

**GERMINATION AND STORAGE OF *DYERA COSTULATA* HOOK.F. AND
MACARANGA GIGANTEA MULL. SEEDS**

By

CHANTHOL SAO

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
fulfilment of the Requirement for the degree of Master of Science**

April 2004

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of
the requirements for the degree of Master of Science

**GERMINATION AND STORAGE OF *DYERA COSTULATA* HOOK.F.
AND *MACARANGA GIGANTEA* MULL. SEEDS.**

By

CHANTHOL SAO

April 2004

Chairman: Jamaluddin Bin Basharuddin, Ph.D.

Faculty: Forestry

Good physiological quality of seed such as high germination percentage and vigor are the most important factors to be considered in the production of high quality tree seeds for commercial plantations. This study therefore was undertaken with the main objective of enhancing the germination and storage condition of commercially important tree seeds, *Dyera costulata* Hook.f. and *Macaranga gigantea* Mull. This study also envisioned of developing tetrazolium test for rapid detection of seed viability. Several germination media such as paper, sand, soil, forest soil, coconut husk and paddy husk were tested for best germination. Whereas, three different storage conditions (refrigerated i.e 10 and 20°C and ambient i.e 30°C) and different storage periods (0, 31, 89 and 181 days); three different plant growth hormones (GA₃, BAP and kinetin) at four different concentrations (0.1, 0.2, 0.5 and 10 mg/l) were used to determine the best conditions for storage and growth promoter for both *D. costulata* and *M. gigantea* seeds. In addition, three different germination media (sand, soil and forest soil) and light regimes (25%, 55% and 75% relative light intensity) were tested to find out the best condition for seedlings growth.

The results of the study indicated that the best germination media for both tree seeds was the sand, followed by forest soil and soil. This study also revealed that the best temperature for storage was at 20°C giving from 85 to 97% seed viability. However,

storage of more than 89 days tends to reduce the germination rate when stored at 10°C, and 20°C, and more 30 days if stored at 30°C. Meanwhile, among the three types of hormones it was found that GA₃ at 10mg/l was most effective not only on the germination but also on excised seedcoat. While, among the concentrations used 10mg/l gave an overall best result for all the hormones in enhancing the germination process. On the other hand, it was found that seedlings grow best when planted in forest-soil and provided with 25% light intensities.

Through tetrazolium test only 57% viability was gained for *M. gigantea* seeds as compared to the control test which resulted to 40% viability. Whereas, the viability of seeds was estimated at 93% compared to the control test, which resulted only to 87.5% viability for *D. costulata* seeds. However, the daily germination percentage was inconsistent, making the germination period longer for those seeds sown in coconut husk and paddy husk as compared to sand and soil treatments. It was found that buried seeds produced better germination rate that resulted vigour seedlings as compared to freshly collected seeds. Overall, hormone treatment using GA₃, BAP and Kinetin at various concentrations was found to enhance the germination rates of *M. gigantea* seeds. However, it was found that GA₃ at 10mg/l was better treatment for germination that gave 65%, even when combined with excised seedcoat that gave 74% viability. From the study upon various media and light intensities, it was found growth was significantly better at 75% light in forest-soil condition.

Comparison of the anatomical structures of buried seeds and fresh seeds of *M. gigantea* were carried out using scanning electron microscopy (SEM). Studies indicated that the buried seeds had a thinner pericarp, full endosperm and had a better-developed embryonic axis compared to the fresh seeds. Differences in buried seeds and fresh seeds were evident their pericarp thickness, between, i.e. 357.6µm and 308.7µm respectively. Differences were also evident within the radicle area where structures from fresh seeds were not as well organized as those of buried seeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

