.05M NaOH and 0.25M NaOH.

0.05M NaOH Fraction

The 0.05M NaOH fraction of protein (glutelin) content in *Hopea ponga* seeds gradually increased during their development up to 24 DAA. This was followed by a gradual decrease up to the final stages of their development. Similar patterns were also found in the cotyledon and embryonic axis of the seeds (Table: 9).

0.25M NaOH Fraction

It is apparent from Table: 9 that the accumulation of glutelin (0.25M NaOH fraction) in *Hopea ponga* seed was comparatively less than 0.05 M NaOH fraction during their development. A slight increase was found at the initial stages (up to 36 DAA) followed by a decline. A minimum accumulation of glutelin (0.25 M NaOH fraction) was recorded at the 48DAA with 10.74 mg/g dwt. Similar patterns in glutelin levels were also observed in the cotyledon and embryonal axis.

Total Proteins

Data in Table 9 showed that total protein accumulation significantly increased from 126.361±1.406 mg/g dwt, at 4 DAA. A gradual decrease was recorded

Days after Anthesis (DAA)	Fruit Length (mm)±SE	Fruit Width (mm)±SE	Fresh weight (mg)±SE	Dry weight (mg) wt./10 Replicates±SE	Moisture Content (%) ±SE
4 WS	$3.106 \pm 0.176^{b^*}$	$0.910 \pm 0.020^{b^*}$	$5.293 \pm 0.352^{a^*}$	$3.456 \pm 0.266^{a^*}$	$34.710 \pm 0.185^{a^*}$
8 WS	$3.813 \pm 0.751^{c^*}$	$1.273 \pm 0.023^{c^*}$	$9.540 \pm 0.070^{b^*}$	$5.476 \pm 0.0384^{ab^*}$	$42.576 \pm 0.811^{b^*}$
12 WS	$4.226 \pm 0.048^{d^*}$	$1.893 \pm 0.023^{d^*}$	$16.026 \pm 0.329^{c^*}$	$8.210 \pm 0.850^{bc^*}$	$48.743 \pm 0.631^{c^*}$
16 WS	$5.560 \pm 0.036^{e^*}$	$2.636 \pm 0.031^{a^*}$	$21.653 \pm 0.572^{d^*}$	$9.143 \pm 0.323^{bc^*}$	$57.736 \pm 0.634^{d^*}$
20 WS	$6.036 \pm 0.048^{f^*}$	$2.710 \pm 0.049^{a^*}$	$34.440 \pm 1.282^{e^*}$	$12.586 \pm 0.386^{c^*}$	$63.420 \pm 0.644^{e^*}$
24 WS	$6.426 \pm 0.058^{g^*}$	$3.203 \pm 0.024^{e^*}$	$54.933 \pm 2.349^{f^*}$	$16.953 \pm 0.676^{d^*}$	$68.363 \pm 0.306^{f^*}$
28 WS	$6.983 \pm 0.026^{h^*}$	$4.120 \pm 0.051^{f^*}$	$78.403 \pm 0.529^{g^*}$	$20.596 \pm 0.272^{de^*}$	$73.72 \pm 0.463^{g^*}$
32 WS	$7.323 \pm 0.023^{i^*}$	$4.57 \pm 0.435^{g^*}$	$93.753 \pm 0.583^{h^*}$	$22.453 \pm 0.364^{e^*}$	$76.046 \pm 0.257^{h^*}$
36 WS	$7.926 \pm 0.035^{j^*}$	$5.116 \pm 0.043^{h^*}$	$119.010 \pm 0.269^{i^*}$	$24.206 \pm 0.228^{e^*}$	$79.656 \pm 0.146^{i^*}$
40 WS	$8.753 \pm 0.070^{a^*}$	$5.296 \pm 0.058^{i^*}$	$164.310 \pm 0.911^{j^*}$	$46.066 \pm 0.246^{f^*}$	$71.926 \pm 0.06^{g^*}$
44WS	$8.894 \pm 0.008^{a^*}$	$5.423 \pm 0.006^{j^*}$	$193.410 \pm 0.585^{k^*}$	$59.156 \pm 0.464^{g^*}$	$69.406 \pm 0.317^{f^*}$
44COT	----------	----------	$45.620 \pm 1.555^{A^*}$	$64.990 \pm 0.938^{A^*}$	$57.26 \pm 0.707^{A^*}$
44 EMB	----------	----------	$41.970 \pm 1.011^{(1)^*}$	$11.96 \pm 0.1908^{(1)^*}$	$71.480 \pm 0.490^{(1)^*}$
48WS	$8.943 \pm 0.008^{a^*}$	$6.040 \pm 0.017^{k^*}$	$243.660 \pm 1.469^{l^*}$	$97.623 \pm 1.067^{h^*}$	$59.926 \pm 0.497^{j^*}$
48 COT	----------	----------	$179.950 \pm 1.209^{B^*}$	$71.410 \pm 1.604^{B^*}$	$60.31 \pm 0.959^{B^*}$
48EMB	----------	----------	$61.703 \pm 0.608^{(2)^*}$	$19.74 \pm 0.4191^{(2)^*}$	$68.01 \pm 0.593^{(2)^*}$
60WS	$9.416 \pm 0.206^{k^*}$	$6.236 \pm 0.064^{l^*}$	$263.710 \pm 0.499^{m^*}$	$118.620 \pm 3.378^{i^*}$	$55.010 \pm 1.366^{k^*}$
60COT	----------	----------	$182.130 \pm 0.878^{B^*}$	$88.580 \pm 0.533^{C^*}$	$51.363 \pm 0.235^{C^*}$
60EMB	----------	----------	$80.107 \pm 0.501^{(3)^*}$	$33.823 \pm 0.671^{(3)^*}$	$57.77 \pm 0.823^{(3)^*}$
80WS	$10.13 \pm 0.202^{l^*}$	$6.576 \pm 0.01^{m^*}$	$280.890 \pm 1.119^{n^*}$	$152.710 \pm 3.986^{j^*}$	$45.620 \pm 1.555^{l^*}$
80COT	----------	----------	$197.010 \pm 0.84^{C^*}$	$113.220 \pm 1.324^{D^*}$	$42.54 \pm 0.429^{D^*}$
80EMB	----------	----------	$89.247 \pm 1.312^{(4)^*}$	$42.467 \pm 0.380^{(4)^*}$	$52.403 \pm 0.27^{(4)^*}$

The factors of the study are represented as WS (Whole Seed), COT(Cotyledon), EMB (Embryonal axes), ±SE: Standard Error of the mean. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. Small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals represent the significant differences. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 7 Dry Matter Accumulation in *H. ponga* seeds During Development

Days after Anthesis (DAA)	Total Soluble Sugars (TSS) mg g $^{-1}$ d. wt.	Starch mg g^{-1}d. wt.	Total Phenol mg g^{-1} d. wt.	Lipid mg g^{-1}d. wt.	Amino Acid mg g^{-1}d. wt.
4WS	$14.603 \pm 1.193^{a^*}$	$19.43 \pm 0.562^{a^*}$	$117.802 \pm 1.39^{a^*}$	$9.503 \pm 0.512^{a^*}$	$0.07 \pm 0.005^{a^*}$
8WS	$24.490 \pm .975^{b^*}$	$26.653 \pm 1.05^{b^*}$	$128.245 \pm .34^{b^*}$	$14.306 \pm 0.98^{b^*}$	$0.160 \pm 0.005^{b^*}$
12WS	$37.30 \pm .625^{c^*}$	$31.953 \pm 0.32^{c^*}$	$154.682 \pm 0.82^{c^*}$	$16.713 \pm 0.21^{c^*}$	$0.20 \pm 0.005^{bc^*}$
16WS	$42.236 \pm .723^{d^*}$	$36.54 \pm 0.274^{d^*}$	$173.602 \pm 2.74^{g^*}$	$20.906 \pm 0.32^{d^*}$	$0.260 \pm 0.005^{c^*}$
20WS	$46.673 \pm .600^{e^*}$	$45.41 \pm 0.624^{e^*}$	$191.475 \pm 1.64^{h^*}$	$24.870 \pm 0.16^{e^*}$	$0.540 \pm 0.015^{d^*}$
24WS	$51.213 \pm .932^{f^*}$	$66.136 \pm 1.974^{f^*}$	$208.201 \pm 2.44^{i^*}$	$27.826 \pm 0.30^{f^*}$	$0.636 \pm 0.021^{e^*}$
28WS	$56.753 \pm .168^{g^*}$	$78.456 \pm 0.62^{g^*}$	$160.972 \pm 1.06^{j^*}$	$32.260 \pm 0.31^{g^*}$	$0.8867 \pm 0.024^{f^*}$
32WS	$61.820 \pm 1.054^{h^*}$	$87.98 \pm 0.662^{h^*}$	$155.27 \pm 0.344^{c^*}$	$48.01 \pm 0.258^{h^*}$	$1.063 \pm 0.060^{g^*}$
36WS	$65.056 \pm .256^{i^*}$	$105.74 \pm 1.383^{i^*}$	$150.331 \pm 0.39^{d^*}$	$54.77 \pm 0.487^{i^*}$	$1.306 \pm 0.023^{h^*}$
40WS	$67.263 \pm .566^{j^*}$	$119.500 \pm 1.11^{j^*}$	$143.18 \pm 0.978^{e^*}$	$62.82 \pm 0.342^{j^*}$	$1.436 \pm 0.024^{i^*}$
44WS	$72.686 \pm 420^{k^*}$	$156.010 \pm 1.35^{k^*}$	$139.31 \pm 0.401^{f^*}$	$82.77 \pm 1.506^{k^*}$	$1.953 \pm 0.039^{j^*}$
44COT	$39.803 \pm 0.955^{A^*}$	$163.25 \pm 0.95^{A^*}$	$125.98 \pm 1.24^{A^*}$	$42.996 \pm 0.92^{A^*}$	$1.80 \pm 0.051^{A^*}$
44EMB	$49.903 \pm 0.77^{(1)^*}$	$131.58 \pm 0.632^{(1)^*}$	$164.73 \pm 0.65^{(1)^*}$	$34.51 \pm 0.86^{(1)^*}$	$2.583 \pm 0.026^{(1)^*}$
48WS	$80.936 \pm .513^{l^*}$	$239.150 \pm 2.19^{l^*}$	$146.375 \pm 0.45^{e^*}$	$93.14 \pm 0.213^{l^*}$	$2.460 \pm 0.036^{k^*}$
48COT	$37.030 \pm 0.367^{B^*}$	$251.51 \pm 1.22^{B^*}$	$132.153 \pm 1.06^{B^*}$	$61.08 \pm 0.97^{B^*}$	$1.763 \pm 0.042^{B^*}$
48EMB	$54.38 \pm 0.337^{(2)^*}$	$191.23 \pm 0.73^{(2)^*}$	$171.27 \pm 0.54^{(2)^*}$	$38.2 \pm 1.19^{(2)^*}$	$3.220 \pm 0.020^{(2)^*}$
60WS	$85.560 \pm .676^{m^*}$	$286.0 \pm 0.796^{m^*}$	$170.781 \pm .183^{g^*}$	$111.8 \pm 1.01^{m^*}$	$2.630 \pm 0.026^{l^*}$
60COT	$35.29 \pm 0.436^{BC^*}$	$261.25 \pm 0.725^{C^*}$	$152.32 \pm 0.38^{C^*}$	$84.09 \pm 0.502^{C^*}$	$1.936 \pm 0.029^{C^*}$
60EMB	$51.11 \pm 0.286^{(1)^*}$	$246.56 \pm 0.62^{(3)^*}$	$181.08 \pm 0.45^{(3)^*}$	$58.13 \pm 0.29^{(3)^*}$	$3.81 \pm 0.062^{(3)^*}$
80WS	$81.503 \pm .499^{m^*}$	$281.25 \pm 0.74^{n^*}$	$180.746 \pm 0.33^{k^*}$	$115.84 \pm .531^{n^*}$	$2.483 \pm 0.020^{k^*}$
80COT	$33.953 \pm 0.14^{C^*}$	$254.79 \pm 0.68^{D^*}$	$158.21 \pm 0.319^{D^*}$	$87.88 \pm 1.06^{D^*}$	$2.126 \pm 0.032^{D^*}$
80EMB	$46.79 \pm 0.249^{(3)^*}$	$238.14 \pm 0.39^{(4)^*}$	$186.85 \pm 0.46^{(4)^*}$	$58.62 \pm 0.42^{(3)^*}$	$4.183 \pm 0.014^{(4)^*}$

The factors of the study are represented as WS (Whole Seed), COT(Cotyledon), EMB (Embryonal axis), ±SE: Standard Error of the mean. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 8 Biochemical changes during seed development in *H. ponga*

Days after Anthesis (DAA)	Albumin mg g $^{-1}$ d. wt.	Globulin mg g^{-1}d. wt.	Prolamin mgg^{-1} d. wt.	Glutelin mgg^{-1}d. wt.		Total Protein mg g^{-1}d. wt.
				NaOH 0.05M	NaOH 0.25M	
4WS	$54.28 \pm 1.196^{a^*}$	$2.90 \pm 0.023^{a^*}$	$26.57 \pm .531^{a^*}$	$26.936 \pm .249^{a^*}$	$15.686 \pm .319^{a^*}$	$126.36 \pm 1.406^{a^*}$
8WS	$63.99 \pm 0.368^{b^*}$	$3.273 \pm .049^{b^*}$	$29.22 \pm .219^{b^*}$	$29.030 \pm .228^{b^*}$	$16.92 \pm .049^{bd^*}$	$142.438 \pm .280^{b^*}$
12WS	$69.90 \pm 0.406^{c^*}$	$4.253 \pm .095^{c^*}$	$31.15 \pm .441^{c^*}$	$32.940 \pm .046^{c^*}$	$17.19 \pm .045^{de^*}$	$155.431 \pm .555^{c^*}$
16WS	$73.08 \pm 0.11^{d^*}$	$4.75 \pm .136^{de^*}$	$33.44 \pm .26^{de^*}$	$34.673 \pm .071^{d^*}$	$17.55 \pm .038^{ef^*}$	$163.492 \pm .466^{d^*}$
20WS	$76.74 \pm 0.489^{e^*}$	$5.046 \pm .04^{ef^*}$	$34.83 \pm .288^{e^*}$	$36.636 \pm .322^{e^*}$	$17.92 \pm .034^{f^*}$	$171.188 \pm .267^{e^*}$
24WS	$79.84 \pm 0.112^{f^*}$	$5.263 \pm .027^{f^*}$	$37.35 \pm .204^{f^*}$	$39.216 \pm .210^{f^*}$	$18.443 \pm .114^{g^*}$	$179.447 \pm .801^{f^*}$
28WS	74.310±.115	$5.186 \pm .214^{f^*}$	$31.96 \pm .87^{cd^*}$	$34.780 \pm .306^{c^*}$	$16.246 \pm .148^{c^*}$	$162.08 \pm 1.102^{dg^*}$
32WS	$76.26 \pm 0.34^{e^*}$	$4.626 \pm .058^{d^*}$	$29.48 \pm 0.44^{b^*}$	$33.33 \pm .380^{c^*}$	$16.46 \pm .294^{bc^*}$	$160.162 \pm .469^{g^*}$
36WS	$80.46 \pm 0.75^{f^*}$	$5.310 \pm .290^{f^*}$	$29.42 \pm 1.41^{b^*}$	$30.990 \pm .785^{g^*}$	$17.71 \pm .128^{f^*}$	$163.899 \pm 1.806^{d^*}$
40WS	$104.14 \pm 0.49^{g^*}$	$6.640 \pm .055^{g^*}$	$32.43 \pm .23^{cd^*}$	$26.696 \pm .278^{a^*}$	$14.970 \pm .334^{h^*}$	$184.871 \pm .592^{h^*}$
44WS	$120.68 \pm 0.43^{h^*}$	$7.506 \pm .083^{h^*}$	$32.83 \pm .11^{cd^*}$	$34.603 \pm .043^{c^*}$	$17.900 \pm .023^{f^*}$	$214.185 \pm 1.11^{i^*}$
44COT	$106.99 \pm .191^{A^*}$	$4.886 \pm .137^{A^*}$	$29.13 \pm .234^{A^*}$	$21.520 \pm .277^{A^*}$	$15.02 \pm .190^{A^*}$	$177.62 \pm .211^{A^*}$
44EMB	$115.05 \pm .34^{(1)^*}$	$8.21 \pm .192^{(1)^*}$	$26.18 \pm .20^{(1)^*}$	$18.24 \pm .249^{(1)^*}$	$14.45 \pm .30^{(1)^*}$	$182.129 \pm .21^{(1)^*}$
48WS	$119.77 \pm 0.29^{h^*}$	$9.360 \pm .064^{i^*}$	$26.92 \pm .358^{a^*}$	$14.003 \pm .02^{h^*}$	$10.863 \pm .052^{i^*}$	$180.913 \pm .68^{f^*}$
48COT	$105.415 \pm .28^{B^*}$	$6.333 \pm .029^{B^*}$	$20.736 \pm .28^{B^*}$	$15.16 \pm .052^{BC^*}$	$11.393 \pm .114^{B^*}$	$159.05 \pm .482^{B^*}$
48EMB	$136.72 \pm .42^{(2)^*}$	$10.14 \pm .04^{(2)^*}$	$33.14 \pm .26^{(2)^*}$	$17.77 \pm .13^{(1)^*}$	$12.33 \pm .225^{(2)^*}$	$210.48 \pm .24^{(2)^*}$
60WS	$131.88 \pm 0.12^{i^*}$	$9.803 \pm .031^{j^*}$	$32.50 \pm .292^{c^*}$	$16.553 \pm .101^{i^*}$	$11.116 \pm .059^{j^*}$	$201.859 \pm .319^{j^*}$
60COT	$128.41 \pm .281^{C^*}$	$7.390 \pm .073^{C^*}$	$21.48 \pm .265^{C^*}$	$15.85 \pm .063^{B^*}$	$11.746 \pm .035^{B^*}$	$184.88 \pm .175^{C^*}$
60EMB	$159.59 \pm .23^{(3)^*}$	12.446±.212	$35.64 \pm .24^{(3)^*}$	$18.64 \pm .16^{(1)(2)^*}$	$13.74 \pm .018^{(3)^*}$	$240.06 \pm .27^{(3)^*}$
80WS	$133.8 \pm 0.18^{j^*}$	$9.320 \pm .02^{i^*}$	$33.05 \pm .052^{d^*}$	$16.633 \pm .102^{i^*}$	$10.820 \pm .06^{k^*}$	$203.627 \pm .229^{j^*}$
80COT	$128.81 \pm .175^{C^*}$	$6.81 \pm .083^{D^*}$	$19.47 \pm .269^{D^*}$	$14.90 \pm .092^{C^*}$	$11.473 \pm .08^{B^*}$	$181.45 \pm .393^{D^*}$
80EMB	$165.75 \pm .34^{(4)^*}$	13.663±.088	$38.22 \pm .24^{(4)^*}$	$19.36 \pm .107^{(2)^*}$	$13.84 \pm .061^{(4)^*}$	$250.85 \pm .55^{(4)^*}$

The factors of the study are represented as WS (Whole Seed), COT(Cotyledon), EMB (Embryonal axes), ±SE: Standard Error of the mean. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. Small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals represent the significant differences. The observations within a column with same inscripts are not significantly distinct with each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 9 Protein content during the seed development period

at 24DAA and 32DAA of the development, followed by an increase up to the final maturity stage. Similar patterns were recorded in the cotyledon and embryonic axis during the entire seed development period in the *Hopea ponga* seeds.

Seed Desiccation Studies

Moisture Content and Germination

Upon harvest, fresh *Hopea ponga* seeds had a moisture content of 45.370%. When stored in open laboratory conditions (28±2°C and 65% RH), both the seed moisture content and the final germination percentage decreased significantly. Initially, the fresh seeds exhibited a 98% germination rate. However, after 72 hours in open laboratory conditions, germination in *H. ponga* seeds dropped to 62%, and a further decrease in moisture content significantly impacted the germination percentage. Complete loss of viability was observed after 168 hours of desiccation in *H. ponga* seeds. Also, the time taken for germination was doubled as the % MC decreased. (Table: 10).

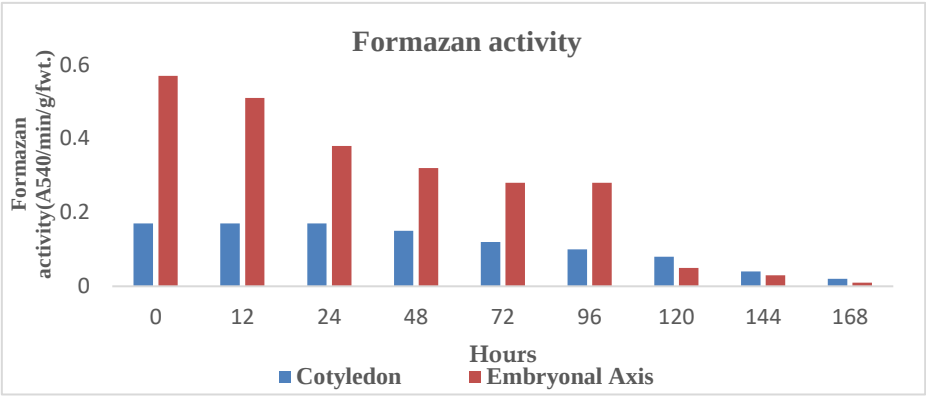
Desiccation Periods (Hours)	Moisture Content (%)	Germination (%)	Days Required for Germination
0	45.370	98	2-3
12	41.603	91	2-3
24	39.220	86	2-3
48	34.816	82	2-5
72	32.643	62	3-4
96	30.452	57	4-5
120	29.452	48	4-7
144	27.681	38	4-8
168	25.532	No germination	---

Table: 10 Moisture content and seed germination rate during desiccation

Tetrazolium Reduction Activity (Formazan Formation)

The decrease in moisture content according to the desiccation period showed the difference in pattern and time required for staining with tetrazolium chloride (TTC) solution. Fresh seeds of *Hopea ponga* (MC 45.370%) showed a deep red colour (in both embryo and cotyledon) on incubation in TTC for 30-45 minutes. With reduced moisture content, the staining pattern showed variations and took more time for colour development.

In *H. ponga* seeds with 25.532% moisture content (MC), the embryo and cotyledon were not stained even after a longer duration. Extraction of formazan formed with acetone justified the decrease in seed viability on desiccation and the subsequent decrease in formazan formation. The absorbance of both cotyledon and embryonic axis of *H. ponga* seeds at 0 hours of desiccation at 540nm are 0.173±0.008 (cotyledon) and 0.566±0.008 (embryonal axes) respectively (Graph: 1). But at 168 hours of desiccation, the absorbance was reduced to 0.023±0.003 (cotyledon) and 0.013±0.003 (Embryonal axis) respectively.



Graph: 1 Formazan activity during desiccation

Chlorophyll Content

During the desiccation, chlorophyll a, chlorophyll b, and total chlorophyll gradually decreased in *Hopea ponga* seeds. Rapid decline was noticed after 24 hours of desiccation followed by almost a stationary phase after 96 hours (Graph: 2). At 0 hours of desiccation the chlorophyll content (Chlorophyll a: 0.316±0.008 mg/g/fwt., Chlorophyll b: 1.483±0.012 mg/g/fwt. and total chlorophyll content: 1.800±.015 mg/g/fwt.) was at its peak and ongoing to the desiccation the pigment level is declined.

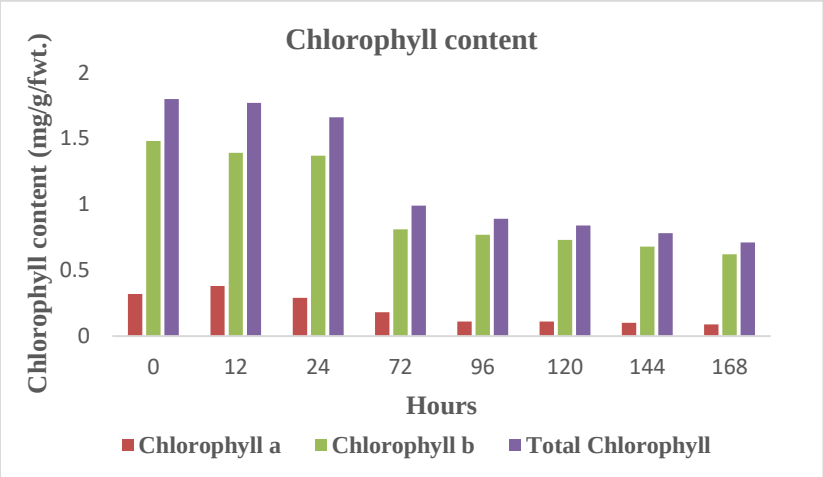
Leachate Conductivity

The electrical conductivity measurement showed an increased solute leachate from the seed tissues. The seed leachate collected from the desiccated *Hopea ponga* seeds at different intervals showed increased leachate conductivity with

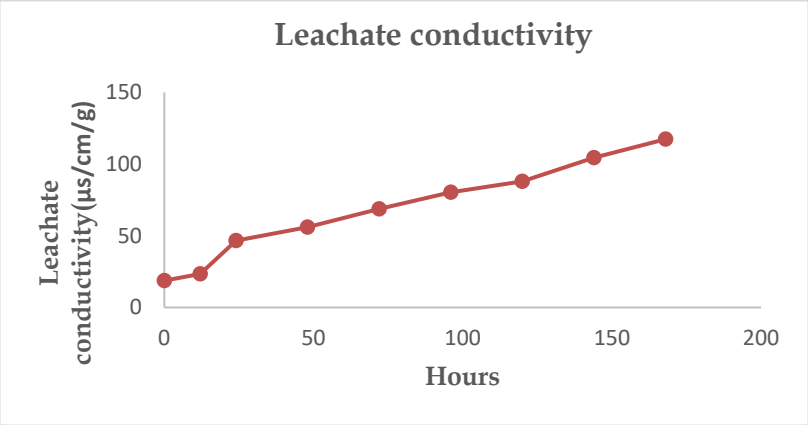
complete loss of viability. This significant increase in leachate conductivity was correlated with the decreasing moisture content and germination. In control seeds of *H. ponga*, the specific conductance was only 18.66 ±1.200 µs/cm/g, but after 168 hours of desiccation, the leachate conductivity increased almost six times (117.33±1.452 µs/cm/g). However, the conductivity rate is closely related to the decline of moisture content and viability of the seeds (Graph: 3).

Lipid Peroxidation

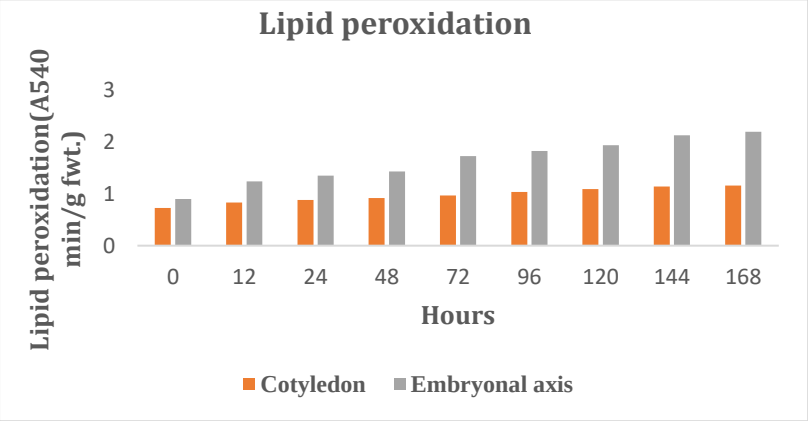
The degree of lipid peroxidation in *Hopea ponga* seeds was assessed by measuring the production of Thiobarbituric Acid Reactive Substances (TBARS) during various desiccation/drying treatments. The increase in TBARS production indicates heightened lipid peroxidation in *H. ponga* throughout the desiccation period, a pattern common to most recalcitrant seeds. Notably, lipid peroxidation was more pronounced in the embryonic axis than in the cotyledonary tissues of *H. ponga* (refer to Graph: 4). The peak level of lipid peroxidation was observed at 168 hours of drying, with cotyledons showing 1.163±0.017 and the embryonic axis showing 2.203±0.014 levels of TBARS in *H. ponga*.



Graph: 2 Chlorophyll content in *Hopea ponga* seeds during desiccation



Graph: 3 Leachate conductivity during desiccation

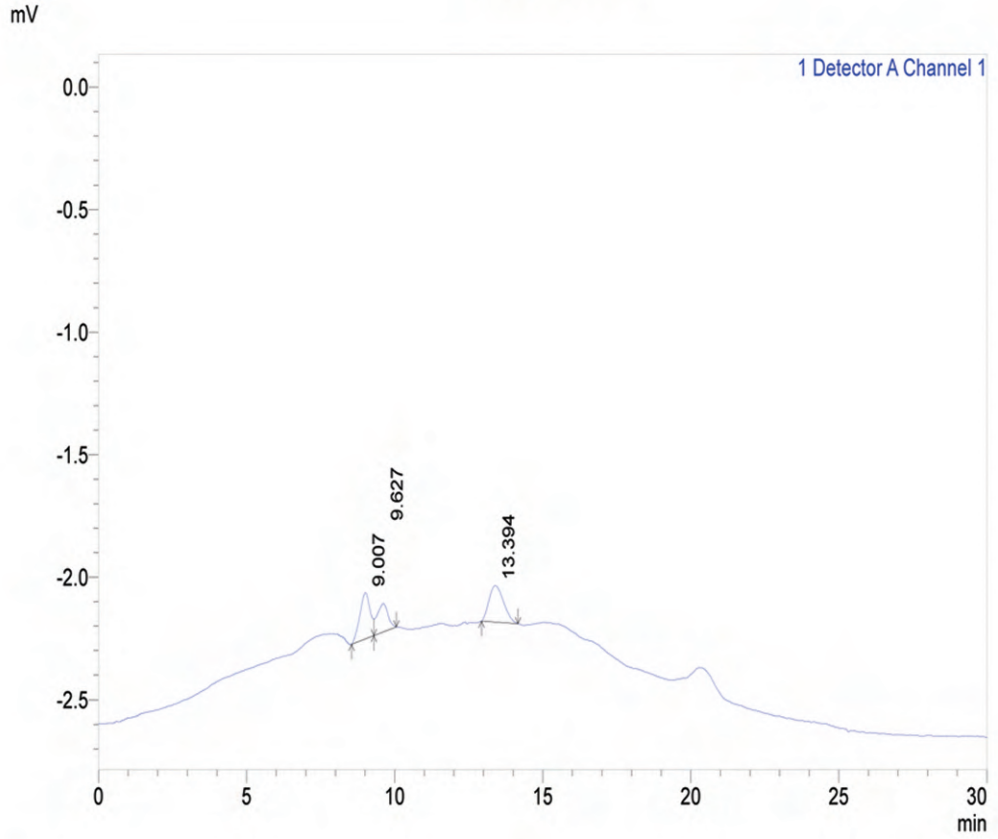


Graph: 4 Lipid peroxidation during desiccation

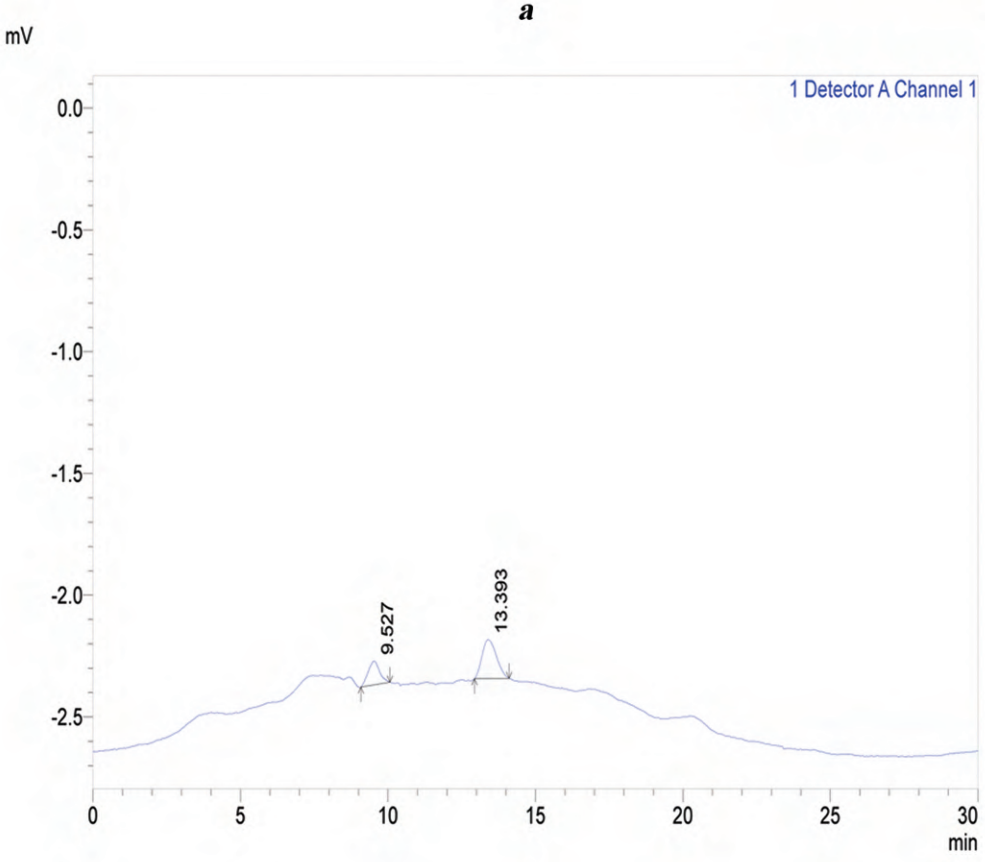
Quantitative Estimation of Soluble Sugars using HPLC Techniques in *Hopea ponga* Seeds During Desiccation

The HPLC data (Table: 11) indicates that sucrose is present in the cotyledonary tissues of *Hopea ponga*, while other sugars like glucose, fructose, arabinose, and raffinose are not detected. Sucrose content increases as moisture content decreases. In the embryonic axis, at the initial (Table: 12) desiccation stage with 45% moisture, sucrose, glucose, and fructose were present. At 39% moisture, only sucrose and glucose are detected, with fructose absent. In the third and fourth desiccation phases (31% and 24% moisture), sucrose content significantly increases. Raffinose and arabinose are not detected at any desiccation phase in both cotyledon and embryonic axis. The chromatogram of the HPLC analysis of cotyledon and embryonal axis is represented in Graph: 5 (i & ii) and Graph: 6 (i & ii).

