



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF TEMPERATURE ON RUBBER  
(HEVEA BRASILIENSIS MUELL.-ARG.) SEED STORAGE**

**NORMAH BT. MOHD. NOOR**

**FP 1987 6**

It is hereby certified that we have read this thesis entitled "Effects of Temperature of Rubber (Hevea Brasiliensis Muell Agr.) Seed Storage" by Normah Mohd. Noor, and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirements for the degree of Doctor of Philosophy



.....  
ALANG P. ZAINUDDIN, Ph.D.  
Assoc. Professor/Dean of Graduate Studies  
Universiti Pertanian Malaysia  
(Chairman Board of Examiners)



.....  
PHILIP STANWOOD, Ph.D.  
Research Agronomist, USDA-ARS  
Colorado State University  
Colorado, USA  
(External Examiner)



.....  
E.H. ROBERTS, Ph.D.  
Professor  
Faculty of Agriculture and Food  
University of Reading  
Reading, ENGLAND  
(External Examiner)



.....  
MOHAMMAD MOHD. LASSIM, Ph.D.  
Associate Professor  
Faculty of Agriculture  
Universiti Pertanian Malaysia  
(Internal Examiner)

*Hor Yue Luan*

.....  
HOR YUE LUAN, Ph.D.

Lecturer

Faculty of Agriculture  
Universiti Pertanian Malaysia  
(Internal Examiner)

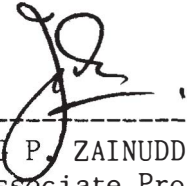
*Chin Hoong Fong*

.....  
CHIN HOONG FONG, Ph. D.

Professor, Faculty of Agriculture  
Universiti Pertanian Malaysia  
(Supervisor)

This thesis was submitted to the Senate of Universiti Pertanian Malaysia and was accepted as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Date: 10 MAR 1988

  
-----  
ALANG P. ZAINUDDIN, Ph. D.  
Associate Professor/  
Dean of Graduate Studies



EFFECTS OF TEMPERATURE ON RUBBER  
(HEVEA BRASILIENSIS MUELL. -ARG.) SEED STORAGE

by

NORMAH BT. MOHD. NOOR

A thesis submitted in partial fulfilment of the  
requirements for the degree of Doctor of Philosophy  
in the Faculty of Agriculture  
Universiti Pertanian Malaysia

October 1987



Dedicated to

FAIZ HADI

ILI FARHANA

MOHAMED



## ACKNOWLEDGEMENTS

I am extremely grateful to Universiti Pertanian Malaysia for enabling this study to be carried out. My sincere appreciation is to Professor Chin Hoong Fong for his advice, supervision, constructive criticisms and guidance.

I am extremely grateful to Universiti Kebangsaan Malaysia for granting me study leave to undertake this study. In particular I wish to express my gratitude to Professor Zakri A. Hamid for his support and encouragement to pursue this study.

My sincere thanks goes to Dr. Hor Yue Luan for his advice and constructive criticisms in completing this study; Professor Yap Thoo Chai for his suggestions on experimental designs and on statistical analysis of data; Dr. Tan Boon Keng, formerly at the Palm Oil Research Institute, who assisted me in the analysis of fatty acid composition; Mr. Ang Boon Beng, Prang Besar Research Station, for arranging supplies of seeds for this study; and Dr. Khatijah Haji Hussin, Department of Botany, Universiti Kebangsaan Malaysia for assisting in the interpretation of the electron micrographs.

I am indebted to Professor Madya Dr. Zaliha Christine Alang for many hours spent on stimulating discussions,



instruction and guidance in the in vitro culture work and in the presentation of this thesis.

My sincere appreciation is extended to Dr. Lai Chooi May and Encik Ho Oi Kuan for their assistance in the electron microscopy study; Puan Siti Ramlah Jaafar, Puan Azmah Mispan, Encik Daud Mustam, Encik Ong Choon Hoe and Puan Nor Rafidah Yusoff for their assistance in the laboratory during the study; Encik Maarof bin Abd. Wahid and Encik Abdul Ghani bin Hashim for their photographic work; and Cik Zulaini bt. Ismail for her assistance in computer analysis.

I also wish to convey my thanks to other members of the Department of Agronomy, Universiti Pertanian Malaysia, who have helped me in various ways throughout this project.

To my husband Mohamed bin Kasim, who encouraged and assisted me throughout this study, I convey my special thanks and appreciation.





## TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT .....	iii
TABLE OF CONTENTS .....	v
LIST OF TABLES .....	viii
LIST OF PLATES .....	x
LIST OF APPENDICES .....	xiii
LIST OF FIGURES .....	xv
ABSTRACT .....	xvi
CHAPTER 1 – INTRODUCTION .....	1
CHAPTER 2 – LITERATURE REVIEW .....	5
CHAPTER 3 – MATERIALS AND METHODS .....	44
SEED MATERIALS .....	45
EXPERIMENTAL PROCEDURES .....	45
Seed Treatment, Packaging and Storage .....	45
Sterilisation of Seeds and Excision of Embryonic Axes .....	46
Preparation of Sections for Observation Under the Light Microscope .....	47
Preparation of Sections for Examination Under the Transmission Electron Microscope .....	50
Preparation of Stock Solutions and Culture Media .....	51
Preparation and Application of Cryoprotectants ..	52
Desiccation of Embryonic Axes .....	53
Cooling and Thawing Procedures .....	53



	PAGE
MEASUREMENT AND OBSERVATIONS .....	54
Moisture Content .....	54
Germination .....	55
Seedling Height and Dry Weight .....	56
Conductivity of Seed Leachate .....	56
Respiration Rate .....	58
Fatty Acid Composition .....	58
EXPERIMENTS .....	60
Effects of Storage Temperature on Physiological, Biochemical and Structural Changes of <u>Hevea</u> Seeds .....	60
Effects of Temperature on Storability of <u>Hevea</u> Seeds .....	62
Effects of Temperature on <u>In vitro</u> Storage of <u>Hevea</u> Embryonic Axes .....	64
STATISTICAL ANALYSIS .....	68
CHAPTER 4 - RESULTS .....	69
EFFECTS OF STORAGE TEMPERATURE ON PHYSIOLOGICAL, BIOCHEMICAL AND STRUCTURAL CHANGES OF <u>HEVEA</u> SEEDS ...	69
Experiment 1. Effects of Temperature on Physiological, Biochemical and Structural Changes of <u>Hevea</u> Seeds During Imbibed Storage ...	69
EFFECTS OF TEMPERATURE ON STORABILITY OF <u>HEVEA</u> SEEDS .....	109
Experiment 1. Effects of Temperature on Imbibed Storage of <u>Hevea</u> Seeds Treated with PEG .....	109
Experiment 2. Effects of Temperature on Dry Storage of <u>Hevea</u> Seeds Treated with Benlate ....	111



	PAGE
EFFECTS OF TEMPERATURE ON <u>IN VITRO</u> STORAGE OF <u>HEVEA</u> EMBRYONIC AXES .....	114
<u>In vitro</u> storage of <u>Hevea</u> Embryonic Axes by the Slow Growth Method .....	114
Experiment 1. <u>In vitro</u> Storage of <u>Hevea</u> Embryonic Axes by the Slow Growth Method .....	114
Cryopreservation of <u>Hevea</u> Embryonic Axes .....	119
Experiment 1. Effects of Cryoprotectants on Cryopreservation of <u>Hevea</u> Embryonic Axes .....	119
Experiment 2. Effects of Cryoprotectants and Subsequent Desiccation on Cryopreservation of <u>Hevea</u> Embryonic Axes .....	122
Experiment 3. Effects of Desiccation on Cryopreservation of <u>Hevea</u> Embryonic Axes .....	125
CHAPTER 5 - DISCUSSION .....	135
EFFECTS OF STORAGE TEMPERATURE ON PHYSIOLOGICAL, BIOCHEMICAL AND STRUCTURAL CHANGES OF <u>HEVEA</u> SEEDS ....	135
EFFECTS OF TEMPERATURE ON STORABILITY OF <u>HEVEA</u> SEEDS .....	144
EFFECTS OF TEMPERATURE ON <u>IN VITRO</u> STORAGE OF <u>HEVEA</u> EMBRYONIC AXES .....	149
CHAPTER 6 - CONCLUSION .....	159
BIBLIOGRAPHY .....	162
APPENDICES	
APPENDIX A : PREPARATION OF CHEMICALS .....	180
APPENDIX B : TABLES OF ANALYSIS OF VARIANCE .....	185
APPENDIX C : VARIABILITY IN MOISTURE CONTENT OF <u>HEVEA</u> SEEDS .....	198