**PERCAMBAHAN DAN PENYIMPANAN BIJI BENIH *DYERA COSTULATA HOOK.F.*
DAN *MACARANGA GIGANTEA***

Oleh

CHANTHOL SAO

April 2004

Pengerusi: **Jamaluddin Bin Basharuddin, Ph.D.**

Fakulti: **Perhutanan**

Kualiti fisiologi yang baik bagi biji benih seperti peratus percambahan yang tinggi dan sihat bertenaga adalah faktor-faktor paling utama yang perlu dipertimbangkan dalam pengeluaran biji-biji benih yang berkualiti tinggi bagi keperluan perladangan secara komersial. Bagi maksud tersebut, kajian ini dijalankan dengan objektif utama untuk menggalakkan percambahan dan keadaan penyimpanan bagi biji-biji benih pokok yang berkomersial iaitu *Dyera costulata* Hook.f. dan *Macaranga gigantea* Mull. Kajian ini juga untuk melihat pembangunan ujian tetrazolium untuk pengesanan yang kerap keatas viabiliti biji benih. Beberapa media percambahan seperti kertas, pasir, tanah, tanah hutan, sekam padi dan gambut padi telah diuji bagi menentukan percambahan yang terbaik. Sementara itu, tiga keadaan penyimpanan yang berbeza (didalam petisejuk iaitu 10 dan 20°C dan dipersekitaran iaitu 30°C) dan jangkamasa yang berbeza (0, 31, 89 dan 181 hari); tiga hormon penggalak tumbesaran yang berbeza (GA₃, BAP dan kinetin) pada

kepekatan yang berbeza (0.1, 0.2, 0.5 dan 10 mg/l) telah digunakan untuk menentukan keadaan yang terbaik bagi simpanan dan penggalak tumbesaran bagi kedua-dua biji benih *D. costulata* dan *M. gigantea*. Seterusnya, tiga media percambahan yang berbeza (pasir, tanah dan tanah hutan) dan rejim cahaya (25%, 55% dan 75% keamatan cahaya relatif) telah diuji untuk mendapatkan keadaan yang terbaik untuk tumbesaran anakpokok.

Keputusan-keputusan daripada kajian menunjukkan media yang terbaik untuk percambahan bagi kedua-dua biji benih pokok berkaitan adalah pasir, diikuti tanah hutan dan tanah. Kajian ini juga mendedahkan bahawa suhu yang terbaik untuk penyimpanan adalah pada suhu 20°C yang memberikan keputusan terhadap viabiliti biji benih dari 87 kepada 97%. Walaubagaimanapun, penyimpanan yang melebihi 89% didapati cenderung kearah pengurangan kadar percambahan apabila disimpan pada 10°C dan 20°C, dan lebih dari 30 hari jika disimpan pada suhu 30°C. Sementara itu, di kalangan tiga jenis hormon, ia didapati hormon GA₃ pada 10mg/l adalah yang paling aktif bukan sahaja pada percambahan tetapi juga dalam "excised" kulit biji benih. Disamping itu, kepekatan 10mg/l telah memberikan keputusan keseluruhan yang terbaik untuk kesemua hormon dalam menggalakkan proses percambahan. Selanjutnya, adalah juga didapati anak-anak pokok tumbuh dengan baik apabila telah ditanam pada tanah hutan dengan menggunakan keamatan cahaya pada 25%.

Melalui ujian tetrazolium hanya 57% viability diperolehi dari biji-biji benih *M. gigantea* jika dibandingkan pada ujian kawalan dimana keputusan viabilitinya adalah 40%. Viabiliti biji-biji benih dianggarkan 93% dibandingkan dengan ujian kawalan dimana

keputusannya hanya 87.5% viabiliti untuk biji-biji benih *D. costulata*. Walaubagaimanapun, peratus percambahan harian adalah tidak menentu menyebabkan tempoh percambahan menjadi panjang bagi biji-biji benih yang disulam dalam sekam padi dan gambut padi berbanding dengan rawatan pasir dan tanah. Adalah didapati biji-biji benih bogel menghasilkan kadar percambahan yang baik dan seterusnya memberikan anak pokok yang sihat berbanding dengan biji-biji benih segar yang dikutip. Keseluruhannya, rawatan hormon menggunakan GA₃, BAP dan Kinetin pada pelbagai kepekatan adalah didapati menggalakan kadar percambahan bagi biji-biji benih *M. gigantea*. Bagaimanapun, didapati juga GA₃ pada 10mg/l adalah rawatan yang terbaik untuk percambahan dimana memberikan 65% walaupun digabungkan dengan 'excised' kulit bijibenih yang memberikan 74% viabiliti. Daripada kajian terhadap pelbagai media dan keamatan cahaya, didapati tumbesaran mempunyai keertian yang terbaik pada cahaya 75% dalam tanah-hutan.