Cotyledon



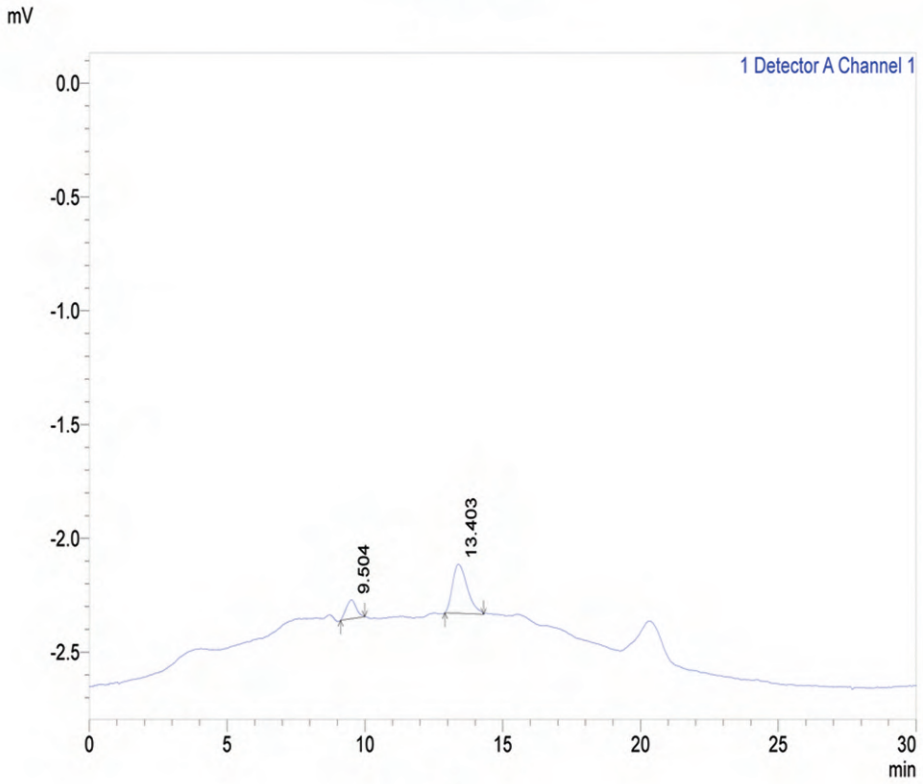
a



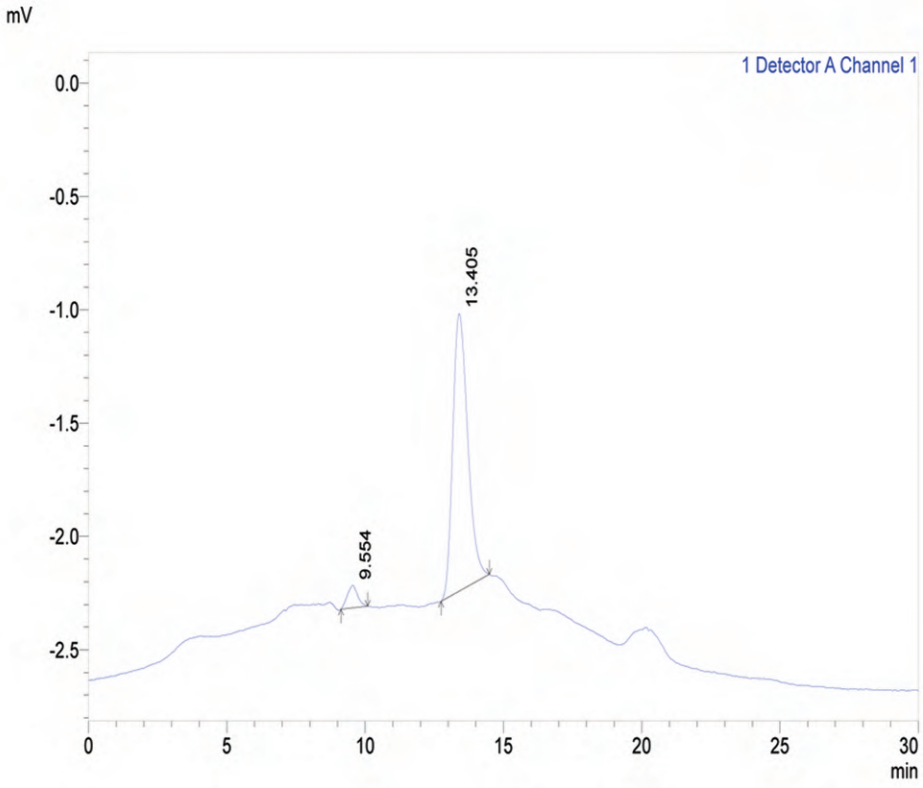
b

Graph 5i HPLC analysis of Cotyledon tissues during desiccation. a. Seed with Moisture content 45%, b. Seed with Moisture content 39%,

Cotyledon



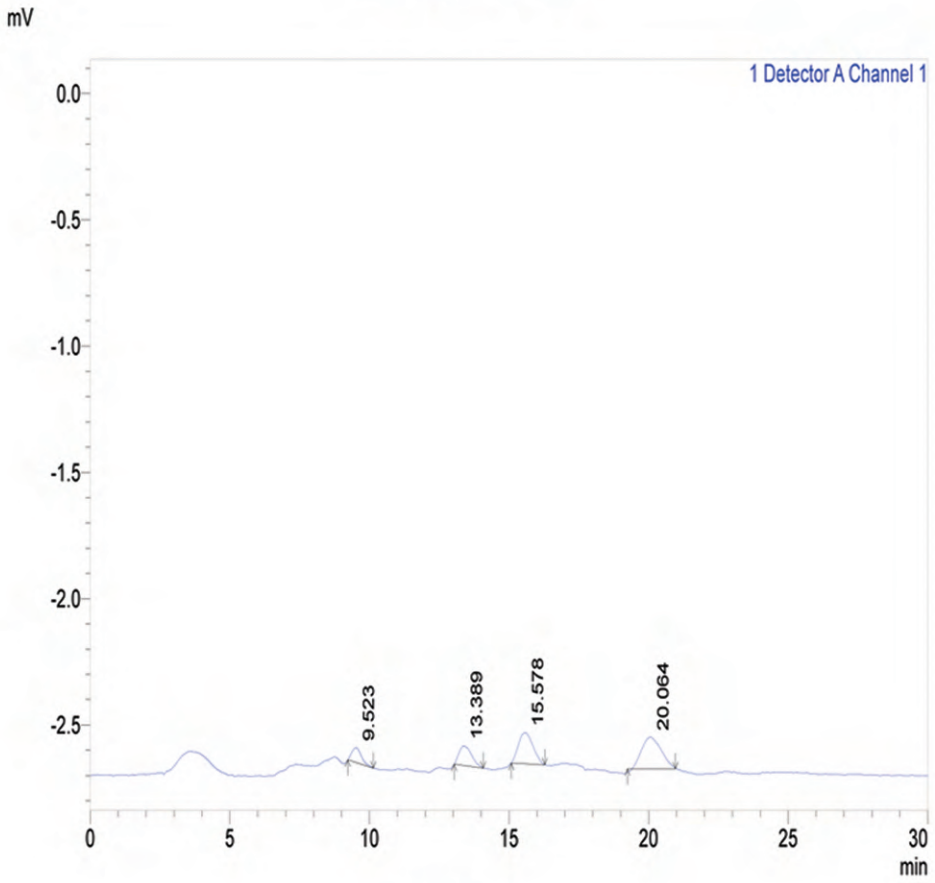
c



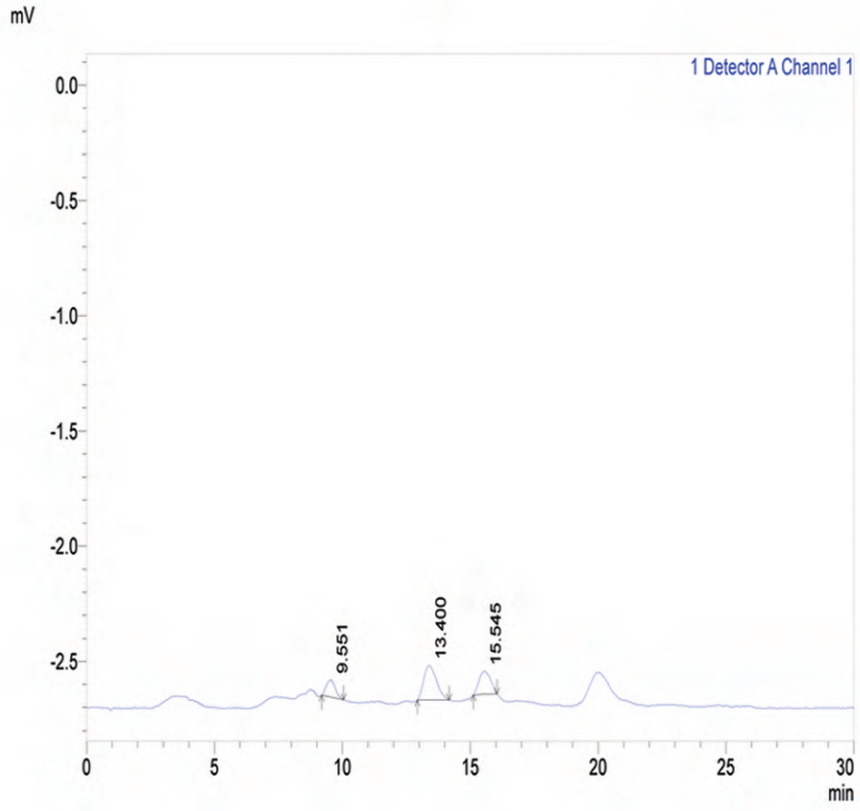
d

Graph 5ii HPLC analysis of Cotyledon tissues during desiccation. c. Seed with Moisture content 31%, d. Seed with Moisture content 24%

Embryonic axis

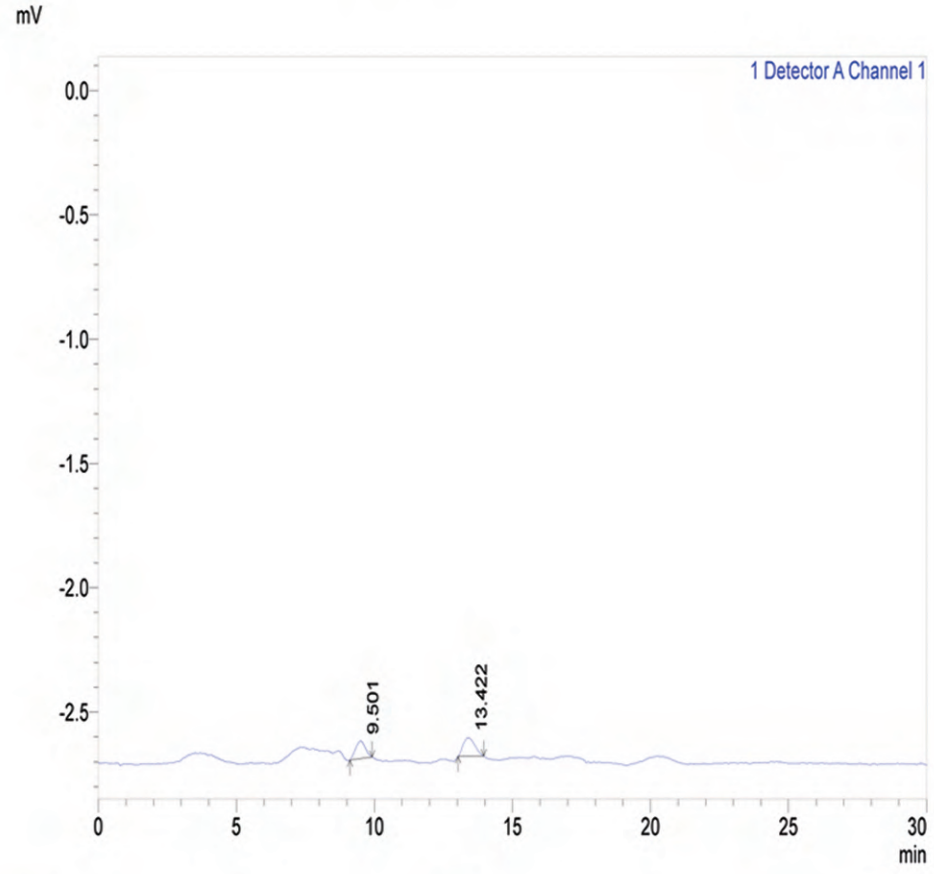


a

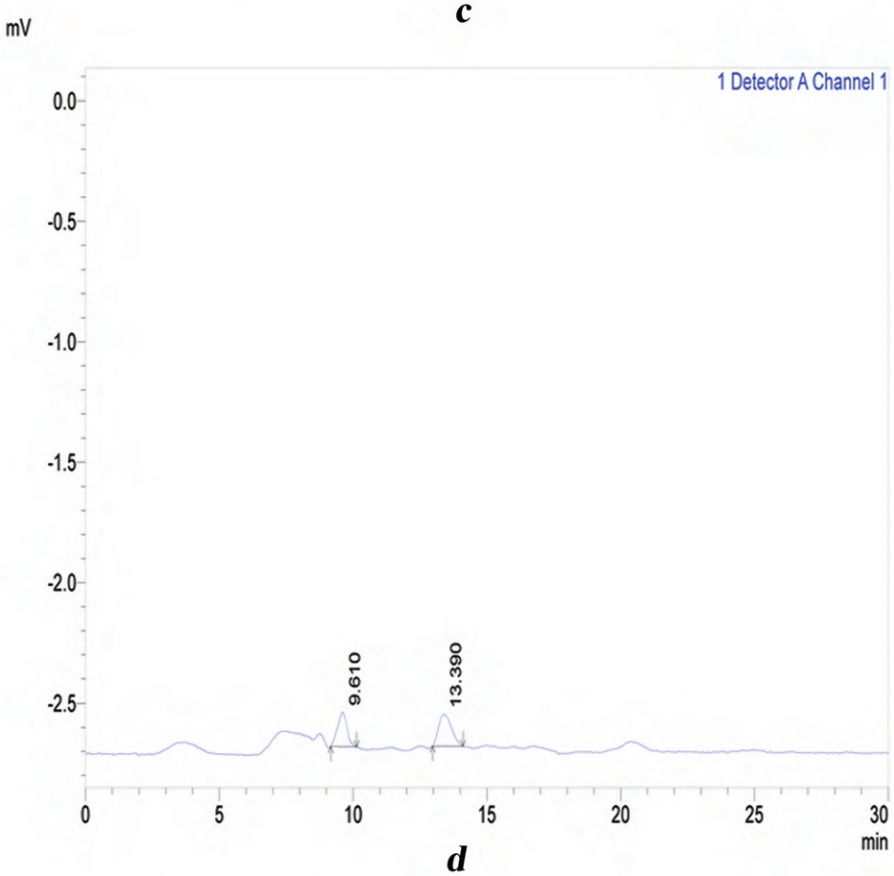


Graph 6i HPLC analysis of Embryonal axis tissues during desiccation. a. Seed with Moisture content 45%, b. Seed with Moisture content 39%,

Embryonic axis



c



d

Graph 6ii HPLC analysis of Embryonal axis tissues during desiccation c. Seed with Moisture content 24%, d. Seed with Moisture content 31%

Moisture Content of Seeds (%)	Sucrose (mg/ml) [Peak area]	Glucose (mg/ml) [Peak area]	Fructose (mg/ml) [Peak area]	Raffinose (mg/ml) [Peak area]	Arabinose (mg/ml) [Peak area]
45	1.160 [5075]	Nil	Nil	Nil	Nil
39	1.191 [5401]	Nil	Nil	Nil	Nil
31	1.435 [8005]	Nil	Nil	Nil	Nil
24	5.001 [45994]	Nil	Nil	Nil	Nil

Table: 11 HPLC Analysis of Cotyledon Tissues

Moisture content of seeds (%)	Sucrose (mg/ml) [Peak area]	Glucose (mg/ml) [Peak area]	Fructose (mg/ml) [Peak area]	Raffinose (mg/ml) [Peak area]	Arabinose (mg/ml) [Peak area]
45	0.912 [2436]	0.266 [4433]	0.333 [6146]	Nil	Nil
39	1.168 [5157]	0.175 [3073]	Nil	Nil	Nil
31	1.102 [4453]	Nil	Nil	Nil	Nil
24	2.665 [21105]	Nil	Nil	Nil	Nil

Table: 12 HPLC Analysis of Embryonal axis Tissues

Biochemical Changes During Seed Desiccation

Total Soluble Sugar (TSS) Content During Desiccation

TSS content in both cotyledons and embryonal axis of *Hopea ponga* seed showed a slight reduction after 12 hours of desiccation. This was followed by a gradual increase up to 168 hours of drying, after which a slight decline was noted. At this stage, the viability of the seeds was completely lost (Table: 13).

Starch Content During Desiccation

A slight increase in starch up to 12 hours of drying followed by gradual reduction was recorded in both the cotyledon and embryonal axis of *Hopea ponga* seeds throughout the desiccation period (Table: 13).

Total Phenol Content During Desiccation

During the initial drying period of up to 48 hours, the total phenolic content in the cotyledon increased. The increase rate was more pronounced in the embryonal axis than in the cotyledonary tissues. An increase of over 80% in the phenolic content of the embryonal axis was noted after 96 hours of

desiccation. Consequently, the highest phenolic content in all tissues was observed during the later stages of drying in *H. ponga* seeds (Table: 13).

Lipid Content During Desiccation

The total lipid content in *Hopea ponga* seeds decreased gradually with desiccation from 87.883±1.062 mg g-1 d. wt. to 20.493±. 225 mg g^{-1} d. wt. (0 to 120 HAH). After 144 hours of desiccation, the level of lipids was almost stabilized (Table: 13).

Amino Acid Content During Desiccation

Amino acid content of both cotyledonary and embryonal axis tissues of *Hopea ponga* seeds gradually increased up to 48 hours of drying followed by a significant reduction throughout the desiccation period (Table: 13).

Protein Fractions During Desiccation

Albumin: The water-soluble protein (albumin) content of *Hopea ponga* seeds during desiccation showed a gradual decrease in both cotyledonary and embryonal axis from 0 hours to 96 hours, followed by a stationary phase until the end of desiccation (Table: 14).

Globulin: There was no significant change in globulin content in the cotyledons of *Hopea ponga* in the first 24 hours of desiccation, which later showed a gradual decrease till 96 hours. The globulin content did not show any change from 96 to 144 hours. A marginal increase in globulin was noticed in the embryonal axis/radicle up to 96 hours of imbibition (Table: 14).

Prolamin: The prolamin content of the cotyledonary tissues of *Hopea ponga* gradually decreased from 19.466±0.269 mg/g dwt. to 13.473±0.040mg/g dwt. from 0 to 120 hours (Table: 14). In embryonal axis/radicle/root prolamin content, a gradual decrease from 0 hours to 96 hours. A minor decrease in prolamin content was recorded from its differentiation at 72 to 168 hours of imbibition.

Glutelin (0.05M NaOH Fraction): Data in Table 14 revealed that the 0.05M NaOH fraction of glutelin in the cotyledon of *Hopea ponga* slowly decreased from 14.900±0.092 to 12.110±0.020 mg/g dwt. from 0 hours to 144 hours of imbibition. A similar pattern was also observed in the embryonal axis throughout the desiccation.

Glutelin (0.25M NaOH Fraction): The level of 0.25M NaOH fraction of glutelin showed much difference between 0 to 96 hours of desiccation in cotyledons of *Hopea ponga* (Table:14). After 96 hours a marginal increase was recorded. The glutelin fraction in the embryonal axis was almost stationary in the 0 desiccation periods up to 24 hours and later a decrease was recorded till 144 hours of imbibition.

Hours After Harvest (HAH)	Moisture content (%) ±SE	Total Soluble Sugars (TSS) mg g $^{-1}$ d. wt. ±SE	Starch mg g^{-1}d. wt .±SE	Total Phenol mg g^{-1} d. wt. ±SE	Lipid mg g^{-1}d. wt. ±SE	Amino acid mg g^{-1}d. wt. ±SE
0WS 0EMB	$42.543 \pm .429^{A^*}$ $52.403 \pm .270^{(1)^*}$	$33.95 \pm .14^{A^*}$ $46.79 \pm .25^{(1)^*}$	$254.79 \pm .68^{A^*}$ $238.14 \pm .40^{(1)(2)^*}$	$158.21 \pm .32^{A^*}$ $186.85 \pm .46^{(1)^*}$	$87.88 \pm 1.06^{A^*}$ $58.62 \pm .424^{(1)^*}$	$2.126 \pm .032^{A^*}$ $4.18 \pm .014^{(1)^*}$
12WS 12EMB	$40.310 \pm .33^{B^*}$ $50.550 \pm .238^{(2)^*}$	$31.173 \pm .06^{B^*}$ $45.81 \pm .089^{(2)^*}$	$275.53 \pm .41^{B^*}$ $240.15 \pm .54^{(1)^*}$	$163.67 \pm .641^{B^*}$ $213.85 \pm 1.07^{(2)^*}$	$85.086 \pm .85^{B^*}$ $47.483 \pm .203^{(2)^*}$	$2.626 \pm .067^{B^*}$ $4.52 \pm .083^{(2)^*}$
24WS 24EMB	$37.466 \pm .19^{C^*}$ $48.346 \pm .161^{(3)^*}$	$35.643 \pm .24^{C^*}$ $47.483 \pm .21^{(3)^*}$	$212.61 \pm 1.21^{C^*}$ $236.60 \pm .34^{(2)^*}$	$178.04 \pm .88^{AB^*}$ $233.20 \pm .676^{(3)^*}$	$46.800 \pm .56^{C^*}$ $41.956 \pm .16^{(3)^*}$	$2.646 \pm .028^{B^*}$ $4.72 \pm .049^{(3)^*}$
48WS 48EMB	$33.106 \pm .23^{D^*}$ $46.400 \pm .228^{(4)^*}$	$37.983 \pm .033^{D^*}$ $49.08 \pm .057^{(4)^*}$	$183.73 \pm 1.14^{D^*}$ $185.6 \pm 1.89^{(3)^*}$	$181.57 \pm .40^{C^*}$ $309.33 \pm 1.84^{(4)^*}$	$35.913 \pm .092^{D^*}$ $32.373 \pm .311^{(4)^*}$	$2.893 \pm .026^{C^*}$ $4.923 \pm .024^{(4)^*}$
72WS 72EMB	$30.260 \pm .17^{E^*}$ $42.280 \pm .15^{(5)^*}$	$40.150 \pm .28^{E^*}$ $54.586 \pm .31^{(5)^*}$	$173.12 \pm .25^{E^*}$ $167.92 \pm .34^{(4)^*}$	$176.76 \pm .55^{A^*}$ $453.18 \pm 2.53^{(5)^*}$	$30.056 \pm .05^{E^*}$ $26.240 \pm .278^{(5)^*}$	$1.683 \pm .02^{D^*}$ $2.790 \pm .05^{(5)^*}$
96WS 96EMB	$29.65 \pm .07^{E^*}$ $40.266 \pm .190^{(6)^*}$	$43.370 \pm .31^{F^*}$ $57.586 \pm .29^{(6)^*}$	$165.86 \pm .51^{F^*}$ $145.81 \pm .61^{(5)^*}$	$178.86 \pm .53`^{B^*}$ $460.24 \pm 1.27^{(6)^*}$	$22.213 \pm .57^{F^*}$ $19.840 \pm .104^{(6)^*}$	$1.076 \pm .027^{E^*}$ $1.476 \pm .03^{(6)^*}$
120WS 120EMB	$28.110 \pm .01^{F^*}$ $38.376 \pm .24^{(7)^*}$	$41.676 \pm .28^{G^*}$ $60.94 \pm .196^{(7)^*}$	$155.23 \pm .64^{G^*}$ $128.95 \pm .46^{(6)^*}$	$181.06 \pm .29^{C^*}$ $462.02 \pm .30^{(6)(7)^*}$	$20.493 \pm .23^{G^*}$ $19.090 \pm .04^{(7)^*}$	$0.953 \pm .028^{F^*}$ $.996 \pm .012^{(7)^*}$
144WS 144EMB	$27.076 \pm .33^{G^*}$ $34.97 \pm .066^{(8)^*}$	$40.806 \pm .34^{E^*}$ $65.92 \pm .121^{(8)^*}$	$150.77 \pm .31^{H^*}$ $121.78 \pm .39^{(7)^*}$	$181.57 \pm .335^{E^*}$ $465.15 .124^{(7)(8)^*}$	$18.063 \pm .031^{H^*}$ $17.503 \pm .25^{(8)^*}$	$.476 \pm .017^{G^*}$ $.363 \pm .014^{(8)^*}$
168WS 168EMB	$25.750 \pm .101^{H^*}$ $31.500 \pm .265^{(9)^*}$	$42.066 \pm .05^{H^*}$ $67.906 \pm .06^{(9)^*}$	$152.85 \pm .036^{I^*}$ $116.29 \pm .31^{(8)^*}$	$185.34 \pm .39^{F^*}$ $467.48 \pm .373^{(8)^*}$	$16.533 \pm .189^{H^*}$ $15.180 \pm .04^{(9)^*}$	$.266 \pm .012^{H^*}$ $.200 \pm .005^{(9)^*}$

The factors of the study are represented as WS (Whole Seed), EMB (Embryonal axis), ±SE: Standard Error of the mean. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 13 Quantitative estimations of biomolecules during desiccation

Hours After Harvest (HAH)	Albumin mg g $^{-1}$ d. wt. ±SE	Globulin mg g^{-1}d. wt. ±SE	Prolamin mg g^{-1} d. wt. ±SE	Glutelin mg g^{-1}d. wt.		Total protein mg g^{-1}d. wt. ±SE
				NaOH 0.05M ±SE	NaOH 0.25M ±SE	
0COT 0EMB	$128.80 \pm .18^{A^*}$ $165.75 \pm .341^{(1)^*}$	$6.810 \pm 0.083^{A^*}$ $13.53 \pm 0.057^{(1)^*}$	$19.466 \pm 0.27^{A^*}$ $38.223 \pm 0.237^{(1)^*}$	$14.900 \pm .0920^{A^*}$ $19.356 \pm .107^{(1)^*}$	$1.473 \pm .080^{A^*}$ $13.84 \pm .061^{(1)^*}$	$181.45 \pm .39^{A^*}$ $250.84 \pm .553^{(1)^*}$
12COT 12EMB	$114.54 \pm .53^{B^*}$ $155.73 \pm .396^{(2)^*}$	$6.633 \pm 0.09^{B^*}$ $11.330 \pm 0.18^{(2)^*}$	$17.613 \pm 0.26^{B^*}$ $32.693 \pm 0.188^{(2)^*}$	$14.663 \pm .064^{A^*}$ $16.270 \pm .128^{(2)^*}$	$10.430 \pm .128^{B^*}$ $12.84 \pm .063^{(2)^*}$	$163.88 \pm .83^{B^*}$ $228.86 \pm .930^{(2)^*}$
24COT 24EMB	$96.30 \pm .18^{C^*}$ $135.79 \pm .32^{(3)^*}$	$4.920 \pm 0.045^{C^*}$ $9.643 \pm 0.04^{(3)^*}$	$16.180 \pm 0.032^{C^*}$ $28.366 \pm 0.185^{(3)^*}$	$13.436 \pm .038^{B^*}$ $15.716 \pm .112^{(3)^*}$	$8.643 \pm .037^{C^*}$ $12.01 \pm .017^{(3)^*}$	$139.48 \pm .126^{C^*}$ $201.53 \pm .029^{(3)^*}$
48COT 48EMB	$81.46 \pm .66^{D^*}$ $101.45 \pm .459^{(4)^*}$	$4.513 \pm 0.058^{D^*}$ $8.400 \pm 0.161^{(4)^*}$	$14.493 \pm 0.26^{D^*}$ $26.523 \pm 0.337^{(4)^*}$	$13.296 \pm .184^{B^*}$ $15.530 \pm .277^{(3)^*}$	$8.233 \pm .041^{D^*}$ $11.13 \pm .063^{(4)^*}$	$122.001 \pm .642^{D^*}$ $163.03 \pm 1.052^{(4)^*}$
72COT 72EMB	$79.41 \pm .41^{E^*}$ $100.48 \pm .632^{(4)^*}$	$4.373 \pm 0.012^{D^*}$ $8.1 \pm 0.070^{(5)^*}$	$13.900 \pm 0.025^{E^*}$ $25.600 \pm 0.028^{(5)^*}$	$12.380 \pm .087^{C^*}$ $14.493 \pm .216^{(4)^*}$	$8.050 \pm .27^{DE^*}$ $10.95 \pm .020^{(5)^*}$	$118.11 \pm .435^{E^*}$ $159.63 \pm .752^{(5)^*}$
96COT 96EMB	$77.47 \pm .15^{F^*}$ $98.04 \pm .315^{(5)^*}$	$4.206 \pm 0.049^{E^*}$ $7.793 \pm 0.026^{(6)^*}$	$13.706 \pm 0.017^{EF^*}$ $23.313 \pm 0.168^{(6)^*}$	$12.420 \pm .032^{C^*}$ $13.990 \pm .032^{(5)^*}$	$7.643 \pm .321^{FG^*}$ $9.603 \pm .043^{(6)^*}$	$115.45 \pm .27^{F^*}$ $152.74 \pm .427^{(6)*}$
120COT 120EMB	$77.36 \pm .055^{F^*}$ $95.83 \pm .268^{(6)^*}$	$4.083 \pm 0.008^{E^*}$ $7.243 \pm 0.037^{(7)^*}$	$13.473 \pm 0.04^{EF^*}$ $22.49 \pm 0.15^{(7)(8)^*}$	$12.266 \pm .020^{CD^*}$ $13.833 \pm .040^{(5)^*}$	$7.816 \pm .046^{EF^*}$ $9.546 \pm .024^{(6)^*}$	$115.001 \pm .099^{G^*}$ $148.94 \pm .346^{(7)^*}$
144COT 144EMB	$75.87 \pm .36^{G^*}$ $93.50 \pm .110^{(7)^*}$	$4.046 \pm 0.02^{E^*}$ $7.080 \pm 0.051^{(8)^*}$	$13.273 \pm 0.04^{F^*}$ $22.163 \pm 0.032^{(8)^*}$	$12.110 \pm .02^{D^*}$ $13.253 \pm .031^{(6)^*}$	$7.476 \pm .023^{FG^*}$ $9.233 \pm .029^{(7)^*}$	$112.78 \pm .32^{G^*}$ $145.23 \pm .151^{(8)^*}$
168COT 168EMB	$74.97 \pm .56^{G^*}$ $93.81 \pm .043^{(7)^*}$	$4.110 \pm 0.011^{E^*}$ $6.800 \pm 0.03^{(9)^*}$	$13.643 \pm 0.034^{EF^*}$ $22.79 \pm 0.02^{(6)(7)^*}$	$12.070 \pm .011^{D^*}$ $13.096 \pm .042^{(6)^*}$	$7.376 \pm .018^{G^*}$ $9.203 \pm .040^{(7)^*}$	$112.18 \pm .523^{G^*}$ $145.70 \pm .073^{(8)^*}$

The factors of the study are represented as COT(Cotyledon), EMB (Embryonal axis), ±SE: Standard Error of the mean. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

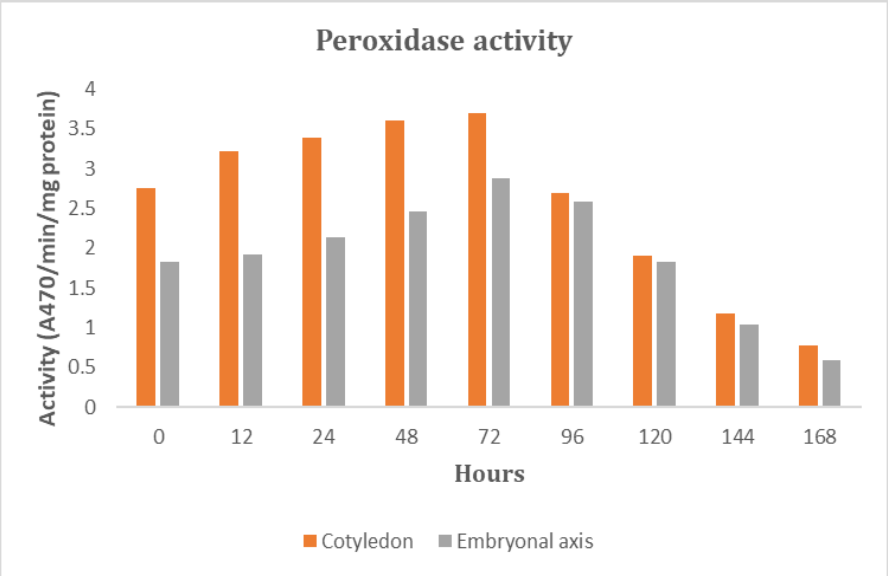
Table: 14 Estimation of protein fractions during desiccation

Total Protein Content

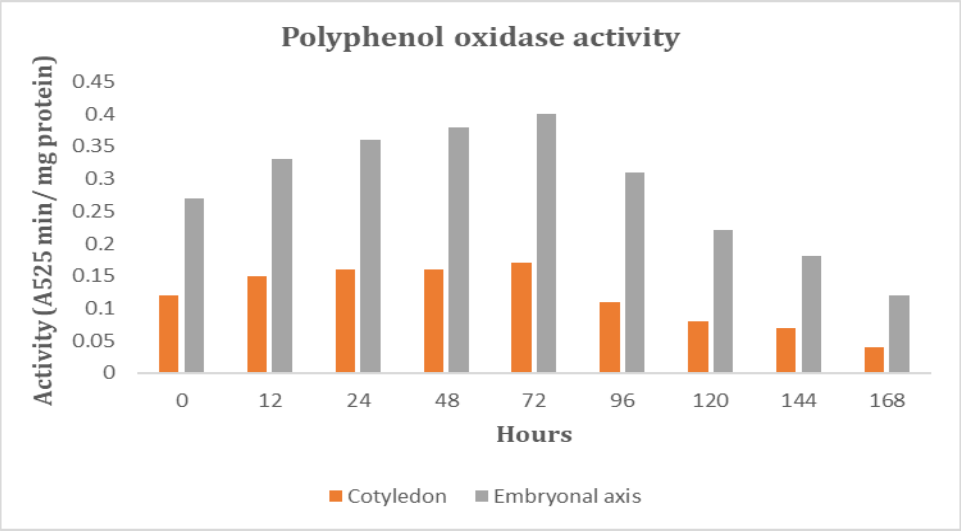
The total protein content of the cotyledonary tissues of seeds showed a significant decline up to 120 hours of desiccation from 181.45±0.393 mg/g dwt. to 115.001±.099mg/g dwt. A stationary phase till 144 hours (Table:14). In the embryonal axis, the content decreased gradually between 0 to 120 hours of desiccation (250.84±0.553 mg/g dwt. to148.94±0.346 mg/g dwt.).

Enzyme Activity During Desiccation

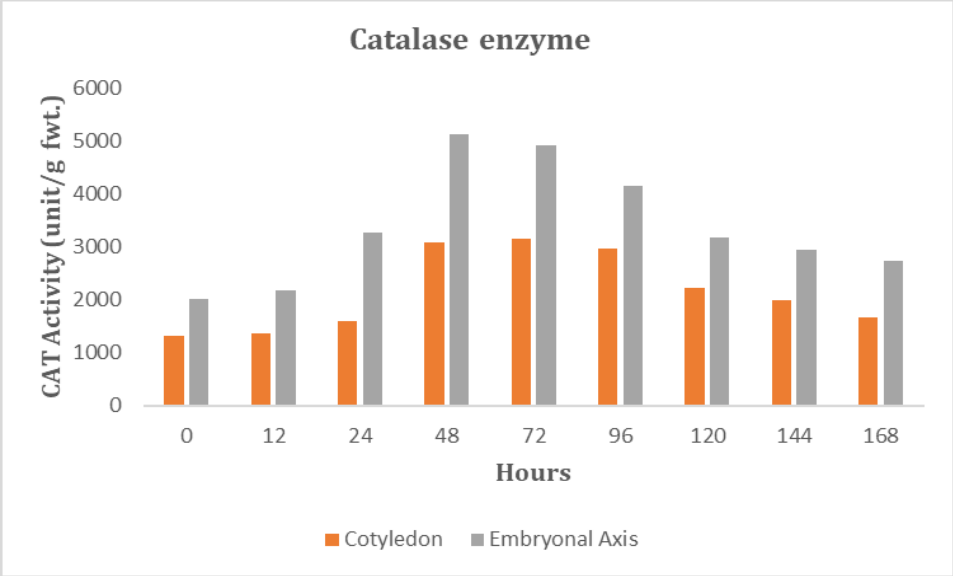
The antioxidant enzyme showed high activity during the initial period of desiccation. The activity of peroxidase (POD) was more in the cotyledon of *Hopea ponga* than in embryonal axis (Graph: 7). Polyphenol oxidase (PPO) activities in *H. ponga* seeds were maximum in embryonal axis than in cotyledon (Graph: 8). Similar patterns were noticed in both cotyledonary and embryonal axis tissues *of H. ponga*. The POD and PPO activities were maximum after 72 hours of drying in *H. ponga* seeds, but Catalase (CAT) activity was maximum after 48 hours of drying (Graph: 9).



