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GERMPLASM PRESERVATION: STORAGE OF SEED, POLLEN AND TISSUE OF SELECTED MALAYSIAN ORCHID SPECIES

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by

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A thesis submitted in partial fulfilment of the
requirements for the degree of Doctor of Philosophy
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Lovingly dedicated to

S H A Z R I N

S H A Z W A N

K A M A R I A H

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LIST OF ABBREVIATIONS

$\overset{\circ}{A}$	:	Angstrom
$^{\circ}C$	:	Degrees Celcius
cm	:	Centimeter
CV	:	Coefficient of variation
DF	:	Degree of freedom
F	:	Calculated F-value
g	:	Gram
Hg	:	Mercury
kg	:	Kilogram
kV	:	Kilo Volt
kPa	:	Kilo Pascal
m	:	Meter
mg	:	Milligram
min	:	Minute
ml	:	Milliliter
mm	:	Millimeter
M	:	Molar
MS	:	Mean square
psi	:	Pound per square inch
REP	:	Replication
SE	:	Standard error
SED	:	Standard error of difference between means
ug	:	Microgram
v/v	:	Volume-to-volume
w/v	:	Weight-to-volume

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

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Supervisor : Professor Chin Hoong Fong

Faculty : Agriculture

In an attempt to develop methods for cheap, long-term preservation of orchid germplasm, seed, pollen and tissue of four local species were subjected to various storage conditions and subsequently determined of their effects on viability. The resultant quality of stored materials varied over the range of conditions and species investigated.

Storage of seeds of Arundina graminifolia, Cymbidium finlaysonianum and Spathoglottis plicata at seed moisture content of less than 5 percent and at storage temperature of less than 5°C gave percentage viability that ranged between 60 and 90 percent for a storage duration of more than twelve weeks. Storage in liquid nitrogen (-196°C) gave the highest

mean among the temperatures tested, confirming the orthodox nature of the seed.

Over the range of storage temperatures tested, storing pollen of A. graminifolia, S. plicata and Vanda hookeriana in liquid nitrogen gave the highest mean of viability (15, 64 and 57 percent respectively) for up to fourteen weeks of storage.

Protocorm tissue of V. hookeriana treated with cryoprotectant and prefrozen to a sub-zero temperature briefly before effecting storage in liquid nitrogen, offers a potential system to long-term preservation of the orchid species. The highest mean of survival (5.6 percent) following liquid nitrogen storage was afforded by cryoprotecting with 5 percent dimethyl sulfoxide in combination with 5 percent sucrose, and subsequent prefreezing to -40°C prior to storage.

Cryopreservation of orchid seed, pollen and tissue presents a number of potential practical advantages. The present study explored this technology as a supplement or extension to existing conventional systems of germplasm preservation.

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

PENGEKALAN JANAPLASMA: PENYIMPANAN BIJI BENIH, DEBUNGA DAN TISU SPESIS PILIHAN ORKID MALAYSIA

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Penyelia : Profesor Chin Hoong Fong

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Dalam usaha mencipta teknik untuk pengekalan janaplasma orkid dalam jangkamasa yang panjang, biji benih, debunga dan tisu dari empat spesis tempatan telah dirawat di dalam beberapa keadaan simpanan dan kemudiannya dikaji kesan-kesan rawatan ke atas daya percambahannya. Mutu bahan yang tersimpan berbeza-beza mengikut keadaan simpanan dan spesis yang dikaji.

Penyimpanan biji benih Arundina graminifolia, Cymbidium finlaysonianum dan Spathoglottis plicata pada paras kelembapan kurang dari 5 peratus dan pada suhu kurang dari 5°C memberi daya percambahan biji benih diantara 60 hingga 90 peratus dalam jangkamasa simpanan melebihi dua belas minggu. Penyimpanan bijibenih dalam cecair nitrogen (-196°C) memberi purata

tertinggi di antara suhu-suhu yang diuji, mengesahkan sifat 'orthodox' biji benih tersebut.

Di antara suhu-suhu yang diuji, penyimpanan debunga A. graminifolia, S. plicata dan Vanda hookeriana dalam cecair nitrogen memberi purata daya percambahan yang tertinggi (15, 64 dan 57 peratus tiap-tiap satu) dalam jangkamasa simpanan selama empat belas minggu.

Tisu protokom V. hookeriana yang telah dirawat dengan 'cryoprotectant' dan dibeku di bawah paras 0°C sebelum disimpan di dalam cecair nitrogen, merupakan satu sistem pengekalan jangkapanjang yang berpotensi bagi spesis orkid. Purata kemandirian tertinggi (5.6 peratus) selepas penyimpanan dalam cecair nitrogen telah diperolehi dari rawatan 5 peratus dwimetil sulfoksida bercampur 5 peratus sukros, dan pembekuan awal ke -40°C sebelum disimpan.

Penyimpanan krio biji benih, debunga dan tisu mempunyai beberapa kebaikan. Penyelidikan ini mengkaji teknologi tersebut sebagai satu tambahan kepada sistem-sistem pengekalan janaplasma yang sedia ada.