## LIST OF TABLES

TABLE		PAGE
1	Moisture Content (%) of <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	70
2	Percentage Germination of <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C (Values are Arcsin Transformed) .....	71
3	Seedling Height (cm) from <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	72
4	Seedling Dry Weight (g) from <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	73
5	Leachate Conductivity ( $\mu$ S) of <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	74
6	Respiration Rate of Embryonic Axes ( $\mu$ l O <sub>2</sub> g dry wt <sup>-1</sup> hr <sup>-1</sup> from <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	77
7	Percentage Lipid Composition (Fresh Weight Basis) of <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	78
8	Percentage Composition of Fatty Acids of <u>Hevea</u> Seeds Stored Imbibed at 10, 22 and 27°C .....	79
9	Means of Percentage Fatty Acid Composition of <u>Hevea</u> Seeds Significant Between Months of Storage .....	80
10	Percentage Germination of <u>Hevea</u> Seeds Treated with PEG and Stored Imbibed at 10, 22 and 27°C (Values are Arcsin Transformed) .....	110
11	Moisture Content (%) of <u>Hevea</u> Seeds Treated with PEG and Stored Imbibed at 10, 22 and 27°C .....	112



TABLE		PAGE
12	Percentage Germination of <u>Hevea</u> Seeds Treated with Benlate and Stored Dry at 10, 22 and 27°C (Values are Arcsin Transformed) .....	113
13	Moisture Content (%) of <u>Hevea</u> Seeds Treated with Benlate and Stored Dry at 10, 22 and 27°C .....	115
14	Percentage Survival of <u>Hevea</u> Seedlings After Slow Growth Storage .....	116
15	Percentage Viability After Cryopreservation of Cryoprotected and Desiccated <u>Hevea</u> Embryonic Axes .....	124
16	Percentage Survival of <u>Hevea</u> Embryonic Axes After Desiccation at Various Moisture Contents .....	126
17	Percentage Viability of <u>Hevea</u> Embryonic Axes After Desiccation and Cryopreservation .....	127
18	Percentage Survival of <u>Hevea</u> Embryonic Axes After Desiccation and Cryopreservation .....	128
19	Mean Square Value for Germination, Moisture Content, Seedling Height and Seedling Dry Weight of <u>Hevea</u> Seeds Stored Imbibed .....	185
20	Mean Square Values for Leachate Conductivity, Respiration and Lipid Composition of <u>Hevea</u> Seeds Stored Imbibed .....	186
21.	Mean Square Values for Fatty Acid Composition (%) of <u>Hevea</u> Seeds Stored Imbibed .....	187
22	Analysis of Variance Table of Germination of <u>Hevea</u> Seeds Treated With PEG and Stored Imbibed .....	188



TABLE	PAGE
23	Analysis of Variance Table of Moisture Content of <u>Hevea</u> Seeds Treated with PEG and Stored Imbibed ..... 189
24	Analysis of Variance Table of Germination of <u>Hevea</u> Seeds Stored Dry ..... 190
25	Analysis of Variance Table of Moisture Content of <u>Hevea</u> Seeds Stored Dry ..... 191
26	Analysis of Variance Table of Percentage Survival of <u>Hevea</u> Embryonic Axes Stored by Slow Growth <u>in vitro</u> Method ..... 192
27	Analysis of Variance of Percentage Viability of Cryoprotected <u>Hevea</u> Embryonic Axes After Cryopreservation ..... 193
28	Analysis of Variance Table of Moisture Contents of <u>Hevea</u> Embryonic Axes After Desiccation ..... 194
29	Analysis of Variance Table of Percentage Survival of <u>Hevea</u> Embryonic Axes After Desiccation ..... 195
30	Analysis of Variance of Percentage Viability of Desiccated <u>Hevea</u> Embryonic Axes after Cryopreservation ..... 196
31	Analysis of Variance of Percentage Survival of Desiccated <u>Hevea</u> Embryonic Axes After Cryopreservation ..... 197
32	Coefficient of Variability of Moisture Content of Different Parts of <u>Hevea</u> Seeds ..... 200
33	Coefficient of Variability of Moisture Contents of Individual Seeds of Recalcitrant (R) and Orthodox (O) Seeds ..... 201
34	Percentage Composition and Moisture Content of Different Parts of <u>Hevea</u> Seeds ..... 202