Graph: 7 Peroxidase activity during seed desiccation



Graph: 8 Polyphenol oxidase activity (PPO) during seed desiccation



Graph: 9 Catalase enzyme activity during seed desiccation

Seed Germination Studies

Germination studies utilized mature, fresh seeds of *Hopea ponga*. The first stage of germination is the absorption of water. Radicle protrusion was observed 24 hours after water uptake and plumule protrusion began at 72 hours. The differentiation of roots stems, and leaves was noted at 168 hours post-water uptake. The emergence of the radicle, followed by the growth of root hairs, was recorded at 96 hours. These germination characteristics were consistent in both laboratory conditions (28±2°C & 65% RH) and field observations. Following radicle protrusion, lab seedlings were subjected to

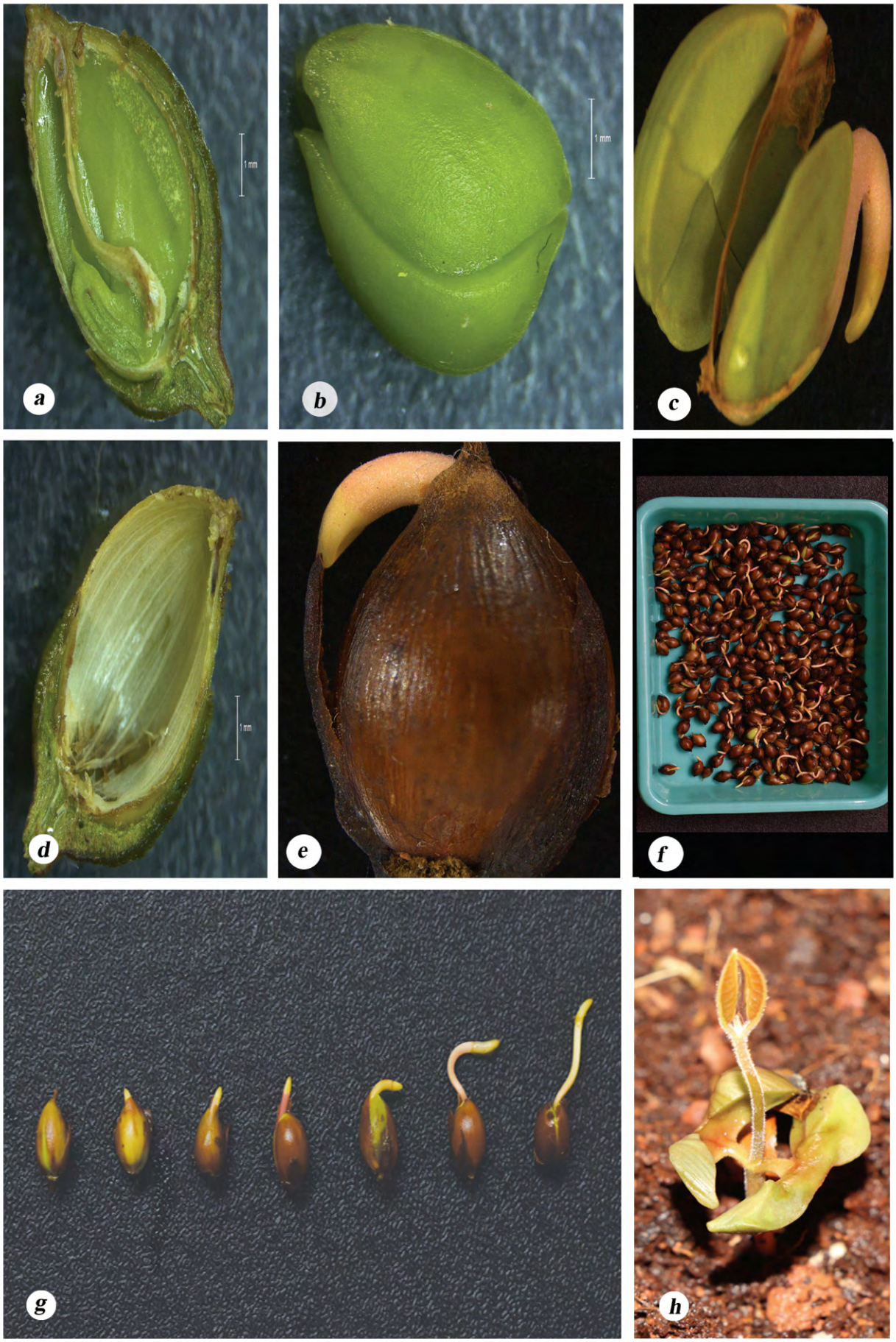


Fig.16 Seed germination stages - a. Fruit split opened, b. Upper surface view of seed, c. Seed germination, d. Fruit coat, e. Germinated seed, , f. Germinated seed lots, g. Different phases of germination (From 0 Hour to 144 hours) h. Seedling

light to promote plumule growth. In the field, seeds were sown in a sandy substrate (treated river sand) within earthen pots and mist chamber beds (32°C & 80% RH). Seeds buried deeper than their size showed inhibited germination, likely due to inadequate light and air exchange. This species exhibits epigeal germination, with cotyledons emerging above ground to commence photosynthesis. Eventually, the cotyledons dry up, and the epicotyl bud forms the primary leaves, influenced by light exposure (Fig.16).

Quantitative Estimation of Biomolecules During Germination

Total Soluble Sugar (TSS) Content: During germination, the Total soluble sugars (TSS) content in the cotyledons of *H. ponga* seeds increased gradually over 120 hours of imbibition, from 33.953±0.140 mg/g dwt. to 47.993±0.264 mg/g dwt. and later decreased. Similarly, the TSS content in the embryonal axis rose steadily, irrespective of the transformation into radicle/root, until 144 hours of imbibition, at which point it stabilized. Meanwhile, the TSS content in the plumule continued to increase throughout the 144 hours of imbibition (Table: 15).

Starch Content: During the germination of *Hopea ponga* seeds, starch content in the cotyledons significantly decreased from 0 to 120 hours of imbibition (from 254.79±0.682 to 168.91±0.780 mg/g dwt), followed by a stationary phase. A considerable reduction in starch content was also observed in the seeds' embryonal axis, radicle/root, and plumule throughout the imbibition periods (Table: 15).

Total Phenol Content: In the cotyledon of *Hopea ponga* seeds, the total phenolic content at the onset of imbibition was recorded at 158.21±0.319 mg/g dry weight. This value decreased significantly to 116.96±0.807 mg/g dry weight after 48 hours of continuous imbibition, before entering a stationary phase. Similarly, the embryonal axis and radicle/ root tissues of *H. ponga* showed a consistent decrease in phenolic content from the start of imbibition to 96 hours, after which it stabilized until the end of the observation period. The plumule tissues also recorded a steady decline in total phenolic content from 72 hours to the final phase at 168 hours of imbibition (Table: 15).

Lipid Content: During the imbibition process, the lipid content in the cotyledons of *Hopea ponga* seeds gradually decreased (from 87.883±1.062 mg/g dwt. to 34.176±0.238 mg/g dwt.). The embryonic axis, radicle, and roots of *H. ponga* seeds exhibited only a slight decrease in lipid content throughout the imbibition period. However, the lipid content in the plumule increased for the first 96 hours, then gradually decreased until 168 hours of imbibition (Table: 15).

Imbibition (Hours)	Total Soluble Sugars (TSS) mg g $^{-1}$ d. wt. ±SE	Starch mg g^{-1}d. wt. ±SE	Total Phenol mg g^{-1} d. wt. ±SE	Lipid mg g^{-1}d. wt. ±SE	Amino Acid mg g^{-1}d. wt. ±SE
0COT	$33.953 \pm .140^{A^*}$	$254.79 \pm .680^{A^*}$	$158.21 \pm .319^{A^*}$	$87.883 \pm 1.062^{A^*}$	$2.126 \pm .032^{A^*}$
0EMB	46.793±.249	238.14±.398	186.85±.459	58.616±.424	4.1833±.014
12COT	$35.706 \pm .457^{B^*}$	$234.8 \pm 2.16^{B^*}$	$142.78 \pm .812^{B^*}$	$75.163 \pm .848^{B^*}$	$2.580 \pm .032^{B^*}$
12EMB	49.440±.340	229.40±.461	172.22±.723	55.553±.417	4.9433±.059
24COT	$39.046 \pm .060^{C^*}$	$227.31 \pm 1.76^{C^*}$	$134.78 \pm 1.04^{C^*}$	$71.983 \pm .200^{C^*}$	$2.793 \pm .021^{C^*}$
24RAD	$52.886 \pm .206^{(a)^*}$	$225.49 \pm .56^{(a)^*}$	$152.70 \pm .488^{(a)^*}$	$54.480 \pm .328^{(a)^*}$	$5.633 \pm 141^{(a)^*}$
48COT	$40.690 \pm .226^{D^*}$	$223.40 \pm .63^{D^*}$	$116.96 \pm .807^{D^*}$	$61.646 \pm .478^{D^*}$	$2.923 \pm .017^{D^*}$
48RAD	$55.770 \pm .219^{(b)^*}$	$189.02 \pm 1.01^{(b)^*}$	$141.75 \pm .542^{(b)^*}$	$52.096 \pm .098^{(b)^*}$	$6.550 \pm .169^{(b)^*}$
72COT	$42.986 \pm .348^{E^*}$	$201.98 \pm .98^{E^*}$	$124.70 \pm .655^{E^*}$	$52.846 \pm .169^{E^*}$	$3.086 \pm .031^{E^*}$
72RAD	$61.090 \pm .38^{(c)^*}$	$168.17 \pm 1.96^{(c)^*}$	$134.34 \pm .363^{(c)^*}$	$44.073 \pm .34^{(c)^*}$	$6.910 \pm .026^{(c)^*}$
72PLU	$21.963 \pm .854^{\mathrm{I}^*}$	$115.010 \pm 1.135^{\mathrm{I}^*}$	$62.010 \pm .906^{\mathrm{I}^*}$	$25.316 \pm .239^{\mathrm{I}^*}$	$4.320 \pm .037^{\mathrm{I}^*}$
96COT	$44.890 \pm .427^{F^*}$	$195.28 \pm .470^{F^*}$	$112.40 \pm .866^{F^*}$	$44.383 \pm .489^{F^*}$	$3.336 \pm .033^{F^*}$
96RAD	$63.643 \pm .230^{(d)^*}$	$132.95 \pm 1.32^{(d)^*}$	$122.79 \pm 1.27^{(d)^*}$	$40.203 \pm .266^{(d)^*}$	$7.226 \pm .054^{(d)^*}$
96PLU	$27.063 \pm .534^{\mathrm{II}^*}$	$102.91 \pm 1.490^{\mathrm{II}^*}$	$52.730 \pm .476^{\mathrm{II}^*}$	$30.043 \pm .391^{\mathrm{II}^*}$	$4.533 \pm .031^{\mathrm{II}^*}$
120COT	$47.993 \pm .264^{G^*}$	$168.91 \pm .780^{G^*}$	$106.31 \pm .321^{A^*}$	$41.173 \pm .325^{G^*}$	$3.653 \pm .043^{G^*}$
120RAD	$69.086 \pm .32^{(e)^*}$	$102.81 \pm 1.05^{(e)^*}$	$116.18 \pm .510^{(e)^*}$	$37.520 \pm .412^{(e)^*}$	$7.640 \pm .055^{(e)^*}$
120PLU	$31.250 \pm .306^{\mathrm{III}^*}$	$86.666 \pm .485^{\mathrm{III}^*}$	$42.460 \pm .837^{\mathrm{III}^*}$	$27.906 \pm .197^{\mathrm{III}^*}$	$4.650 \pm .025^{\mathrm{III}^*}$
144COT	$40.873 \pm .299^{D^*}$	$167.14 \pm .73^{G^*}$	$104.58 \pm .762^{A^*}$	$36.510 \pm .260^{H^*}$	$3.890 \pm .023^{H^*}$
144RAD	$72.890 \pm .39^{(f)^*}$	$95.23 \pm .23^{(f)^*}$	$112.76 \pm .444^{(f)^*}$	$36.430 \pm .349^{(f)^*}$	$7.883 \pm .032^{(e)^*}$
144PLU	$33.826 \pm .150^{\mathrm{IV}^*}$	$81.303 \pm .539^{\mathrm{IV}^*}$	$40.556 \pm .290^{\mathrm{III}^*}$	$23.510 \pm .351^{\mathrm{IV}^*}$	$5.563 \pm .060^{\mathrm{IV}^*}$
168COT	$38.843 \pm .147^{C^*}$	$168.12 \pm .83^{G^*}$	$106.68 \pm .246^{A^*}$	$34.176 \pm .238^{I^*}$	$4.263 \pm .08^{I^*}$
168RAD	$71.020 \pm .32^{(g)^*}$	$93.363 \pm .36^{(f)^*}$	$116.44 \pm .378^{(e)^*}$	$33.503 \pm .306^{(g)^*}$	$8.223 \pm .069^{(f)^*}$
168PLU	$30.813 \pm .364^{\mathrm{III}^*}$	$169.453 \pm .408^{\mathrm{V}^*}$	$38.073 \pm .066^{\mathrm{IV}^*}$	$21.156 \pm .128^{\mathrm{V}^*}$	$7.556 \pm .187^{\mathrm{V}^*}$

The factors of the study are represented as COT(Cotyledon), EMB (Embryonal axis), RAD (Radicle), PLU (Plumule), ±SE: Standard Error of the mean. ANOVA is used to study the effect of different characteristics, and Duncan's Multiple Range Test is used post-hoc. Small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals represent the significant differences. The observations within a column with the same inscriptions are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 15 Quantitative Estimations of Biomolecules During Germination

Imbibition (Hours)	Albumin mg g $^{-1}$ d. wt. ±SE	Globulin mg g^{-1}d. wt. ±SE	Prolamin mg g^{-1} d. wt. ±SE	Glutelin mgg^{-1}d. wt. ±SE		Total protein mg g^{-1}d. wt. ±SE
				NaOH 0.05M	NaOH 0.25M	
0COT	$127.44 \pm .36^{A^*}$	$6.793 \pm .034^{A^*}$	$18.66 \pm .172^{A^*}$	$14.71 \pm .101^{A^*}$	$11.673 \pm .141^{A^*}$	$179.28 \pm .264^{A^*}$
0EMB	166.30±.567	13.510±.135	36.673±.578	19.220±.095	13.630±.052	249.34±.255
12COT	$122.78 \pm .132^{B^*}$	$6.660 \pm .045^{A^*}$	$17.55 \pm .216^{B^*}$	$13.743 \pm .11^{B^*}$	$11.623 \pm .190^{A^*}$	$172.36 \pm .408^{B^*}$
12EMB	169.29±.195	13.853±.050	38.366±.301	16.876±.055	13.340±.034	251.73±.502
24COT	$120.88 \pm .115^{C^*}$	$6.300 \pm .023^{B^*}$	$13.41 \pm .229^{C^*}$	$13.26 \pm .032^{C^*}$	$10.550 \pm .040^{B^*}$	$164.40 \pm .317^{C^*}$
24 RAD	$168.01 \pm .153^{(a)^*}$	$14.936 \pm .150^{(a)^*}$	$35.910 \pm .503^{(a)^*}$	$14.593 \pm .189^{(a)^*}$	$13.936 \pm .024^{(a)^*}$	$247.37 \pm .316^{(a)^*}$
48COT	$113.03 \pm .476^{D^*}$	$4.970 \pm .101^{C^*}$	$11.030 \pm .196^{D^*}$	$10.206 \pm .26^{D^*}$	$10.036 \pm .144^{B^*}$	$149.27 \pm .822^{D^*}$
48RAD	$172.61 \pm .586^{(b)^*}$	$15.803 \pm +.334^{(b)^*}$	$32.056 \pm .263^{(b)^*}$	$12.810 \pm .091^{(b)^*}$	$13.720 \pm .128^{(a)^*}$	$247.01 \pm 1.21^{(a)^*}$
72COT	$108.71 \pm .334^{E^*}$	$3.36 \pm .052^{D^*}$	$8.753 \pm .239^{E^*}$	$6.55 \pm .132^{E^*}$	$10.113 \pm .023^{B^*}$	$137.49 \pm .345^{E^*}$
72RAD	$176.02 \pm .086^{(c)^*}$	$16.863 \pm .069^{(c)^*}$	$31.230 \pm .16^{(c)^*}$	$11.523 \pm .159^{(c)^*}$	$12.830 \pm .128^{(b)^*}$	$248.47 \pm .195^{(a)^*}$
72 PLU	$63.960 \pm .741^{\mathrm{I}^*}$	$8.643 \pm .218^{\mathrm{I}^*}$	$17.770 \pm .597^{\mathrm{I}^*}$	$9.650 \pm .132^{\mathrm{I}^*}$	$17.090 \pm .410^{\mathrm{I}^*}$	$117.11 \pm .303^{\mathrm{I}^*}$
96COT	$104.08 \pm .284^{F^*}$	$2.063 \pm .052^{E^*}$	$6.876 \pm .049^{F^*}$	$4.696 \pm .129^{F^*}$	$9.230 \pm .081^{C^*}$	$126.95 \pm .477^{F^*}$
96RAD	$171.73 \pm .528^{(b)^*}$	$18.043 \pm .269^{(d)^*}$	$28.663 \pm .314^{(d)^*}$	$9.646 \pm .12^{(d)^*}$	$11.926 \pm .12^{(b)^*}$	$240.01 \pm .591^{(b)^*}$
96PLU	$68.866 \pm .437^{\mathrm{II}^*}$	$10.326 \pm .373^{\mathrm{II}^*}$	$21.160 \pm .258^{\mathrm{II}^*}$	$12.533 \pm .208^{\mathrm{II}^*}$	$18.376 \pm .246^{\mathrm{I}^*}$	$131.26 \pm 1.084^{\mathrm{II}^*}$
120COT	$106.36 \pm .237^{G^*}$	$1.773 \pm .029^{F^*}$	$6.230 \pm .101^{G^*}$	$3.840 \pm .034^{G^*}$	$9.050 \pm .045^{C^*}$	$127.25 \pm .155^{F^*}$
120RAD	$169.23 \pm .648^{(a)^*}$	$15.993 \pm .092^{(e)^*}$	$31.213 \pm .294^{(c)^*}$	$8.456 \pm .067^{(e)^*}$	$11.110 \pm .064^{(c)^*}$	$236.00 \pm 1.01^{(c)^*}$
120PLU	$75.676 \pm .453^{\mathrm{III}^*}$	$16.160 \pm .418^{\mathrm{III}^*}$	$26.280 \pm .355^{\mathrm{III}^*}$	$13.896 \pm .112^{\mathrm{III}^*}$	$19.270 \pm .293^{\mathrm{I}^*}$	$151.28 \pm 1.232^{\mathrm{III}^*}$
144COT	$107.98 \pm .113^{E^*}$	$1.646 \pm .028^{F^*}$	$7.253 \pm .057^{F^*}$	$3.120 \pm .075^{H^*}$	$13.156 \pm .527^{D^*}$	$133.11 \pm .321^{G^*}$
144RAD	$165.06 \pm .537^{(d)^*}$	$14.883 \pm .11^{(a)^*}$	$28.766 \pm .473^{(d)^*}$	$7.476 \pm .226^{(f)^*}$	$10.793 \pm .170^{(c)^*}$	$226.98 \pm .94^{(d)^*}$
144PLU	$85.283 \pm .949^{\mathrm{IV}^*}$	$18.763 \pm .058^{\mathrm{IV}^*}$	$28.056 \pm .345^{\mathrm{IV}^*}$	$11.006 \pm .426^{\mathrm{IV}^*}$	$20.656 \pm .064^{\mathrm{II}^*}$	$163.77 \pm 1.641^{\mathrm{IV}^*}$
168COT	$109.01 \pm .095^{E^*}$	$1.160 \pm .032^{G^*}$	$6.870 \pm .045^{F^*}$	$2.673 \pm .076^{I^*}$	$14.190 \pm .080^{E^*}$	$133.90 \pm .149^{G^*}$
168RAD	$160.21 \pm 1.07^{(e)^*}$	$13.016 \pm .086^{(f)^*}$	$27.006 \pm .083^{(e)^*}$	$7.073 \pm .027^{(f)^*}$	$11.816 \pm .09^{(b)^*}$	$219.13 \pm 1.04^{(e)^*}$
168 PLU	$82.393 \pm .840^{\mathrm{V}^*}$	$19.346 \pm .193^{\mathrm{IV}^*}$	$29.103 \pm .084^{\mathrm{IV}^*}$	$9.833 \pm .120^{\mathrm{I}^*}$	$21.973 \pm .375^{\mathrm{III}^*}$	$162.65 \pm .758^{\mathrm{IV}^*}$

The factors of the study are represented as COT(Cotyledon), EMB (Embryonal axis), RAD (Radicle), PLU (Plumule), ±SE: Standard Error of the mean. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 16 Quantitative estimations of protein fractions during germination of *Hopea ponga* seeds

Amino Acid Content

The lowest amino acid content was observed at the onset of imbibition in *Hopea ponga* (across all cotyledons, embryonal axis, radicles/roots, and plumules), with levels rising in correlation with the duration of imbibition (Table: 15).

Estimation of Protein Fraction During Germination

According to Osborne's method of protein fractionation, the water extract represents the albumin fraction, the NaCl extract corresponds to the globulin fraction, the ethanol (80%) extract is indicative of the prolamin fraction, and the 0.05M and 0.25M NaOH extracts signify the glutelin fractions, respectively.

Albumin

Approximately 70% of the total protein in the dormant seed of *Hopea ponga* (at 0 hours of imbibition) was albumin. Upon imbibition, the albumin content in *H. ponga* (cotyledon) slightly decreased up to 120 hours, followed by an increase to 168 hours in the cotyledonary tissues. However, the albumin content in the embryonal axis/radicle rose until 72 hours of imbibition, after which it declined until 168 hours. In the plumule, there was a steady increase in albumin content from 72 hours to 168 hours of imbibition (Table: 16).

Globulin

In the cotyledons of *Hopea ponga*, the globulin content did not change significantly in the first 24 hours of imbibition but later exhibited a gradual decline until the final hours. The embryonal axis/radicle showed a slight increase in globulin during the first 96 hours of imbibition (from 13.510±0.135 to 18.043±0.269 mg/g dwt). However, between 96 and 168 hours, there was a slight decrease in globulin levels from 18.043±0.269 to 13.016±0.086 mg/g dwt. A notable increase in globulin content was observed in the plumule tissues of *H. ponga* from 72 to 168 hours of imbibition (Table: 16).

Prolamin

The prolamin content in the cotyledonary tissues of *Hopea ponga* showed a gradual decrease from 18.663±0.172 mg/g dwt to 6.230±0.101 mg/g dwt over 0 to 120 hours (Table: 16). Conversely, the embryonal axis/radicle/root tissues exhibited a steady decline in prolamin content from 12 to 96 hours. Post 120 hours of imbibition, the prolamin levels in both the cotyledon and radicle/root tissues of *H. ponga* nearly equalized. Meanwhile, the plumule experienced a consistent increase in prolamin content from the onset of differentiation at 72 hours to the final phase at 168 hours of imbibition.

Glutelin

0.05M NaOH Fraction

The concentration of the 0.05M NaOH-soluble glutelin fraction in the cotyledon of *Hopea ponga* steadily declined from 14.706±0.101 to 2.673±0.076 mg/g dry weight throughout 0 to 168 hours of imbibition (Table: 13). The same pattern was also noted in the embryonal axis, radicle, and root tissues during imbibition. Conversely, the plumule's 0.05M NaOH fraction showed a slight increase between 72 and 120 hours of imbibition before showing a small decrease (Table: 16).

0.25M NaOH Fraction

The 0.25M NaOH-soluble glutelin fraction showed little variation from 0 to 96 hours of imbibition in the cotyledons of *Hopea ponga*, with only a slight increase noted after 96 hours (Table: 16). In the embryonic axis/radicle, the glutelin levels remained nearly constant during the initial 24 hours of imbibition, followed by a decrease up to 168 hours. On the other hand, the glutelin content in the plumule showed an increase from 72 to 168 hours of imbibition.

Total Protein Content

The whole protein fractions are combined into the total protein which confirms that the highest protein content in cotyledon is 179.28±.264 mg g^{-1}d. wt.

251.73±.502 mg g^{-1}d. wt. is the highest content in the embryo, 248.47±.195 mg g^{-1}d. wt. in the radicle and 162.65±.758 mg g^{-1}d. wt. in plumule.

Amylase Activity

The α-amylase activity in the cotyledonary tissues of seeds showed a gradual increase from 1.830±0.005 to 4.583±0.026 mg maltose/15 min/g fwt. from 0 to 96 hours of imbibition (Graph: 10). Subsequently, a gradual decrease in activity was observed up to 168 hours of imbibition.

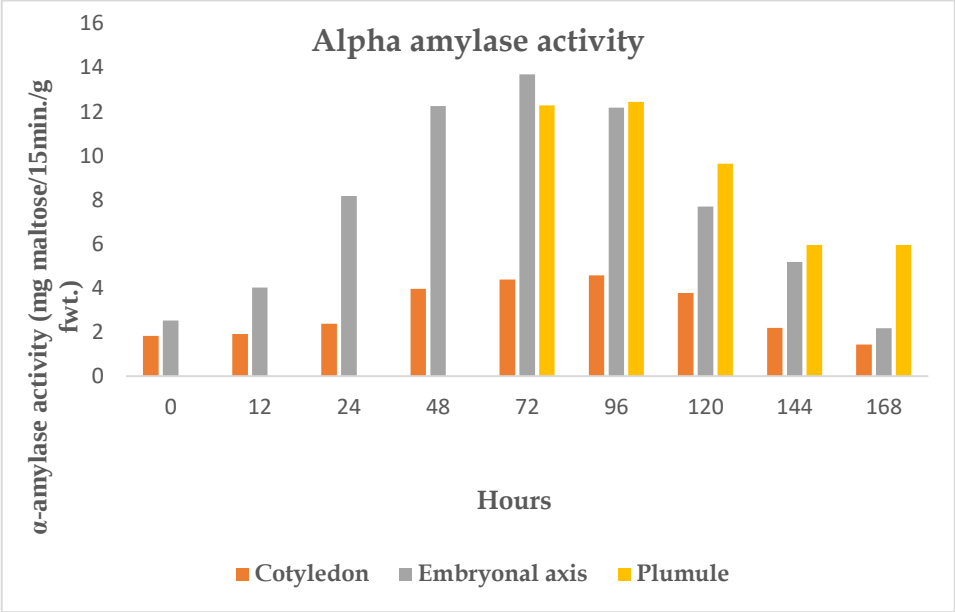
The activity of β-amylase in cotyledons showed a pattern akin to that of α-amylase. During the first 72 hours of imbibition, there was an increase in the activities of both α and β-amylase in the embryonic axis, radicle, and root tissues. Subsequently, there was a consistent decline until 144 hours of imbibition, followed by a sharp decrease at 168 hours in both the cotyledon and embryonal axis (Graph: 11).

In plumule tissues, the activities of both α and β amylase exhibited a slight increase from 72 to 96 hours of imbibition, followed by a notable decrease. The amylase activity, for both α and β, was comparatively higher in the embryonal axis/radicle/root than in the cotyledon for up to 72 hours, with the plumule showing high amylase activity.

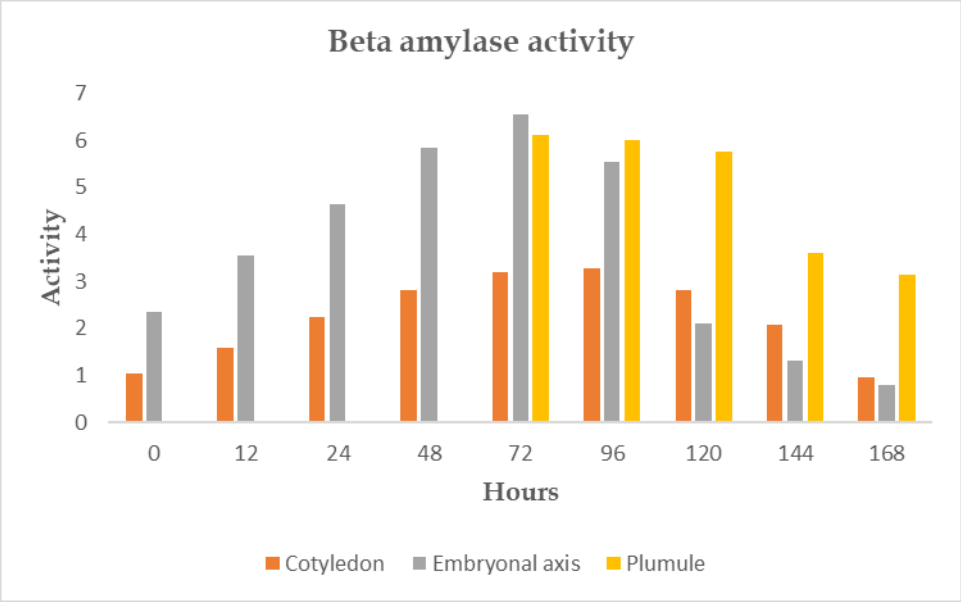
Protease Activity

The protease activity of cotyledons of seed gradually increased from 12.556±.072 to 17.150±.105 mg/g fwt. (from 0 to 96 hours of imbibition) and followed by a gradual decrease of up to 168 hours of imbibition. Similarly, protease activity in the embryonal axis/radicle/root showed an increasing pattern during the initial imbibitional period from 0 to 24 hours of imbibition and later a slight decline which was followed by an increase between 48 to 96 hours of imbibition. After that, a significant reduction was noticed between 96 to 168 hours of imbibition (17.150±.105 to 6.233±.089 mg/g fwt. in cotyledon). The maximum protease activity was recorded in the embryonal axis followed by radicle/roots. Plumule tissues of seeds also showed an

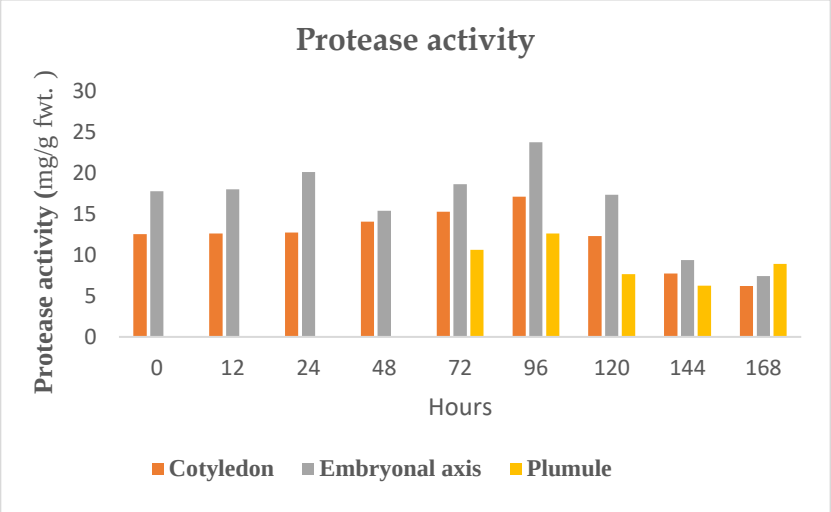
increasing pattern in protease activity from 72 hours to 96 hours of imbibition followed by a decrease up to 168 hours of imbibition (Graph: 12).



Graph: 10 α-amylase enzyme activity during germination



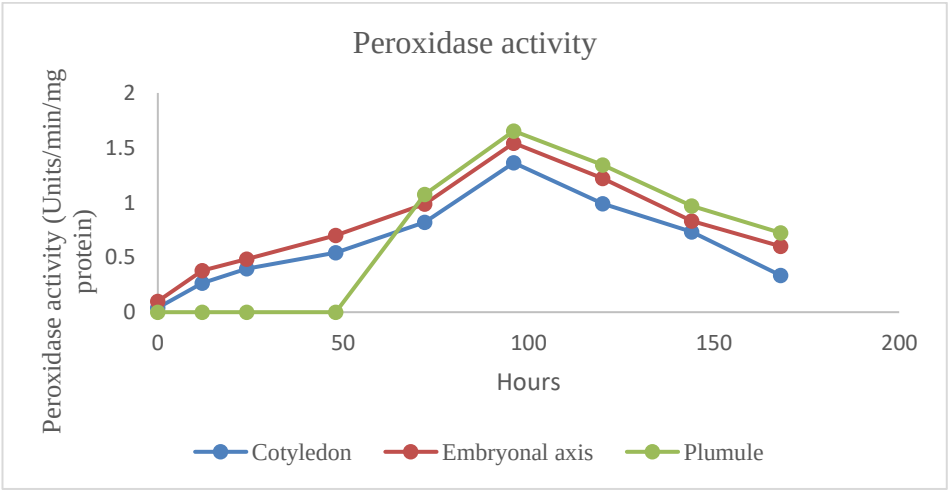
Graph: 11 β-amylase enzyme activity during germination



Graph: 12 Protease enzyme activity during germination

Peroxidase Activity

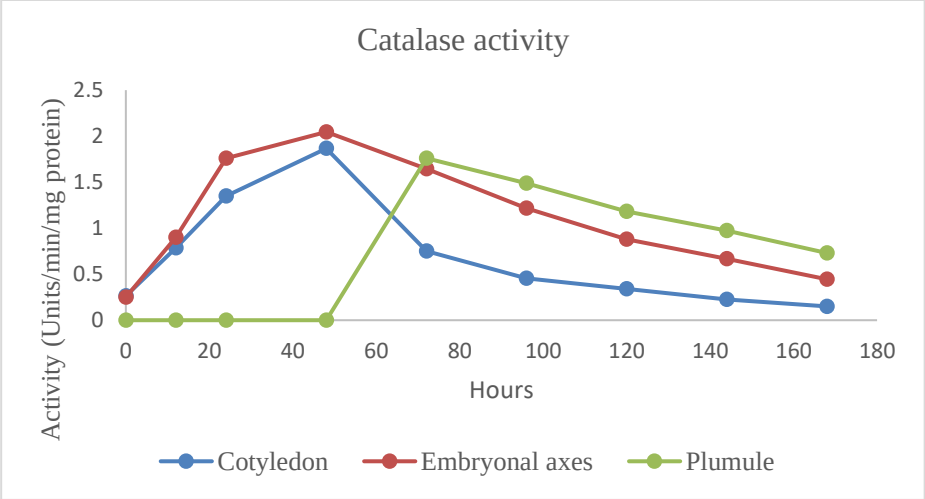
The peroxidase activity of cotyledons of seed gradually increased from 0.040±.005 to 1.363±.038 (from 0 to 96 hours of imbibition) followed by a gradual decrease of up to 168 hours of imbibition (Graph: 13). Similarly, peroxidase activity in the embryonal axis/radicle/root showed an increase during the initial imbibitional period from 24 to 96 hours of imbibition. Then a slight decline was followed by a decrease from 120 to 168 hours of imbibition. Plumule tissues of seeds also showed an increasing pattern in peroxidase activity from 72 hours to 96 hours of imbibition followed by a decrease up to 168 hours.



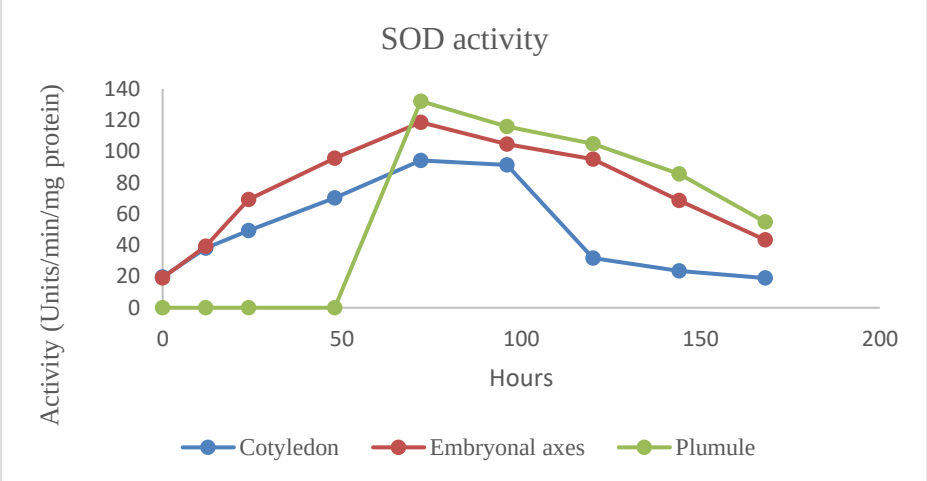
Graph: 13 Peroxidase activity during germination

Catalase Activity

The catalase activity of cotyledons of seed gradually increased from 0.263±.020 to 1.866±.044 (from 0 to 48 hours of imbibition) followed by a gradual decrease of up to 168 hours of imbibition (Graph: 14). Similarly, catalase activity in the embryonal axis/radicle/root showed an increase during the initial imbibitional period of 24 and 48 hours, and then a decline was followed by a decrease from 72 to 168 hours of imbibition. Plumule tissues of seeds showed a decreasing pattern in catalase activity from 72 hours to 168 hours of imbibition.



Graph: 14 Catalase activity during germination



Graph: 15 SOD activity during germination

Superoxide Dismutase (SOD) Activity

The SOD activity of cotyledons of seed gradually increased from 19.720±.549 to 94.240±.960 Units/min/mg protein (from 0 to 72 hours of imbibition) followed by a gradual decrease of up to 168 hours of imbibition (Graph: 15). Similarly, SOD activity in the embryonal axis/radicle/root showed an increase during the initial imbibitional period from 24 to 72 hours, and then a gradual decline was followed by a decrease from 96 to 168 hours of imbibition. Plumule tissues of seeds showed a decreasing pattern in SOD activity from 72 hours to 168 hours of imbibition.

Seed Storage Studies

Many tropical plant species generate recalcitrant seeds, characterized by high moisture content and rapid deterioration. The species under investigation in this study also produces recalcitrant seeds, which rapidly lose viability when subjected to ambient conditions. Consequently, this study aims to determine the optimal storage conditions for these seeds. Mature *Hopea ponga* seeds were found to have an initial moisture content of 45.37% and a germination rate of 98±1%. When stored at 0°C in sealed polycarbonate containers, the seeds retained their moisture content after 24 hours but were no longer viable. Storage at -80°C, -20°C, 5°C, and in liquid nitrogen (-196°C) also failed to yield any viable seeds (Table 17). The seeds even though maintained their moisture content, but no germination was recorded. At 10°C, seeds maintained their initial moisture content in closed polycarbonate bottles (PB) and polythene bags (PC), but no germination was recorded. Storage at 20±2°C and 40% RH (in closed PB), *Hopea ponga* seeds retained their initial moisture content for up to 7 days but their germination percentage showed a slight reduction of 5%. After 30 days of storage, the seeds were viable with 81.66% germination. During storage, the moisture content of the seeds slightly increased up to 180 days may be due to the respiration of the seeds, and thereafter the moisture content decreased. After 210 days of storage, the moisture content reduced to 32.38% and a parallel decrease in germination

scaled down to 25.33%. Radicle emergence was also observed during storage conditions. At the final stages of storage, the cotyledons became brownish green, and the embryonal axis tum pinkish green in color. After 240 days of storage, the seeds lost viability completely as evidenced by germination percentage. Hence, the maximum period of seed storage was recorded at 20±2°C and 40% RH in closed polycarbonate bottles for 210 days with 25.33% germination. During the initial period of storage, the moisture content of the seeds increased which may be due to the respiration of seeds inside the closed container. But seeds stored in open plastic trays under the same temperature condition showed viability for 14 days with 40% germination and the moisture content reduced to 33.02%. Seeds stored in closed polycarbonate bottles (PB) kept at laboratory conditions (28±2° C and 65% RH showed viability for 14 days with 27 % germination and the moisture content reduced to 28.6%. Seeds stored in a Polyethene bag (PC) and Open tray (OT) kept at laboratory conditions (28±2° C) show viability for 14 days and 7 days respectively (Table: 17).

