UNIVERSITI PUTRA MALAYSIA

SOMATIC EMBRYOGENESIS,ORGANOGENESIS AND ASSESSMENT OF SOMACLONAL VARIATIONS IN MANGOSTEEN (*Garcinia mangostana* L.)

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DOCTOR OF PHILOSOPHY

UNIVERSITI PUTRA MALAYSIA

2011

This thesis is dedicated to:

My husband, Epijanto Anggariadi

My mother, Siti Aisjah and my father, Amrie Anwar

My mother in law, Soemiani

My sons, Muhammad Ammar Aiman Majid and

Muhammad Zidni Ilman Tafsiri

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

SOMATIC EMBRYOGENESIS, ORGANOGENESIS AND ASSESSMENT OF SOMACLONAL VARIATIONS IN MANGOSTEEN (*Garcinia mangostana* L.)

By

INNAKA AGENG RINEKSANE

May 2011

Chairman : Associate Professor Mihdzar Abdul Kadir, PhD

Faculty : Agriculture

Mangosteen is one of the most delicious tropical fruits which has an increasing demand due to its wide range of uses. It is used for its medicinal properties such as an antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal and antiviral. One of the problems related to the establishment of mangosteen plantation is to obtain seedlings throughout the year, which can be solved by micropropagation.

In attempts to establish the embryogenic calli of *G. mangostana*, the potential of uncoated and coated seed explants in forming embryogenic callus was examined in the basal Linsmaier and Skoog (LS) medium supplemented with different auxins at various concentrations. Combinations of cytokinins and 2,4-D in two different media [Murashige and Skoog (MS) and LS] were assessed to improve embryogenicity of calli in mangosteen.

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Addition of glutamine at various concentrations into MS medium containing 8 mg/L 2,4-D and 0.1 mg/L BAP was also carried out to induce embryogenic callus of mangosteen. A study was also carried out to determine the growth and multiplication of cells in suspension cultures and the effect of cytokinins on the advanced formation of embryogenic stages of mangosteen. BAP and NAA were considered for shoot regeneration and the rooting of mangosteen shoots. The most favorable medium for acclimatization of mangosteen plantlets was also produced. The assessment of somaclonal variations among mangosteen plantlets by using RAPD was performed.

Uncoated seed explants produced a lower percentage of callus formation (44.23 %), callus score (1.66), fresh weight of callus (59.98 mg) and a lower percentage of contaminated explants (9.7 %) compared to coated seed explants. Among the highest percentage of callus formation (93.3 %) and callus score (3.06) were obtained when uncoated seed explants were cultured on basal LS medium containing 8 mg/L 2,4-D. The calli were yellowish, compact and nodular compared to the spongy loose, whitish and yellowish calli produced on media containing IAA, IBA or NAA. The highest percentage of callus formation (80 %) and the lowest percentage of callus browning (53.53 %) occurred on MS medium supplemented with 8 mg/L 2,4-D and 0.1 mg/L BAP. Although glutamine did not increase the growth of calli, the texture (more friable) and color of callus (more yellowish) were improved.

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The cells were able to divide and proliferate eventhough cultured in half strength MS liquid medium without 2,4-D. After six months of culture, the heart embryogenic stage was obtained only on medium supplemented with 1 mg/L BAP. The globular and torpedo embryogenic stages were obtained on media supplemented with 1, 3 and 9 mg/L TDZ after five months of culture.

Mass production of adventitious shoots was achieved by culturing seed segments of mangosteen on MS solid medium supplemented with 5 mg/L BAP + 0.1 mg/L NAA which produced the highest shoot number (31.7 shoots). Forty-one percent of shoots were successfully rooted in MS liquid medium supplemented with 1 mg/L IBA, 60 g/L sucrose and 5 g/L activated charcoal after 4 months. During acclimatization, plantlets grown in medium consisting of organic matter only (A6) showed the highest height difference (7 mm) as compared with other treatments.

Stunted shoots with narrow leaves were produced in abundance in MS solid medium supplemented with 5 mg/L BAP + 0.1 mg/L NAA. These shoots were morphologically and genetically different from shoots of other treatments as detected by RAPD marker. RAPD marker effectively recognized the genetic difference among the *in vitro* shoots and from their mother plant with high level of similarity (80 %). Among the acclimatized plantlets and *in vitro* shoots, 72 % level of similarity was obtained. The lowest level of similarity (55%) was found between *in vivo* samples from Serdang and Pahang.

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

SOMATIK EMBRIOGENESIS, ORGANOGENESIS DAN PENGKAJIAN VARIASI SOMAKLONAL PADA MANGGIS (*Garcinia mangostana* L.)

Oleh

INNAKA AGENG RINEKSANE

Mei 2011

Pengerusi : Profesor Madya Mihdzar Abdul Kadir, PhD

Fakulti : Pertanian

Manggis merupakan antara spesies buah tropika yang paling lazat, mempunyai permintaan yang semakin bertambah kerana ianya mempunyai keberkesanan sebagai anti oksidan, anti tumor, anti-inflamasi, anti alergi, anti bakteria, anti jamur dan agen anti virus. Permasalahan yang berlaku adalah tidak cukupnya anak pokok untuk penanaman skala besar. Salah satu pilihan untuk pembiakan secara besar-besaran ialah dengan kultur tisu.

