MICROPROPAGATION OF TONGKAT ALI (*EURYCOMA LONGIFOLIA* JACK) VIA SOMATIC EMBRYOGENESIS AND DIRECT PLANT REGENERATION TECHNIQUES

By

SOBRI BIN HUSSEIN

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

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Especially dedicated to:

My parents, Brothers, Sisters

&

Dr. Anna Ling

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

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Chairman: **Professor Maziah Mahmood, Ph.D.**

Faculty: **Biotechnology and Biomolecular Sciences**

Eurycoma longifolia Jack or Tongkat Ali is well known among the local communities mainly for its aphrodisiac properties and its effectiveness as the cytotoxic, anti-malarial, anti-ulcer, anti-tumor promoting and anti-parasitic agent. In view of its potential commercial value as a plantation crop as well as to conserve its germplasm, the somatic embryogenesis and direct plant regeneration of *E. longifolia* were carried out as these *in vitro* micropropagation protocols had not been reported.

In attempts to establish the somatic embryos of *E. longifolia*, the potential of cotyledon, zygotic embryo, leaf, petiole, stem and taproot in forming embryogenic callus were examined in the basal Murashige and Skoog (MS) medium supplemented with different auxins at various concentrations. Only cotyledon explants were able to form embryogenic callus in the presence of 1.0 mg/L (w/v) of 2,4-dichlorophenoxyacetic (2,4-D) at 30%. A higher yield (60%) of

embryogenic callus was obtained when the Type 4 method dissected cotyledon explants were cultured in basal MS medium containing 0.5 mg/L (w/v) of kinetin and 1.0 mg/L (w/v) of 2,4-D. The highest number of somatic embryos (45 ± 2) was observed in the same medium formulation with the addition of 1.0 g/L (w/v) activated charcoal. Subsequent transfer of these mature somatic embryos in basal MS media supplemented with 1.0 mg/L (w/v) of kinetin produced a 90% of plantlet regeneration. Addition of activated charcoal, casein hydrolysate, abscisic acid, proline and polyethylene glycol (PEG) at various concentrations into the regeneration medium did not stimulate the conversion of *E. longifolia* somatic embryo into plantlet. The differences between the embryogenic and non-embryogenic callus were also determined based on histological studies.

Successful direct plant regeneration was obtained from the root, stem, shoot tip, axillary and adventitious bud explants. Each explant generally requires different combinations of media and plant growth regulators to produce the highest regeneration percentage. In the root explant, the best medium formulation determined was basal Juglans medium (DKW) supplemented with 1.0 mg/L (w/v) of kinetin + 1.0 mg/L (w/v) of zeatin whereas in the stem explants, basal woody plant medium (WPM) enriched with 2.0 mg/L (w/v) of BAP and 2.0 mg/L (w/v) of zeatin was found to be the best medium formulation in increasing the regeneration rate and healthy plantlets formation. Stem and root explants that were 2 cm distant from one another as has been identified as the most suitable position for attaining the maximum percentage of direct plant regeneration. Successful direct plant regeneration from *in vitro* and *in vivo* shoot tip explants of

E. longifolia was achieved in basal MS medium supplemented with 3.0 and 5.0 mg/L (w/v) of kinetin, respectively. As for the direct plant regeneration from axillary bud explant, explants produced the highest regeneration capability (90%) in basal Nitsch medium (NM) supplemented with 10.0 mg/L (w/v) of zeatin while basal NM supplied with 6.0 mg/L (w/v) of zeatin produced the highest regeneration percentage in adventitious bud explants. Rooting induction of *in vitro* plantlets of *E. longifolia* was also achieved and the highest induction rate was attained in basal MS medium supplemented with 0.5 mg/L (w/v) of Indole-3-butyric acid (IBA). Acclimatization of *in vitro* plantlets regenerated from somatic embryogenesis and direct plant regeneration survived well with no morphological differences from the parent plants after two months of transplantation to the soil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

MCROPROPAGASI TONGKAT ALI (*EURYCOMA LONGIFOLIA* JACK) MELALUI KAEDAH SOMATIK EMBRIOGENESIS DAN REGENERASI SECARA TERUS

Oleh

SOBRI BIN HUSSEIN

Oktober 2004

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Eurycoma longifolia Jack atau Tongkat Ali lebih dikenali oleh penduduk tempatan kerana ianya mempunyai ciri afrodisiak dan keberkesanannya sebagai sitotoksik, anti malaria, anti ulser, anti tumor dan agen anti parasit. Memandangkan potensi nilai komersial sebagai tanaman estet dan juga untuk pemuliharaan germplasma, pembentukan embrio somatik dan regenerasi secara terus *E. longifolia* telahpun dijalankan kerana cara micropropagasi secara *in vitro* tidak pernah dilaporkan sebelum ini.