Temperature Treatments	Storage Days	Moisture Content (%) ±SD	Germination Rate (%)	Findings
-80°C (in closed bottles and Aluminium foil)	1	45.436±.245	0	Seeds showing chilling sensitivity
	7	45.353±.350	0	
-20°C (in closed bottles and Aluminium foil)	1	45.233±.385	0	Seeds showing chilling sensitivity
	7	44.783±.755	0	
0°C (in closed bottles and Polyethene bags)	1	45.083±.177	0	Seeds showing chilling sensitivity
	7	45.406±.230	0	
5°C (in closed bottles and Polyethene bags)	1	44.236±.559	0	No response in germination
	7	42.690±.144	0	
10°C (in closed bottles and open trays)	1	44.863±.707	0	No response in germination
	7	41.420±.291	0	
	1	44.333±.295	97.666±.577	
	7	45.196±.051	95.666±.577	
	14	45.893± .047	84.000±1.000	
	30	46.193± .032	81.666±.577	

20±2°C (40% RH) In Closed bottle	60	41.720±.193	63.000±1.000	
	90	40.510±.357	58.333±1.154	
	120	38.383±.223	54.000±1.000	
	150	35.476±.488	50.333±.577	
	180	33.120±.034	42.000±2.000	
	210	32.386±.433	25.333±1.527	
	240	30.496±.609	0	
20±2°C (40% RH) In open tray	1	41.740±1.097	87.333±.577	
	7	36.076±.300	71.666±.527	
	14	33.020±.816	40.000±2.00	
	30	28.846±1.517	0	
Laboratory condition 28±2°C (65% RH) in polythene bag	1	39.963±.213	88.000±1.000	
	7	34.716±.374	68.333±1.527	
	14	30.483±.279	39.666±1.527	
	30	29.063±.418	0	
Laboratory condition 28±2°C (65% RH) In closed bottle	1	40.586±.195	88.00±1.000	
	7	34.336±.787	67.666±2.516	
	14	30.303±.094	38.333±1.527	
	30	27.603±.964	0	
Laboratory condition 28±2°C (65% RH) In open tray	1	38.140±.642	68.000±3.000	
	7	32.896±.624	36.333±5.686	
	14	25.826±.947	0	
-196°C (Liquid nitrogen)	1	45.636±.165	0	Seeds showing chilling sensitivity
	7	45.860±.055	0	

Table: 17 Effect of storage and different temperature treatments

*±SD- Standard deviation, RH- Relative humidity

Biochemical Aspects of Stored Seeds

Total Soluble Sugar Content (TSS)

Fresh seeds of *Hopea ponga* contain 37.4 mg/g dwt. of TSS in cotyledons and 51.4 mg/g dwt. in embryonal axes. In seeds stored at 0°C in PB and PG, the TSS content of cotyledon and embryonal axes remained the same as in control/fresh seeds. TSS in *H. ponga* seeds stored at 10°C, in closed polycarbonate bottles and polythene bags recorded a slight increase both in cotyledon and embryonal axes (after 7 days). Seeds stored in closed polycarbonate bottles at 20±2°C and 40% RH showed a linear increase of TSS content in cotyledon and embryonal axes till the end of the storage. Seeds stored in open plastic trays (at 20±2°C and 40% RH) showed an increase in TSS content for up to 2 weeks. TSS content significantly increased in seeds stored under laboratory conditions in closed polycarbonate bottles, closed

polythene bags, and open plastic trays at 28±2°C and 65 % RH. However, in seeds stored under 10°C, 5°C, -80°C, -20°C and -196°C, no significant changes were recorded in TSS content (Table: 18).

Starch Content

The fresh seeds of *Hopea ponga* contained 257.74 mg/g dwt. of starch in the cotyledon and 236.87 mg/g dwt. in the embryonal axis. After 24 hours of storage at different temperatures (0°C, 5°C, 10°C, -80°C, -20°C, -196°C, 20±2°C, and 28±2°C), the starch content did not change significantly from the initial levels. However, with the progression of the storage period, there was a significant decrease in the starch content in all storage conditions (Table: 19). At 20±2°C, the starch content of cotyledons in closed polycarbonate bottles (PB) was 223.618 mg/g dwt. after 240 days of storage. Similarly, the embryonal axis recorded a starch content of 209.391 mg/g dwt. after 240 days of storage. There was a noticeable reduction in starch content in the cotyledons of seeds stored in open plastic trays after 1 week, as compared to the embryonal axis at 20±2°C. A similar reduction in starch content was observed in all the seed lots under 28±2°C storage.

Phenol Content

The phenol content of fresh *Hopea ponga* seeds was found to be 151.0 mg/g dwt in the cotyledon and 186.0 mg/g dwt in the embryonal axis. There was no significant change in phenolic content after 24 hours of storage at various temperatures ranging from 0°C to -196°C. However, seeds stored at 20±2°C showed a linear increase in phenolic content over time. Additionally, significant increases in phenolic contents were also recorded in seeds stored in closed polythene bags and open plastic trays at room temperature. Overall, the phenolic content gradually increased in all stored seeds, with the rate of increase being higher in the embryonal axis than in cotyledons. However, the increase was minimal in seeds stored at 20±2°C and 40% RH in closed polycarbonate bottles (Table: 20).

Lipid Content

The lipid content remains unchanged after 24 hours of storage at -80, -20, 0, 5, 10, and -196°C. But at 20±2°C and 28±2°C in open trays, a rapid decrease is noted after 7 days of storage. A marginal reduction in lipid content was found in seeds stored at 20±2°C in closed polycarbonate bottles. The reduction rate in lipid content was higher at 28±2°C either in polycarbonate bottles or polythene bags (Table: 21).

Amino Acid Content

Fresh *Hopea ponga* seeds have 2.10 mg/g dwt. of amino acids in cotyledon and 4.35 mg/g dwt. in the embryonal axis. At 24 hours of storage in 0,10,5, -80, -20 and -196°C no significant variation in amino acid level was noticed in seeds. But in all the other storage conditions (20±2°C) the amino acid content showed a slight increase in both cotyledonary and embryonal axis tissues up to 4 months of storage followed by gradual reduction till the end of their storage period (Table: 22).

Protein Content

The protein content remains unchanged after 24 hours of storage at -80, -20, 0, 5, 10, and -196°C. But at 20±2°C and 28±2°C in open trays, a rapid decrease is noted after 7 days of storage. A marginal reduction in protein content was found in seeds stored at 20±2°C in closed polycarbonate bottles. The reduction rate in protein content was higher at 28±2°C either in polycarbonate bottles or polythene bags (Table: 23).

LEA proteins were absent up to 44 DAA and found in the subsequent periods of maturity (60 DAA to 80 DAA). It remained stable after 30 to 210 days of postharvest storage. On the 240th storage day, the expression of the LEA ceased, and seeds became non-viable. During seed storage studies the moisture content of the seed is reduced with the progress of the storage. The presence of the LEA protein or intensity of the bands shown in the zymogram

(Fig. 17) is reduced when moving from 30 days of storage to 240 days of storage. On the 240th day, the intensity of the band is completely diminished.

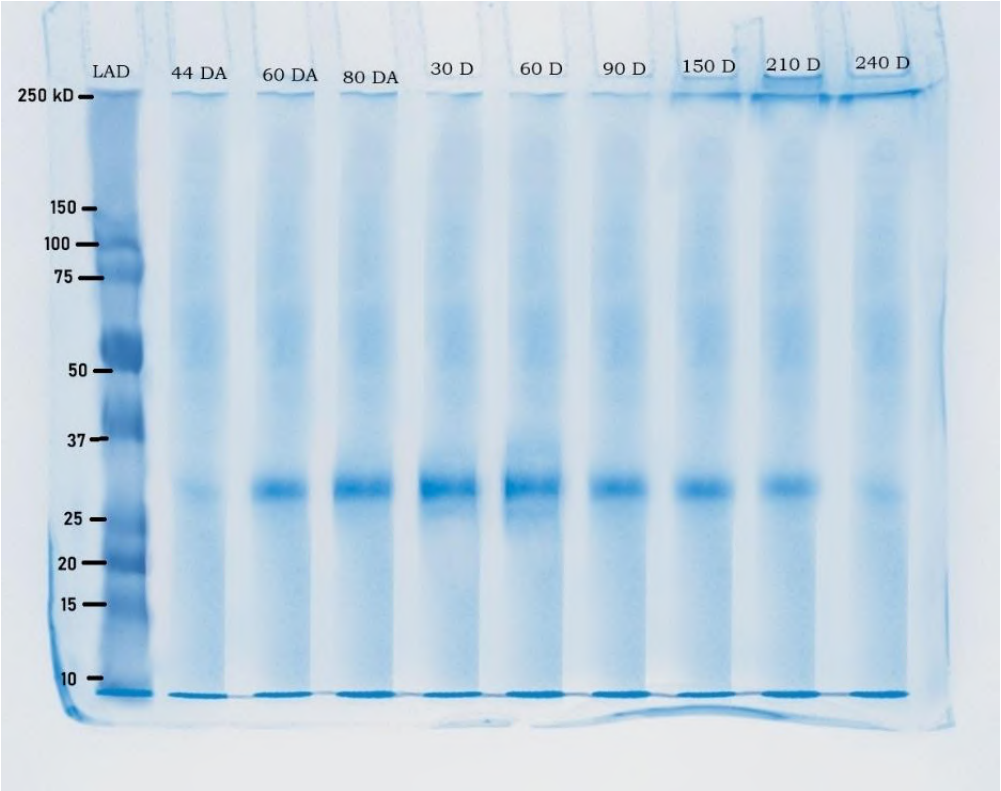


Fig. 17 Zymogram of LEA Protein Expression

Lipid Peroxidation

The lipid peroxidation rate in fresh *Hopea ponga* seed is 0.78 mg/g fwt. in cotyledon and 0.92 mg/g fwt. in embryonal axes. Seeds stored at 0, -80, -20, 5, 10, and -196 °C did not show any variation in the rate of lipid peroxidation after 24 hours of storage. However, in all the other storage conditions, lipid peroxidation showed a linear increase from a minimum during the initial to a maximum at the end of the storage period (Table: 24). However, maximum lipid peroxidation was noticed in seeds stored in open plastic trays both at 20±2°C and 28±2°C.

Treatments			Storage Periods (Days)										
			1	7	14	30	60	90	120	150	180	210	240
-80 °C	PB	COT	37.570 ±1.074										
		EMB	51.023 ±2.202										
	AF	COT	35.970 ±1.227										
		EMB	49.930 ±2.276										
-20 °C	PB	COT	35.793 ±0.912										
		EMB	51.393 ±0.445										
	AF	COT	37.553 ±0.612										
		EMB	51.273 ±0.406										
0 °C	PB	COT	35.600 ±1.132										
		EMB	51.216 ±0.760										
	PC	COT	36.346 ±0.965										
		EMB	53.030 ±0.795										
5°C	PB	COT	37.053 ±0.855										
		EMB	51.960 ±1.040										
	PC	COT	36.533 ± 0.595										
		EMB	53.220 ±0.430										
10°C	PB	COT	36.713 ±0.315	38.320 ±1.120									
		EMB	52.583 ±1.133	54.183 ±0.961									

	PC	COT	36.803 ±0.748	39.480 ±0.599									
		EMB	53.410 ±0.707	55.523 ±0.592									
20±2°C (40% RH)	PB	COT	37.266 ± .267$^{a^*}$	41.666 ± .976$^{b^*}$	43.710 ± 1.023$^{c^*}$	47.156 ± .990$^{d^*}$	49.030 ± .64$^{e^*}$	51.256 ± .153$^{f^*}$	53.160 ± .94$^{g^*}$	56.290 ± .56$^{h^*}$	58.096 ± .412$^{i^*}$	60.896 ± .74$^{j^*}$	62.926 ± .548$^{k^*}$
		EMB	51.170 ± .38$^{A^*}$	53.430 ± .614$^{B^*}$	55.516 ± .69$^{C^*}$	57.593 ± .725$^{D^*}$	59.606 ± .50$^{E^*}$	61.076 ± .654$^{F^*}$	63.806 ± .545$^{G^*}$	66.080 ± .549$^{H^*}$	68.556 ± .73$^{I^*}$	72.090 ± .63$^{J^*}$	74.776 ± 1.38$^{K^*}$
	OT	COT	36.110 ± .55$^{a^*}$	37.943 ± .23$^{a^*}$	39.926 ± .34$^{b^*}$	42.250 ± .81$^{c^*}$							
		EMB	50.956 ± .66$^{A^*}$	53.556 ± 1.01$^{B^*}$	55.683 ± .57$^{C^*}$	59.036 ± .67$^{D^*}$							
Laboratory condition 28±2°C (65% RH)	PB	COT	36.986 ± .71$^{a^*}$	40.063 ± .37$^{b^*}$	41.990 ± .49$^{b^*}$	46.277 ± .86$^{c^*}$							
		EMB	51.310 ± .49$^{A^*}$	55.090 ± .29$^{B^*}$	57.636 ± .40$^{C^*}$	58.713 ± .61$^{C^*}$							
	PC	COT	36.283 ± .44$^{a^*}$	38.973 ± .42$^{b^*}$	41.983 ± .67$^{c^*}$	44.633 ± .55$^{d^*}$							
		EMB	51.326 ± .36$^{A^*}$	53.043 ± .11$^{B^*}$	54.670 ± .46$^{C^*}$	57.366 ± 1.21$^{D^*}$							
	OT	COT	36.056 ± .33$^{a^*}$	38.943 ± .23$^{b^*}$	40.486 ± .516$^{c^*}$								
		EMB	51.450 ± .44$^{A^*}$	54.146 ± 1.05$^{B^*}$	55.423 ± .61$^{C^*}$								
-196 °C	CV	COT	36.080 ±.969										
		EMB	51.303 ±1.939										

COT- Cotyledon, EMB- Embryonal axis, AF- Aluminium Foil, PB- Plastic bottle, PC- Polythene cover, OT- Open tray, CV- Cryo vial. ANOVA is used to study the effect of different characteristics, and Duncan's Multiple Range Test is used post-hoc. Small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals represent the significant differences. The observations within a column with the same inscriptions are not significantly distinct. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01.

Table: 18 Total Soluble Sugar content in various storage conditions

Treatments			Storage Periods (Days)										
			1	7	14	30	60	90	120	150	180	210	240
-80°C	PB	COT	257.632 ±2.867										
		EMB	235.471 ±.657										
	AF	COT	254.390 ±1.308										
		EMB	235.672 ±1.032										
-20°C	PB	COT	257.34 ±2.504										
		EMB	237.257 ±.775										
	AF	COT	258.983 ±2.364										
		EMB	236.594 ±1.945										
0°C	PB	COT	258.761 ±2.325										
		EMB	235.908 ±2.115										
	PC	COT	259.133 ±1.436										
		EMB	237.012 ±1.112										
5°C	PB	COT	257.45 ±.860										
		EMB	236.973 ±1.667										
	PC	COT	256.308 ±2.443										
		EMB	236.045 ±1.316										
10°C	PB	COT	255.226 ±1.88072	251.663 ±.76501									
		EMB	235.405	233.396									

			±1.337	±.810									
	PC	COT	254.613 ±2.67877	252.235 ±1.64710									
		EMB	236.231 ±1.166	234.807 ±1.480									
20±2°C (40% RH)	PB	COT	254.743 $\pm 1.57^{a^*}$	250.274 $\pm .56^{b^*}$	245.724 $\pm 2.14^{c^*}$	245.216 $\pm .65^{d^*}$	241.886 $\pm .44^{e^*}$	237.175 $\pm .54^{f^*}$	233.816 $\pm .475^{g^*}$	231.188 $\pm .82^{h^*}$	228.249 $\pm .57^{i^*}$	226.014 $\pm .52^{j^*}$	223.618 $\pm 1.12^{k^*}$
		EMB	236.018 $\pm .42^{A^*}$	234.896 $\pm .72^{B^*}$	233.189 $\pm .59^{B^*}$	230.523 $\pm .93^{C^*}$	228.247 $\pm .27^{D^*}$	225.712 $\pm .92^{E^*}$	223.675 $\pm 1.12^{F^*}$	220.167 $\pm .72^{G^*}$	217.024 $\pm 1.82^{H^*}$	213.610 $\pm 1.04^{I^*}$	209.391 $\pm 1.28^{J^*}$
	OT	COT	256.022 $\pm .81^{a^*}$	250.567 $\pm .83^{b^*}$	240.886 $\pm 2.17^{c^*}$	232.052 $\pm 1.07^{d^*}$							
		EMB	236.115 $\pm .51^{A^*}$	230.376 $\pm .78^{B^*}$	226.768 $\pm 1.02^{C^*}$	217.787 $\pm 1.47^{D^*}$							
Laboratory condition 28±2°C (65% RH)	PB	COT	255.378 $\pm 1.18^{a^*}$	249.142 $\pm 1.40^{b^*}$	239.827 $\pm .51^{c^*}$	234.609 $\pm 1.75^{d^*}$							
		EMB	235.289 $\pm 1.22^{A^*}$	228.140 $\pm 3.14^{B^*}$	222.946 $\pm 2.27^{C^*}$	212.627 $\pm 2.30^{D^*}$							
	PC	COT	255.305 $\pm 1.34^{a^*}$	247.717 $\pm 2.64^{b^*}$	236.891 $\pm 1.44^{c^*}$	231.70 $\pm 1.01^{d^*}$							
		EMB	235.500 $\pm 1.17^{A^*}$	229.749 $\pm .88^{B^*}$	221.863 $\pm 1.92^{C^*}$	214.896 $\pm 1.51^{D^*}$							
	OT	COT	256.153 $\pm .49^{a^*}$	240.462 $\pm 1.002^{b^*}$	218.735 $\pm 2.13^{c^*}$								
		EMB	235.467 $\pm .86^{A^*}$	225.771 $\pm .74^{B^*}$	214.603 $\pm .800^{C^*}$								
-196 °C	CV	COT	257.263 ±2.353										
		EMB	239.316 ±1.125										

COT- Cotyledon, EMB- Embryonal axis, AF- Aluminium Foil, PB- Plastic bottle, PC- Polythene cover, OT- Open tray, CV- Cryo vial. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. Small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals represent the significant differences. The observations within a column with the same inscripts are not significantly distinct. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 19 Starch content in various storage conditions

Treatments			Storage Periods (Days)										
			1	7	14	30	60	90	120	150	180	210	240
-80 °C	PB	COT	151.220 ±.580										
		EMB	186.657 ±.93516										
	AF	COT	152.100 ±1.597										
		EMB	185.738 ±.295										
-20 °C	PB	COT	152.773 ±.996										
		EMB	186.231 ±.332										
	AF	COT	152.001 ±1.289										
		EMB	186.378 ±.410										
0 °C	PB	COT	152.521 ±.771										
		EMB	186.306 ±.570										
	PC	COT	153.187 ±.549										
		EMB	181.809 ±.880										
5°C	PB	COT	152.495 ±.95922										
		EMB	182.217 ±.604										
	PC	COT	152.424 ±.30746										
		EMB	181.759 ±.899										
10°C	PB	COT	152.163 ±.95631	159.267 ±.44298									
		EMB	185.378	192.132									

			±1.75586	±.93376									
	PC	COT	152.558 ±1.104	159.796 ±.795									
		EMB	186.126 ±.742	194.849 ±1.513									
20±2°C (40% RH)	PB	COT	151.95 ± .46$^{a^*}$	157.668 ± 1.18$^{b^*}$	168.243 ± 1.06$^{c^*}$	184.751 ± 1.21$^{d^*}$	190.867 ± .63$^{e^*}$	198.419 ± 1.21$^{f^*}$	206.627 ± .88$^{g^*}$	214.558 ± 2.34$^{h^*}$	248.514 ± 1.51$^{i^*}$	244.765 ± .63$^{j^*}$	252.929 ± 1.31$^{k^*}$
		EMB	185.853 ± .85$^{A^*}$	188.754 ± .27$^{B^*}$	194.318 ± 1.305$^{C^*}$	206.615 ± 2.041$^{D^*}$	217.78 ± 2.61$^{E^*}$	227.47 ± 1.01$^{F^*}$	234.092 ± 2.292$^{G^*}$	264.503 ± 3.358$^{H^*}$	292.649 ± 2.166$^{I^*}$	320.847 ± 1.477$^{J^*}$	353.800 ± .94$^{K^*}$
	OT	COT	152.803 ± 1.115$^{a^*}$	157.587 ± 1.064$^{b^*}$	187.958 ± 2.081$^{c^*}$	237.415 ± .734$^{d^*}$							
		EMB	184.984 ± .711$^{A^*}$	191.726 ± .786$^{B^*}$	253.735 ± 1.25$^{C^*}$	304.753 ± 2.402$^{D^*}$							
Laboratory condition 28±2°C (65% RH)	PB	COT	153.178 ± 1.238$^{a^*}$	165.908 ± 2.361$^{b^*}$	207.320 ± 3.946$^{c^*}$	280.58 ± 3.572$^{d^*}$							
		EMB	186.013 ± .740$^{A^*}$	206.821 ± 3.816$^{B^*}$	273.450 ± 4.876$^{C^*}$	300.504 ± 3.389$^{D^*}$							
	PC	COT	151.723 ± .79563$^{a^*}$	172.106 ± 1.575$^{b^*}$	207.653 ± 5.152$^{c^*}$	290.648 ± 4.373$^{d^*}$							
		EMB	186.114 ± .396$^{A^*}$	205.068 ± 3.035$^{B^*}$	259.729 ± 5.504$^{C^*}$	305.532 ± 7.173$^{D^*}$							
	OT	COT	152.421 ± .71393$^{a^*}$	174.284 ± 3.994$^{b^*}$	206.113 ± 5.565$^{c^*}$								
		EMB	186.257 ± .452$^{A^*}$	202.793 ± .920$^{B^*}$	246.695 ± 5.587$^{C^*}$								
-196 °C	CV	COT	151.069 ±.674										
		EMB	186.588 ±.758										

COT- Cotyledon, EMB- Embryonal axis, AF- Aluminium Foil, PB- Plastic bottle, PC- Polythene cover, OT- Open tray, CV- Cryo vial. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 20 Phenol content in various storage conditions

Treatments			Storage Periods (Days)										
			1	7	14	30	60	90	120	150	180	210	240
-80 °C	PB	COT	87.883 ±1.840										
		EMB	58.616 ±.735										
	AF	COT	87.970 ±1.197										
		EMB	57.880 ±.694										
-20 °C	PB	COT	88.710 ±1.006										
		EMB	58.130 ±.212										
	AF	COT	88.943 ±.679										
		EMB	57.740 ±.599										
0 °C	PB	COT	88.410 ±1.035										
		EMB	58.130 ±.212										
	PC	COT	86.306 ±1.002										
		EMB	57.613 ±1.617										
5°C	PB	COT	87.743 ±2.192										
		EMB	56.443 ±.819										
	PC	COT	87.256 ±1.045										
		EMB	58.090 ±.867										
10°C	PB	COT	88.403 ±.902	89.046 ±.813									
		EMB	58.480	59.483									

			±.963	±.565									
	PC	COT	87.496 ±2.409	88.500 ±1.756									
		EMB	58.493 ±.836	58.983 ±.660									
20±2°C (40% RH)	PB	COT	88.353 ± .777$^{a^*}$	91.046 ± .751$^{b^*}$	92.306 ± .84$^{c^*}$	94.216 ± .160$^{d^*}$	95.950 ± .412$^{e^*}$	97.566 ± .897$^{f^*}$	98.430 ± .096$^{g^*}$	96.376 ± 1.024$^{h^*}$	92.603 ± .563$^{i^*}$	87.440 ± 1.64$^{j^*}$	79.780 ± .736$^{k^*}$
		EMB	57.906 ± 1.207$^{A^*}$	58.906 ± 1.099$^{B^*}$	62.196 ± .934$^{C^*}$	63.943 ± 1.657$^{D^*}$	65.623 ± .2$^{E^*}$	66.306 ± .26$^{F^*}$	67.010 ± .43$^{G^*}$	61.48 ± 1.04$^{H^*}$	57.72 ± 1.04$^{I^*}$	53.196 ± .228$^{J^*}$	47.443 ± 1.231$^{K^*}$
	OT	COT	88.896 ± .335$^{a^*}$	84.256 ± 1.242$^{b^*}$	72.926 ± 2.623$^{c^*}$	58.616 ± 2.075$^{d^*}$							
		EMB	58.773 ± .295$^{A^*}$	54.566 ± .995$^{B^*}$	44.130 ± 3.826$^{C^*}$	37.166 ± 1.876$^{D^*}$							
Laboratory condition 28±2°C (65% RH)	PB	COT	87.706 ± .400$^{a^*}$	69.160 ± .523$^{b^*}$	51.210 ± 1.943$^{c^*}$	35.986 ± 4.806$^{d^*}$							
		EMB	58.400 ± .883$^{A^*}$	51.603 ± 1.828$^{B^*}$	45.976 ± 1.557$^{C^*}$	28.833 ± .853$^{D^*}$							
	PC	COT	87.786 ± .621$^{a^*}$	69.716 ± 1.582$^{b^*}$	51.943 ± 1.822$^{c^*}$	32.230 ± 1.722$^{d^*}$							
		EMB	57.233 ± .806$^{A^*}$	53.590 ± .486$^{B^*}$	47.740 ± 1.542$^{C^*}$	26.166 ± 2.769$^{D^*}$							
	OT	COT	87.963 ± .527$^{a^*}$	71.713 ± 1.351$^{b^*}$	37.410 ± 2.585$^{c^*}$								
		EMB	57.186 ± 1.05$^{A^*}$	49.353 ± 1.82$^{B^*}$	21.270 ± 1.635$^{C^*}$								
-196 °C	CV	COT	88.010 ±1.433										
		EMB	88.010 ±1.433										

COT- Cotyledon, EMB- Embryonal axis, AF- Aluminium Foil, PB- Plastic bottle, PC- Polythene cover, OT- Open tray, CV- Cryo vial. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 21 Lipid content in various storage conditions

Treatments			Storage Periods (Days)										
			1	7	14	30	60	90	120	150	180	210	240
-80 °C	PB	COT	2.173 ±.104										
		EMB	4.326 ±.117										
	AF	COT	2.160 ±.030										
		EMB	4.216 ±.037										
-20 °C	PB	COT	2.130 ±.036										
		EMB	4.296 ±.083										
	AF	COT	2.276 ±.168										
		EMB	4.203 ±.040										
0 °C	PB	COT	2.196 ±.040										
		EMB	4.283 ±.075										
	PC	COT	2.303 ±.060										
		EMB	4.296 ±.105										
5°C	PB	COT	2.283 ±.086										
		EMB	4.260 ±.052										
	PC	COT	2.283 ±.092										
		EMB	4.283 ±.025										
10°C	PB	COT	2.340 ±.052	2.436 ±.020									
		EMB	4.293	4.370									

			±.015	±.010									
	PC	COT	2.363 ±.025	2.443 ±.041									
		EMB	4.296 ±.032	4.363 ±.015									
20±2°C (40% RH)	PB	COT	2.093 ± .065$^{a^*}$	2.190 ± .010$^{b^*}$	2.346$^{c^*}$ ±.085	2.610 ± .043$^{d^*}$	2.663 ± .028$^{e^*}$	2.783 ± .02$^{f^*}$	2.886 ± .015$^{g^*}$	2.493 ± .136$^{h^*}$	2.183 ± .015$^{i^*}$	1.873 ± .03$^{j^*}$	1.660 ± .036$^{k^*}$
		EMB	4.350 ± .06$^{A^*}$	4.896 ± .04$^{A^*}$	5.103$^{B^*}$ ±.08	5.300 ± .045$^{C^*}$	5.930 ± .04$^{D^*}$	6.156 ± .07$^{D^*}$	6.390 ± .04$^{D^*}$	5.016 ± .04^{B*}	4.363 ± .105$^{A^*}$	4.026 ± .08$^{E^*}$	3.493 ± .136$^{F^*}$
	OT	COT	2.776 ± .1$^{a^*}$	2.446 ± .185$^{b^*}$	1.950$^{c^*}$ ±.055	1.493 ± .126$^{d^*}$							
		EMB	4.440 ± .05$^{A^*}$	3.736 ± .105$^{B^*}$	2.530$^{C^*}$ ±.10	1.530 ± .055$^{D^*}$							
Laboratory condition 28±2°C (65% RH)	PB	COT	2.343 ± .094$^{a^*}$	2.896 ± .025$^{b^*}$	2.276$^{c^*}$ ±.080	1.916 ± .056$^{d^*}$							
		EMB	4.226 ± .03$^{A^*}$	4.820 ± .12$^{B^*}$	3.060$^{C^*}$ ±.07	2.136 ± .25$^{D^*}$							
	PC	COT	2.366 ± .083$^{a^*}$	2.920 ± .026$^{b^*}$	2.320$^{c^*}$ ±.108	1.846 ± .02$^{d^*}$							
		EMB	4.380 ± .07$^{A^*}$	4.790 ± .124$^{B^*}$	3.170$^{C^*}$ ±.026	2.313 ± .085$^{D^*}$							
	OT	COT	2.3867 ± .021$^{a^*}$	2.5833 ± .134$^{b^*}$	1.8633 ± .065$^{c^*}$								
		EMB	4.443 ± .14$^{A^*}$	4.640 ± .09$^{B^*}$	2.843$^{C^*}$ ±.115								
-196 °C	CV	COT	2.220 ±.147										
		EMB	4.293 ±.136										

COT- Cotyledon, EMB- Embryonal axis, AF- Aluminium Foil, PB- Plastic bottle, PC- Polythene cover, OT- Open tray CV- Cryo vial. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 22 Amino acid content in various storage conditions

Treatments			Periods (Interval)	Albumin (mg g dwt.) $^{-1}$	Globulin (mg g dwt.) $^{-1}$	Prolamin (mg g dwt.) $^{-1}$	Glutelin (mg g dwt.) $^{-1}$		Total Proteins (mg g dwt.) $^{-1}$
							0.05M NaOH	0.25M NaOH	
-80°C	PB	COT	1st Day	127.118±1.547	6.896±.025	19.913±.716	14.650±.238	11.356±.077	179.925±1.851
		EMB		165.906±.157	13.333±.581	35.166±.591	19.103±.990	13.870±.393	247.377±.368
	AF	COT	1st Day	127.842±1.418	6.703±.151	19.406±.587	14.790±.252	11.533±.557	180.271±1.410
		EMB		166.393±.909	13.076±.489	35.570±.905	19.180±.540	14.480±.73137	248.701±1.120
-20°C	PB	COT	1st Day	127.314±1.136	6.316±.100	18.580±.232	14.133±.161	10.553±.512	176.895±1.495
		EMB		161.208±.751	12.256±.176	34.420±.695	18.803±.205	14.320±.190	241.009±.871
	AF	COT	1st Day	25.254±.584	6.170±.036	18.143±.076	14.046±.466	10.273±.040	173.897±.926
		EMB		159.225±.84388	12.056±.037	32.920±.317	13.793±.243	14.243±.061	232.248±.795
0°C	PB	COT	1st Day	126.319±.556	6.673±.201	19.243±.623	14.560±.270	11.203±.032	177.991±.333
		EMB		166.509±.496	12.613±.073	33.863±.189	18.570±.211	13.250±.244	244.891±.032
	PC	COT	1st Day	124.598±1.598	6.450±.052	18.520±.425	14.176±.041	11.090±.036	174.839±1.880
		EMB		162.997±1.397	12.150±.125	30.576±.445	18.210±.087	13.090±.045	237.024±1.528
5°C	PB	COT	1st Day	126.449±.531	6.786±.075	18.653±.363	14.583±.083	11.516±.015	177.984±.47173
		EMB		166.053±.080	13.680±.147	37.310±.330	19.310±.294	13.626±.083	249.962±.510
	PC	COT	1st Day	125.267±.488	6.796±.041	18.140±.088	14.433±.235	11.266±.061	175.906±.45826
		EMB		164.349±1.589	13.213±.098	36.080±.578	19.086±.140	13.136±.140	245.853±1.692
10°C	PB	COT	1st Day	126.604±.852	6.723±.115	18.340±.257	14.303±.244	11.100±.193	177.073±.960
		EMB		166.4681±.22859	13.686±.132	36.803±.613	19.320±.115	13.236±.951	249.518±1.238
		COT	7th Day	124.670±.924	5.820±.020	17.286±.207	13.143±.065	9.086±.070	170.018±.84500
		EMB		159.410±.72831	12.596±.208	33.120±1.898	17.573±.040	12.560±.494	235.267±1.928
	PC	COT	1st Day	126.386±1.287	6.763±.055	18.206±.030	14.423±.268	11.166±.075	176.949±1.634
		EMB		166.347±.611	13.573±.147	36.600±.633	19.436±.200	13.023±.220	248.988±1.514
		COT	7th Day	123.923±.176	5.870±.095	17.020±.085	12.856±.125	9.140±.045	168.809±.273
		EMB		159.748±.885	12.333±.061	31.043±.596	17.033±.085	11.950±.113	232.108±.9254
20±2°C (40% RH)	PB	COT	1st Day	126.668±.897 ^{A*}	6.750±.062 ^{A*}	18.726±.530 ^{A*}	14.623±.268 ^{A*}	11.263±.066 ^{A*}	178.035±1.626 ^{A*}
		EMB		166.564±.625 ^{a*}	13.736±.081 ^{a*}	36.513±.706 ^{b*}	19.590±.125 ^{a*}	14.343±.497 ^{a*}	250.749±.548 ^{a*}
		COT	7th Day	125.007±.196 ^{B*}	6.656±.040 ^{B*}	18.563±.397 ^{AB*}	14.446±.215 ^{AB*}	10.530±.176 ^{B*}	175.209±.766 ^{B*}
		EMB		164.797±.861 ^{b*}	12.806±.146 ^{b*}	34.866±.145 ^{b*}	18.246±.115 ^{b*}	13.466±.300 ^{b*}	244.156±.740 ^{b*}
		COT	14th Day	124.687±.841 ^{C*}	6.553±.040 ^{C*}	18.160±.036 ^{C*}	14.103±.058 ^{C*}	9.826±.035 ^{C*}	173.322±.761 ^{C*}
		EMB		164.659±.098 ^{b*}	12.796±.045 ^{c*}	33.483±.647 ^{c*}	18.043±.051 ^{c*}	12.920±.045 ^{c*}	241.906±.731 ^{c*}
		COT	30th Day	121.324±.673 ^{D*}	6.450±.030 ^{D*}	18.036±.030 ^{D*}	13.996±.032 ^{D*}	9.496±.056 ^{D*}	169.301±.724 ^{D*}
		EMB		162.060±.096 ^{c*}	12.686±.040 ^{d*}	33.143±.406 ^{d*}	17.900±.040 ^{d*}	12.550±.055 ^{d*}	238.349±.450