Perbandingan struktur anatomi biji-biji benih bogel dan biji-biji benih segar *M. gigantea* telah dijalankan menggunakan imbasan "scanning electron microscope (SEM)". Kajian menunjukkan biji-biji benih bogel mempunyai perikarp yang nipis, enosperm penuh dan mempunyai pembentukan embryonic axis yang lebih baik jika dibandingkan dengan biji benih daripada pokok. Perbezaan dalam biji-biji benih bogel dan biji-biji benih segar adalah pada bukti ketebalan perikarp masing-masing iaitu diantara 357.6 μ m and 308.7 μ m. Perbezaan adalah juga dibuktikan diantara kawasan radikal dimana struktur daripada biji-biji benih segar adalah tidak tersusun dengan baik berbanding biji-biji benih yang tertimbus dalam tanah.

ACKNOWLEDGEMENTS

This study was carried out as a part of the scholarship financed by CTSP (Cambodia Tree Seed Project).

I am very grateful to my main supervisor, Dr. Jamaluddin Bin Basharuddin for his helpful and valuable advice, comments, guidance and encouragement throughout the process of my research study at UPM. His patience, and moral and technical supports are highly appreciated. I am also grateful and sincerely thankful to my advisory committee members, Dr. Adam Puteh, and Dr. Marzalina Marsor for their valuable advice, suggestions and constructive comments that substantially improved my work.

I greatly acknowledge the Department of Forestry and wildlife for granting me the study leave. Much appreciation and thanks also goes to Mr. Arvid Sloth, advisor of Cambodia Tree Seed Project (CTSP), for supported during my study. Special thanks are also due to the Director, Mr. Ty Sokhun, the Deputy Director, Mr. Chea Sam Ang, the Head of Forest Management office, Mr. Ccheng Kimsun and the head of reforestation office, Mr. Ma soktha who enable and incessantly encouraged me to accomplish this study.

I would like to express my great thanks and gratitude to Dr. Nor Aini Ab. Shukor for her guidance and helped throughout my study. Thanks are also due to Boung Long, Anut Chantiratikul, Dang Thinh Trieu and all staff of Faculty of Forestry for their help and sharing during my study in Malaysia.

Finally, I express my deepest appreciation to my mother, sister Davi, Chantha, my brother in law and all of my nephews and my niece for their love, sustainable support, encouragement that makes me feel of having a great power in succeeding my graduate study.

I certify that an Examination Committee met on 21st September 2004 conduct the final examination of Sao Chanthol on her Master of Science thesis entitled “Germination and Storage of *Dyera costulata* Hook.F. and *Macaranga gigantea* Mull. Seed” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Mohd Zaki Hamzah, Ph.D.

Lecturer
Faculty of forestry
Universiti Putra Malaysia
(Chairman)

Ahmad Ainuddin Nuruddin, Ph.D.

Associate Professor
Faculty of Forestry
Universiti Putra Malaysia
(Member)

Mohamed Azani Alias, Ph.D.

Lecturer
Faculty of Forestry
Universiti Putra Malaysia
(Member)

Chin Hoong Fong, Ph.D.

Professor
International Plant Genetic Resources Institute
(Independent Examiner)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Jamaluddin Bin Basharuddin, Ph.D

Faculty of Forestry
Universiti Putra Malaysia
(Chairman)

Adam Puteh, Ph.D

Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Marzalina Marsor, Ph.D

Forest Research Institute Malaysia
(Member)

AINI IDERIS, Ph.D.

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia.

Date:

DECLARATION

I hereby declare that the thesis is based on original work except for quotations and citations that have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

CHANTHOL SAO

Date:

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi LIST
OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF PLATES	xix LIST
OF ABBREVIATIONS	xx
 CHAPTER	
I GENERAL INTRODUCTION	1
1.1 Objectives of the study	6
II LITERATURE REVIEW	7
2.1 Taxonomy and Botanical	7
2.1.1 <i>Dyera costulata</i> Hook.f.	7
2.1.2 <i>Macaranga gigantea</i> Mull.	8
2.2 Seed storage	9
2.3 Type of dormancy	18
2.3.1 Nikolaeva and Gordon & Rowe	20
2.3.2 Harper and Bewley & Black	24
2.4 Seed germination	35
2.5 Media	37
2.6 Pre-treatment	38
2.7 Moisture content	40
2.8 Seed viability	41
2.9 Plant growth regulators	44
2.10 Scanning Electron Microscopy	46
III MATERIALS AND METHODS	49
3.1 Seed source	49
3.2 Seed collection	49
3.3 Seed extraction	50
3.4 Sterilization	51
3.5 Pre-treatment	51
3.6 Germination conditions	51
3.7 Germination test	52
3.8 Substratum	52
3.8.1 Paper medium	52
3.8.2 Sand medium	53
3.8.3 Soil medium	54

