EFFECT OF CRYOPRESERVATION ON MICRO STRUCTURE OF RAMBUTAN EMBRYONIC AXIS

By

CHUA CHIN KOK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Specially dedicated to

my beloved family

and Sooi Ping

Abstract of thesis to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Nephelium lappaceum or locally known as rambutan is a valuable fruit species in Malaysia with export potential. Due to its recalcitrant seed characteristic, it cannot be preserved under classical storage conditions and thus, cryopreservation offers a promising tool for long-term storage and conservation of its genetic resources. The study on the morphology and anatomy of excised embryonic axes from seeds of rambutan was undertaken to provide a scientific understanding of the material to be cryopreserved and to elucidate the basis of cryopreservation-associated injuries. Histology of the embryonic axis, both longitudinal and transverse sections were carried out which allowed the reconstruction of three-dimensional model. The embryonic axis consisted of conical shaped epicotyl and dome shaped radicle. The recommended size for excision of the embryonic axis would be a 3mm cubical block that is made up of an oblong structured inner axis (1.8mm in length) attached to some amount of cotyledonary tissue. Retaining part of the cotyledon with the embryonic axis helped to provide the minimal nutrient supply for the embryonic axis. The three-dimensional model showed the connection of the embryonic axis to the cotyledons. The cotyledonary vessel

from the procambium of the radicle appeared to be the umbilical cord of the embryonic axis to the cotyledon. The embryonic axis, *in* and *ex vivo*, shared similar growth and development pattern. With adequate moisture, it was able to undergo the normal germination process. Shoot development in *in* and *ex vivo* embryonic axis was normal and rapid. Within four days of moisture imbibition, the conical shaped epicotyl had expanded into initial shoot. Growth of trichomes or hairy structures, which presumably could protect the embryonic axis from rapid desiccation and injury, also ensures germination. Dissimilarity occurred when the axis that germinated within the seed (*in vivo*) developed root cap while those cultured on MS media (*ex vivo*) did not. However, this characteristic had no adverse effect against a normal germination route.

Cryopreservation of recalcitrant seed species is difficult and is often not reproducible. Results reported by Hiew (1991) and Ginibun (2001) were not reproducible in this study in spite of close adherence to the protocol used. Even minor modification of the successful recipe and protocols reported by them did not produce surviving cryopreserved embryonic axis. It is evident in the study that the cells of the embryonic axis were possibly killed by the subzero temperature of the liquid nitrogen. As compared to the severe damage of fresh embryonic axis when directly exposed to liquid nitrogen, the structural damage of the cryopreserved embryonic axis appeared to be minimised after pretreatment by vitrification. This study suggested that the recipe of the vitrification solution used by Ginibun (2001) was not sufficient to reproduce the results reported. However, the potential of vitrification as a pretreatment prior to cryopreservation in liquid nitrogen cannot be discounted. Further study needs to be pursued on the effects of

liquid nitrogen on the vitrified cryopreserved embryonic axis of recalcitrant rambutan at the ultrastructural level. Fundamental studies through microscopic work have provided new insight and understanding of the plant material.