		COT	60th Day	117.578±.682 E*	6.196±.056 E*	17.720±.212 E*	13.813±.117 E*	9.156±.092 E*	164.466±.798 E*
		EMB		158.235±.661 d*	12.130±.081 e*	31.493±.506 e*	17.293±.140 e*	12.103±.049 e*	231.256±.474 e*
		COT	90th Day	113.443±.427 F*	6.026±.015 F*	14.526±.183 F*	12.190±.652 F*	9.006±.094 F*	155.198±.805 F*
		EMB		152.855±.961 e*	11.613±.080 f*	29.630±.546 f*	16.210±.081 f*	8.623±.112 f*	218.934±.459 f*
		COT	120th Day	111.868±.647 G*	5.840±.105 G*	13.750±.091 G*	11.056±.025 G*	8.696±.145 G*	151.207±.457 G*
		EMB		147.681±.966 f*	11.046±.035 g*	27.753±.870 g*	15.986±.040 g*	8.233±.040 g*	210.709±1.918 g*
		COT	150th Day	107.531±1.195 H*	5.540±.124 H*	13.156±.109 H*	10.603±.155 H*	8.103±.075 H*	144.939±1.617 H*
		EMB		144.327±.559 g*	10.753±.075 h*	26.536±.070 h*	13.146±.123 h*	8.043±.051 h*	202.805±.525 h*
		COT	180th Day	106.078±.687 I*	5.176±.040 I*	12.690±.134 I*	10.183±.045 I*	7.743±.060 I*	141.860±.687 I*
		EMB		139.648±1.364 h*	10.453±.066 i*	26.103±.025 i*	10.450±.240 i*	7.860±.062 i*	194.516±1.293 i*
		COT	210th Day	101.885±.659 J*	5.040±.052 J*	12.076±.041 J*	8.903±.834 J*	7.116±.080 J*	135.029±.874 J*
		EMB		135.477±1.123 i*	10.076±.056 j*	25.600±.325 j*	9.363±.211 j*	7.570±.055 j*	188.089±.992 j*
		COT	240th Day	96.906±1.455 K*	4.726±.092 K*	11.503±.290 K*	6.886±.416 K*	6.603±.498 K*	126.632±.567 K*
		EMB		130.989±.482 j*	9.666±.102 k*	23.456±.452 k*	7.5567±.274 k*	6.856±.073 k*	178.524±.213 k*
	OT	COT	1st Day	126.469±.729 A*	6.920±.043 A*	19.736±.884 A*	14.510±.151 A*	11.343±.056 A*	178.977±.072 A*
		EMB		166.390±.889 a*	13.463±.629 a*	35.093±.525 a*	19.453±.426 a*	14.670±.153 a*	249.065±1.279
		COT	7th Day	117.197±.624 B*	6.510±.026 B*	17.770±.325 B*	14.086±.020 B*	11.056±.037 B*	166.619±.564 B*
		EMB		158.236±.612 b*	11.716±.281 b*	31.680±.381 b*	18.036±.463 b*	12.480±.390 b*	232.147±.641 b*
		COT	14th Day	113.279±.603 C*	6.210±.026 C*	16.580±.694 C*	12.883±.178 C*	10.600±.060 C*	159.546±.470 C*
		EMB		153.409±.406 c*	11.226±.178 c*	29.543±.414 c*	16.053±.417 c*	10.563±.474 c*	220.783±1.393 c*
		COT	30th Day	109.071±.610 D*	5.880±.045 D*	14.263±.493 D*	11.993±.145 D*	10.220±.091 D*	151.424±1.077 D*
		EMB		147.704±.422 d*	10.196±.147 d*	26.663±.140 d*	12.196±.194 d*	8.836±.761 d*	205.608±1.017 d*
Laboratory condition 28±2°C (65% RH)	PB	COT	1st Day	117.625±.895 A*	6.580±.078 A*	18.773±.142 A*	13.246±.326 A*	11.040±.036 A*	167.264±.802 A*
		EMB		152.298±1.696 a*	11.730±.205 a*	31.416±.672 a*	18.056±.064 a*	14.100±.055 a*	227.609±1.765 a*
		COT	7th Day	112.782±.533 B*	6.110±.075 B*	17.863±.189 B*	12.790±.283 B*	10.510±.141 B*	160.058±.809 B*
		EMB		146.775±2.351 b*	11.363±.281 b*	27.603±1.002 b*	17.790±.145 ab*	13.613±.094 b*	217.145±3.123 b*
		COT	14th Day	109.071±.660 C*	5.816±.155 C*	16.836±.262 C*	11.110±.380 C*	9.000±.640 C*	151.836±1.205 C*
		EMB		141.085±.448 c*	10.836±.105 c*	26.356±.190 c*	16.196±.394 c*	10.733±.716 c*	205.202±.488 c*
		COT	30th Day	104.940±.211 D*	5.236±.072 D*	14.710±.886 D*	10.420±.127 D*	8.040±.105 D*	143.329±.906 D*
		EMB		137.747±1.038 d*	10.223±.075 d*	24.946±.115 d*	12.483±.322 d*	8.550±.481 d*	193.951±1.295 d*
	PC	COT	1st Day	116.807±.613 A*	6.526±.083 A*	18.590±.140 A*	13.200±.351 A*	11.053±.025	166.173±1.016
		EMB		153.121±1.582 a*	11.390±.217 a	31.683±1.038 a*	18.120±.091 a*	14.066±.055 a*	228.387±1.735 a*
		COT	7th Day	112.653±.075 B*	6.0±.035 B*	17.666±.148 B*	12.226±.184 B*	10.750±.081 B*	159.348±.210 B*
		EMB		146.747±.930 b*	11.080±.0400 b*	28.416±.231 b*	17.493±.417 b*	13.533±.087 b*	217.265±.887 b*
		COT	14th Day	107.669±.686 C*	5.786±.061 C*	16.996±.041 C*	11.620±.127 C*	9.556±.073 C*	151.629±.917 C*
		EMB		141.356±.508 c*	10.640±.110 c*	26.526±.485 c*	16.130±.105 c*	10.900±.511 c*	205.550±1.025 c*
		COT	30th Day	104.476±.489 D*	5.266±.058 D*	14.763±.041 D*	10.110±.036 D*	7.656±.141 D*	142.265±.566 D*
		EMB		133.308±2.196 d*	10.046±.047 d*	24.3967±.402 d*	12.090±.026 d*	8.096±.058 d*	187.934±2.586 d*

	OT	COT	1st Day	107.441±.265 A*	5.753±.068 A*	17.586±.487 A*	11.636±.200 A*	9.553±.025 A*	151.978±.4493 A*
		EMB		143.147±.692 a*	10.830±.105 a*	26.266±.241 a*	16.166±.041 a*	11.583±.055 a	207.996±.659 a
		COT	7th Day	104.245±.447 B*	5.276±.030 B*	14.690±.144 B*	10.436±.270 B*	7.626±.170 B*	142.273±.7784 B*
		EMB		133.745±2.652 b*	10.333±.186 b*	25.150±.508 b*	12.530±.410 b*	8.526±.310 b*	190.289±2.496 b
		COT	14th Day	100.630±.601 C*	5.160±.055 C*	14.013±.076 C*	9.666±.176 C*	7.160±.036 C*	136.635±.5688 C*
		EMB		130.322±.681 c	9.750±.296 c*	22.716±.342 c*	11.673±.317 c*	8.063±.035 c*	182.521±.820 c*
-196°C	CV	COT	1st Day	126.928±1.779	6.846±.095	20.333±.467	14.860±.157	11.543±.168	180.506±1.801
		EMB		166.439±1.227	13.203±.350	35.470±.285	19.603±.629	13.853±.197	248.561±.381

EMB-Embryonic axis, COT- Cotyledon, CV-Cryo-vial, PB- Polycarbonate bottle, PC- Polythene cover, AF- Aluminium foil. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. Small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals represent the significant differences. The observations within a column with the same inscripts are not significantly distinct with each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

Table: 23 Different Protein fractions in various storage conditions

Treatments			Storage Periods (Days)										
			1	7	14	30	60	90	120	150	180	210	240
-80 °C	PB	COT	.776±.005										
		EMB	.910±.043										
	AF	COT	.760±.028										
		EMB	.915±.021										
-20 °C	PB	COT	.756±.035										
		EMB	.970±.010										
	AF	COT	.806±.025										
		EMB	.956±.030										
0 °C	PB	COT	.756±.025										
		EMB	.950±.026										
	PC	COT	.753±.030										
		EMB	.980±.010										
5 °C	PB	COT	.766±.032										
		EMB	.976±.015										
	PC	COT	.743±.015										
		EMB	.980±.010										

10°C	PB	COT	.750±.010	.810±.020									
		EMB	.970±.010	.876±.098									
	PC	COT	.760±.010	.810±.020									
		EMB	.973±.020	1.026±.015									
20±2°C (40% RH)	PB	COT	.773.02$^{a^*}$	.803 ±.01$^{b^*}$	.830 ±.01$^{c^*}$	.840 ±.036$^{d^*}$	.906 ±.015$^{e^*}$	.933 ±.00$^{f^*}$	.980 ±.01$^{g^*}$	1.013 ±.015$^{h^*}$	1.056 ±.015$^{i^*}$	1.080 ±.01$^{j^*}$	1.106 ±.02$^{k^*}$
		EMB	.930 ±.010$^{A^*}$	.966 ±.015$^{B^*}$	.976 ±.015$^{C^*}$	1.020 ±.010$^{D^*}$	1.040 ±.01$^{E^*}$	1.073 ±.005$^{F^*}$	1.110 ±.010$^{G^*}$	1.176 ±.015$^{H^*}$	1.220 ±.036$^{I^*}$	1.293 ±.025$^{J^*}$	1.400 ±.036$^{K^*}$
	OT	COT	.810 ±.020$^{a^*}$	1.103 ±.025$^{b^*}$	1.373 ±.041$^{c^*}$	1.736 ±.040$^{d^*}$							
		EMB	.980 ±.010$^{A^*}$	1.696 ±.051$^{B^*}$	1.820 ±.026$^{C^*}$	2.116 ±.077$^{D^*}$							
Laboratory condition 28±2°C (65% RH)	PB	COT	.776 ±.015$^{a^*}$	.840 ±.050$^{b^*}$	.963 ±.0416$^{c^*}$	1.176 ±.055$^{d^*}$							
		EMB	.923 ±.015$^{A^*}$	.973 ±.015$^{B^*}$	1.296 ±.090$^{C^*}$	1.516 ±.056$^{D^*}$							
	PC	COT	.780 ±.020$^{a^*}$	.776 ±.035$^{b^*}$	.963 ±.015$^{c^*}$	1.190 ±.055$^{d^*}$							
		EMB	.940 ±.01$^{A^*}$	1.063 ±.02$^{B^*}$	1.366 ±.03$^{C^*}$	.676 ±.035$^{D^*}$							
	OT	COT	.816 ±.124$^{a^*}$	1.226 ±.061$^{b^*}$	1.926 ±.066$^{c^*}$								
		EMB	.936 ±.020$^{A^*}$	1.476 ±.065$^{B^*}$	2.113 ±.025$^{C^*}$								
-196 °C	CV	COT	.773±.015										
		EMB	.913±.015										

COT- Cotyledon, EMB- Embryonal axis, AF- Aluminium Foil, PB- Plastic bottle, PC- Polythene cover, OT- Open tray, CV- Cryo vial. The test used to study the effect of different characteristics is ANOVA and Duncan's Multiple Range Test is used as post-hoc. The significant differences are represented by small alphabets, capital alphabets, numbers in parenthesis, alphabets in parenthesis and Arabic numerals. The observations within a column with same inscripts are not significantly distinct from each other. All other observations are statistically significant at 1% level of significance. *Significant at P=0.01

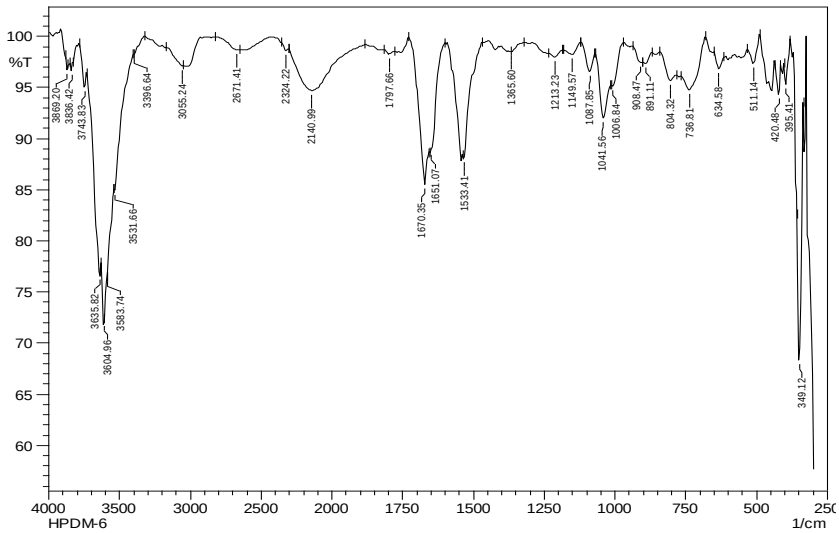
Table: 24 Lipid peroxidation content in various storage conditions

FT-IR Analysis

In FT-IR spectroscopy, three solvents are selected based on their polarity to analyze samples: Hexane, Acetone and Methanol. This analysis is conducted on both dried and fresh samples using each of the three solvents. The dried samples include: 1. Dried seeds with Methanol (HPDM), 2. Dried seeds in Acetone (HPDA) and 3. Dried seeds in Hexane (HPDH). Similarly, the fresh seed samples are prepared as follows: 4. Fresh seeds in Methanol (HPFM), 5. Fresh seeds in Acetone (HPFA), and 6. Fresh seeds in Hexane (HPFH). It is well established that FT-IR spectroscopy is a powerful tool that can be used to identify the type of bonding particularly functional groups present in organic compounds

1. **Dried Seeds in Methanol Fraction (HPDM)**

   Hydroxy group, Common inorganic ions, Amino-related components, Transition metal carbonyls, Ketone compound, Phosphate ions, Aliphatic bromo compounds, and Aryl disulfides are the compounds identified from the samples through FT-IR analysis (Table: 25).



Graph: 16 FTIR spectrum of methanol dried sample

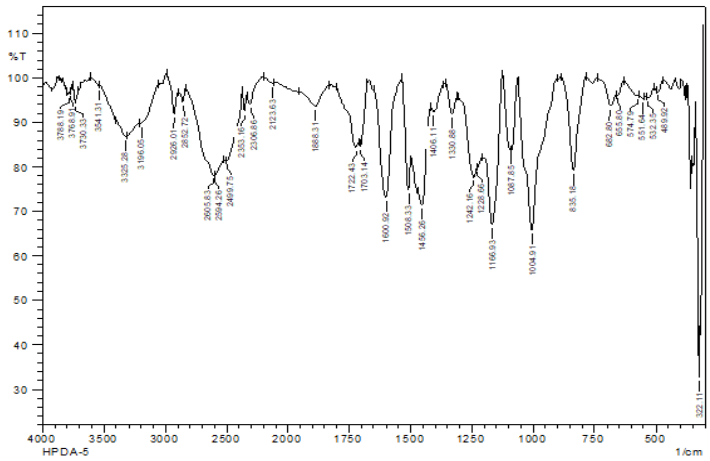
Sl. No.	Wave Number cm^{-1} [Test sample]	Wave Number cm^{-1} [Reference article]	Functional Group Assignment	Phyto Compounds Identified
1	3869.20	>3500	Non-bonded, O-H stretch	Hydroxy group

2	3836.42	>3500	Non-bonded, O-H stretch	Hydroxy group
3	3743.83	>3500	Non-bonded, O-H stretch	Hydroxy group
4	3635.82	>3500	Non-bonded, O-H stretch	Hydroxy group
5	3604.96	>3500	Non-bonded, O-H stretch	Hydroxy group
6	3583.74	>3500	Non-bonded, O-H stretch	Hydroxy group
7	3531.66	3570-3200	O-H stretch, H bonded	Hydroxy compound
8	3396.64	3570-3200	O-H stretch, H bonded	Hydroxy compound
9	3055.24	3300–3030/1430–13905)	Ammonium ion	Common inorganic ions
10	2324.22	2332 about 2350	NH component	Amino-related component
11	2140.99	2100-1800	Carbonyl compound frequency	Transition metal carbonyls
12	1670.35	1650-1600	C=O stretching	Ketone compound
13	1651.07	1650-1600	C=O stretching	Ketone compound
14	1087.85	1100-1000	PO3 stretch	Phosphate ion
15	1041.56	1100-1000	PO3 stretch	Phosphate ion
16	1006.84	1100-1000	PO3 stretch	Phosphate ion
17	736.81	700-600	C-Br stretch	Aliphatic bromo compounds
18	634.58	700-600	C-Br stretch	Aliphatic bromo compounds
19	511.14	500-430	S-S stretch	Aryl disulfides
20	420.48	500-430	S-S stretch	Aryl disulfides

Table: 25 FTIR Interpretation of Compounds in Methanol-dried Samples

2. Dried Seeds in Acetone Fraction (HPDA)

Poly Hydroxy compound, Hydroxy compound, Common inorganic ions, Methylene (>CH2), Carboxylic acids, Nitrogen multiple and cumulated double-bond compound, Carbonyl compound, Thiols and thio-substituted compounds, Aliphatic organo halogen compound, Common inorganic ions, Primary amino, Simple hetero-oxy compounds, Ether and oxy compound, are the compounds identified from the sample (Table: 26).



Graph: 17 FTIR spectrum of acetone dried sample

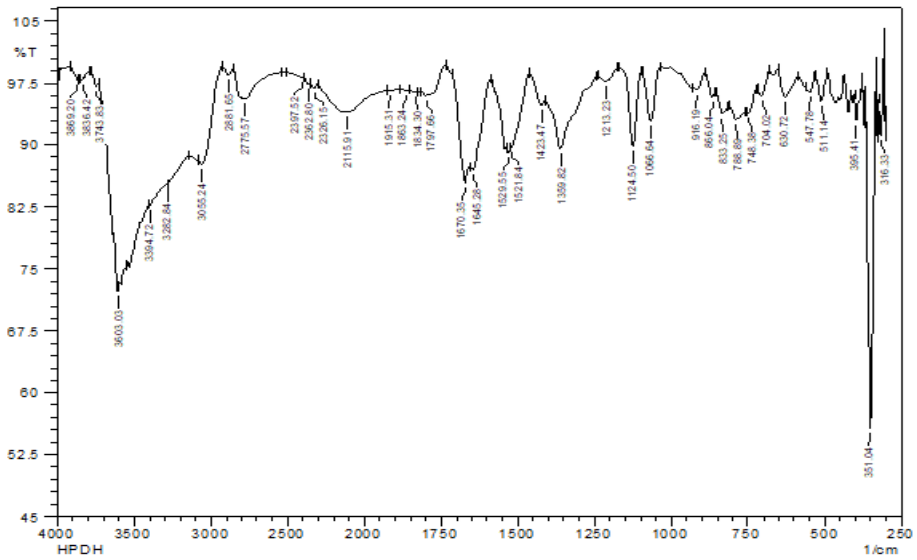
Sl No.	Wave number cm-1 [Test sample]	Wave number cm-1 [Reference article]	Functional group assignment	Phyto compounds Identified
1	3788.19	>3500	Non-bonded, O-H stretch	Hydroxy group
2	3768.91	>3500	Non-bonded, O-H stretch	Hydroxy group
3	3730.33	>3500	Non-bonded, O-H stretch	Hydroxy group
4	3541.31	3570-3200	O-H stretch, Hydroxy group, H-bonded	Poly Hydroxy compound
5	3325.28	3400-3200	O-H stretch, Polymeric OH	Hydroxy compound
6	3196.05	3300–3030	Ammonium ion	Common inorganic ions
7	2926.01	2935-2915	Methylene C-H asym. /sym. stretch	Methylene (>CH_2)
8	2852.72	2865-2845	Methylene C-H asym. /sym. stretch	Methylene (>CH_2)
9	2605.83	3500-2400	O-H stretch, Carboxylic group	Carboxylic acids
10	2594.26	2600-2550	Thiols (S-H stretch)	Thiols and thio-substituted compounds
11	2499.75	2600-2550	Thiols (S-H stretch)	Thiols and thio-substituted compounds
14	2123.63	2150-1990	Isothiocyanate (-NCS)	Nitrogen multiple and cumulated double-bond compound
15	1888.31	2100-1800	Transition metal carbonyls	Carbonyl compound
16	1722.43	1815-1770	Acid (acyl) halide	Carbonyl compound
17	1703.14	1725-1700	Carboxylic acid	Carbonyl compound
18	1600.92	1610-1550	Carboxylate (carboxylic acid salt)	Carbonyl compound
19	1508.33	1555-1485	Aromatic nitro compounds	Simple hetero-oxy compounds

20	1456.26	1490-1410	Carbonate ion	Common inorganic ions
21	1406.11	1430-1390	Ammonium ion	Common inorganic ions
22	1330.88	1335-1300	Sulfur-oxy compounds (dialkyl/aryl sulfones)	Simple hetero-oxy compounds
22	1242.16	1270-1230	Aromatic ethers, aryl-O stretch	Ether and oxy compound
23	1228.66	1240-1190	Phosphorous-oxy compounds (aromatic phosphates (P-O-C stretch))	Simple hetero-oxy compounds
24	1166.93	1170-1135	Sulfur-oxy compounds (dialkyl/aryl sulfones)	Simple hetero-oxy compounds
25	1087.85	1090-1020	Primary amine C-N stretch	Primary amino
26	1004.91	1100-900	Silicate ions	Common inorganic ions
27	835.18	840-815	Nitrate ions	Common inorganic ions
28	682.80	600-700	Aliphatic bromo compound C-Br stretch	Aliphatic organo halogen compound
29	655.80	600-700	Aliphatic bromo compound C-Br stretch	Aliphatic organo halogen compound
30	574.79	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
31	551.64	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
32	532.35	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
33	489.92	500-430	Aryl disulfides (S-S stretch)	Thiols and thio-substituted compounds

Table: 26 FTIR interpretation of compounds in acetone-dried samples

3. Dried Seeds in Hexane Fraction (HPDH)

Hydroxy group, Alcohol and hydroxy compound, Saturated Aliphatic (alkene/alkyl), Acetylenic (alkyne), Aromatic ring (aryl), Carbonyl compound, Olefinic (alkene), Simple hetero-oxy compounds, Ether and oxy compound, Primary amino, Common inorganic ions, Aromatic ring (aryl), Aliphatic organo halogen compound, Thiols and thio substituted compounds, Aliphatic organo halogen compound are the compounds identified through the FT-IR analysis (Table: 27).



Graph: 18 FTIR spectrum of hexane-dried sample

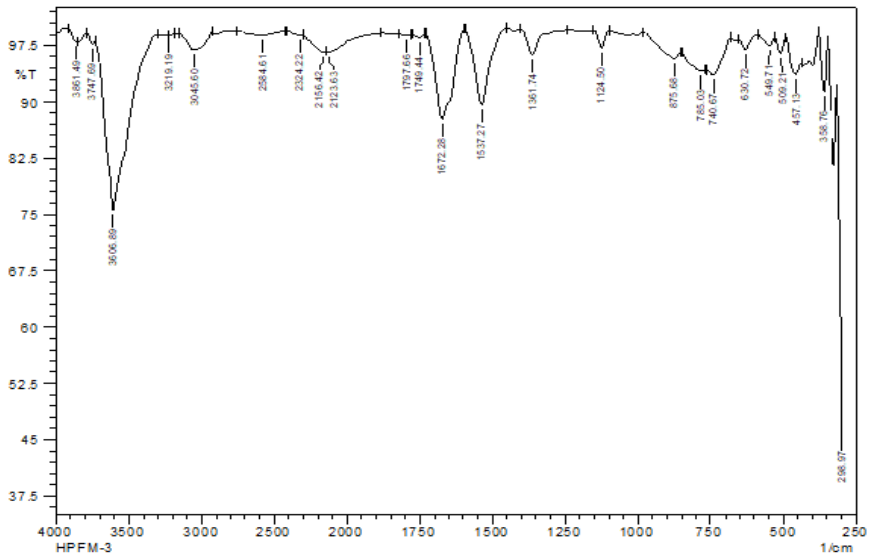
Sl No.	Wave number cm^{-1} [Test sample]	Wave number cm^{-1} [Reference article]	Functional group assignment	Phyto compounds Identified
1	3869.20	>3500	Non-bonded, O-H stretch	Hydroxy group
2	3836.42	>3500	Non-bonded, O-H stretch	Hydroxy group
3	3743.83	>3500	Non-bonded, O-H stretch	Hydroxy group
4	3603.03	3640–3530	Phenols, OH stretch	Alcohol and hydroxy compound
5	3394.72	3400–3200	Normal ‘‘polymeric’’ OH stretch	Alcohol and hydroxy compound
6	3282.84	3400–3200	Normal ‘‘polymeric’’ OH stretch	Alcohol and hydroxy compound
7	3055.24	3300–3030	Ammonium ion	Common inorganic ions
8	2881.65	2880–2860	Methyl C-H asym. /sym. Stretch	Saturated Aliphatic (alkene/alkyl)
9	2775.57	2600–2550	Thiols (S-H stretch)	Thiols and thio-substituted compounds
10	2115.91	2140–2100	C≡C Terminal alkyne (monosubstituted)	Acetylenic (alkyne)
11	1915.31	2000–1660	Aromatic combination bands	Aromatic ring (aryl)
12	1863.24	1870-1820	Five-membered ring anhydride	Carbonyl compound
13	1834.30	1870-1820	Five-membered ring anhydride	Carbonyl compound
14	1797.66	1820–1775	Aryl carbonate	Carbonyl compound

15	1670.35	1680-1620	Alkenyl C=C stretch	Olefinic (alkene)
16	1645.28	1680-1620	Alkenyl C=C stretch	Olefinic (alkene)
17	1529.55	1555-1485	Aromatic nitro compounds	Simple hetero-oxy compounds
18	1521.84	1555-1485	Aromatic nitro compounds	Simple hetero-oxy compounds
19	1423.47	1490-1410	Carbonate ion	Common inorganic ions
20	1359.82	1365-1340	Sulfur-oxy compounds (Sulfonates)	Simple hetero-oxy compounds
21	1213.23	1240-1190	Phosphorous-oxy compounds (aromatic phosphates (P-O-C stretch))	Simple hetero-oxy compounds
22	1124.50	1150-1050	Alkyl-substituted ether, C-O stretch	Ether and oxy compound
23	1066.64	1090-1020	Primary amine C-N stretch	Primary amino
24	916.19	1100-900	Silicate ions	Common inorganic ions
25	866.04	880-860	Carbonate ions	Common inorganic ions
26	833.25	840-815	Nitrate ions	Common inorganic ions
27	788.89	810-750	C-H 1,3-disubstitution (meta)	Aromatic ring (aryl)
28	748.38	770-735	C-H 1,3-disubstitution (ortho)	Aromatic ring (aryl)
29	704.02	800-700	Aliphatic chloro compounds, C-Cl stretch	Aliphatic organo halogen compound
30	630.72	660-630	Thioethers, CH_3-S- (C-S stretch)	Thiols and thio substituted compounds
31	547.78	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
32	511.14	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound

Table: 27 FTIR interpretation of compounds in hexane-dried samples

4. Fresh Seeds in Methanol Fraction (HPFM)

Hydroxy group, Poly Hydroxy compound, Carboxylic acids, Carbonyl compound, Nitrogen multiple and cumulated double-bond compound, Simple hetero-oxy compounds, Ether and oxy compound, Aromatic ring (aryl), Aliphatic organo halogen compound, Thiols and thio-substituted compounds are the phytocompounds identified from the sample (Table: 28).



Graph: 19 FTIR spectrum of methanol fresh sample

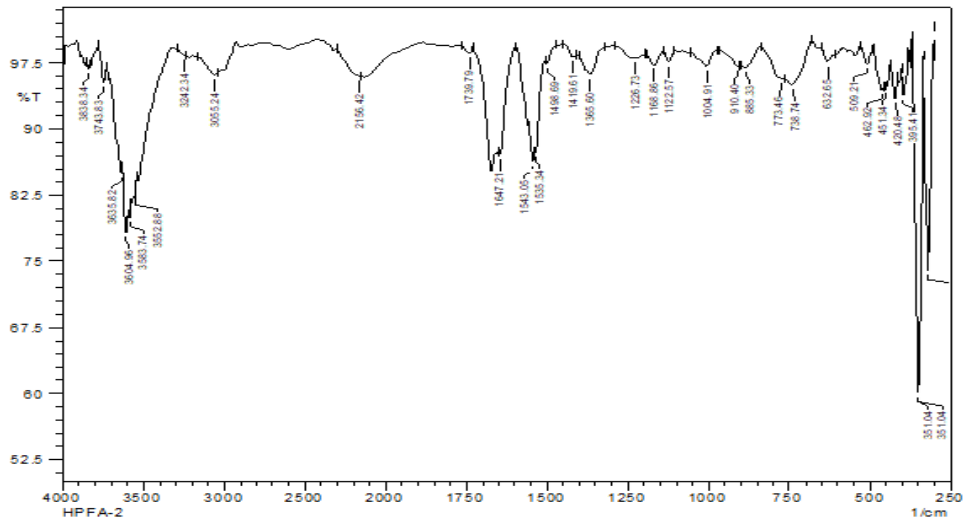
Sl No.	Wave number cm-1 [Test sample]	Wave number cm-1 [Reference article]	Functional Group Assignment	Phyto Compounds Identified
1	3661.49	>3500	Non-bonded, O-H stretch	Hydroxy group
2	3747.69	>3500	Non-bonded, O-H stretch	Hydroxy group
3	3606.89	>3500	Non-bonded, O-H stretch	Hydroxy group
4	3219.19	3400-3200	O-H stretch	Poly Hydroxy compound
5	3045.60	3500-2400	O-H stretch, Carboxylic group	Carboxylic acids
6	2584.61	3500-2400	O-H stretch, Carboxylic group	Carboxylic acids
7	2156.42	2175–2140	Thiocyanate (-SCN)	Nitrogen multiple and cumulated double-bond compound
8	2123.63	2150–1990	Isothiocyanate (-NCS)	Nitrogen multiple and cumulated double-bond compound
9	1797.66	1820–1775	Aryl carbonate	Carbonyl compound
10	1749.44	1760-1740	Alkyl carbonate	Carbonyl compound
11	1672.28	1690-1590	Open-chain imino (-C=N-)	Nitrogen multiple and cumulated double-bond compound
12	1537.27	1555-1485	Aromatic nitro compounds	Simple hetero-oxy compounds
13	1361.74	1365-1340	Sulfur-oxy compounds (Sulfonates)	Simple hetero-oxy compounds
14	1124.50	1150-1050	Alkyl-substituted ether, C-O stretch	Ether and oxy compound
15	875.68	890-820	Peroxides, C-O-O-stretch	Ether and oxy compound

16	785.03	810-750	C-H 1,3-disubstitution (meta)	Aromatic ring (aryl)
17	740.67	770-735	C-H 1,3-disubstitution (ortho)	Aromatic ring (aryl)
18	630.72	660-630	Thioethers, CH_3-S- (C-S stretch)	Thiols and thio substituted compounds
19	549.71	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
20	509.21	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
21	457.13	500-430	Aryl disulfides (S-S stretch)	Thiols and thio-substituted compounds

Table: 28 FTIR interpretation of compounds in methanol-fresh samples

5. Fresh Seeds in Acetone Fraction (HPFA)

Hydroxy group, Alcohol and hydroxy compounds, Poly Hydroxy compounds, Carboxylic acids, Nitrile compounds, Aldehyde compounds, Nitrogen multiple and cumulated double-bond compounds, Simple hetero-oxy compounds, Common inorganic ions, Ether and oxy compounds, Aliphatic organo halogen compounds, Thiols and thio-substituted compounds are identified from the sample (Table: 29).



Graph: 20 FTIR spectrum of acetone fresh sample

Sl No.	Wave number cm-1 [Test sample]	Wave number cm-1 [Reference article]	Functional group assignment	Phyto compounds Identified
1	3838.34	>3500	Non-bonded, O-H stretch	Hydroxy group

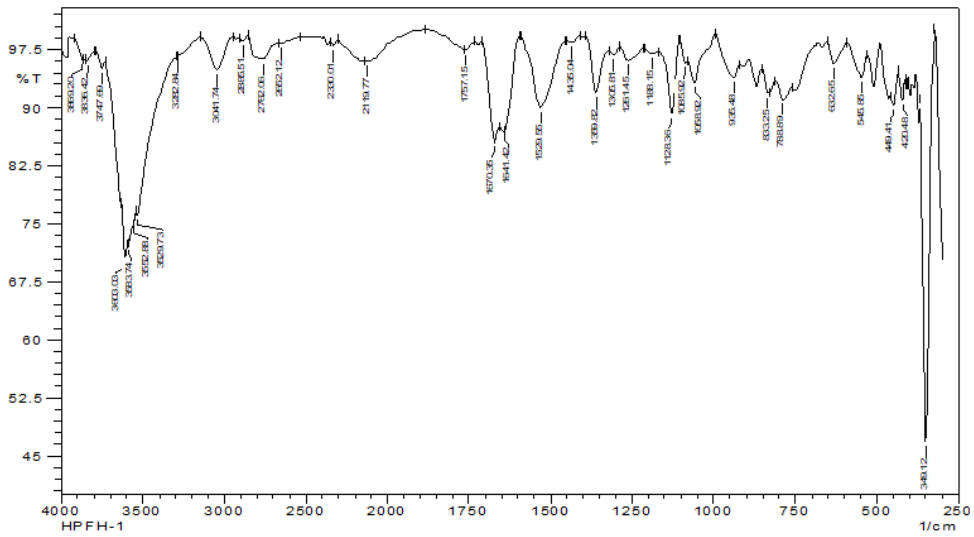
2	3743.83	>3500	Non-bonded, O-H stretch	Hydroxy group
3	3635.82	>3500	Non-bonded, O-H stretch	Hydroxy group
4	3604.96	>3500	Non bonded, O-H stretch	Hydroxy group
5	3583.74	3640–3530	Phenols, OH stretch	Alcohol and hydroxy compound
6	3552.88	3640–3530	Phenols, OH stretch	Alcohol and hydroxy compound
7	3242.34	3400-3200	O-H stretch	Poly Hydroxy compound
8	3055.24	3500-2400	O-H stretch, Carboxylic group	Carboxylic acids
9	2156.42	2300–1990	Multiple bonding	Nitrile compounds
10	1739.79	1740-1725	C=O stretch	Aldehyde compound
11	1647.21	1690-1590	Open-chain imino (-C=N-)	Nitrogen multiple and cumulated double-bond compound
12	1543.05	1560-1540	Nitrogen-oxy compounds (aliphatic nitro compounds)	Simple hetero-oxy compounds
13	1535.34	1555-1485	Nitrogen-oxy compounds (aromatic nitro compounds)	Simple hetero-oxy compounds
14	1498.69	1555-1485	Nitrogen-oxy compounds (aromatic nitro compounds)	Simple hetero-oxy compounds
15	1419.61	1490-1410	Carbonate ion	Common inorganic ions
16	1365.60	1380-1350	Nitrate ion	Common inorganic ions
17	1226.73	1240-1190	Phosphorous-oxy compounds (aromatic phosphates (P-O-C stretch))	Simple hetero-oxy compounds
18	1168.86	1170-1135	Sulfur-oxy compounds (dialkyl/aryl sulfones)	Simple hetero-oxy compounds
19	1122.57	1130-1080	Sulfate ion	Common inorganic ions
20	1004.91	1100-1000	Phosphate ion	Common inorganic ions
21	910.40	1100-900	Silicate ion	Common inorganic ions
22	885.33	890-820	Peroxides, C-O-O-stretch	Ether and oxy compound
23	773.46	800-700	Aliphatic chloro compounds, C-Cl stretch	Aliphatic organo halogen compound
24	738.74	800-700	Aliphatic chloro compounds, C-Cl stretch	Aliphatic organo halogen compound
25	632.65	700-600	Aliphatic bromo compounds, C-Br stretch	Aliphatic organo halogen compound
26	509.21	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound

27	462.92	500-430	Aryl disulfides (S-S stretch)	Thiols and thio-substituted compounds
28	451.34	500-430	Aryl disulfides (S-S stretch)	Thiols and thio-substituted compounds

Table: 29 FTIR interpretation of compounds in acetone-fresh samples

6. Fresh Seeds in Hexane Fraction (HPFH)

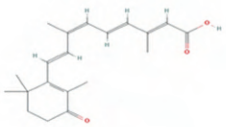
Hydroxy group, Alcohol and hydroxy compound, Alcohol, and hydroxy compounds, Poly Hydroxy compounds, Carboxylic acids, Acetylenic (alkyne), Carbonyl compounds, Simple hetero-oxy compounds, Ether and oxy compound, Aliphatic organo halogen compound, Thiols, and thio-substituted compounds are the phytocompounds identified (Table:30).



