The Micromorphology of Rambutan (*Nephelium lappaceum*) and Durian (*Durio zibethinus*)

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Introduction

Germplasm conservation of species producing recalcitrant seeds is a problem and poses a great challenge. Presently, conservation of such materials is made *via* the field gene bank methods whereby the plants are maintained in the field. However, this method of conservation is costly, labor intensive and occupies a large area of valuable land, besides the risk of pests, diseases and natural disasters. It is now well recognized that for any given gene pool, a number of different complementary approaches and methods are necessary for a safe, efficient and cost-effective conservation. Cryopreservation has been proposed as the only viable option for conservation of recalcitrant seeds and vegetatively propagated materials. Despite it being mentioned as the only possible method for conservation of such materials success has been limited and the sporadic survival is often not reproducible. The approach to cryopreservation is usually through empiric means by screening for tolerance to various cryoprotectant cocktails and testing for the best exposure duration. This results in little understanding of the effect of the treatment on the material used for cryopreservation. We hereby propose that the understanding of the material used for cryopreservation is an important factor for success as it would increase our fundamental understanding of the materials and allow elucidation of cryoprotective mechanism and cryo-associated injuries.

Materials and Methods

Mature rambutan fruits of clone R162 were collected from Department of Agriculture orchard, Serdang. The seeds were cleaned of the sarcotesta, washed under running water and blotted dry. The embryonic axes were cut into block with each cotyledon attached, for optical microscopy. The embryonic axes were fixed for 24 hours in formaldehyde acetic acid (FAA), dehydrated through graded series of alcohol and embedded in wax. After embedding, serial transverse and longitudinal sections were cut at 12 µm in a rotary microtome and sections were stained with Toluidine blue O. Similarly, a landrace of durian fruit was obtained from the local night market. The fruit was split open and the fleshy aril around the seed was removed manually. The seed was then cleaned under running tap water, blotted dry and carefully dissected to reveal the embryo still attached to part of the cotyledonary tissue. In addition to the preparation for histology studies as mentioned for rambutan, these tissues were fixed in Bouin's fixative, post fixed in 1% cocadylate buffered osmium tetroxide, dehydrated in graded series of ethanol, critical point dried in Balzer CD 30, sputter coated and observed under JOEL 6400 scanning electron microscope with an acceleration voltage of either 10 or 15 kV.

Results and Discussion

The rambutan embryonic axis is located at the distal end of the seed, away from the hilum and is bridged by two large cotyledons which are not proportionate in size. The embryonic axis appears as a small oblong shaped structure, formed by the dome shaped rudimentary radicle and conical shaped epicotyl, which is not connected. The embryonic axis connects to the two cotyledon halves through tissue extension at the junction of radicle and epicotyl. Mophologically, the conical shaped epicotyl is formed by two slightly raised regions, which differ from the single peak in the embryo of jackfruit. These two-raised regions in the epicotyl cone are the first leaf primordial, which are supported internally by the procambial tissue. The radicle region of the embryonic axis is dome shaped, protrudes slightly beyond the surface of the seed. It is made up of three distinct layers, namely a thick cortical region, a thin-layered intermediate provascular tissue and inner ground meristematic tissue. Transversely, the shape of embryonic axis changes from the apex to the point of attachment to the cotyledon, with gradual transformation of the shape from two small lobes to peanut-like shape joined at the apical plane and finally to an ovular structure in which the size gradually reduces. The continuity of the embryonic axis procambium is disrupted at the region adjoining the cotyledonary tissue and the embryonic axis tissue. At this region of attachment, the cotyledonary vessel progressively fused to the procambium. Presumable forming a food mobilization network. The surface of the rambutan embryonic axis is formed by columnar cells, which develop into single trichome during germination.

In the case of durian the embryo is located below the hilum and is adpressed between two massive cotyledons. It is wedge shaped, with the broad side below the hilum and tapering towards the apical end attached to the cotyledonary tissue. Transversely, the shape changes from round to diamond and finally to two isosceles triangles joined at the base, rather different to that observed for rambutan. The embryo is completely separated by a narrow space throughout the entire length from the surrounding cotyledon except at the apical end of the embryo. The cotyledonary tissue adjoining the embryo at the apical end contains numerous mucilaginous bodies. The separation between the cotyledon and the embryo is maintained by

flattened, round or oval shaped spongy tissues or polyps on the surface of the embryo with numerous pores interspersed between the spongy polyps. These polyps develop into stellate type trichome during germination again differing with the single trichome observed on the surface of rambutan embryonic axis during germination.

Conclusions

Based on the structural understanding of the two materials used in this study, it is clear that variations occur among seeds in many forms such as seed and axis size, location of the embryo within the seed, attachment between the embryo and the cotyledon and also on the surface morphology of the embryonic axis. This demonstrates the need for unique handling method for each species especially when parts such as the embryo or embryonic axis is involved. The currently employed trial and error technique of cryopreservation may remain empirical if sound understanding of the material and method of handing does not exist. This study will also provide avenue for understanding the nature of desiccation and freezing damages, which frequently occurs during the cryopreservation.

Benefits from the study

This study has shown that due to the orientation and connection between the embryo and the cotyledon, excision of embryos are to be done accordingly. Durian embryo can be easily popped out of the cotyledons whereby one of the isosceles triangles would snap of at the apical end with no cotyledonary tissues attached with it. However, rambutan embryos are more tedious to handle as a block consisting of embryonic axis and part of the cotyledonary tissues have to be excised in order to obtain survival. This is due to the extension of the radical procambial tissue into the cotyledonary tissue at the sides around the area of the junction between epicotyl and radicle. As the embryonic axis is the material to be cryopreserved, it is important to ensure that the material is in good condition and is highly regenerable.

Patent(s), if applicable :

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

1. Sinniah U R and. Razak AR. 2000. Comparative Micromorphology Of Durian Embryo (*Durio Zibethinus*) During Germination. In: Proceedings of the 7th Asia-Pacific Electron Microscopy Conference "Perspective Imaging", Singapore, Pp. 90 – 92.

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Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Chua Chin Kok	**Structural biology of rambutan embryonic axis in relation to cryopreservation**	**Cryopreservation, structural biology**	**MSc (final stages of completion)**	

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