Dalam usaha untuk mendapatkan embrio somatik daripada *G. mangostana*, kalus yang berpotensi daripada eksplan biji berkulit dan tidak berkulit telah diuji dalam medium basal Linsmaier dan Skoog (LS) yang dibekalkan dengan pelbagai jenis auksin pada kepekatan yang berbeza. Kombinasi sitokinin dan 2,4-D yang dibekalkan dalam dua media yang berbeza [Murashige dan Skoog (MS) dan LS] telah diuji untuk merangsang kalus embriogenik pada manggis. Penambahan glutamin pada kepekatan yang berbeza ke dalam medium MS

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yang mengandungi 8 mg/L 2,4-D dan 0.1 mg/L BAP juga dikenalpasti untuk merangsang kalus embriogenik manggis. Penyelidikan juga telah dijalankan untuk mengenalpasti regenerasi dan multiplikasi sel dalam kultur suspensi dan mengenalpasti kesan daripada sitokinin untuk merangsang pembentukan tahap embriogenik daripada manggis. BAP dan NAA diuji untuk regenerasi pucuk dan akar. Media aklimatisasi yang paling berkesan untuk planlet manggis juga telah dikenalpasti. Pengenalpastian variasi somaklonal di antara plantlet manggis melalui kaedah RAPD juga telah dikaji.

Biji tidak berkulit menghasilkan peratusan lebih rendah pada regenerasi kalus (44.23%), skor kalus (1.66), berat segar kalus (59.98 mg) dan peratusan lebih rendah pada eksplan terkontaminasi (9.7%) apabila dibandingkan dengan biji berkulit. Antara peratusan regenerasi kalus (93.3%) dan skor kalus paling tinggi (3.06) telah diperolehi apabila eksplan biji tidak berkulit dikulturkan di dalam medium basal LS yang mengandungi 8 mg/L 2,4-D. Kalus yang dihasilkan adalah padat kekuningan dan bernodul apabila dibandingkan dengan kalus berspan, putih dan kekuningan yang dihasilkan daripada media yang mengandungi IAA, IBA atau NAA. Peratusan regenerasi kalus tertinggi (80%) dan peratusan pemerangan kalus terendah (53.53%) dihasilkan daripada medium MS yang dibekalkan dengan 8 mg/L 2,4-D dan 0.1 mg/L BAP. Walaupun glutamin tidak merangsang regenerasi kalus, tekstur kalus yang lebih rapuh (friable) dan warna kalus yang lebih kekuningan telah ditingkatkan.

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Sel dapat membahagi dan mengganda walaupun dikulturkan di dalam medium MS separuh cecair yang tidak mengandungi 2,4-D. Enam bulan selepas pengkulturan, embrio somatik jantung telah diperolehi hanya pada medium yang mengandungi 1 mg/L BAP. Embrio somatik bulat dan torpedo telah diperolehi pada medium yang mengandungi 1, 3 dan 9 mg/L TDZ selepas 5 bulan pengkulturan.

Pengeluaran pucuk adventitius secara besar-besaran telah diperolehi dengan pengkulturan segmen biji manggis ke dalam medium padat MS yang dibekalkan dengan 5 mg/L BAP dan 0.1 mg/L NAA yang telah menghasilkan bilangan pucuk tertinggi (31.7 pucuk). Empat puluh satu peratus pucuk telah berjaya menghasilkan akar pada medium cecair MS yang mengandungi 1 mg/L IBA, 60 g/L sukrosa dan 5 g/L arang teraktif selepas 4 bulan. Untuk aklimatisasi, plantlet yang dipindahkan ke dalam medium organik (A6) sahaja yang menghasilkan perbezaan ketinggian tertinggi (7 mm) apabila dibandingkan dengan rawatan yang lain.

Pucuk terbantut dengan daun kecil telah dihasilkan dalam bilangan besar daripada medium padat MS yang mengandungi 5 mg/L BAP dan 0.1 mg/L NAA. Ianya menunjukkan perbezaan dari segi morfologi dan genetik dengan pucuk-pucuk yang dihasilkan daripada rawatan lainnya selepas dikenalpasti melalui kaedah RAPD. Kaedah RAPD telah berjaya mengenalpasti variasi genetik di antara pucuk *in vitro* dan pokok induk dengan tahap persamaan yang tinggi (80%). Di antara plantlet aklimatisasi dan pucuk-pucuk *in vitro*,

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tahap persamaan 72% telah diperolehi. Tahap persamaan terendah (55%) telah dikenalpasti di antara aksesi *in vivo* dari Serdang dan Pahang.

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I certify that a Thesis Examination Committee has met on 19 May 2011 to conduct the final examination of Innaka Ageng Rineksane on her thesis entitled "Somatic Embryogenesis, Organogenesis and Assessment of Somaclonal Variation in Mangosteen (*Garcinia mangostana* L.)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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Date: 26 July 2011

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of **Doctor of Philosophy**. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

INNAKA AGENG RINEKSANE

Date: 19 May 2011

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TABLE OF CONTENTS

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