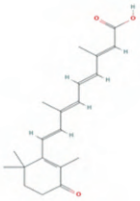
Graph: 21 FTIR spectrum of hexane fresh sample

Sl No.	Wave number cm-1 [Test sample]	Wave number cm-1 [Reference article]	Functional group assignment	Phyto compounds Identified
1	3869.20	>3500	Non bonded, O-Hstretch	Hydroxy group
2	3836.42	>3500	Non bonded, O-Hstretch	Hydroxy group
3	3747.69	>3500	Non bonded, O-Hstretch	Hydroxy group
4	3603.03	>3500	Non bonded, O-Hstretch	Hydroxy group

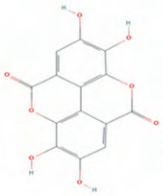
5	3583.74	3640–3530	Phenols, OH stretch	Alcohol and hydroxy compound
6	3552.88	3640–3530	Phenols, OH stretch	Alcohol and hydroxy compound
7	3529.73	3570-3200	O-H stretch, Hydroxy group, H-bonded	Poly Hydroxy compound
8	3282.84	3400-3200	O-H stretch	Poly Hydroxy compound
9	3041.74	3500-2400	O-H stretch, Carboxylic group	Carboxylic acids
10	2885.51	3500-2400	O-H stretch, Carboxylic group	Carboxylic acids
11	2762.06	3500-2400	O-H stretch, Acidic	Carboxylic acid
12	2652.12	3500-2400	O-H stretch, Acidic	Carboxylic acid
13	2330.01	3570-3200	O-H stretch, H-bonded	Hydroxy compound
14	2119.77	2140-2100	C≡C Terminal alkyne (monosubstituted)	Acetylenic (alkyne)
15	1757.15	1760-1740	Alkyl carbonate	Carbonyl compound
16	1670.35	1680-1630	Amide	Carbonyl compound
17	1641.42	1680-1630	Amide	Carbonyl compound
18	1529.55	1555-1485	Aromatic nitro compounds	Simple hetero-oxy compounds
19	1435.04	1490-1410	Carbonate ion	Common inorganic ions
20	1359.82	1380-1350	Nitrate ion	Common inorganic ions
21	1305.81	1335-1300	Sulfur-oxy compounds (dialkyl/aryl sulfones)	Simple hetero-oxy compounds
22	1261.45	1270-1230	Aromatic ethers, aryl-O stretch	Ether and oxy compound
23	1188.15	1190-1130	Secondary amino (Secondary amine, CN stretch)	Ether and oxy compound
24	1128.36	1130-1080	Sulphate ion	Common inorganic ions
25	1085.92	1130-1080	Sulphate ion	Common inorganic ions
26	1058.92	1130-1080	Sulphate ion	Common inorganic ions
27	935.48	1100-900	Silicate ion	Common inorganic ions



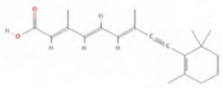
4-Keto 9-cis Retinoic Acid



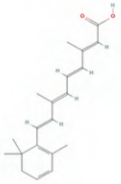
4-Ketoretinoic acid



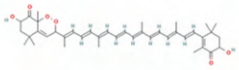
Ellagic Acid



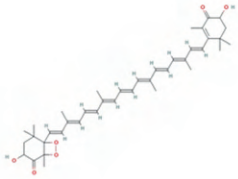
7,8-Didehydroretinoic acid



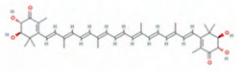
3,4-Didehydroretinoic acid



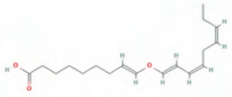
Astaxanthin-5, 8-peroxide



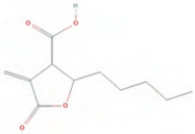
Astaxanthin-5,6-peroxide



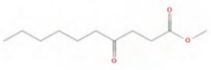
2,2'Dihydroxy astaxanthin



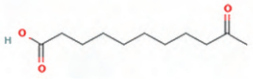
Colnelenic acid



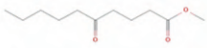
Methylenolactocin



Methyl 4-oxodecanoate

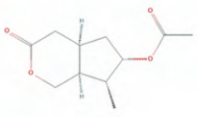


10-Oxoundecanoic acid



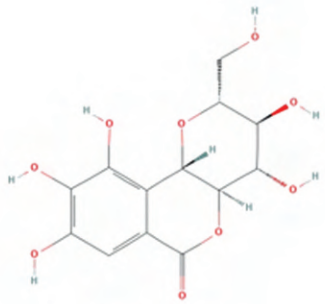
Methyl 5-oxodecanoate

4-Hydroxybenzaldehyde

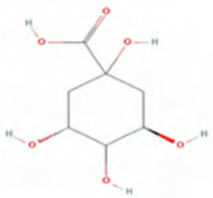


Isoboonein acetate

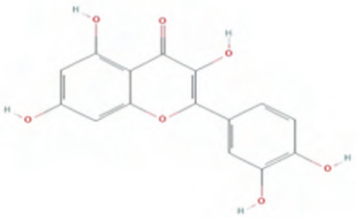
Fig. 18 Compounds present in the hexane extract of the fresh samples *H. ponga*



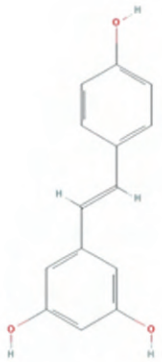
Demethylbergenin
(Norbergenin)



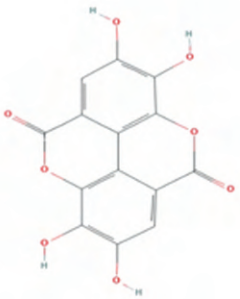
Quinic acid



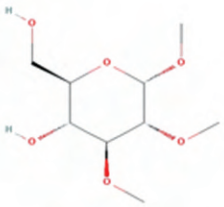
Quercetin



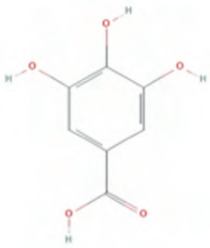
Resveratrol



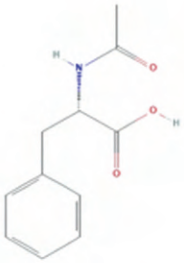
Ellagic Acid



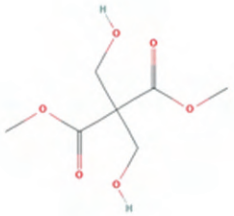
Methyl 2,3-
di-O-methyl-alpha-
D-glucopyranoside
(1,2,3-Tri-O-methyl-alpha-
D-glucopyranose)



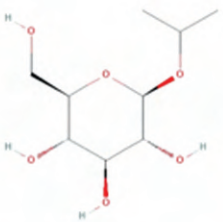
Gallic Acid



N-Acetyl-L-phenylalanine



Dimethyl 2,2-bis
(hydroxymethyl)malonate



Isopropyl beta-D-glucopyranoside

Fig. 19 Compounds present in the acetone extract of the fresh samples *H. ponga*

28	833.25	890-820	Peroxides, C-O-Stretch	Ether and oxy compound
29	788.89	800-700	Aliphatic chloro compounds, C-Cl stretch	Aliphatic organo halogen compound
30	632.65	680-610	Alkyne C-H bend	Acetylenic (alkyne)
31	545.85	600-500	Aliphatic iodo compounds, C-I stretch	Aliphatic organo halogen compound
32	449.41	500-430	Aryl di sulfides (S-S stretch)	Thiols and thio-substituted compounds

Table: 30 FTIR interpretation of compounds in hexane-fresh samples

LC-MS Analysis

LC-MS analysis identified the phytochemical compounds present in the hexane, acetone, and methanol of the seeds from the fresh and dried samples of *Hopea ponga.*

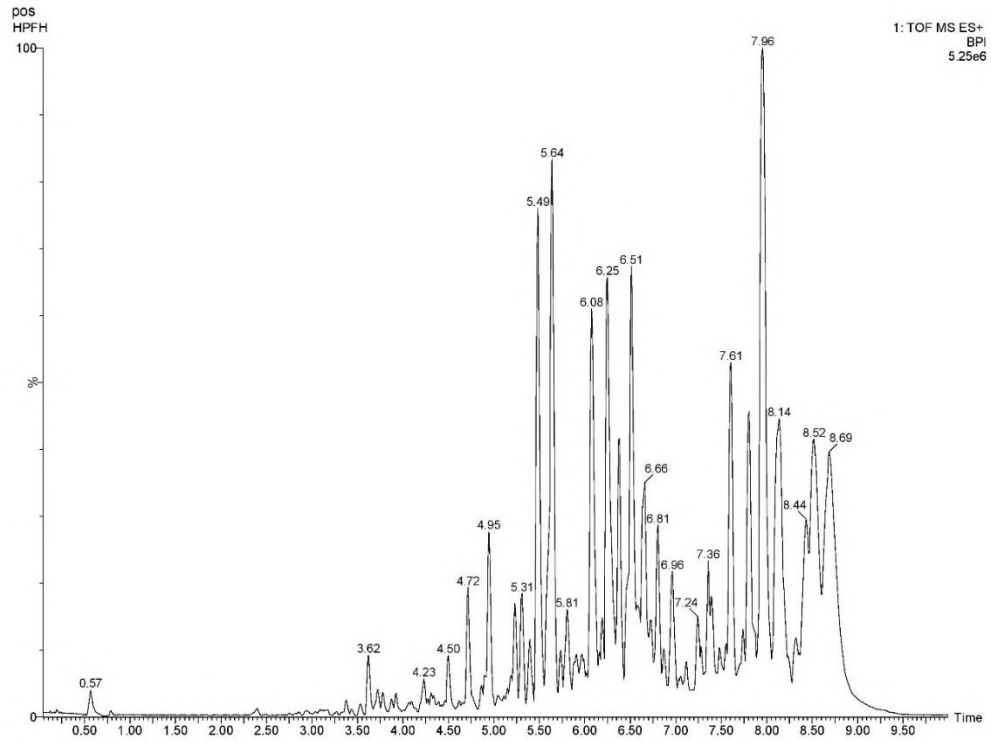
LC/MS Analysis of Hexane Extract of Fresh Seed Samples

Fifteen compounds were identified from the hexane extract of the fresh *Hopea ponga* seeds and are presented in Table: 31 and Fig.18. The plant samples revealed the synthesis of 4-Keto 9-cis Retinoic Acid, Ellagic Acid, 3,4-Didehydroretinoic acid, Astaxanthin-5,6-peroxide, Colnelenic acid, Isoboonein acetate, etc. The LC-MS chromatogram of hexane extract is shown in Fig.18. 4-Keto 9-cis Retinoic Acid is a versatile compound that interacts with receptors, influences cellular processes, and holds promise for various applications. This compound supports dopamine cell survival and induces neuroprotection, making it relevant in neurodegenerative disorders like Parkinson's disease. It promotes neuronal differentiation and may have therapeutic potential in treating stroke and influences cytokine secretion and lymphocyte proliferation. 4-Ketoretinoic acid is a derivative of retinoic acid, a type of vitamin A. It is involved in various biological processes, including cell growth, differentiation, and development. 4-Ketoretinoic acid may have potential anticancer properties and could be used in cancer treatment.

Ellagic acid is a naturally occurring polyphenol that has potential health benefits, including anti-inflammatory, antioxidant, and anticancer properties. Ellagic acid has been shown to have anti-inflammatory effects by inhibiting pro-inflammatory enzymes and cytokines. It also acts as an antioxidant, scavenging free radicals and protecting cells from oxidative damage. Due to these properties, ellagic acid may have potential anticancer properties, it may inhibit the growth of cancer cells and induce apoptosis (cell death) in cancer cells. 7,8-Didehydroretinoic acid, also known as 7,8-DHRA and is related to retinoic acid (RA), a derivative of vitamin A. RA plays a crucial role in cell differentiation, growth, and development. Reductions in cellular retinoic acid-binding protein 2 (CRABP2) and augmentations in fatty acid-binding protein 5 (FABP5) can alter RA signalling, impacting physiological growth inhibition and potentially leading to pathological states, such as neoplastic breast cancer growth.

3,4-Didehydroretinoic acid, also known as vitamin A2 acid, belongs to the class of organic compounds called retinoids. These are oxygenated derivatives of 3,7-dimethyl-1-(2,6,6-trimethylcyclohex-1-enyl) nona-1,3,5,7-tetraene and its derivatives. Astaxanthin has a distinctive structure, featuring polar moieties at both ends of its polyene chain. This unique arrangement contributes to its potent antioxidant activity and allows it to interact with cell membranes for various biological functions such as anti-inflammation activities, diabetes, cardiovascular diseases, etc. 2,2’-Dihydroxyastaxanthin is a red diketocarotenoid with remarkable health-promoting properties such as antioxidant, anti-inflammatory, and antitumor, and used as an immune booster. Colnelenic acid is a long-chain, divinyl ether fatty acid composed of 8-nonenoic acid in which the E-hydrogen at position 9 is substituted by a (1E,3Z,6Z)-nona-1,3,6-trien-1-yloxy group. Methylenolactocin is a chemical compound having antitumor property. Methyl 4-oxodecanoate is a chemical compound and not typically associated with specific biological properties. It is more commonly used in organic synthesis and as a flavoring agent. 10-

Oxoundecanoic acid, also known as 9-acetyl pelargonic acid, has antimycotic properties. It inhibits the production of exocellular keratinase, lipase, and the biosynthesis of several phospholipids. Methyl 5-oxodecanoate is a chemical compound used in the production of flavours and fragrances. 4-Hydroxybenzaldehyde is a compound that has shown various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Isoboonein acetate is a compound that has been found to possess various biological properties, including anti-inflammatory and antioxidant effects and have potential anticancer properties.



Graph: 22 LC/MS profiling of hexane extract of fresh seed samples

Sl. No.	Compound Name	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)
1	4-Keto 9-cis Retinoic Acid	3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohexen-1-yl) nona-2,4,6,8-tetraenoic acid	$C_{20}H_{26}O_3$	314.4
2	4-Ketoretinoic acid	3,7-dimethyl-9-(2,6,6-trimethyl-3-oxocyclohexen-1-yl) nona-2,4,6,8-tetraenoic acid	$C_{20}H_{26}O_3$	314.4

3	Ellagic Acid	6,7,13,14-tetrahydroxy-2,9-dioxatetracyclo [6.6.2.04,16.011,15] hexadeca 1(15),4,6,8(16),11,13-hexaene-3,10-dione	$C_{14}H_6O_8$	302.19
4	7,8-Didehydroretinoic acid	3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl) nona-2,4,6-trien-8-ynoic acid	$C_{20}H_{26}O_2$	298.4
5	3,4-Didehydroretinoic acid	3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl) nona-2,4,6,8-tetraenoic acid	$C_{20}H_{26}O_2$	298.4
6	Astaxanthin-5,8-peroxide	7-hydroxy-3-[(2E,4E,6E,8E,10E,12E,14E,16E)-17-(4-hydroxy-2,6,6-trimethyl-3-oxocyclohexen-1-yl)-6,11,15-trimethylheptadeca 2,4,6,8,10,12,14,16-octaen-2-yl]-5,5,8a-trimethyl-6,7-dihydro-3H-1,2-benzodioxin-8-one	$C_{40}H_{52}O_6$	628.8
7	Astaxanthin-5,6-peroxide	3-hydroxy-6 [(1E,3E,5E,7E,9E,11E,13E,15E,17E)-18-(4-hydroxy-2,6,6-trimethyl-3-oxocyclohexen-1-yl)-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-1,5,5-trimethyl-7,8-dioxabicyclo[4.2.0]octan-2-one	$C_{40}H_{52}O_6$	628.8
8	2,2' Dihydroxyastaxanthin	(5R,6S)-3-[(1E,3E,5E,7E,9E,11E,13E,15E,17E)-18-[(4S,5R)-4,5-dihydroxy-2,6,6-trimethyl-3-oxocyclohexen-1-yl]-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-5,6-dihydroxy-2,4,4-trimethylcyclohex-2-en-1-one	$C_{40}H_{52}O_6$	628.8
9	Colnelenic acid	E)-9-[(1E,3Z,6Z)-nona-1,3,6-trienoxy] non-8-enoic acid	$C_{18}H_{28}O_3$	292.4
10	Methylenolactocin	4-methylidene-5-oxo-2-pentyloxolane-3-carboxylic acid	$C_{11}H_{16}O_4$	212.24
11	Methyl 4-oxodecanoate	methyl 4-oxodecanoate	$C_{11}H_{20}O_3$	200.27
12	10-Oxoundecanoic acid	10-oxoundecanoic acid	$C_{11}H_{20}O_3$	200.27

13	Methyl 5-oxodecanoate	methyl 5-oxodecanoate	$C_{11}H_{20}O_3$	200.27
14	4-Hydroxybenzaldehyde	4-hydroxybenzaldehyde	$C_7H_6O_2$	122.12
15	Isoboonein acetate	[(4aR,6S,7R,7aS)-7-methyl-3-oxo-4,4a,5,6,7,7a-hexahydro-1H-cyclopenta[c]pyran-6-yl] acetate	$C_{11}H_{16}O_4$	212.24

Table: 31 Compounds identified from the Hexane extract of the fresh samples *H. ponga*

LC/MS Analysis of Acetone Extract from Fresh Seed Samples

A total of ten compounds were identified from the acetone extract of the *Hopea ponga* seeds are presented in Table: 32 and Fig.19. The plant samples revealed the synthesis of Demethylbergenin, Quinic acid, Quercetin, Methyl 2,3-di-O-methyl-alpha-D-glucopyranoside, Ellagic Acid, Gallic acid, etc. The LC-MS chromatogram of acetone extract is shown in Figure. Demethylbergenin is a natural compound that has potential biological properties, including anti-inflammatory, antioxidant, and antitumor effects. Quinic acid (QA) is a cyclohexanecarboxylic acid having the potential for antioxidant, antidiabetic, anticancer, antimicrobial, antiviral, anti-nociceptive, and analgesic effects. Quercetin is a plant flavanol from the flavonoid group of polyphenols. Quercetin has antioxidant properties, which help protect cells from oxidative damage. It can reduce inflammation by inhibiting certain enzymes and pathways and may help regulate blood sugar levels. Quercetin has been investigated for its potential in Alzheimer's disease prevention and is also used as anti-arthritic. Quercetin intake affects mitochondrial function and energy production, and it may promote wound healing.

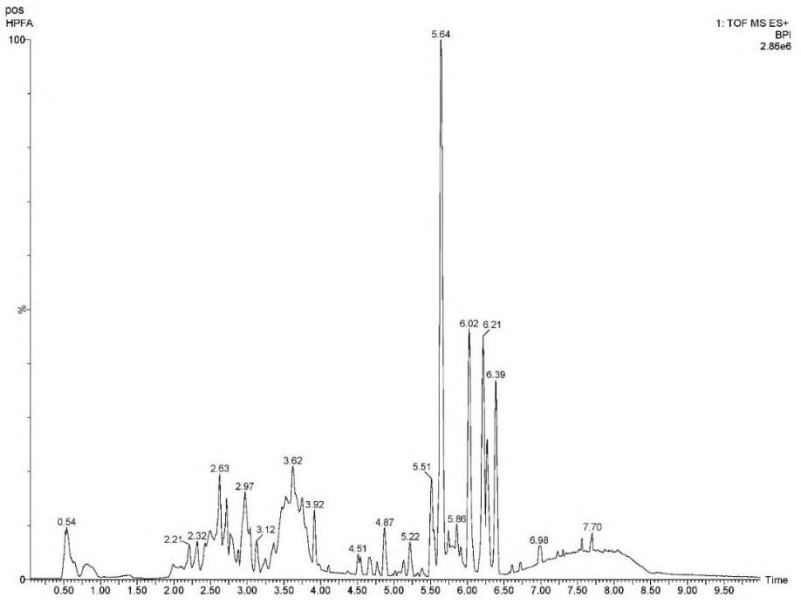
Resveratrol is a natural stilbene compound found in plants. It possesses several interesting biological properties such as antioxidant activity, anti-inflammatory effects, metabolic regulation (helps improve insulin sensitivity, manage blood sugar level, and support weight management), cardiovascular protection, anti-cancer potential, and neuroprotective properties. Methyl 2,3-

di-O-methyl-alpha-D-glucopyranoside is a synthetic derivative of the naturally occurring sugar, glucose. This compound has garnered significant attention in the scientific community due to its diverse and intriguing biological properties. Effectively inhibit the production of pro-inflammatory mediators, such as nitric oxide and prostaglandin E2, in various cell types. This suggests its potential therapeutic application in the management of inflammatory disorders, including arthritis, chronic pain, and certain autoimmune conditions.

Gallic acid, a naturally occurring polyphenolic compound, has garnered significant attention in the scientific community due to its diverse and remarkable biological properties. As a ubiquitous plant-derived substance, gallic acid is found in various fruits, vegetables, and plant-based beverages, making it a prevalent component in our daily dietary intake. N-Acetyl-L-phenylalanine is a chemical compound used as a derivative of the amino acid phenylalanine, this compound has demonstrated a wide range of potential applications in various fields, including medicine, biochemistry, and pharmacology. One of the most notable characteristics of N-Acetyl-L-phenylalanine is its ability to act as a neuroprotective agent. This compound can effectively protect neuronal cells from oxidative stress and apoptosis, making it a potential therapeutic candidate for the treatment of neurodegenerative diseases. Additionally, N-Acetyl-L-phenylalanine has been observed to exhibit anti-inflammatory properties, suggesting its potential utility in the management of inflammatory conditions. This compound has been investigated for its role in regulating metabolism and energy homeostasis.

Dimethyl 2,2-bis(hydroxymethyl)malonate lies in its potential therapeutic applications. It may exhibit antimicrobial and antifungal activities, for the development of novel pharmaceutical interventions. Additionally, its hydroxymethyl functional groups have been explored for their ability to interact with various biological targets, potentially leading to the modulation

of cellular processes and the regulation of specific pathways. Isopropyl beta-D-glucopyranoside, commonly referred to as IPBG, is a synthetic compound derived from the monosaccharide glucose and acts as a selective inhibitor of various enzymes. Its efficacy in inhibiting the activity of several glycosidases, a class of enzymes responsible for the hydrolysis of glycosidic bonds. This inhibitory action has implications in the management of various metabolic disorders, as well as in the regulation of carbohydrate metabolism. Moreover, IPBG has been explored for its potential antimicrobial properties.



Graph: 23 LC/MS profiling of acetone extract of fresh seed samples

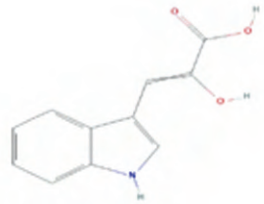
Sl. No.	Compound name	IUPAC name	Molecular Formula	Molecular Weight (g/mol)
1	Demethylbergenin (Norbergenin)	(2R,3S,4S,4aR,10bS)-3,4,8,9,10-pentahydroxy-2-(hydroxymethyl)-3,4,4a,10b-tetrahydro-2H-pyrano[3,2-c] isochromen-6-one	$C_{13}H_{14}O_9$	314.24
2	Quinic acid	(3R,5R)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid	$C_7H_{12}O_6$	192.17
3	Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	$C_{15}H_{10}O_7$	302.23
4	Resveratrol	5-[(E)-2-(4-hydroxyphenyl) ethenyl] benzene-1,3-diol	$C_{14}H_{12}O_3$	228.24

5	Ellagic Acid	6,7,13,14-tetrahydroxy-2,9-dioxatetracyclo [6.6.2.04,16.011,15] hexadeca 1(15),4,6,8(16),11,13-hexaene-3,10-dione	$C_{14}H_6O_8$	302.19
6	Methyl 2,3-di-O-methyl-alpha-D-glucopyranoside (1,2,3-Tri-O-methyl-alpha-D-glucopyranose)	(2R,3R,4S,5R,6S)-2-(hydroxymethyl)-4,5,6-trimethoxyoxan-3-ol	$C_9H_{18}O_6$	222.24
7	Gallic Acid	3,4,5-trihydroxybenzoic acid	$C_7H_6O_5$	170.12
8	N-Acetyl-L-phenylalanine	(2S)-2-acetamido-3-phenylpropanoic acid	$C_{11}H_{13}NO_3$	207.23
9	Dimethyl 2,2-bis(hydroxymethyl)malonate	dimethyl 2,2-bis(hydroxymethyl)propanedioate	$C_7H_{12}O_6$	192.17
10	Isopropyl beta-D-glucopyranoside	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-propan-2-yloxyoxane-3,4,5-triol	$C_9H_{18}O_6$	222.24 g

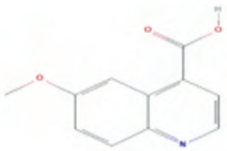
Table: 32 Compounds identified from the acetone extract of the fresh samples *Hopea ponga* seeds

LC/MS Analysis of Methanol Extract from Fresh Seed Samples

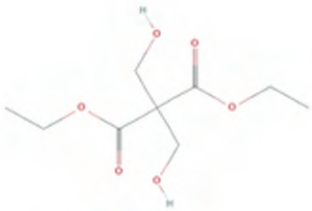
A total of eleven compounds were identified from the methanol extract of the *Hopea ponga* seeds are presented in Table: 33 and Fig. 20. The plant samples revealed the synthesis of Indole 3-pyrubate, Quininic acid, Dimethyl bis(hydroxymethyl)malonate, Quinic acid, Methyl gallate, Polydatin, Ellagic acid, etc. The LC-MS chromatogram of methanol extract is shown in Figure. Indole 3-pyruvate, a naturally occurring compound, has its potential as a neuroprotective agent. It exhibits the ability to modulate neurotransmitter systems, particularly the serotonergic and dopaminergic pathways, which are crucial for maintaining cognitive function and emotional well-being. Furthermore, indole 3-pyruvate has been found to possess antioxidant properties, contributing to its protective effects against oxidative stress-induced neuronal damage. Additionally, indole 3-pyruvate has been investigated for its role in regulating metabolic processes and may play a role in glucose and lipid metabolism, potentially influencing energy homeostasis and potentially contributing to the management of metabolic disorders, such as obesity and type 2 diabetes. Beyond its neurological and metabolic implications, indole 3-pyruvate has also demonstrated promising anti-



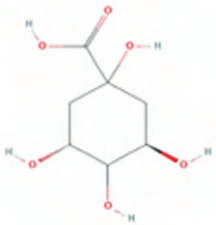
Indole 3-pyrubate



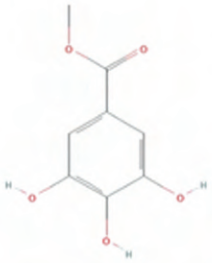
Quininic acid



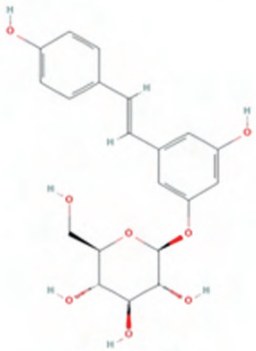
Dimethylbis
(hydroxymethyl)malonate



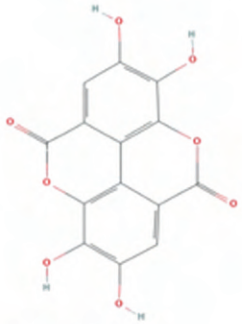
Quinic acid



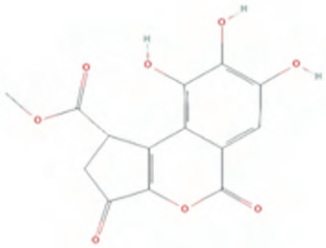
Methyl gallate



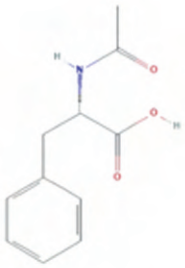
Polydatin



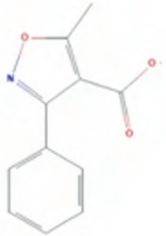
Ellagic Acid



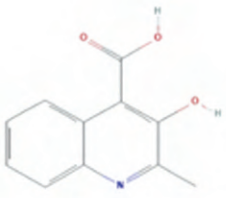
Methyl
brevifolincarboxylate



N-Acetyl-
L-phenylalanine

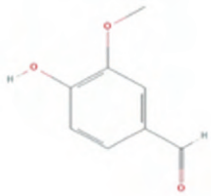


5-Methyl-3-phenyl-1,2
-oxazole-4-carboxylate

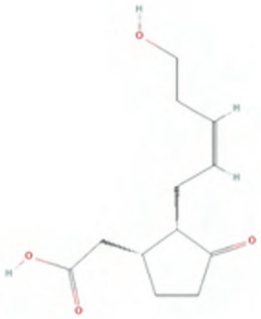


3-Hydroxy-2
-methylquinoline-4-
carboxylic acid

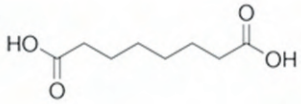
Fig. 20 Compounds present in the methanol extract of the fresh samples *H. ponga*



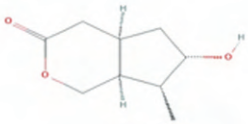
Vanillin



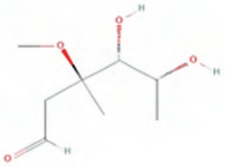
Tuberonic acid



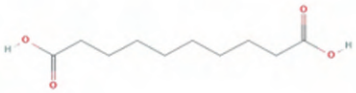
Suberic acid



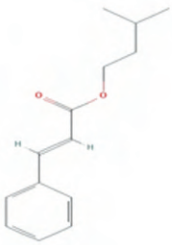
Isoboonein



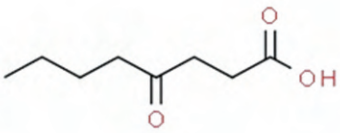
Cladinose



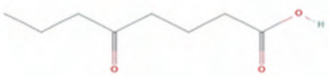
Sebacic Acid



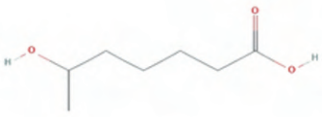
Isopentyl cinnamate



4-Oxooctanoate



5-Oxooctanoic acid



6-Hydroxyheptanoic acid

Fig. 21 Compounds present in the hexane extract of the dried samples *H. ponga*

inflammatory and immunomodulatory properties. Its ability to modulate the activity of various inflammatory mediators and immune cells has garnered attention in the context of various inflammatory and autoimmune conditions. Quininic acid, a naturally occurring organic compound, as a precursor to the well-known antimalarial drug quinine, quininic acid has demonstrated its effectiveness in the treatment of malaria, a global health concern that affects millions of individuals annually. The compound's ability to disrupt the life cycle of the malaria-causing parasite has made it a valuable tool in the ongoing fight against this debilitating disease. Quininic acid has exhibited promising neuroprotective properties, suggesting its potential in the management of neurodegenerative disorders.

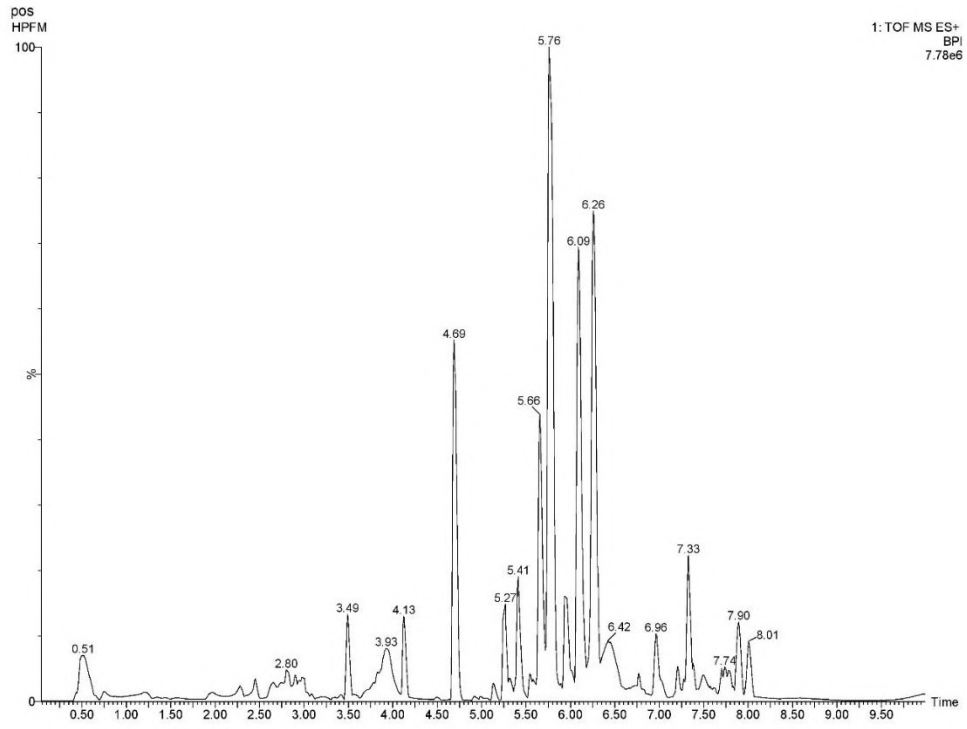
Methyl gallate, a naturally occurring phenolic compound, has potent antioxidant activity. As a free radical scavenger, it can neutralize harmful reactive oxygen species, which are implicated in the development of various chronic diseases, such as cardiovascular disorders, neurodegenerative conditions, and cancer. This antioxidant potential has led researchers to explore the use of methyl gallate as a protective agent against oxidative stress-induced cellular damage. Methyl gallate has exhibited promising antimicrobial properties, exhibiting inhibitory effects against a range of pathogenic bacteria, fungi, and viruses. Polydatin, a naturally occurring glucoside of resveratrol, possesses potent free radical scavenging abilities, effectively neutralizing harmful reactive oxygen species (ROS) that can contribute to oxidative stress and various disease states. This antioxidant capacity has been linked to polydatin's ability to modulate the expression and activity of key antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, thereby enhancing the body's natural defence mechanisms against oxidative insults. Additionally, Polydatin has demonstrated impressive anti-inflammatory properties. It has been found to inhibit the production of proinflammatory mediators, such as cytokines and chemokines, which play

crucial roles in the initiation and propagation of inflammatory responses. This anti-inflammatory activity has made Polydatin a potential therapeutic candidate for the management of various inflammatory conditions, including cardiovascular diseases, neurodegenerative disorders, and autoimmune disorders.

The antioxidant capacity of methyl brevifolincarboxylate is attributed to its unique chemical structure, which allows it to interact with and neutralize reactive oxygen species. In addition to its antioxidant properties, methyl brevifolincarboxylate has also exhibited promising antimicrobial and anti-inflammatory activities. The compound has been found to inhibit the growth of various pathogenic bacteria and fungi, suggesting its potential as a natural antimicrobial agent. Furthermore, its anti-inflammatory effects have been observed in various in vitro and in vivo models, indicating its ability to modulate the inflammatory response and potentially alleviate inflammation-related diseases. 5-Methyl-3-phenyl-1,2-oxazole-4-carboxylate exhibits promising antimicrobial activity, particularly against a wide spectrum of bacterial and fungal pathogens. The underlying mechanisms involve the disruption of essential cellular processes, making this compound for the development of novel antimicrobial agents. Furthermore, the molecule has been explored for its potential in the field of cancer research. Emerging evidence suggests that 5-Methyl-3-phenyl-1,2-oxazole-4-carboxylate possesses antiproliferative properties, demonstrating the ability to inhibit the growth and proliferation of various cancer cell lines.

In addition to its pharmacological applications, 5-Methyl-3-phenyl-1,2-oxazole-4-carboxylate is used as a synthetic intermediate. The unique structural features of this molecule make it a valuable building block in organic synthesis, enabling the construction of more complex molecules and the exploration of diverse chemical transformations.

3-Hydroxy-2-methylquinoline-4-carboxylic acid, heterocyclic compound consisting of a quinoline core with a hydroxyl group at the third position and a methyl substituent at the second position. 3-hydroxy-2-methylquinoline-4-carboxylic acid exhibits potent inhibitory effects against a range of pathogenic bacteria, including both Gram-positive and Gram-negative strains. This attribute makes it is used for the development of novel antibacterial agents, particularly in the context of the growing concern over antibiotic resistance. Additionally, this compound has been investigated for its antiviral properties. Beyond its antimicrobial and antiviral activities, 3-hydroxy-2-methylquinoline-4-carboxylic acid has also demonstrated promising anticancer properties.



Graph: 24 LC/MS profiling of methanol extract of fresh seed samples

Sl No.	Compound Name	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)
1	Indole 3-pyrubate	2-Hydroxy-3-(1H-indol-3-yl)-2-propenoic acid	$C_{11}H_9NO_3$	203.19
2	N-Acetyl-L-phenylalanine	2S)-2-acetamido-3-phenylpropanoic acid	$C_{11}H_{13}NO_3$	207.23

3	Dimethyl bis(hydroxymethyl)malonate	dimethyl 2,2-bis(hydroxymethyl)propanedioate	$C_7H_{12}O_6$	192.17
4	Ellagic Acid	6,7,13,14-tetrahydroxy-2,9-dioxatetracyclo [6.6.2.04,16.011,15] hexadeca 1(15),4,6,8(16),11,13-hexaene-3,10-dione	$C_{14}H_6O_8$	302.19
5	Methyl gallate	methyl 3,4,5-trihydroxybenzoate	$C_8H_8O_5$	184.15
6	Polydatin	(2S,3R,4S,5S,6R)-2-[3-hydroxy-5-[(E)-2-(4-hydroxyphenyl) ethenyl] phenoxy]-6 (hydroxymethyl)oxane-3,4,5-triol	$C_{20}H_{22}O_8$	390.38
7	Quinic acid	(3R,5R)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid	$C_7H_{12}O_6$	192.17
8	Methyl brevifolincarboxylate	methyl 7,8,9-trihydroxy-3,5-dioxo-1,2-dihydrocyclopenta[c]isochromene-1-carboxylate	$C_{14}H_{10}O_8$	306.22
9	Quininic acid	6-methoxyquinoline-4-carboxylic acid	$C_{11}H_9NO_3$	203.19
10	5-Methyl-3-phenyl-1,2-oxazole-4-carboxylate	5-methyl-3-phenyl-1,2-oxazole-4-carboxylate	$C_{11}H_8NO_3$	202.19
11	3-Hydroxy-2-methylquinoline-4-carboxylic acid	3-hydroxy-2-methylquinoline-4-carboxylic acid	$C_{11}H_9NO_3$	203.19

Table: 33 Compounds identified from the methanol extract of the fresh samples *Hopea ponga* seeds.

