



UNIVERSITI PUTRA MALAYSIA

**THE EFFECTS OF MOISTURE CONTENT AND TEMPERATURE ON
THE STORABILITY OF AN ORTHODOX, INTERMEDIATE AND A
RECALCITRANT FOREST TREE SEED**

JAYANTHI NADARAJAN

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**THE EFFECTS OF MOISTURE CONTENT AND TEMPERATURE ON THE
STORABILITY OF AN ORTHODOX, INTERMEDIATE AND A
RECALCITRANT FOREST TREE SEED**

By

JAYANTHI NADARAJAN

**Thesis Submitted in Fulfillment of the Requirements for the
Degree of Master in Agricultural Science in the
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DEDICATED

To My Beloved

Husband

Ponmuthu

Parents

Sisters and Brothers

And Above All

To

LORD SIVA



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LIST OF ABBREVIATIONS

NAA	α -Naphthalene acetic acid
BAP	Benzylaminopurine
DNMRT	Duncan's New Multiple Range Test
RH	Relative Humidity
Min.	Minutes
$^{\circ}$ C	Degree Centigrade
mg	milligram
g	gram
l	litre
ml	millilitre
g/l	grams per litre
mg/l	milligram per litre
mm	millimetre
mg/ml	milligram per millilitre
MS	Murashige and Skoog Medium formulation
GI	Germination index
%	percentage
KG	kilogram
fw	fresh weight
Kg/cm ²	kilogram per square centimetre
cm/min	centimetre per minute



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JAYANTHI NADARAJAN

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Chairman: Associate Professor Hor Yue Luan, Ph.D.

Faculty: Agriculture

The effects of seed moisture and storage temperature on storability of an orthodox (*Instia palembanica*), an intermediate (*Swietenia macrophylla*) and a recalcitrant (*Hopea odorata*) forest tree seed were studied. The objectives were to determine the critical and optimum moisture and storage temperature for storage of each of the three species, and to evaluate the potential of these seeds for cryopreservation at ultra-low temperatures of liquid nitrogen. The study was divided into four parts of three experiments, each being for one of the three species.

In Part I, the effects of desiccation on seed viability were evaluated. The orthodox seed, *I. palembanica*, has low shedding moisture of 10%. It was tolerant to desiccation as its critical and optimum moistures were low at 6% and 8-10% respectively. For the intermediate seed, *S. macrophylla*, it was more sensitive to desiccation. Its shedding moisture was high at 37% and it could not withstand



excessive desiccation as its critical and optimum moistures were high at 15% and 25% respectively. The recalcitrant seed, *H. odorata*, has very high shedding moisture of 48% and was highly sensitive to even slight desiccation. Its critical and optimum moistures were very high at 29% and 32-35% respectively.

In Part II, the effects of storage temperature were evaluated. The orthodox seed *I. palembanica*, could withstand storage at sub-freezing temperature of -20 °C as high viability was maintained over 4 weeks. However, its optimum temperatures was higher at 5 °C. For the intermediate seed, *S. macrophylla*, it could also withstand -20 °C storage with minimum injury, but its optimum storage temperature was higher than the orthodox seed at 15 °C. For the recalcitrant seed, *H. odorata*, it was highly sensitive to low temperature as all seeds were killed at 5 °C. Its optimum temperature was relatively high at 15-20 °C.

The interaction between near optimum seed moisture and storage temperature was evaluated in Part III to establish more accurately the optimum storage conditions for the three seed species. For the orthodox seed, *I. palembanica*, low seed moisture and temperature of 8-10% and 5 °C respectively were optimum. For the intermediate seed, *S. macrophylla*, the optimum seed moisture and temperature were higher at 25% and 15 °C respectively. Recalcitrant seed like *H. odorata* was not amenable to conventional storage as nearly all seeds were killed within 3 months even at the optimum seed moisture and temperature of 32-35% and 20 °C respectively.

The last part, Part IV, evaluated the potential of cryopreservation for the three forest tree seeds. For the orthodox species, *I. palembanica*, their embryos can

be directly frozen in liquid nitrogen at 10-11.9% moistures with 75% survival. For the intermediate seed, *S. macrophylla*, its embryo can also be easily cryopreserved especially at lower moistures of 18% and below when more than 70% survival is possible. However, cryopreservation of the recalcitrant embryos of *H. odorata* is not possible as none of them survived irrespective of their moisture.

In conclusion, orthodox species like *I. palembanica* can store well at low moisture and temperature and the embryos can be cryopreserved easily even at shedding moisture of 10%. The intermediate species, *S. macrophylla*, needs higher moisture and temperature for conventional storage and the embryos can only be cryopreserved after desiccation to low moistures of 18% and below. Storage of recalcitrant species like *H.odorata* is very difficult as they could not withstand even 3 months conventional storage and all their embryos were killed on exposure to liquid nitrogen irrespective of their moisture.

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

**KESAN KANDUNGAN KELEMBAPAN DAN SUHU KE ATAS
PENYIMPANAN BIJI BENIH POKOK HUTAN BERSIFAT ORTODOKS,
INTERMEDIAT DAN REKALSITRAN**

Oleh

JAYANTHI NADARAJAN

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Pengerusi: Profesor Madya Hor Yue Luan, Ph.D.