3.8.4	Forest soil medium	54
3.8.5	Coconut husk	54
3.8.6	Paddy-husk	54
3.9	Viability Study	55
3.9.1	Experiment 1: Viability of <i>D. costulata</i> and <i>M. gigantea</i> seeds by the biochemical test.	55
3.9.2	Experiment 2: To investigate the influence of different types of media on <i>D. costulata</i> and <i>M. gigantea</i> seeds.	56
3.9.3	Experiment 3: To examine the viability of seed obtained from mature pods of <i>D. costulata</i> .	58
3.9.4	Experiment 4: To determine the germination rate of <i>D. costulata</i> seeds collected from the ground and buried seeds of <i>M. gigantea</i> on various media.	59
3.9.5	Experiment 5: To investigate the influence of light on the germination of <i>D. costulata</i> seeds.	61
3.9.6	Experiment 6: To investigate the effects of storage duration on the seed germination of <i>D. costulata</i> and <i>M. gigantea</i> .	61
3.10	Plant Growth Regulators Study	63
3.10.1	Experiment 7: The effects of exogenous application of various concentrations GA ₃ , BAP and Kinetin on <i>D. costulata</i> and <i>M. gigantea</i> seeds.	63
3.10.2	Experiment 8: Comparative of different types of hormone treatments of excised seedcoat of <i>D. costulata</i> and <i>M. gigantea</i> .	65
3.10.3	Experiment 9: The effects of GA ₃ concentration on the germination percentage of buried seeds of <i>M. gigantea</i> .	66
3.10.4	Experiment 10: To investigate the seedlings growth of <i>D. costulata</i> and <i>M. gigantea</i> under three different media and different light regimes.	67
3.11	Anatomical Study of <i>M. gigantea</i> seeds.	68
3.11.1	Experiment 11: Use Scanning Electron Microscope to identify a difference of embryo on buried seeds and fresh seeds of <i>M. gigantea</i> .	68
3.12	Data Analysis	69
IV	RESULTS	70
4.1	Viability Study	70
4.1.1	Experiment 1: Viability of <i>D. costulata</i> and <i>M. gigantea</i> seeds by the biochemical test.	70
4.1.2	Experiment 2: To investigate the influence of different types of media on <i>D. costulata</i> and <i>M. gigantea</i> seeds.	79
4.1.3	Experiment 3: To examine the viability of seed obtained from mature pods of <i>D. costulata</i> .	84
4.1.4	Experiment 4: To determine the germination rate of seeds of <i>D. costulata</i> collected from the ground and buried seeds of <i>M. gigantea</i> on various media.	86
4.1.5	Experiment 5: To investigate the influence of light on	

the germination of <i>D. costulata</i> seeds.	90
4.1.6 Experiment 6: To investigate the effects of storage duration on the seed germination of <i>D. costulata</i> and <i>M. gigantea</i> .	92
4.2 Plant Growth Regulators Study	98
4.2.1 Experiment 7: The effects of exogenous application of various concentrations GA3, BAP and Kinetin on <i>D. costulata</i> and <i>M. gigantea</i> seeds.	98
4.2.2 Experiment 8: Comparison of different types of hormone treatments of excised seedcoat of <i>D. costulata</i> and <i>M. gigantea</i> seeds.	105
4.2.3 Experiment 9: The effects of GA3 concentration on the germination percentage of buried seeds of <i>M. gigantea</i> .	112
4.2.4 Experiment 10: To investigate the seedlings growth of <i>D. costulata</i> and <i>M. gigantea</i> under three different media and different light regimes.	115
4.3 Anatomical Study of <i>M. gigantea</i> seeds.	120
4.3.1 Experiment 11: Use Scanning Electron Microscope to identify the difference in embryos on buried seeds and fresh seeds of <i>M. gigantea</i> .	120
V DISCUSSION	126
5.1 Biochemical Test	126
5.2 Media and Maturity pods	127
5.3 Seeds collected from the ground	130
5.4 Storage	131
5.5 Plant Growth Regulators	135
5.6 Seedlings Growth	140
5.7 Scanning Electron Microscope	142
VI CONCLUSIONS AND RECOMMENDATIONS	144
6.1 Conclusions	144
6.2 Recommendations	147
REFERENCES	149
APPENDICES	174
BIODATA OF THE AUTHOR	182