LC/MS Analysis of Hexane Extract from Dry Seed Samples

A total of ten compounds were identified from the hexane extract of the *Hopea ponga* seeds are presented in Table: 34 and Fig. 21. The plant samples revealed the synthesis of Vanillin, Tuberonic acid, Suberic acid, Isoboonein, Cladinose, Sebacic acid, etc. The LC-MS chromatogram of the hexane extract of the dried sample is shown in Figure. Vanillin, the primary component responsible for the distinctive aroma and flavour of vanilla, is a naturally occurring compound with a diverse range of biological properties. As a result of its widespread use in various industries, including food, cosmetics, and pharmaceuticals, the study of vanillin's biological characteristics has become increasingly important. Anillin possesses potent free radical scavenging abilities, which can help mitigate the harmful effects of oxidative stress on

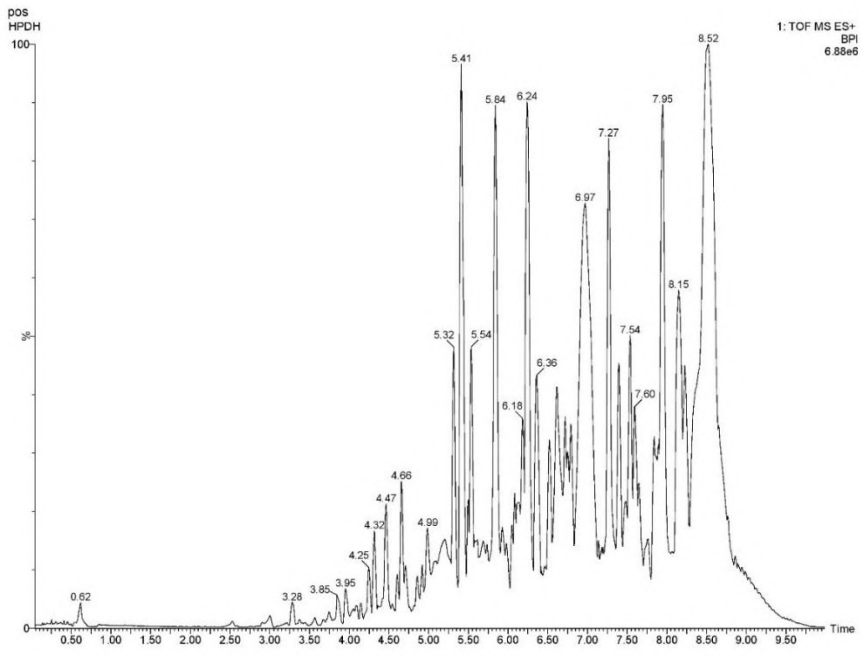
cellular structures and functions. This antioxidant capacity has been linked to potential health benefits, such as the prevention of various chronic diseases, including cancer and cardiovascular disorders. In addition to its antioxidant properties, vanillin has also been shown to exhibit antimicrobial and anti-inflammatory effects. The compound's ability to inhibit the growth of certain pathogenic bacteria and fungi has garnered attention in the development of natural preservatives and antimicrobial agents. Furthermore, vanillin's anti-inflammatory activities may contribute to its potential therapeutic applications in the management of inflammatory conditions.

Tuberonic acid has its role as a plant growth regulator. It can influence the growth and development of various plant species, playing a crucial part in processes such as seed germination, root elongation, and leaf expansion. This ability to modulate plant physiology makes tuberonic acid a valuable tool in the field of agriculture, where it can be utilized to enhance crop productivity and resilience. Tuberonic acid has exhibited promising antimicrobial and antifungal properties. Furthermore, tuberonic acid has demonstrated anti-inflammatory and antioxidant activities, which hold significant implications for human health. These properties suggest the compound's potential in the treatment and management of various inflammatory conditions, as well as its ability to mitigate oxidative stress, a key factor in the development of chronic diseases. Suberic acid, a dicarboxylic acid with the chemical formula $CH_2(CH_2)_6COOH$, is a naturally occurring compound found in various plant and animal sources. One of the primary biological functions of suberic acid is its role in the synthesis of lipids, particularly in the formation of cuticular waxes in plants. These waxes serve as a protective barrier against water loss, pathogen invasion, and environmental stresses, making them crucial for the plant's survival and adaptation. Additionally, suberic acid has been identified as a precursor to produce other valuable compounds, such as adipic acid, a widely used industrial chemical. Furthermore, suberic acid has demonstrated therapeutic potential in the field of medicine.

Cladinose, a unique monosaccharide unit, a versatile compound found in various natural sources, has biological properties and potential applications. One of the primary areas of interest surrounding cladinose lies in its role within complex biomolecules, particularly in the context of bacterial cell walls and glycosylated natural products. The presence of cladinose has been observed in the structure of various antibiotics, conferring them with unique characteristics and functionalities. This potential therapeutic application of cladinose-containing compounds explores their antimicrobial activities and potential as lead compounds in drug development. Sebacic acid, a dicarboxylic acid with the chemical formula $CH_3(CH_2)_8COOH$, is a naturally occurring compound, derived from castor oil, this long-chain fatty acid has found applications in various industries, including pharmaceuticals, cosmetics, and lubricants. One of the primary applications of sebacic acid lies in the field of biomedicine. Due to its biocompatibility and biodegradability, sebacic acid has been extensively used in the development of drug delivery systems and biomedical materials. Its ability to form polymers, such as polyesters and polyamides, makes it a valuable component in the fabrication of implantable devices, wound dressings, and tissue engineering scaffolds. Furthermore, sebacic acid has demonstrated promising antimicrobial properties, making it a potential candidate for the development of antimicrobial agents.

Isopentyl cinnamate, is a potent antioxidant. Isopentyl cinnamate has been found to exhibit anti-inflammatory properties. By modulating the activity of key inflammatory mediators, this compound has the potential to alleviate the symptoms associated with various inflammatory disorders, such as arthritis, asthma, and certain skin conditions. This makes isopentyl cinnamate a promising candidate for the development of anti-inflammatory agents that could provide relief without the adverse side effects commonly associated with traditional pharmaceutical treatments. In addition to its antioxidant and anti-inflammatory properties, isopentyl cinnamate has also been investigated

for its potential antimicrobial and antifungal activities. 4-Oxooctanoic acid belongs to the class of organic compounds known as medium-chain keto acids and derivatives. These keto acids have a side chain with 6 to 12 carbon atoms. 5-oxooctanoic acid, also known as 5-keto-n-caprylic acid, belongs to the class of organic compounds known as medium-chain fatty acids. 6-Hydroxyheptanoic acid is an (ω^{-1})-hydroxy fatty acid, which means it is derived from heptanoic acid by replacing one of the hydrogens at position 6 with a hydroxy group. 6-Hydroxyheptanoic acid may have specific roles in lipid metabolism or signaling pathways, but further research is needed to understand its biological functions fully.



Graph: 25 LC/MS profiling of hexane extract of dried seed samples

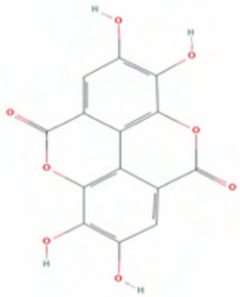
Sl. No.	Compound name	IUPAC name	Molecular Formula	Molecular Weight (g/mol)
1	Vanillin	4-hydroxy-3-methoxybenzaldehyde	$C_8H_8O_3$	152.15
2	Tuberonic acid	2-[(1R,2S)-2-[(Z)-5-hydroxypent-2-enyl]-3-oxocyclopentyl] acetic acid	$C_{12}H_{18}O_4$	226.27
3	Suberic acid	Octanedioic acid	$C_8H_{14}O_4$	174.19

4	Isoboonein	(4aR,6S,7R,7aS)-6-hydroxy-7-methyl-4,4a,5,6,7,7a-hexahydro-1H-cyclopenta[c]pyran-3-one	$C_9H_{14}O_3$	170.21
5	Cladinose	(3S,4R,5R)-4,5-dihydroxy-3-methoxy-3-methylhexanal	$C_8H_{16}O_4$	176.21
6	Sebacic Acid	Decanedioic acid	$C_{10}H_{18}O_4$	202.25
7	Isopentyl cinnamate	3-methylbutyl (E)-3-phenylprop-2-enoate	$C_{14}H_{18}O_2$	218.29
8	4-Oxooctanoate	4-oxooctanoic acid	$C_8H_{13}O_3$	157.19
9	5-Oxooctanoic acid	5-oxooctanoic acid	$C_8H_{14}O_3$	158.19
10	6-Hydroxyheptanoic acid	6-hydroxyheptanoic acid	$C_7H_{14}O_3$	146.18

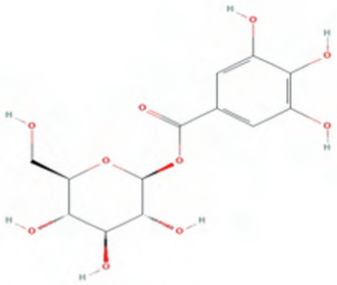
Table: 34 Compounds identified from the hexane extract of the dried samples *Hopea ponga* seeds

LC/MS Analysis of Acetone Extract from Dry Seed Samples

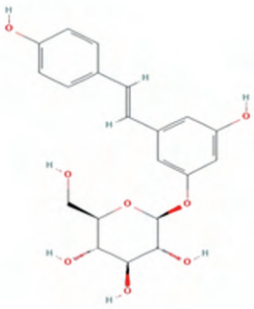
A total of ten compounds were identified from the acetone extract of the *Hopea ponga* seeds are presented in Table: 35 and Fig.22. The plant samples revealed the synthesis of Ellagic acid, Beta-glucogallin, Polydatin, Xanthyletin, Ethyl 2-naphthylacetate, etc. The LC-MS chromatogram of the acetone extract of the dried sample is shown in Figure. β-Glucogallin, also known as 1-Galloylglucose, is a chemical compound formed from gallic acid and β-D-glucose. β-Glucogallin exhibits several pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, cataract prevention, anti-glaucoma, and UV protection. Polydatin is a natural potent stilbenoid polyphenol and a derivative of resveratrol. It exhibits various biological activities by modulating essential signalling pathways related to inflammation, oxidative stress, and apoptosis. The various properties are cardioprotective, anti-diabetic, gastroprotective, hepatoprotective, neuroprotective, anti-microbial, etc. Xanthyletin is a coumarin compound that has antifungal activity, antioxidant properties, anti-cancerous effects, and anti-inflammatory properties. Ethyl 2-naphthylacetate is an ester compound present in various biological molecules and plays essential roles. Isopropyl beta-D-glucopyranoside (also known as 1-O-isopropyl-beta-D-glucopyranoside) is a glycoside that is often involved in various metabolic pathways and cellular functions. Quinic acid (QA) is a cyclohexanecarboxylic



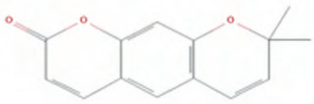
Ellagic Acid



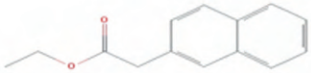
beta-Glucogallin



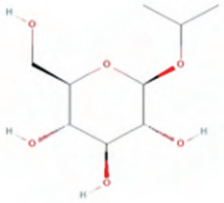
Polydatin



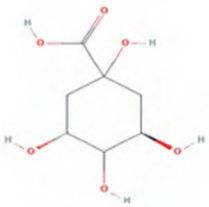
Xanthyletin



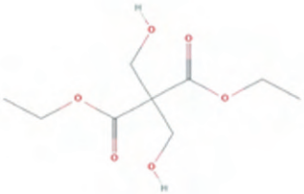
Ethyl 2-naphthylacetate



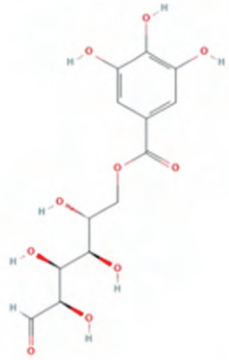
Isopropyl beta-D-glucopyranoside



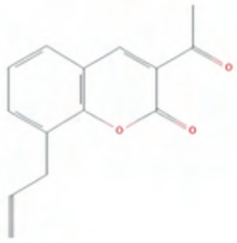
Quinic acid



Dimethylbis(hydroxymethyl) malonate

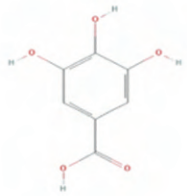


6-O-Galloylglucose

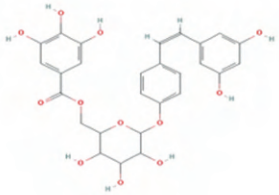


3-Acetyl-8-allyl-2 H-chromen-2-one

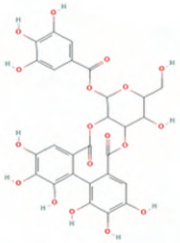
Fig. 22 Compounds present in the acetone extract of the dried samples *H. ponga*



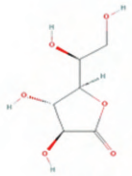
Gallic Acid



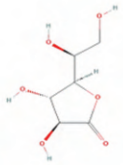
Resveratrol 4'-
(6-galloylglucoside)



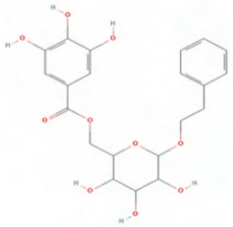
Sanguiin H4



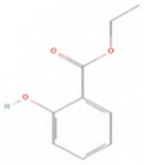
L-Galactono-
1,4-lactone



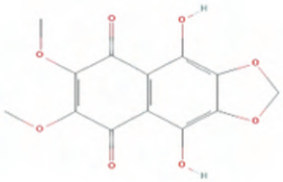
Vitisin C



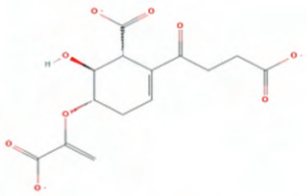
Phenethyl 6-
galloylglucoside



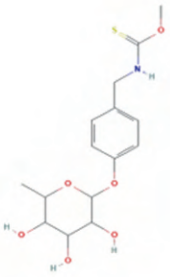
Ethyl salicylate



Tricrozarin A



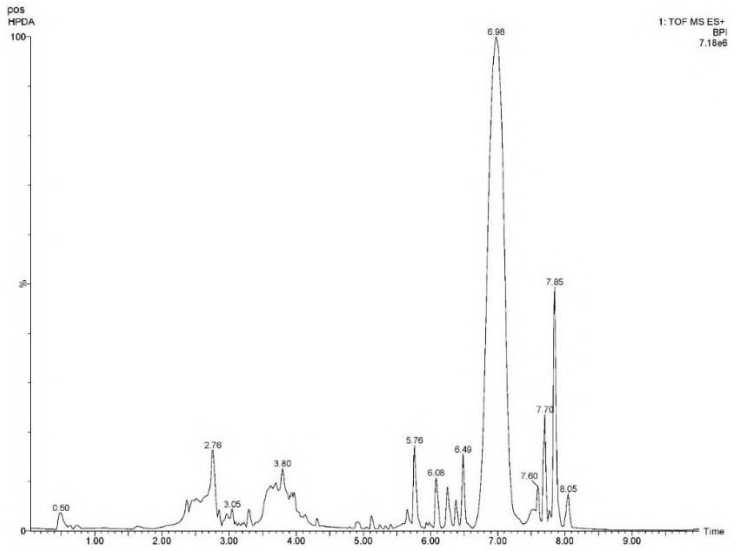
2-Succinyl-5-enolpyruvoyl-6-
hydroxy-3-cyclohexene-
1-carboxylate



Niazinin A

Fig. 23 Compounds present in the methanol extract of the dried samples *H. ponga*

acid found in extracts from various medicinal plants. QA stimulates insulin secretion by mobilizing calcium ions from intracellular reserves and increasing the NAD(P)H/NAD(P)+ ratio. QA promotes apoptosis (programmed cell death) and inhibits activator protein 1 (AP-1) and certain signalling pathways involved in cancer progression. Dimethyl bis(hydroxymethyl)malonate is a compound with the formula $C_9H_{16}O_6$. It has a molecular weight of approximately 220.22 g/mol. 6-O-Galloylglucose, also known as 6-O-galloyl-D-glucose, is a compound with biological properties antioxidant activity, anti-inflammatory properties, antimicrobial effects, and anti-cancer potential. 3-acetyl-2H-chromen-2-one (coumarin) is used as a green, fluorescent dye in luminescent materials, chemical sensors, and biomolecules after modification. It's a biochemical compound used in proteomics research.



Graph: 26 LC/MS profiling of acetone extract of dried seed samples

Sl. No.	Compound Name	IUPAC Name	Molecular Formula	Molecular Weight (g/mol)
1	Ellagic Acid	6,7,13,14-tetrahydroxy-2,9-dioxatetracyclo [6.6.2.04,16.011,15] hexadeca 1(15),4,6,8(16),11,13-hexaene-3,10-dione	$C_{14}H_6O_8$	302.19

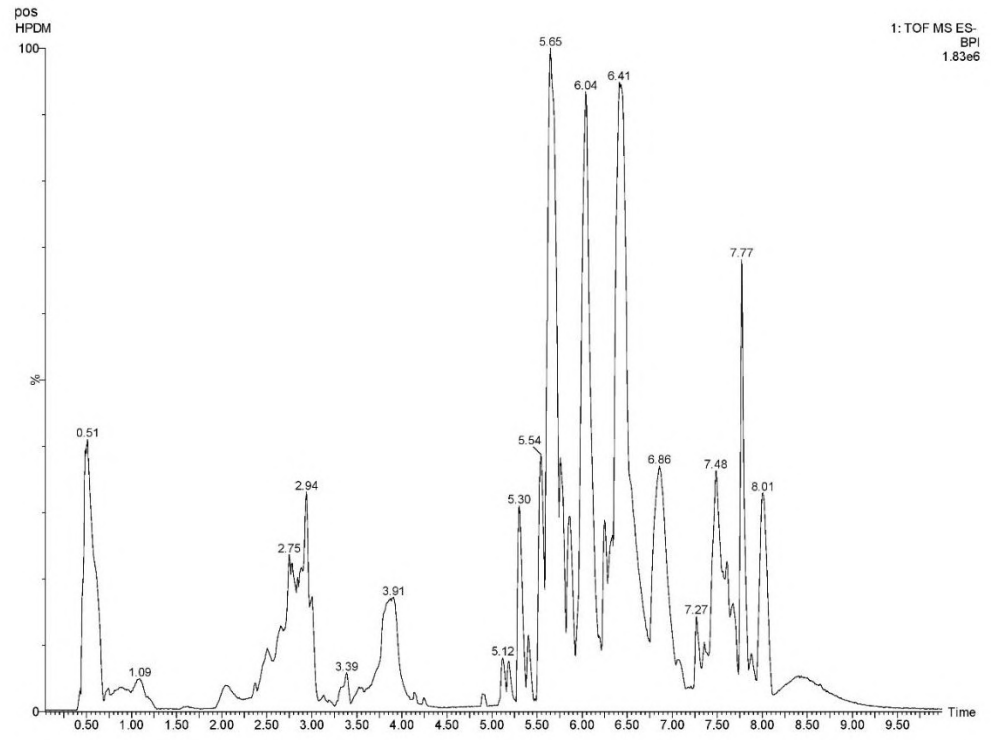
2	beta-Glucogallin	[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3,4,5-trihydroxybenzoate	$C_{13}H_{16}O_{10}$	332.26
3	Polydatin	(2S,3R,4S,5S,6R)-2-[3-hydroxy-5-[(E)-2-(4-hydroxyphenyl) ethenyl] phenoxy]-6 (hydroxymethyl)oxane-3,4,5-triol	$C_{20}H_{22}O_8$	390.38
4	Xanthyletin	2,2-dimethylpyrano[3,2-g] chromen-8-one	$C_{14}H_{12}O_3$	228.24
5	Ethyl 2-naphthylacetate	ethyl 2-naphthalen-2-ylacetate	$C_{14}H_{14}O_2$	214.26
6	Isopropyl beta-D-glucopyranoside	(2R,3S,4S,5R,6R)-2-(hydroxymethyl)-6-propan-2-yloxyoxane-3,4,5-triol	$C_9H_{18}O_6$	222.24
7	Quinic acid	(3R,5R)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid	$C_7H_{12}O_6$	192.17
8	Dimethyl bis(hydroxymethyl)malonate	dimethyl 2,2-bis(hydroxymethyl)propanedioate	$C_7H_{12}O_6$	192.17
9	6-O-Galloylglucose	[(2R,3R,4S,5R)-2,3,4,5-tetrahydroxy-6-oxohexyl] 3,4,5-trihydroxybenzoate	$C_{13}H_{16}O_{10}$	332.26
10	3-Acetyl-8-allyl-2H-chromen-2-one	3-acetyl-8-prop-2-enylchromen-2-one	$C_{14}H_{12}O_3$	228.24

Table: 35 Compounds identified from the acetone extract of the dried samples *Hopea ponga* seeds

LC/MS Analysis of Methanol Extract from Dry Seed Samples

A total of ten compounds were identified from the methanol extract of the *Hopea ponga* seeds are presented in Table: 36 and Fig.23. The plant samples revealed the synthesis of Gallic acid, Resveratrol 4'-(6-galloylglucoside), Sanguiin H4, L-Galactono-1,4-lactone, Vitisin C, etc. The LC-MS chromatogram of the methanol extract of the dried sample is shown in Figure 23. Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound with numerous biological properties such as antioxidant activity, anti-cancer potential, anti-inflammatory effects, and antimicrobial properties. Gallic acid is an astringent in cosmetic preparations and a preservative in food products. It also protects against the harmful effects of UV radiation. Resveratrol 4'-(6-galloylglucoside) is a natural compound classified as a stilbenoid and a glycoside. It has potential health benefits, including anti-cancer effects. Sanguiin H4, a dimer of sanguinarine, has been studied for its potential health benefits as well. The biological properties have antimicrobial

activity, antiviral, anti-inflammatory, anticancer, and osteoclast genesis inhibition. L-galactono-1,4-lactone acts as a precursor, being converted into ascorbic acid through the catalytic action of the enzyme L-galactono-1,4-lactone dehydrogenase. This conversion is essential for maintaining optimal levels of vitamin C in living organisms, which is crucial for a wide range of physiological processes, including immune function, collagen production, and antioxidant protection. It possesses antioxidant and anti-inflammatory properties, which could make it a valuable therapeutic agent in the management of various health conditions. Vitisin C is a compound having molecular formula $C_{56}H_{42}O_1$. Phenethyl 6-galloylglucoside has demonstrated potent antioxidant capabilities. The antioxidant activity of Phenethyl 6-galloylglucoside is attributed to its chemical structure, which includes the presence of a galloyl moiety and a glucoside substituent. Ethyl salicylate, a derivative of salicylic acid, is a chemical compound that has garnered significant attention due to its diverse biological properties. This ester compound, formed by the esterification of salicylic acid with ethanol, exhibits a range of intriguing characteristics that have made it a subject of extensive research and application. Tricrozarin A's potent ability to scavenge free radicals and mitigate oxidative stress within biological systems. It has exhibited promising antimicrobial and antiviral properties. 2-Succinyl-5-enolpyruvoyl-6-hydroxy-3-cyclohexene-1-carboxylate may serve as an intermediate or a regulatory element in the biosynthesis of essential biomolecules, such as certain amino acids or secondary metabolites. The unique structural features of the target compound, including the presence of the cyclohexene ring and the enolpyruvate moiety, have led researchers to hypothesize its potential participation in enzymatic reactions or signalling cascades that are central to cellular metabolism. Niazinin A has a potent antioxidant capacity and antimicrobial activity.



Graph: 27 LC/MS profiling of methanol extract of dried seed samples

Sl. No.	Compound name	IUPAC name	Molecular Formula	Molecular Weight (g/mol)
1	Gallic Acid	3,4,5-trihydroxybenzoic acid	$C_7H_6O_5$	170.12
2	Resveratrol 4'-(6-galloylglucoside)	[6-[4-[(Z)-2-(3,5-dihydroxyphenyl)ethenyl]phenoxy]-3,4,5-trihydroxyoxan-2-yl]methyl 3,4,5-trihydroxybenzoate	$C_{27}H_{26}O_{12}$	542.5
3	Sanguiin H4	[3,4,5,14,20,21,22-heptahydroxy-13-(hydroxymethyl)-8,17-dioxo-9,12,16-trioxatetracyclo[16.4.0.0^{2,7}.0^{10,15}]docosa-1(22),2,4,6,18,20-hexaen-11-yl] 3,4,5-trihydroxybenzoate	$C_{27}H_{22}O_{18}$	634.5
4	L-Galactono-1,4-lactone	(3S,4S,5R)-5-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyoxolan-2-one	$C_6H_{10}O_6$	178.14
5	Vitisin C	5-[(2S,3S)-4-[(E)-2-[(2R,3S)-3-[(2S,3S)-3-(3,5-dihydroxyphenyl)-6-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1-benzofuran-4-yl]-2-(4-hydroxyphenyl)-2,3-dihydro-1-benzofuran-5-yl]ethenyl]-6-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1-benzofuran-3-yl]benzene-1,3-diol	$C_{56}H_{42}O_{12}$	906.9

Fig.24 Conservation activities - a. Saplings raised from germinated seeds, b. Miniature Arboretum (To promote the significance of conservation), c. Planting drive of saplings at Fathima Matha U.P School in Peruvannamuzhi, Kozhikode, d. Planting drive of saplings at GVHSS, Cheruvannur, Kozhikode

6	Phenethyl 6-galloylglucoside	[3,4,5-trihydroxy-6-(2-phenylethoxy)oxan-2-yl]methyl 3,4,5-trihydroxybenzoate	$C_{21}H_{24}O_{10}$	436.4
7	Ethyl salicylate	ethyl 2-hydroxybenzoate	$C_9H_{10}O_3$	166.17
8	Tricrozarin A	4,9-dihydroxy-6,7-dimethoxybenzo[f][1,3]benzodioxole-5,8-dione	$C_{13}H_{10}O_8$	294.21
9	2-Succinyl-5-enolpyruvoyl-6-hydroxy-3-cyclohexene-1-carboxylate	(1R,5S,6S)-5-(1-carboxylatoethenoxy)-2-(3-carboxylatopropanoyl)-6-hydroxycyclohex-2-ene-1-carboxylate	$C_{14}H_{13}O_9^{-3}$	325.25
10	Niazinin A	O-methyl N-[[4-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyphenyl]methyl]carbamothioate	$C_{15}H_{21}NO_6S$	343.4

Table: 36 Compounds identified from the methanol extract of the dried samples *Hopea ponga* seeds

Conservation Activities with Seeds of *Hopea ponga*

The germinated seedlings from the experiments are transferred to a mist chamber, providing an ideal environment for growth. A mini arboretum concept is designed to raise awareness among the public (Fig.24). Along with these, more than 250 saplings of *Hopea ponga* are reared through this program and distributed in schools along with awareness and conservation-oriented classes. Fathima UP School, Peruvannamuzhi, Kozhikode, and GVHSS Cheruvannur, Kozhikode, have started the conservation program, including *H. ponga* as one of their focal species.

DISCUSSION

An extensive field survey explored 35 viable populations *of Hopea ponga*. There are some studies conducted in the Western Ghats regarding *H. ponga*. Sasikumar et al., (2019) collected specimens from the Western Ghats' Wayanad district, while Hari et al., (2022) conducted anatomical characterization of the *H. ponga* leaf and stem with samples taken from the CMS College campus in Kottayam. Irudhyaraj & Ramasubbu (2018) gathered specimens from Iringole Kavu (Kerala) and Tamil Nadu. Chandrashekara & Sankar (1998) identified the *H. ponga* population in S.N. Puram Kavu (Thrissur) and Iringole Kavu (Ernakulam) in Kerala. The current study also includes these locations and reports that the population is decreasing due to social, anthropological, and developmental impacts.

Phenology has achieved a prominent position in current scenarios of global change research, given its role in monitoring and predicting the timing of recurrent life cycle events. However, the implications of phenology to environmental conservation and management remain poorly explored. Plant phenology, the study of recurring events in the life cycle of plants, has gained increasing public and scientific attention over the last few decades. Species within a plant community will likely not respond to climate change using the same phenological strategies, which may have significant consequences for resource (i.e., light and nutrients) competition, food webs, ecosystem productivity, carbon cycling, and other ecosystem functions and services. Plant phenology frequently impacts animal populations by inducing temporal shifts in the availability of resources. Phenological research on *Hopea ponga* encompasses both vegetative and reproductive stages. The studies commence with leaf flushing, identifying and monitoring flowering times, and calculating reproductive efficiency using various reproductive parameters. This includes observing different fruiting phases from development to maturation, premature fruit drops, pest infestations in fruits and flowers, and identifying

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Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

factors contributing to habitat reduction for the species. Such factors are both biological and anthropogenic. For *H. ponga*, leaf flushing begins in the last week of September and continues until December. From the initial tender yellowish-green foliage to the mature dark green leaves, a higher level of infestation is observed, persisting into the mature stage. Numerous irregularities are noted from flower-to-fruit initiation and throughout the flowering to gregarious fruiting stages. Similar patterns are reported in *Vateria indica* by Kamurudeen et al., (2017a) and *Hopea parviflora* by Kamarudeen et al., (2017). Singh & Kushwaha (2005) pointed out that leaf flushing occurs in semi-evergreen species of *Shorea robusta* from March to April. Putri et al., (2020) reported that the flowering and fruiting periods may not occur annually, mainly for Dipterocarpacae species. In the present study, the flowering and fruiting periods are irregular in all 35 locations studied.

In the present study, leaf flushing in *H. ponga* was observed from September to December, and mass flowering and fruiting (February-March), with seeds maturing by the onset of the Southeast monsoon (May-June), due to their short lifespan and sensitivity to desiccation. Though the leaf flushing occurred every year, flowering was observed only at an interval of 2-4 years. Okimori & Permono (1991) investigated the flowering and fruiting phenologies of 18 trees, 11 Dipterocarp species are included in this study such as *Dryobalanops lanceolata, Dipterocarpus cornutus*, *Dipterocarpus humeratus*, *Shorea ovalis*, *S. smithiana*, *S. gibbosa*, *S. parvifolia*, *S. pauciflora*, *S. laevis*, *S. lamellata*, and *Anisoptera costata*. They noticed that flowering and fruiting phases show many variations. The flowering periods of individual trees were between one and three months long, and their fruiting periods were between 42 and approximately 200 days. Their observations were unlikely to miss episodes of flowering and fruiting. Kiyono & Hastaniah (2000) also reported that phenological fluctuations are common in *S. laevis, S. smithiana, Dipterocarpus cornutus, S. pauciflora, S. johorensis, S. parvifolia, S. seminist,*

Dipterocarpus humeratus, S. leprosula, Cotylelobium lanceolatum, S. gibbose, S. ovalis, Dryobalanops beccarii, Dipterocarpus tempehes, Dipterocarpus confertus, Dipterocarpus gracilis, Hopea mengarawan, S. bracteolate, S. lamellate, Vatica javanica var. scaphifolia, Anisoptera costata, Hopea rudiformi, Parashorea sp., and *S. parvistipulata*. Thapliyal & Phartyal (2005) state that the winged seeds of *Adina cordifolia* and *Holoptelea integrifolia* mature in the summer, weeks before the monsoon rains begin. The germination of seeds from these species progressively increases, reaching peak germination during the heaviest rainfall.

In nature, plants are closely associated with a diversity of micro- and macro-organisms, both within and outside their tissues. Animal pollination is a foundational ecosystem service, with an estimated 80% of flowering plants requiring or benefitting from pollination. A diversity of animals provides pollinator services, but most pollinators are insects; bees are the most effective and important of these anthophilic insects. Dyer (1985) reported that *Apis dorsata* is a predominant pollinator in two species of Dryobalanops of Dipterocarpaceae. Similarly, in our study, *A. dorsata* is one of the important pollinators in *H. ponga*. Kobayashi et al., 2017 reported that red-bellied squirrel (*Callosciurus erythrieus*) is pollinating the flowers of *Mucuna macrocarpa*. In *H. ponga*, a three-stripped palm squirrel (*Funambulus palmarum*) is also observed as an efficient pollinator. Numerous insects have been observed feeding on newly sprouted leaves.

Immature fruits suffer insect infestations, leading to a significant decrease in seed availability. According to Ribeiro-Costa & Almeida, (2012), insect consumption of seeds is crucial as seeds are abundant in proteins, carbohydrates, and lipids, providing more nutrients than other plant parts. However, some insects can have detrimental effects by intensely consuming seeds, thereby reducing the availability and viability of seeds. Pests and diseases mainly infested seeds, saplings, trees, and their products. Natawiria

et al., 1986 observed that Curculionidae weevils damaged 40% to 90% of the seeds of *Shorea pauciflora, S. ovalis, S. laevis, S. smithiana,* and *Diperocarpus cornutus*. In *S. macrophylla*, the important pests were recorded as Colytidae (Daljeet-Singh, 1974). Additionally, the impact can be indirect, such as diminishing seedling quality or inflicting damage that leads to the rejection of fruit and/or seeds by dispersal agents. The study by Xu et al., (2015) indicates that pre-dispersal seed predation (PDSP) frequently occurs in woody plants and is acknowledged as a significant factor influencing the variability of seed production, which is vital for effective regeneration. PDSP was observed in 17 of the 44 woody plant species studied, with insect predators accounting for three-quarters of the PDSP events. There was considerable variation in annual seed production at the community level. Gopal et al., (2021) state that seed predation significantly influences the plant population and community structure within forests. The study notes that forest fragmentation and habitat loss impact seed predation, affecting tree recruitment. However, these effects are highly variable and context-dependent, with scant data available from South Asia. The study observed that in *Hopea ponga*, an *Anthonomous* sp. weevil severely predated young and mature fruits.

In *Araucaria angustifolia* seeds, high contamination by fungi was observed during both storage and germination tests. This may be one of the factors related to higher respiratory activity at the end of the storage period of RS provenance and the concomitant loss of seed viability (Gasparin et al., 2020). According to Bewley et al., 2013, Storage fungi may cause several damaging effects in seeds, such as viability loss, discoloration, production of mycotoxins, and heat. In the current study, 6 fungal species have been identified from *Hopea ponga* seeds which cause potential damage at the time of storage and germination.

Galls are the peculiar characteristics of *H. ponga* and it is observed in all populations in the current study. Raman & Takagi (1992) observed and identified the activity of the *Mangalorea hopeae* in induced leaf axil galls of *Hopea ponga*. In *Dipterocarp tuberculatus*, Chrysanthemum flower-like galls are developed on the shoots, and it is induced by *Beesonia diptercarpi* (Raman & Singh, 2014). Takagi & Hodgson, 2005 studies confirmed that galls with elongated overlapping scales are present in different species of Shorea induced by *Gallacoccus anthonyae*.

Survival in the dry state is a result of a series of molecular and cellular processes during seed development that start with the acquisition of desiccation tolerance, defined as the ability to survive a loss of water that rapidly brings the seed moisture content to equilibration with that of ambient air (*i.e.* the relative humidity (RH) of the air). The time component of survival in the dry state originates from the nature and speed of chemical and physical reactions that cause the slow deterioration of seeds during storage, despite the extremely severe limitations in molecular diffusion and motion brought about by the glassy state. That the threshold (desiccation tolerance) and time (longevity) components are acquired separately during seed development implies the existence of different regulatory factors acting sequentially and/or different mechanisms of regulation if the molecular players are identical.

Seeds of *H. ponga* matured at 80 DAA with a high % moisture content of 45.62 ± 1.55% indicating the recalcitrant nature and losing viability within 7 days in open laboratory conditions. Berjak et al., (2004) reported that just after the dispersal, recalcitrant seeds may go to germination, it is because of the highly hydrated state of the seeds (higher amount of moisture). The longevity is short due to the reduction of seed vigour during subsequent storage. Analysis of seed development parameters started at 4 DAA in case of seed length, width, fresh and dry weight, and % moisture content as the seeds were not large enough for measurement till 4 DAA. Similarly, biochemical and

enzyme analysis started at 12 DAA before which sufficient samples could not be obtained due to small size and weight. Seed length, width, and fresh and dry weights followed the typical sigmoid growth pattern of development and reached the maximum maturity at 80 DAA. The parameters of seed development, including the accumulation of dry matter and alterations in metabolite content, etc. are critical and dictate seed storage behaviour.

Key factors in seed development, such as the accumulation of dry matter and shifts in metabolite content, are vital in determining seed storage characteristics. In *H. ponga*, there has been a consistent rise in dry weight accumulation. The peak of dry weight was reached after the maturation phase, indicating that the seeds amassed storage substances throughout their development. Despite a significant reduction in moisture content, it remained above 40%, suggesting that the seeds are recalcitrant. The ongoing increase in dry weight suggests an absence of maturation drying within the seeds. Orthodox seeds exhibit a distinct pattern of development, marked by an increased rate of dry matter accumulation during the second phase, which then transitions into maturation drying. This process results in a substantial decrease in moisture content, as noted by Bewley & Black (1994) and further supported by Coelho & Benedito (2008). The growth habit, natural habitat, and pattern of dry matter accumulation in seeds during their development indicate that *H. ponga* is a recalcitrant seed species, like other Dipterocarpaceae in the tropical Western Ghats. Yulianti et al., (2020) classify both *Hopea sp.* and *Shorea sp.* within the tropical recalcitrant seed category and noted their susceptibility to desiccation and damage at low temperatures.