Dalam usaha untuk mendapatkan embrio somatik daripada *E. longifolia*, kalus yang berpotensi daripada eksplan kotilidon, embrio zigotik, daun, petiol, batang dan akar tunjang telah dianalisis dalam basal media Murashige and Skoog (MS) yang dibekalkan dengan pelbagai jenis auksin pada kepekatan yang berbeza. Hanya eksplan kotilidon sahaja yang mampu menghasilkan kalus embriogenik sebanyak 30% dengan kehadiran 1.0 mg/L (b/i) 2,4-dichlorophenoxyacetic (2,4-

D). Sebanyak 60% kalus embriogenik dihasilkan bila kaedah pemotongan jenis ke-4 dikulturkan di dalam basal media yang mengandungi 0.5 mg/L (b/i) kinetin dan 1.0 mg/L (b/i) 2,4-D. Sebanyak 45 ± 2 embrio somatik dapat diperhatikan di dalam formulasi medium yang sama dan telah ditambah dengan 1.0 g/L (b/i) arang teraktif. Seterusnya, pemindahan embrio somatik yang matang ke dalam basal MS medium yang telah dibekalkan dengan 1.0 mg/L (b/i) kinetin boleh menghasilkan regenerasi anak pokok sebanyak 90%. Penambahan arang teraktif, kasein hidrolisat, asid absisik, prolin dan polietilene glicol (PEG) pada kepekatan yang berbeza ke dalam medium regenerasi tidak berjaya merangsang regenerasi embrio somatik kepada anak pokok. Perbezaan di antara kalus embriogenik dan kalus bukan embriogenik juga dapat dikenalpasti melalui analisis histologi.

Regenerasi secara terus telah berjaya diperolehi daripada eksplan akar, batang, tunas ketiak dan juga tunas sisi. Setiap eksplan memerlukan kombinasi medium dan hormon perangsang tumbuhan yang berbeza untuk menghasilkan peratus regenerasi yang tinggi. Bagi eksplan akar, formulasi medium terbaik telah dikenalpasti di dalam basal Juglans medium DKW yang dibekalkan dengan 1.0 mg/L (b/i) kinetin + 1.0 mg/L (b/i) zeatin, manakala bagi eksplan batang, basal medium WPM yang dibekalkan dengan 2.0 mg/L (b/i) BAP dan 2.0 mg/L (b/i) zeatin telah dikenalpasti sebagai formulasi terbaik kerana menghasilkan anak pokok yang sihat serta peratus regenerasi yang tinggi. Eksplan akar dan batang yang berada pada jarak 2 cm di antara satu sama lain telah dikenalpasti sebagai posisi yang paling sesuai untuk menghasilkan peratus regenerasi yang maksima. Regenerasi secara terus daripada eksplan pucuk *in vitro* dan *in vivo E. longifolia*

telah diperolehi di dalam basal medium MS yang dibekalkan masing-masing dengan 3.0 dan 5.0 mg/L (b/i) kinetin. Bagi regenerasi secara terus daripada eksplan tunas ketiak, sebanyak 90% kadar regenerasi diperolehi di dalam basal medium Nitsch (NM) yang dibekalkan dengan 10.0 mg/L (b/i) zeatin manakala basal medium NM yang dibekalkan dengan 6.0 mg/L (b/i) zeatin telah menghasilkan peratus regenerasi yang tinggi bagi eksplan tunas sisi. Pengakaran daripada anak pokok *in vitro E. longifolia* juga berjaya dihasilkan dan kadar penghasilan yang tertinggi telah diperolehi di dalam basal media MS yang dibekalkan dengan 0.5 mg/L (b/i) Indole-3-butyric acid (IBA). Anak pokok *in vitro* yang terbentuk daripada embrio somatik dan regenerasi secara terus tumbuh dengan baik dan tidak mennujukkan sebarang perbezaan dari segi morfologi dengan pokok induknya selepas dua bulan dipindahkan ke dalam tanah.

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I certify that an Examination Committee met on 4th October 2004 to conduct the final examination of Sobri Bin Hussein on his Doctor of Philosophy thesis entitled "Micropropagation of Tongkat Ali (*Eurycoma longifolia* Jack) via Somatic Embryogenesis and Direct Plant Regeneration Techniques" in accordance with Universiti Pertanian Malaysia (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 Doctor of Philosophy. 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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SOBRI BIN HUSSEIN

Date:

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