Fakulti: Pertanian

Kesan kandungan kelembapan dan suhu ke atas penyimpanan bijibenih pokok hutan bersifat ortodoks (*Instia palembanica*), intermediat (*Swietenia macrophylla*) dan rekalsitran (*Hopea odorata*) telah dikaji. Objektif kajian ini adalah untuk menentukan kandungan kelembapan dan suhu yang kritikal dan optimum untuk penyimpanan ke semua tiga species tersebut dan untuk mengkaji kebolehan bijibenih ini untuk dikrioawetkan pada suhu ultra-rendah cecair nitrogen. Kajian ini telah dibahagikan kepada 4 bahagian yang mengandungi 3 eksperimen masing-masing untuk 3 spesies yang dikaji.

Dalam Bahagian I, kesan pengeringan kepada kemandirian bijibenih telah dikaji. Bijibenih ortodoks, *I. palembanica* yang mempunyai kandungan kelembapan awal yang rendah iaitu 10%, didapati tidak sensitif terhadap pengeringan. Kandungan kelembapan kritikal dan optimum bagi bijibenih ini adalah serendah 6%

dan 8-10% masing-masing. Bijibenih intermediat, *S. macrophylla* pula didapati lebih sensitif kepada pengeringan. Kandungan kelembapan awal bijibenih ini adalah tinggi, 37% serta kandungan kelembapan kritikal dan optimum juga adalah tinggi iaitu 15% dan 25% masing-masing. Bijibenih rekalsitran, *H. odorata* mempunyai kandungan kelembapan awal yang sangat tinggi iaitu 48% dan didapati sangat sensitif kepada pengeringan. Kandungan kelembapan kritikal dan optimum bagi bijibenih ini adalah 29% dan 32-35% masing-masing.

Di dalam Bahagian ke-II, kesan suhu penyimpanan telah dikaji. Bijibenih ortodoks, *I. palembanica* didapati boleh disimpan pada suhu di bawah takat beku iaitu -20°C dengan peratus kemandirian yang tinggi selepas 4 minggu disimpan. Walaubagaimana pun, suhu optimum bagi bijibenih ini didapati adalah lebih tinggi iaitu 5°C . Bijibenih intermediat, *S. macrophylla* juga didapati boleh disimpan pada suhu -20°C dengan kecederaan yang minimum, tetapi suhu penyimpanan optimum adalah lebih tinggi daripada bijibenih ortodoks iaitu 15°C . Bijibenih rekalsitran, *Hopea odorata*, didapati sangat sensitif kepada suhu rendah di mana kesemua bijibenih telah mati pada 5°C . Suhu penyimpanan optimum bagi bijibenih ini adalah tinggi iaitu $15-20^{\circ}\text{C}$.

Kesan interaksi di antara kandungan kelembapan optimum dan suhu penyimpanan optimum telah dikaji dalam Bahagian ke-III untuk menentukan keadaan penyimpanan optimum yang lebih tepat bagi ketiga-tiga species tersebut. Bagi bijibenih ortodoks, *I. palembanica*, kandungan kelembapan dan suhu yang rendah pada 8-10% dan 5°C masing-masing didapati optimum untuk penyimpanan. Bagi bijibenih intermediat, *S. macrophylla*, kandungan kelembapan dan suhu yang

optimum adalah 25% dan 15 °C masing-masing. Bijibenih *H. odorata* didapati tidak boleh disimpan secara konvensional kerana kesemua bijibenih didapati mati selepas penyimpanan selama 3 bulan walaupun disimpan pada kandungan kelembapan dan suhu optimum mereka iaitu 32-35% dan 20 °C masing-masing.

Di dalam bahagian terakhir iaitu Bahagian ke-IV, keupayaan ketiga-tiga jenis bijibenih ini untuk dikrioawetkan telah dikaji. Embrio bijibenih ortodoks, *I. palembanica* didapati boleh dikrioawetkan secara langsung di dalam cecair nitrogen dengan 75% kemandirian pada kandungan kelembapan 10-11.9%. Bagi bijibenih intermediat, *S. macrophylla*, embrionya juga didapati boleh dikrioawetkan terutamanya pada kandungan kelembapan rendah iaitu 18% dan ke bawah yang mencatatkan kemandirian lebih 70% pada kelembapan 5-8%. Walaubagaimana pun, embrio *H. dorata* didapati tidak boleh dikrioawetkan kerana tiada kemandirian dicatatkan pada mana-mana kandungan kelembapan yang dikaji.

Sebagai kesimpulannya, bijibenih ortodoks seperti *I. palembanica* boleh disimpan dengan baiknya pada kandungan kelembapan dan suhu yang rendah dan embrio mereka didapati boleh dikrioawetkan dengan mudah walaupun pada kandungan kelembapan awal. Bijibenih intermediat seperti *S. macrophylla* pula memerlukan kandungan kelembapan dan suhu yang lebih tinggi untuk penyimpanan konvensional dan embrionya hanya boleh dikrioawetkan setelah dikeringkan kepada kandungan kelembapan 18% dan ke bawah. Bijibenih rekalsitran, *Hopea odorata*, didapati tidak boleh disimpan lebih dari 3 bulan secara konvensional dan kesemua embrio mereka telah mati apabila disimpan dalam cecair nitrogen.