## LIST OF PLATES

PLATE		PAGE
1	<u>Hevea</u> Seed With and Without Testa .....	48
2	<u>Hevea</u> Seed Showing the Endosperm (E), Cotyledons (C) and the Embryonic Axis Attached to the Cotyledons (EA) and Excised (A) .....	48
3	Normal Seedlings of <u>Hevea</u> (B and C) and Dead Seeds (A) .....	57
4	Fresh (A) and Deteriorated (B) <u>Hevea</u> Seeds .....	82
5	The Structure of <u>Hevea</u> Seeds Fresh (A) and Deteriorated (B) .....	82
6	Structure of the Embryonic Axis of <u>Hevea</u> Seed .....	83
7	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Fresh Seeds .....	84
8	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 10°C for One Month .....	86
9	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 10°C for Two Months .....	87
10	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 10°C for Three Months .....	88
11	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 22°C for One Month .....	89



PLATE		PAGE
12	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 22°C for Two Months .....	90
13	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 22°C for Three Months .....	91
14	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 27°C for One Month .....	92
15	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 27°C for Two Months .....	93
16	Cells of Epicotyl (A) and Ground Tissue of Radicle (B) Regions of <u>Hevea</u> Embryonic Axes from Seeds Stored at 27°C for Three Months .....	94
17	Ultrastructure of Radicle Cells From Fresh <u>Hevea</u> Seeds .....	96
18	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 10°C After One Month .....	97
19	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 10°C After Two Months .....	98
20	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 10°C After Three Months.....	100
21	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 22°C After One Month .....	101
22	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 22°C After Two Months .....	103





PLATE		PAGE
23	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 22°C After Three Months .....	104
24	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 27°C After One Month .....	105
25	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 27°C After Two Months .....	107
26	Ultrastructure of Radicle Cells From <u>Hevea</u> Seeds Stored at 27°C After Three Months .....	108
27	Dead <u>Hevea</u> Seedling After Five Months at 18°C .....	117
28	<u>Hevea</u> Seedlings Stored at 20°C After Five Months .....	118
29	Development of <u>Hevea</u> Embryonic Axis on MS Medium With (A) and Without (B) Charcoal After Two Weeks of Culture in the Light .....	120
30	Dead <u>Hevea</u> Embryonic Axis .....	121
31	<u>Hevea</u> Embryonic Axis With Root Formation .....	123
32	Examples of Abnormal Seedlings which Developed from <u>Hevea</u> Embryonic Axes After Cryopreservation .....	130
33	Growth of Cryopreserved <u>Hevea</u> Embryonic Axis .....	131
34	Seedling from Cryopreserved Embryonic Axis (Left) and Seedling From Non-Cryopreserved Embryonic Axis (Right) After Two Weeks in Culture .....	132
35	Normal Seedling from Cryopreserved <u>Hevea</u> Embryonic Axis .....	134



## LIST OF APPENDICES

APPENDIX	PAGE
A Preparation of Chemicals .....	180
B Tables of Analysis of Variance .....	185
C Variability in moisture content of <u>Hevea</u> Seeds .....	198



LIST OF FIGURES

FIGURE		PAGE
1	Moisture Content of Cotyledons and Endosperm of Individual <u>Hevea</u> Seed .....	203
2	Moisture Content of Embryonic Axis of Individual <u>Hevea</u> Seed .....	204
3	Moisture Content of Individual <u>Hevea</u> Seed .....	205
4	Moisture Content of Individual Seed of <u>H. brasiliensis</u> , <u>N. lappaceum</u> and <u>Z. mays</u> .....	206



An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia as partial fulfilment of the requirements for the Degree of Doctor of Philosophy

THE EFFECTS OF TEMPERATURE ON RUBBER (HEVEA BRASILIENSIS  
MUELL. -ARG.) SEED STORAGE

by

NORMAH BT. MOHD. NOOR

October 1987

Supervisor : Professor Chin Hoong Fong, Ph. D.

Co-Supervisor : Hor Yue Luan, Ph. D.

Faculty : Agriculture

The effects of temperature on the storage of Hevea seeds and embryonic axes were studied.

The first part of the study was on the effects of temperature (10, 22 and 27°C) on physiological, biochemical and structural changes during imbibed storage of Hevea seeds. The second part of the study involved the effects of temperature (10, 22 and 27°C) on storability of Hevea seeds using a modified imbibed storage method incorporating the application of 10 percent and 20 percent concentrations of a germination inhibitor (PEG) and a dry storage method in the presence of a fungicide (0.3 percent Benlate). The third part of the study was on in vitro storage of Hevea embryonic axes including a



slow growth method (at 10, 15, 18 and 20°C) and cryopreservation in liquid nitrogen (-196°C).