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

KESAN KRIOAWETAN TERHADAP STRUKTUR MIKRO EMBRIO RAMBUTAN

Oleh

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Nephelium lappaceum atau lebih dikenali dengan nama tempatan iaitu rambutan merupakan salah satu spesis buah-buahan di Malaysia yang penting dan mempunyai nilai potensi eksport. Oleh kerana biji benihnya yang bersifat rekalsitran, ia tidak boleh disimpan dalam keadaan simpanan klasik, maka teknik krioawetan merupakan satu kaedah untuk penyimpanan jangkamasa panjang serta pemuliharaan sumber genetiknya. Paksi embrio yang diasingkan daripada biji benih rambutan telah dikaji dari segi morfologi dan anatomi untuk memberi satu kefahaman saintifik ke atas bahan yang dikrioawet dan juga untuk menjelaskan asas kecederaaan yang dikaitkan dengan proses krioawetan. Histologi terhadap paksi embrio telah dibuat pada bahagian-bahagian memanjang dan melintang bagi memudahkan pembentukan model tiga dimensi. Paksi embrio terdiri daripada epikotil yang berbentuk kon dan radikel yang berbentuk kubah. Saiz pemotongan paksi embrio yang dicadangkan ialah blok kubus sepanjang 3mm yang terdiri daripada paksi dalaman persegi bujur (panjang 1.8mm) yang bersambung dengan sebahagian kecil tisu kotiledon. Pengekalan sebahagian tisu kotiledon dengan paksi embrio ini membantu membekalkan nutrien yang minima

kepada paksi embrio. Model tiga dimensi menunjukkan sambungan diantara paksi embrio dan kotiledon. Saluran kotiledon yang berasal daripada prokambium radikel merupakan tali pusat yang menyambung paksi embrio dengan kotiledon. Paksi embrio '*in*' dan '*ex vivo*' mempunyai persamaan dari segi corak pertumbuhan dan perkembangan. Dalam keadaan kelembapan yang mencukupi, ia dapat menjalani proses percambahan yang normal. Perkembangan pucuk dalam paksi embrio '*in*' dan '*ex vivo*' adalah normal dan cepat. Semasa imbibisi kelembapan selama masa empat hari, epikotil yang berbentuk kon telah berkembang kepada pucuk peringkat awalan. Pertumbuhan 'trichomes' atau struktur berambut yang diandaikan dapat melindungi paksi embrio daripada pengeringan yang terlalu cepat dan kecederaaan juga berlaku semasa percambahan. Perbezaan berlaku apabila paksi embrio yang bercambah di dalam biji benih membentuk jidal akar manakala paksi embrio yang dikultur dalam media MS tidak. Walau bagaimanapun, sifat ini tidak memberi kesan negatif terhadap proses percambahan yang normal.

Krioawetan spesis biji benih rekalsitran adalah sukar dan sering tidak dapat diulangi. Keputusan yang dilaporkan oleh Hiew (1991) dan Ginibun (2001) tidak dapat dihasilkan semula dalam kajian ini meskipun protokol mereka dipatuhi langkah demi langkah. Walaupun resipi dan protokol yang berjaya tersebut diubahsuaikan tetapi paksi embrio yang dikrioawet masih tidak dapat hidup. Jelas terbukti dalam kajian ini bahawa sel-sel paksi embrio mungkin telah terjejas oleh suhu terlampau sejuk di dalam cecair nitrogen. Berbanding dengan kecederaan teruk yang dialami oleh paksi embrio yang segar apabila didedah kepada cecair nitrogen, kecederaan struktur pada paksi embrio yang telah diberi prarawatan

vitrifikasi sebelum dikrioawet kelihatan lebih minima. Kajian ini mencadangkan resipi larutan vitrifikasi yang digunakan oleh Ginibun (2001) tidak mencukupi untuk menghasilkan semula keputusan yang dilaporkan. Walaupun demikian, potensi vitrifikasi sebagai pra-rawatan sebelum krioawetan di dalam cecair nitrogen tidak boleh dinafikan. Kajian yang mendalam di tahap ultrastruktur tentang kesan cecair nitrogen ke atas paksi embrio rambutan yang rekalsitran perlu diteruskan. Pengajian asas melalui kerja-kerja mikroskopik telah memberikan gambaran dan kefahaman yang menakjubkan terhadap bahan tumbuhan.

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I certify that an Examination Committee met on 13 March 2004 to conduct the final examination of Chua Chin Kok on his Master of Science thesis entitled "Effect of Cryopreservation on Micro Structure of Rambutan Embryonic Axis" in accordance with Universiti Pertanian (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:

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This thesis submitted to the Senate of 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:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which 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.

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CHUA CHIN KOK

Date:

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