The deposition of storage substances is one of the key processes of zygotic embryogenesis, providing compounds that will be used from the early stages of embryonic development until autotrophy after germination (Merkle et al., 1995). As sugars may be a major component as biochemical precursors, an understanding of the changes in sugar content during maturation is important.

In the present study, sugar content tended to increase throughout the maturation, and the increase was significant at 1% P level. According to Aguirre et al., (2018), carbohydrates play an important role in seed development; starch is the main polysaccharide stored in seeds. Starch, the carbohydrate reserve in seeds, recorded accumulation during seed development in *H. ponga* till 60 DAA, and later it stabilized and reduced at 80 DAA. The polynomial curve fitted was significant at 0.01% P level and showed a sigmoid pattern. The possible role of the starch reserve is to provide a source during germination. Phenol content recorded a typical sigmoidal pattern as that of starch during seed development in *H. ponga*. Weidnera et al., (2000) reported that both phenolic acids and total phenolic compounds reach the highest levels at the initial stage of development of cereal caryopses and decrease considerably at the final stage of grain maturation. Most higher plants respond to various environmental stimuli by activating secondary metabolic pathways such as phenylpropanoid metabolism. Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) is the first and one of the key regulatory enzymes of this pathway (Hahlebrock & Scheel, 1989).

The increase in the dry weight of the seed during seed development results from synthesizing and depositing storage substances (Table 5&6). The accumulation of storage proteins in conifer somatic embryos is influenced by ABA and water stress, by the addition of an osmoticum into the culture medium (Attree & Fowke, 1993). According to Warsame et al., 2022 in the early growth stages, proteins related to cell growth, cell division, and metabolism were most abundant that are 45 days after pollination. In the present study of *H. ponga*, proteins follow the same pattern. Many of the physiological and biochemical effects brought about in developing embryos by ABA can also be induced by low osmotic potentials (Bewley & Black, 1994). The continuous increase in starch content and increase in dry matter may result in the proportional reduction of osmotic potential (though in the

present work not evaluated). Probably, the increase in starch content during the seed development was due to triacylglycerols breakdown during seed development. Stone & Gifford in *P. taeda* reported changes in starch accumulation (Stone & Gifford 1999), during early seedling growth and the role of triacylglycerols breakdown and subsequent carbohydrate metabolism. It has been suggested that endogenous ABA plays a role in the stimulation of specific storage proteins, in dehydration at the end of development, and in the prevention of precocious germination (Groot et al., 1991).

Tetrazolium reduction activity is effectively used to calculate seed viability. At the time of incubation, there is a correlation developed between formazan activity and moisture content. Embryos of the fresh seeds stained fast and developed a uniform deep red colour. But in the case of desiccated seeds the staining or intensity of staining is predominantly reduced. This finding in *H.ponga* is in line with the findings of Anilkumar et al., 2002 done in *Myristica malabarica*. Similarly, Varghese & Naithani (2000) calculated the seed viability rate for the *Azadiracta indica* tree. In the case of micro-seeds of the endangered plant, *Deinanthe caerulea* TTC staining method is used (Xu et al., 2024). According to Green et al., 1998, the chlorophyll content is negatively correlated with the moisture content of the seeds. Here, in the case of *H. ponga*, chlorophyll content is reduced by the progress of the desiccation period because a maximum value of total chlorophyll was recorded at 0 hours of the desiccation period, and a lower value is observed on the tail end of the 168th hour of desiccation. In *H. ponga* the leachate conductivity shows a significant increase, and it was correlated with a decrease in moisture content and germination. Generally, electrolyte leakage is measured to check the seed viability and its desiccation sensitivity (Becwar et al., 1982; McKersie & Stinson, 1980).

Enzymes that scavenge reactive oxygen species are crucial for protecting cell membranes from free radicals generated during metabolism impairment due

to desiccation stress. According to King & Roberts, 1979, the altered metabolism observed in the ageing of seeds leads to the loss of seed viability which is attributed to the reduced enzyme activity and breakdown of macromolecular structures. Enzymes like peroxidase and polyphenyl oxidase play a vital role in eliminating harmful products from the degradative reactions thereby preserving seed viability. An increase in antioxidant enzymes like PO, PPO and CAT plays a role in protecting the embryo and embryonic axis from free radicals produced during desiccation and exerts pressure in protecting the viability of the seeds. After a certain period when the desiccation is high, the metabolism is also affected, thereby, a reduction in these enzymes. Similar reports have been noted in the seeds of *Mesua ferrea* and *Elaeocarpus tuberculatus* by Babu (2015).

During desiccation, the total sugar content was increased; it may be due to hydrolyses of starch, which was supported by the gradual decrease of starch content noted during the desiccation. The pattern of reduction in the starch content is justified by Bewley & Black, (1994) and Berjak et al., (1989). Nkang et al. (2003) conducted a study on the metabolites *of Telfairia occidentalis*, revealing that the starch content diminishes as the soluble carbohydrates rise periodically. This is a valuable understanding of the metabolic shifts in this tropical plant, which may illuminate its growth patterns, nutritional profile, and potential uses. A similar pattern is depicted in Chandel et al., 1995 as in the case of *Theobroma cacao* and *Atrocarpus heterophyllus*. The protective function of sugars in desiccation has been explored in terms of total soluble sugar content and specific sugars by numerous researchers, including Koster & Leopold (1988) and Leprince et al., 1993. The total soluble sugar content increases gradually from 0 to 168 hrs. in *H.ponga* seeds during desiccation. It is inversely correlated with starch, lipid, and amino acid content and directly correlated with total phenol content. According to Bernal-Lugo & Leopold, 1995 stated that sucrose plays a key

role in maintaining the liquid crystalline structure of cell membranes in the dry state. Together with sucrose, raffinose contributes to the vitrification process that takes place in the living systems during the time of cell dehydration. In the present study, sucrose content is increasing gradually, and the major contributor to the vitrification process, raffinose, was absent throughout the desiccation period. This may lead to the viability loss.

Seed germination is a critical phase in the plant life cycle, affected by multiple factors such as moisture content and the duration of desiccation. The correlation between moisture content and seed germination has been extensively studied, offering a significant understanding of the physiological and molecular mechanisms that play.

Bareke (2018) discussed the significance of moisture content in seed development and germination physiology. The author highlighted the importance of optimal moisture levels for successful seed germination. The present finding emphasizes the critical role of moisture content in providing the necessary hydration for the activation of metabolic processes during germination. Furthermore, the study underscored the impact of moisture content on seed viability and the ability of seeds to withstand desiccation.

Sukesh & Chandrashekar (2011) observed that *H. ponga* seeds stored in open conditions lost their viability within 11 days. Generally, the seeds of Dipterocarpaceae species are short-lived and sensitive to desiccation, which is why the failure of the monsoon plays a crucial role in significantly reducing *H. ponga* seed germination. The current study reported that seed viability in open conditions was lost within 7 days of storage. Additionally, irregular flowering and fruiting patterns lead to off-season fruit maturation, and the monsoon failure contributes to a substantial decrease in germination and seedling establishment.

The study confirmed that the seed was recalcitrant, losing its viability shortly after storage. Additionally, germination studies indicated that for *H. ponga*,

seed germination begins immediately post-detachment, with a 98% rate, decreasing to 62% after 72 hours. These findings on the seed biology of *H. ponga* differ from those of Sukesh & Chandrashekar (2011), where the germination percentage was slightly higher. Previous reports indicated that the germination of *H. ponga* fails when the critical moisture content drops below 27% (Dayal & Kaveriappa, 2000) this current study aligns with that threshold.

Satyanarayana et al., (2011) studied the biochemical changes occurring during the seed germination of *Sterculia urens*. It examines the levels of proteins, total amino acids, reducing sugars, total soluble sugars, and lipids across different stages of seed germination. The seeds of *S. urens* are abundant in proteins, lipids, and carbohydrates, which diminish in quantity as germination advances, signifying their crucial function in the development of the embryonic axis. In *H. ponga*, all biomolecules such as Total Soluble Sugars, Starch, Total Phenol, Lipids, and Amino acids are abundant at the initial stage of germination but decrease as germination progresses, and these may be used for the initial development of seedlings. Similarly, the content of Albumin, Globulin, and Prolamin also diminishes gradually through the stages of germination. While the 0.05M NaOH fraction of Glutelin decreases, the 0.25M NaOH fraction does not follow this pattern.

During imbibition, the amino acid levels in *Hopea ponga* seeds consistently increased. These findings align with Heldt's observations in 1999, who proposed that germination initiates with the degradation of storage proteins into amino acids. These amino acids are then used to synthesize enzymes that mobilize storage molecules. Waterworth et al., 2001 also noted that the enzymatic breakdown of storage proteins during germination leads to the production of amino acids supported by the increase in the protease enzyme activity during the initial germination period, followed by *H. ponga* seed germination. These amino acids are then translocated to provide organic nitrogen to the germinating embryo.

Yue et al., 2019, state that alpha-amylase (AMY) and beta-amylase (BAM) are crucial in starch degradation, which is central to carbohydrate metabolism and significantly influences various biological processes such as growth, development, and stress response in *Camellia sinensis*. The present study indicates that starch degradation occurs during germination, and the activity of both α-amylase and β-amylase increases up to 96 hours of germination. Later, a decrease was noted; by then, the reserved proteins decreased as germination progressed.

In *Hopea ponga*, protease activity increased in the cotyledon and embryonal axis from 0 to 96 hours of imbibition. In plumule, it increases till 72 hours to 96 hours of imbibition. The result of the present study agrees with the result reported by Misra & Kar (1990), who suggested that a rapid rise in protease activity accompanies the most rapid degradation of storage proteins. Gupta & Mukherjee (1988) also reported that the protease activity increased during the germination of *Albizzia* seeds. Typically, storage proteins are hydrolyzed by proteolytic enzymes, which supply nutrients essential for the growth and development of seedlings (Wang et al., 2012; Rahman et al., 2007). Botcha & Prattipati (2020) observed a consistent pattern of changes during the seed germination of *S. urens*, where there was a gradual increase in proteolytic activity in the initial stages, a significant increase after that, and a subsequent decline in activity.

Kamerhe et al. (2012) found that in the germinating seeds of *Pyrus glabra* (wild pear), catalase activity rises during the initial stages of imbibition and falls in the final phase. *Hopea ponga*, recorded a steady climb in catalase activity within the seed cotyledons from 0 to 48 hours of imbibition. In the embryonic axis, catalase activity increased from 24 to 48 hours, then decreased from 72 to 168 hours of imbibition. Moreover, the plumule tissues of the seeds showed reduced catalase activity from 72 to 168 hours of imbibition.

SOD exhibits a similar pattern to Catalase in *Hopea ponga*. Reactive oxygen species (ROS) are critical molecules central to plant signalling, playing roles in alleviating seed dormancy and germination. These molecules interact with seed hormones such as ABA and GA, influencing the ABA/GA signalling pathways. They also serve as environmental sensors, allowing seeds to germinate or stay dormant in response to environmental cues. Pergo & Ishii-Iwamoto (2011) proposed that the combined activities of SOD and CAT are essential in preserving the oxidative status of seeds during germination. Both the SOD and CAT in the present study also reported an increase during germination may be preserving the oxidative status of the seed by scavenging the free radicles produced during the metabolic process.

Hopea ponga fresh or fast-dried seeds did not survive exposure to liquid nitrogen for even 24 hours. Cryopreserving whole or excised embryos is an efficient, convenient, and cost-effective method for conserving germplasm outside of its natural habitat (Engelmann, 2004). Various factors influence the metabolic imbalance that occurs during cryo-storage. Therefore, further studies should explore different moisture contents and drying rates to conserve the seeds effectively. Alternatively, storing the excised embryonic axis in liquid nitrogen is also viable. Pammenter & Berjak (2014) state that mild drying can inhibit germination during storage (sub-imbibed storage) and cause a quicker loss of viability. Cryopreservation is used for long-term storage of germplasm from species with recalcitrant seeds, which necessitates partial drying to avert ice crystal damage. The outcome of drying, however, is contingent on the rate of drying: slow drying leads to loss of viability at higher water contents, while rapidly dried material can endure (temporarily) to water contents sufficiently low to allow for vitrification of the remaining intracellular water, thus preventing ice crystal formation, provided the cooling rates are sufficiently fast. But here, fast-dried *H. ponga* seeds did not retain viability after one day of storage.

Soluble sugars typically exhibited an increasing pattern throughout storage, although seed viability declined. No significant differences were noted when seeds were stored at low temperatures (0°C, -80°C, -20°C, and -196°C). Similarly, the sugar content in seeds stored at 5°C and 10°C showed no variation compared to fresh seeds. The sugar accumulation during storage could be attributed to the hydrolysis of starch in moist conditions. At low temperatures, seeds might experience extreme chilling stress, potentially causing intra-cellular ice formation, leading to mechanical cell damage, membrane collapse, and consequent solute leakage. These impacts might exceed the protective capacity of sugars, even at elevated concentrations. These observations indicate that sugars alone cannot confer desiccation tolerance to stored tissues.

In seeds, lipids are stored as triacylglycerols (TAGs), produced through an extension of the membrane-lipid biosynthetic pathway ubiquitous across plant tissues. Unlike the conserved fatty acid (FA) composition of membrane lipids, there is a significant divergence in the acyl chain composition of seed oils across various species (Voelker & Kinney, 2001). In *Hopea ponga* seeds during storage, the lipid content remains stable after 24 hours of storage at -80, -20, 0, 5, 10, and -196°C. However, a rapid decline is observed after 7 days of storage at 20±2°C and 28±2°C in open trays. Seeds stored at 20±2°C in sealed polycarbonate bottles show slightly decreased lipid content. The rate of lipid reduction is more pronounced at 28±2°C, regardless of whether the seeds are in polycarbonate bottles or polyethylene bags.

At 20±2°C in polycarbonate bottles, there is a gradual increase in total soluble sugars within both the embryo and cotyledon. The embryo exhibits a relatively higher rate of increase, likely due to its high initial sugar content. Concurrently, as sugar content increases, starch content shows a decreasing pattern. Kumar et al., (2011) observed a similar pattern in *Pongamia pinnata* under varying storage conditions, akin to *Hopea ponga*, where total soluble

sugars increased gradually while total soluble proteins and enzyme activity decreased over the storage period in all treatments (4°C and 20°C). Lipid peroxidation levels rose alongside a decline in seed viability and vigour (Shahzad et al., 2000), suggesting membrane destruction due to lipid peroxidation during deterioration. The current study similarly notes an increase in lipid peroxidation corresponding with reduced seed viability across all storage conditions.

Phenolics, a diverse group of secondary metabolites, influence metabolic processes such as respiration, ATP synthesis, and the activity of plant growth regulators. No significant change in phenolic content was observed after 24 hours of storage at temperatures of 0, 5, 10, -80, -20, and -196°C. However, seeds of *H. ponga* stored at 20±2°C exhibited a linear increase in phenolic content as storage time progressed. This general rise in phenol content could be linked to the loss of seed viability due to inhibited metabolic activities. Additionally, the increase in phenolic content might result from the reduced availability of oxygen in sealed containers, which diminishes polyphenol oxidase activity. These findings are consistent with Sgherri et al., (2004), who studied the role of phenolics in the antioxidative status of *Ramonda serbica* seeds during dehydration and rehydration, indicating potential new phenolic synthesis during catabolic reactions in storage. Similarly, Taylor et al., (1988) reported viability loss in cabbage, and the current results in *H. ponga* align with these observations.

The protein content in *Hopea ponga* seeds is higher in the embryo than in the cotyledons. During storage, a decrease in protein content leads to the degradation of structural and enzymatic proteins, causing a loss of viability. Lower temperatures exacerbate protein degradation and halt protein synthesis. An optimum storage temperature of 20±2°C for *H. ponga* seeds may minimize such degradation. Nautiyal et al., (1985) observed that proteins with high

mobility in *Shorea robusta* are denatured with age. Chaitanya et al., (2000) also noted a similar declining pattern in the protein content in *S. robusta*.

According to Kalemba & Pukacka (2007), Late embryogenesis abundant (LEA) proteins are synthesized in seeds during maturation and in response to various stressors. Their production is either developmentally regulated or triggered by conditions such as drought, salinity, cold, oxidative stress, and abscisic acid (ABA). The protective roles of LEA proteins have been extensively studied in numerous plant seeds, encompassing orthodox, recalcitrant, and intermediate types. Martinez et al., (2019) designed to assess how fruit maturity and postharvest storage affect the physiological properties of chilli seeds during development. They observed the expression of LEA proteins and associated this with the lower molecular weight proteins, as indicated by bands in SDS-PAGE. Vidigal et al., (2009) also evaluated the impact of fruit maturity and post-harvest storage on the physiological quality and LEA protein activity in *Capsicum annuum* L. (sweet pepper) seeds. The presence and expression of LEA proteins were linked to the presence and intensity of bands in SDS-PAGE. Similarly, in *Hopea ponga*, fruits were collected at 44, 60, and 80 DAA, and the seeds were stored for 30 to 240 days post-harvest. The band intensity increased from 44 DAA to 80 DAA, but diminished gradually during storage, disappearing completely by the 240th day. Various studies, including those by Galau et al., (1987), Kermode (1997), Walters et al., (1997), Faria et al., (2004), and Rosa et al., (2005), have shown that mature embryos have high levels of LEA proteins, which are believed to play a role in developing desiccation tolerance during the final phases of seed maturation.

In the present study, the major compounds recognized from the LC/MS analysis were 4-Keto 9-cis Retinoic acid, ellagic acid, and resveratrol. Sasikumar et al., 2019, isolated and characterized the resveratrol oligomers from the stem bark of *Hopea ponga* acetone and ethanol extracts of the stem

bark of *Hopea ponga* (Dennst.) Mabb. afforded six resveratrol oligomers. *E*-resveratrol, *ε*-viniferin, *α*-viniferin, trihydroxyphenanthrene glucoside (THPG)**,** vaticaphenol A, hopeaphenol, along with four phytosterols. Rose et al., (2013) documented the antioxidant and antibacterial properties of methanol and aqueous extracts of *H. ponga*. Plants of the Dipterocarpaceae family are known to elaborate mainly on resveratrol oligomers (Keylor et al., 2015). Resveratrol oligomers from Dipterocarpaceae have diverse biological activities, including antibacterial (Zetterstrom et al., 2013), anti-HIV (Dai et al., 1998), anti-inflammatory (Annabi et al., 2012), and anti-proliferative (Empl et al., 2014) properties. *H. ponga* was reportedly used in traditional medicine for treating diabetes, piles, and snake bites (Muralikrishnan & Chandrashekar, 1997). Beyond these, detailed phytochemical and pharmacological evaluations of *H. ponga* have not been reported.

Many species have been found to contain ellagitannin-forming dilactone, ellagic acid, in their leaves and seeds. Even now, it has been possible to isolate bergenin, a remarkable gallic acid derivative, from individuals belonging to the genera Dipterocarpus, Shorea, Stemonoporus, and Vateria (Bhrara & Seshadri, 1966; Desai, 1967; Bandaranayake et al., 1977). Both substances will probably demonstrate favorable chemical properties of the Dipterocarpaceae family. Hopea-phenol, a phenolic component found in barks and heartwoods that is currently only known to exist in Hopea and Shorea species, may exhibit similar characteristics (Sutopo & Noviany, 2009). It was demonstrated that four molecules of the trihydroxystilbene resveratrol condense to form hopea-phenol. It shares chemical similarities with *Vitis vinifera*'s phytoalexins of the viniferin type. About the components of phenolic leaves, the frequency of vicinal trihydroxylation (Ellagic acid, Gallic acid, B-ring in the flavonoids myricetin and prodelphinidin) in dipterocarps was emphasized by Bate-Smith & Whitmore, 1959. As far as research has allowed, stearic and oleic acid predominate strongly in Dipterocarpaceae seed fats

(oils). Recent research has revealed that Sal fat, or the oil from *Shorea robusta* seed kernels, also contains trace amounts of 9,10-epoxy stearic acid (Reddy & Prabhakar, 1987). While focusing on substances of chemotaxonomic interest elsewhere, Bate-Smith & Whitmore (1959) investigated the phenols of fresh leaves in 28 species across 8 genera. Although grouping the Shorea species according to their established sections was impossible, Neobalanocarpus was found to closely resemble Hopea. A grouping of genera was determined based on the leucoanthocyanins present and their abundance, which little reflected grouping established by traditional means.

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MITHUN VENUGOPAL, PhD THESIS
Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

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List of Publications

Research Papers

- **Mithun V**, Pradeep NS, Krishnan PN. Physiological and Biochemical Changes during Seed Development in *Hopea ponga* (Dennst.) Mabberley: An Endemic Endangered Tree Species of Western Ghats. *Journal of Plant Science and Research*. 2020;7(2): 193.
- **Mithun V,** Babu KP, Pradeep NS, Krishnan PN. Phenology and Seed Development in *Mesua ferrea* L., A Rare Medicinal Tropical Tree Species. *Tropical Plant Research.* 2020;7(2): 406–414.
- **Mithun V,** Pradeep NS, Krishnan PN. Seed Storage Studies in *Mesua ferrea* L. A Medicinal Tree of Indo-Malayan Region. *Plant Science Today*. 2021;8(3): 712–716.
- **Mithun V,** Pradeep NS, Krishnan PN. Biochemical Changes During the Seed Storage of *Mesua ferrea* L., A Medicinal Tropical Tree Species. *Annals of Plant Sciences*. 2023;12(3): 5773-5782.
- Ranjith AP, Quick DLJ, **Mithun V**, Pradeep NS, Butcher BA, Priyadarsanan DR. Two New Species of Conobregma van Achterberg, 1995 (Hymenoptera: Braconidae, Rogadinae) from India and Thailand. *Journal of Asia-Pacific Entomology*. 2024;27: 102213.

Books

- **Mithun Venugopal**, Pooja Pushkaran, NS Pradeep, S Pradeep Kumar (2022) Biodiversity Conservation. KSCSTE- Malabar Botanical Garden and Institute for Plant Sciences, Calicut, Kerala, ISBN: 978-81-931285-5-8.
- NS Pradeep, R Ansari, **Mithun Venugopal**, S Pradeep Kumar (2023) Field Guide for Aquatic Plants. KSCSTE- Environment Impact Assessment Capacity Building Program (EIACP) Hub, KSCSTE, Kerala, ISBN: 978-81-931285-7-2.

Book Chapter

- **Mithun Venugopal**, Pradeep Kumar S, Pradeep NS (2023) Conservation of RET Plants: Strategies and Plans. In: Sukumaran, S.T., T R, K. (eds) Conservation and Sustainable Utilization of Bioresources. Sustainable Development and Biodiversity. Springer, Singapore.

Papers in Seminars/Conference Proceedings

- **Mithun V**, Pradeep NS & Krishnan PN. 2018. Phenology and seed characterization of *Mesua ferrea* L. a rare medicinal tree species. In: International Biodiversity Congress, Forest Research Institute, Dehradun, India.

- **Mithun V**, Pradeep NS & Krishnan PN. 2019. Seed Characterization of *Vatica Chinensis* L., A Critically Endangered Tree Species and Its Conservation. In: XL11 All India Botanical Conference of the Indian Botanical Society and National Symposium on Innovations and Inventions in Plant Science Research, University of Calicut, Kerala.
- **Mithun V**, Pradeep NS & Krishnan PN. 2022. Phenological and Biochemical Changes During Seed Development in *Hopea ponga* (Dennst.) Mabberley: An Endemic Threatened Tree Species of Western Ghats. In: International Conference on Recent Innovations in Biological Research (ICRIBR-2022) March-17&18.
- **Mithun V**, Pradeep NS & Krishnan PN. 2022. Phenology, Seed Development and Seed Storage in *Mesua ferrea* L., A Medicinal Tree of Indo-Malayan Region. In: 34th Kerala Science Congress, Trivandrum. Kerala State Council for Science, Technology and Environment, Kerala,385. Available at: 34th-KSC-Proceedings-1.pdf (kerala.gov.in)
- **Mithun V**, Pradeep NS & Krishnan PN. 2023. Seed Development, Storage, and Predation of *Hopea erosa* (Bedd.) Slooten, A Critically Endangered Tree Species of Western Ghats. In: 35th Kerala Science Congress, Idukki. Kerala State Council for Science, Technology and Environment, Kerala, 264-265. Available at: 35KSC_f.pdf (kerala.gov.in)
- **Mithun V**, Pooja P, Pradeep NS & Krishnan PN. 2024. Phenology, Seed Development, Seed Germination and Seed Characterization in *Humboldtia brunonis* Wall., A Small Tree Species Endemic to Southern India. In: 36th Kerala Science Congress, Kasaragod. Kerala State Council for Science, Technology and Environment, Kerala, 168-169.

SUMMARY & CONCLUSION

The Western Ghats of India are broadly divided into three subdivisions: the northern, central and southern, separated by the Goa Gap and the Palghat Gap, respectively. Due to their climatic stability and terrain ruggedness, they are a museum and cradle of species diversity. Over the years, a series of studies on various habitats in the Western Ghats have shown how drought, rainfall, and elevation influence evolutionary processes and play an important role in shaping this rich diversity. Habitat preservation and restoration are vital to conserve and enhance biodiversity, which can boost the various ecosystem services they provide. Trees constitute these forests' primary structural and functional foundation and can be reliable indicators of ecological changes and stressors on a landscape level. The spatial heterogeneity of diversity could stem from underlying patterns or processes like environmental variability, biotic regulation, and the interaction between abiotic and biotic factors.

This self-sustained equilibrium is now very much concerning due to the immense pressures from various sources, especially those of anthropogenic origin. Over-exploitation of the resources, felling, recreation, and all such activities caused the regeneration potential and, hence, dwindling of the flora in the region. Both *in-situ* and *ex-situ* conservation practices are paramount for preserving the germplasm that has existed here from the past. Many of the endemic, threatened, and economically important tree species of Western Ghats produce recalcitrant seeds that cannot be stored longer than orthodox seeds. Many Dipterocarpaceae species produce such seeds, and Hopea species are no exception.

Of the ex-situ conservation practices, the storage of intact seeds demands less space, convenience, and economic efficiency. However, a better understanding of the storage behaviour of the seeds is imperative. Therefore, careful and planned strategies should be developed to store the seeds successfully. Storage behaviour of the seeds is determined by multiple factors

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Mithun Venugopal. Seed biology of Hopea ponga (Dennst.) Mabb.(Dipterocarpaceae): An endemic and threatened tree species of the western ghats. Thesis.2024. KSCSTE-Malabar botanical garden and institute for plant sciences Calicut. University of Calicut.

from flower initiation to harvest. The seeds are classified into recalcitrant, intermediate and orthodox types based on the storage behaviour. Moreover, for the effective storage of the seeds, careful handling of the seeds is essential as the decrease in moisture content reduces viability unpredictably.

Understanding seed germination and seedling establishment is crucial for successfully restoring endangered tropical forests that produce recalcitrant seeds. The ecological needs at the seed stage encompass knowledge of seed viability, dormancy, germination patterns, and seedling behaviour of tropical tree species.

The objectives of the present study are (a) to the Seed biology of the selected species, *Hopea ponga* on the line of phenology, seed development, desiccation, and storage behaviour, (b) To analyze the physiological changes including synthesis and accumulation of metabolites and their role in the development of desiccation sensitivity or tolerance and (c) To compare the role of specific sugars, Late embryogenesis abundant protein (LEA protein), phenols, lipid peroxidation, and the role of oxidative enzymes and the proteins in desiccation sensitivity or tolerance.

An extensive field survey explored 35 viable populations of *Hopea ponga*, concluding that the population is decreasing due to social, anthropological, and developmental impacts. In the present study, leaf flushing in H. ponga was observed from September to December, and mass flowering and fruiting (February-March), with seeds maturing by the onset of the Southeast monsoon (May-June) due to their short lifespan and sensitivity to desiccation. Though the leaf flushing occurred every year, flowering was observed only at an interval of 2-4 years.

A diversity of animals provides pollinator services, but most pollinators are insects; the most effective and important of these anthophilic insects are bees and *Apis dorsata* is one of the important pollinators in *H. ponga*. Additionally, a three-stripped palm squirrel (*Funambulus palmarum*) is observed as one of

the efficient pollinators. Immature fruits suffer insect infestations, leading to a significant decrease in seed availability of *H.ponga*. The study observed that in *Hopea ponga*, an *Anthonomous sp.* weevil severely predated young and mature fruits. Storage fungi may cause several damaging effects in seeds, such as viability loss, discolouration, production of mycotoxins, and heat. In the current study, 6 fungal species have been identified from *Hopea ponga* seeds, which cause potential damage during storage and germination. Galls are the peculiar characteristics of *H. ponga* and are observed in all populations in the current study.

Seeds of *H. ponga* matured at 80 DAA with a high % moisture content of 45.62 ± 1.55% and lost viability within 7 days in open laboratory conditions. Analysis of seed development parameters started at 4 DAA in the case of seed length, width, fresh and dry weight, and % moisture content, and biochemical and enzyme analysis started at 12 DAA. Seed length, width, and fresh and dry weights followed the typical sigmoid growth pattern of development and reached the maximum maturity at 80 DAA. The parameters of seed development, including the accumulation of dry matter and alterations in metabolite content, etc. are critical and dictate seed storage behavior and its recalcitrant nature. In *H. ponga*, there has been a consistent rise in dry weight accumulation. The peak of dry weight was reached after the maturation phase, indicating that the seeds amassed storage substances throughout their development. Seeds of *Hopea ponga* are dispersed from the parent plant with a moisture content of 45.37% and a 10 % reduction in moisture content. The associated complete loss of viability poses substantial challenges to the conservation efforts of this species.

Deposing storage substances is one of the key processes of zygotic embryogenesis, providing compounds that will be used from the early stages of embryonic development until autotrophy, after germination. As sugars may be a major component as biochemical precursors, understanding the changes

in sugar content during maturation is important. In the present study sugar content tended to increase throughout the maturation and was significant at 1% P level. Starch, the carbohydrate reserve in seeds, recorded accumulation during seed development in *H. ponga* till 60 DAA, and later it stabilized and reduced at 80 DAA. The polynomial curve fitted was significant at 0.01% P level and showed a sigmoid pattern. Phenol content recorded a typical sigmoidal pattern, like that of starch, during seed development in *H. ponga*. Most higher plants respond to environmental stimuli by activating secondary metabolic pathways such as phenylpropanoid metabolism.

The desiccation sensitivity and brief viability of recalcitrant seeds limit the environmental conditions under which they can successfully reproduce. Such seeds are typically found in moist tropical forests, where they germinate and establish a seedling bank, in contrast to contributing to a soil seed bank. Seed desiccation in *Hopea ponga* was measured by changes in fresh weight and moisture content relative to viability, exhibiting a pattern characteristic of recalcitrant seeds. Desiccation stress leads to a sharp decline in germination percentage, triggering abnormal metabolic pathways that result in cell death. During prolonged desiccation of *H. ponga*, leachate conductivity increases linearly, indicating solute leakage possibly due to compromised membrane integrity. Ionic leakage results from the physical disruption of cell membranes caused by water outflow during dehydration. This damage to the cell membrane leads to the loss/leaching out of essential ions and metabolites from the cytoplasm, which is necessary for maintaining viability and enabling seed germination. The duration and intensity of tetrazolium staining indicate viability loss due to desiccation. The study shows that *H. ponga* seeds with high moisture content stain more quickly than desiccated seeds. After seven days of desiccation under open laboratory conditions (28±2°C and 65% RH), *H. ponga* seeds showed no staining with tetrazolium chloride, confirming the

loss of viability due to desiccation as evidenced by the formazan concentration during different desiccation periods.

The study indicated chlorophyll degradation in *Hopea ponga* seeds during desiccation, a trait typical of recalcitrant seeds reflecting various cellular deteriorations. Total soluble sugars (TSS) content increased as starch content decreased during desiccation. High-performance liquid chromatography analysis verified the presence of sucrose, glucose, and fructose, with an increase in sucrose concentration during desiccation, whereas raffinose was not detected in the species.

Based on the results, it is known that the decreasing concentration of oligosaccharides during desiccation affects the membrane stability of the seeds, resulting in the loss of vitrification that causes desiccation sensitivity in *H. ponga* seeds. This leads to a loss of vitrification, which in turn causes desiccation sensitivity in seeds of *H. ponga*. It is therefore deduced that alterations in Total Soluble Sugars (TSS) in seeds might be the principal factor contributing to the loss of desiccation tolerance in the species observed during desiccation, suggesting its significance in recalcitrant seeds; however, additional research is necessary to ascertain its definitive role.

Seed germination encompasses a series of intricate metabolic processes, including respiration, synthesis of nucleic acids and proteins, and the mobilization of metabolites, all of which contribute to cell differentiation and growth. This study offers experimental support for the hypothesis that reducing sugars (TSS) in the species exhibit an initial increase during the early imbibition periods, followed by a subsequent decrease. This trend could be attributed to the consumption of soluble sugars for differentiation and growth. Additionally, the starch content in the seeds demonstrates an initial decline, proceeding to a stationary phase. Starch is the main carbohydrate reserve in *H. ponga*, and its breakdown during germination primarily occurs via the amylolytic pathway. The germination of *H. ponga* seeds involves the

degradation of storage proteins, marked by a swift increase in protease activity. Consequently, the current study focused on the roles of α-amylase, β-amylase, protease, peroxidase, catalase, and superoxide dismutase in the germination. The lower kDa protein, Late embryogenesis abundant (LEA) protein, showed the presence during the final phases of seed maturation 60 DAA and 80 DAA, which concluded that it plays a pivotal role in developing desiccation tolerance.

Ether and oxy compounds Polyhydroxy compounds, hydroxy compounds, aliphatic organic halogen compounds, thiols, and thio substituted compounds, acetylenic, aromatic ring compounds, and primary amino are the major functional groups present in the seeds. Different economically important compounds are present in the seeds of *Hopea ponga.* The hexane extracts of both the fresh and dry samples show the versatile compound 4-Keto 9-cis Retinoic acid, ellagic acid, which has the properties of anti-inflammatory, antioxidant and anti-cancerous. The natural stilbene compound, resveratrol, is present in the acetone extract of the seed samples. The presence of quercetin, a potential ingredient in the preparation of medicine for Alzheimer's disease was recognized from the acetone extract.

The seed behaviour of *Hopea ponga* is biologically programmed to align with their specific habitat requirements. They display typical traits of recalcitrant seeds, including rapid viability loss under natural atmospheric conditions, sensitivity to desiccation and chilling, and sprouting during storage, which leads to poor storability. This study has standardized optimal seed storage conditions at 20±2°C and 40% RH in sealed polycarbonate bottles to facilitate effective and prolonged germplasm preservation for up to 210 days. Additionally, *H. ponga* faces challenges such as irregular flowering and fruiting, which affect the population.

Human invasion, the endemic nature of the tree, over-exploitation, recalcitrance, habitat specificity, biological threats (pests, fungi, etc.), and

irregular fruiting cycles have disrupted the balance of species in their natural habitats. Consequently, from a conservation perspective, developing a scientific strategy to preserve these precious germplasm resources effectively is imperative. The current study endeavours to comprehend the various factors influencing seed development, maturation, desiccation, and germination and establish optimal storage conditions. Phenological study outcomes have further elucidated the reasons behind the scarcity of these species. The metabolic imbalances observed through various analyses during different treatments offer valuable insights into the characteristics of multiple biomolecules and their functional behaviour. These could serve as indicators of seed viability and inform the development of appropriate conservation strategies through seed banking. In essence, this study serves as a prototype for future research into the seed physiology and biochemistry of other significant and threatened recalcitrant seed species, fulfilling the dual goals of diversity conservation and sustainable use, particularly concerning the endemic trees of the Southern Western Ghats.

RECOMMENDATIONS

- Members of the Dipterocarpaceae family found in tropical forests are under threat due to their recalcitrant seeds and timber extraction. Therefore, this research will contribute to strategic conservation planning and implementing sustainable restoration programs.
- As specific LEA proteins correlate with the seed longevity of this species and its relative's, detailed studies are required to identify particular proteins responsible for seed longevity.
- Long-term storage methods, akin to those used for orthodox seeds, are crucial for maximizing storage duration. Therefore, it is necessary to standardize cryopreservation techniques using appropriate vitrification agents in embryos, shoot tips etc.
- Molecular level studies are necessary to analyse gene-level expression/blocking to improve seed viability in recalcitrant seeds.
- Seed pest attacks are common in the target species because of the high total soluble sugar and starch content. Therefore, it presents an opportunity to study the interaction between pests and seeds and the life cycle of the pests involved.
- Development and standardization of control mechanisms are necessary for pests and other fungal pathogens.
- Comprehensive population studies are necessary across the Western Ghats to identify the earlier population locations and present distribution and confirm the species' venerable status.
- Developing a community-based seed bank that leverages indigenous knowledge can enhance the populations of *Hopea ponga* and related species.
- Molecular docking studies are essential to predict the optimal orientation of one molecule to another when binding a ligand to a target, resulting in a stable complex in the seeds.
- Awareness of conservation and seed propagation techniques is necessary for the public, forest officials, and students.
- Elevate the densely populated regions of *H.ponga* to a biodiversity heritage site with the involvement of the community and the respective Biodiversity Management Committee (BMC). This initiative will transition conservation efforts into a legal framework.