In the first part of the study, there was a decrease in percentage germination, seedling height, seedling dry weight, respiration rate and total lipid composition and an increase in leachate conductivity as duration of storage increased. Membrane degeneration appeared to be the most common ultrastructural feature of deterioration. Viability of imbibed Hevea seeds was maintained longest when the seeds were stored in perforated black polythene bags at 27°C (ambient temperature).

Ambient temperature (27°C) was also the best storage temperature in the modified imbibed storage and for dry storage. Ten percent and 20 percent PEG did not improve the storability of seeds. The dry storage method in the presence of Benlate as a fungicide resulted in better storability of Hevea seeds than imbibed storage at 27°C (13 months, percentage germination 20 percent).

In vitro storage of Hevea embryonic axes was a new method investigated. The slow growth method using temperatures of 10, 15 and 18°C was not successful. Storage at 20°C resulted in survival period of about five months. However, the potential for long term storage of Hevea embryonic axes was shown, when desiccated axes survived liquid nitrogen storage (-196°C) using



various cooling and thawing treatments. Normal growth and development of seedlings from the cryopreserved axes were observed.



Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi sebahagian daripada syarat-syarat keperluan untuk ijazah Doktor Falsafah.

KESAN SUHU KE ATAS PENYIMPANAN BIJI BENIH GETAH  
(HEVEA BRASILIENSIS MUELL. - ARG)

oleh

NORMAH BT. MOHD. NOOR

Oktober 1987

Penyelia : Profesor Chin Hoong Fong, Ph. D.

Penyelia Bersama : Hor Yue Luan, Ph. D.

Fakulti : Pertanian

Kesan suhu ke atas penyimpanan biji benih dan paksi embrio Hevea telah dikaji.

Bahagian pertama kajian ini adalah kesan suhu (10, 22 dan 27°C) ke atas pertukaran fisiologi, biokimia dan struktur semasa penyimpanan pedap biji benih Hevea. Bahagian kedua meliputi kesan suhu (10, 22 dan 27°C) ke atas penyimpanan biji benih Hevea dengan menggunakan cara penyimpanan pedap terubah-suai dengan penggunaan perencat percambahan (PEG) pada kepekatan 10 peratus dan 20 peratus dan dengan menggunakan cara penyimpanan kering beserta rawatan racun kulat (0.3 peratus Benlate). Bahagian ketiga ialah kajian ke atas penyimpanan in



in vitro paksi embrio Hevea yang meliputi cara pertumbuhan perlahan (pada 10, 15, 18 dan 20°C) dan krioawetan dalam nitrogen cecair (-196°C).

Dalam bahagian pertama kajian ini peratus percambahan, ketinggian dan berat kering anak benih, kadar respirasi dan komposisi lipid menyusut sementara kekonduksian luluh larut meningkat semasa penyimpanan. Pemosotan membran nampaknya adalah satu ciri ultrastruktur kerosakan yang paling biasa. Daya hidup biji benih Hevea dapat dipertahankan paling lama dengan penyimpanan pedap dalam beg politena hitam berlubang pada suhu 27°C (suhu ambien).

Suhu ambien (27°C) juga merupakan suhu yang paling sesuai untuk cara penyimpanan pedap terubahsuai dan cara penyimpanan kering. PEG dengan kepekatan 10 peratus dan 20 peratus tidak memperbaiki penyimpanan. Penyimpanan kering dengan rawatan racun kulat Benlate telah menghasilkan daya penyimpanan biji benih Hevea yang lebih baik dari penyimpanan pedap pada suhu 27°C (13 bulan, peratus percambahan 20 peratus).

Penyimpanan in vitro paksi embrio Hevea adalah kajian cara penyimpanan yang baru. Cara pertumbuhan perlahan pada 10, 15 dan 18°C tidak berjaya. Penyimpanan pada suhu 20°C menghasilkan masa kemandirian selama lima bulan. Walau bagaimanapun, keupayaan penyimpanan jangka panjang paksi embrio Hevea telah



ditunjukkan apabila paksi-paksi embrio yang telah dikeringkan mandiri penyimpanan dalam nitrogen cecair ( $-196^{\circ}\text{C}$ ) selepas berbagai perlakuan pendinginan dan pencairan. Pertumbuhan dan perkembangan anak benih yang normal daripada paksi embrio yang dikrioawetkan telah dicerap.