UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF *IN VITRO* PROPAGATION TECHNIQUES FOR *ENDOSPERMUM MALACCENSE* M.A AND *SHOREA PARVIFOLIA* DYER

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By

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Chairman: **Associate Prof. Dr. Nor Aini Ab. Shukor**

Faculty: **Forestry**

Endospermum malaccense M.A and *Shorea parvifolia* Dyer are two commercially important timber species identified as potential plantation species. The procurement and storage of their seeds are difficult and is a major hindrance to plantation establishment. Development of micropropagation techniques is being pursued to provide an alternative in planting stock production.

Micropropagation of *E. malaccense* was achieved through *in vitro* production of plants through axillary shoots development from nodal segment explants of an elite tree. The explants were initially washed in 10 changes of sterile distilled water, followed by 5 changes of 0.05% (v/v) Tween 20 solution for 10 minutes each. This was then followed by rinsing in 10 changes of sterile distilled water and subsequently sterilised in a solution comprising 60 % (v/v) Clorox and 0.05% v/v Tween 20 for 10 minutes. After

which they were rinsed 10 times in 300ml sterile distilled water and finally immersed in 70% (v/v) ethanol for 1 minute. Shoots were induced on the nodal segment explants in MS basal salts supplemented with 22.2 x 10^{-6} M or 44.4 x 10^{-6} M. For shoot multiplication, the best medium is MS supplemented with 44.4 x 10^{-6} M BAP and solidified with a mixture of 1.7 g Gelrite and 4g Bacto agar per liter. *In vivo* rooting with Seradix 3 was more suitable for *in vitro* produced shoots of *E. malaccense*, compared with an *in vitro* rooting technique.

For *S. parvifolia*, high contamination was observed in all explant types. Multiple shoots were induced on nodal segments culture in WPM solid medium supplemented with 10^{-5} M BAP or 10^{-6} TDZ but were non-amenable to further subculture. Callus developed from immature seeds with gelatinous endosperm termed as embryonic masses in WPM supplemented with 10^{-4}M CPA induced callus formation. Globular shaped callus developed upon subculture. Histological examination of the globular shaped callus showed no evidence of somatic embryos formation. The globular structure was similar to the development of nodules.

The contaminants found on the immature seeds included a fungus, *Collectotrycum* spp. and a range of bacteria which are as follows: -*Kleibsella planticola*, *Enterobacter agglomerans*, *Erwina* spp. (*E. uredora* or *E. herbicola*), *Serratia odorifera*, *Serratia marcesens*, *Serratia proteomaculans*, *Morganella moragnii*, *Kluyera ascorbata*. A fern, *Asplenium nidus* was found contaminating the nodal segment cultures.

Abstrak tesis yang dikemukakan kepada Senat Universityi Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

PEMBENTUKAN TEKNIK MIKRO PERAMBATAN UNTUK *ENDOSPERMUM MALACCENSE* M.A DAN *SHOREA PARVIFOLIA* DYER

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April 2003

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Endospermum malaccense M.A dan S*horea parvifolia* Dyer, merupakan dua sepsis balak yang berpotensi ditanam secara ladang. Masalah pengutipan dan penyimpanan bijibenih menghindar kemajuan peladangan hutan secara komersial. Pembentukan teknik mikroperambatan dapat mengatasi masalah pembekalan bahan tanaman.

Mikroperambatan *E. Malaccense* tercapai apabila pembentukan pucuk aksil terbentuk ke atas eksplan bahagian nod pokok elit. Eksplan dibasmikuman dengan mencuci 10 kali dengan air suling steril, diikuti dengan 5 pencucian dalam larutan 0.05% (isipadu/isipadu) Tween 20 selama 10 minit, diikuti pula dengan 10 pertukaran air suling steril. Kemudian dicuci pula dalam larutan 60% (isipadu/isipadu) Clorox

dicampur dengan 0.05% (isipadu/isipadu) Tween 20 selama 10 minit. Kemudian dicuci dengan 10 kali dengan 300ml air suling steril dan akhir sekali direndam didalam 70% (isipadu/isipadu) etanol. Media MS dicampur dengan 22.2 x 10^{-6} M or 44.4 x 10^{-6} M sesuai dalam pengaruhan pembentukan pucuk. Gandaan pucuk tercapai dalam campuran medium MS dan 44.4 x 10^{-6} M BAP yang dipepejalkan dengan jel campuran 1.7 g Gelrite and 4g Bacto agar se liter. Teknik pengakaran *in vivo* dengan Seradix 3 lebih sesuai dibandingkan dengan teknik *in vitro*.

Untuk *S. parvifolia*, kadar kontaminasi tinggi dihadapi dalam kultur semua jenis eksplan. Pembentukan pucuk berbilang tercapai apabila bahagian nod di kulturkan didalam medium WPM yang ditambah dengan 10^{-5} M BAP atau 10^{-6} M TDZ. Pertambahan pucuk tidak tercapai dalam semua jenis medium. Pembentukan kalus terjadi ke atas eksplan endoperma cair di dalam medium WPM ditambah dengan 10^{-4} M CPA. Pengsubkulturan menghasilkan kalus berbentul globul. Kajian histology menunjukkan bahawa struktur ini adalah nodul.

Bijibenih muda dikontaminasikan oleh sejenis kulat, *Collectotrycum* spp. Dan pelbagai jenis bacteria seperti berikut:--*Kleibsella planticola, Enterobacter agglomerans, Erwina spp. (E. uredora or E. herbicola), Serratia odorifera, Serratia marcesens, Serratia proteomaculans, Morganella moragnii, Kluyera ascorbata*. Sejenis paku penumpang *Asplenium nidus* juga didapati menkontaminasikan kultur.

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