UNIVERSITI PUTRA MALAYSIA

# EFFECTS OF SELECTED PLANT GROWTH REGULATORS ON REGENERATION OF MANGOSTEEN (GARCINIA MANGOSTANA L. ) USING TISSUE CULTURE TECHNIQUE

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*In The Name of Allah, the Most Gracious and the Most Merciful*

*Specially Dedicated*

*To*

*My heaven dwelling father:*

*Mohammad Torabi Sirchi*

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

**EFFECTS OF SELECTED PLANT GROWTH REGULATORS ON REGENERATION OF MANGOSTEEN (*GARCINIA MANGOSTANA* L.) USING TISSUE CULTURE TECHNIQUE**

By

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The *in-vitro* plantlet regeneration system via organogenesis and callus induction was developed from various mangosteen explants (seed, shoot tip, stem and leaf) using various plant growth regulators (PGR). For shoot induction, the mangosteen half seed, leaf and stem explants sufficiently achieved shoot regeneration rates above 60% of total explants cultured (70%, 60% and 60% respectively) on various auxin and cytokinin supplementation into the basal MS medium [4.0 $mgL^{-1}$ (w/v) BAP with 0.2 $mgL^{-1}$ (w/v) NAA for seed; 1.0 $mgL^{-1}$ (w/v) BAP in combination with 0.05 $mgL^{-1}$ (w/v) KIN for stem; 2.0 $mgL^{-1}$ (w/v) of BAP for leaf]. Shoot induction from the shoot tip explant was not successful. However,

among the three explants, the leaf explant gave the highest mean number of shoots at 46.5 (seed and stem at 9.3 and 8.9 respectively). Mean shoot height however, was 0.48 cm for leaf, while it was higher for shoots generated from stem and seed explants (0.7 cm and 0.8 cm respectively). The shoots rooted successfully on full strength MS medium without IBA with high percentage of root formation (87%) and number of roots (3.8) (seven months after culture). The plantlets were then successfully potted and acclimatized on medium containing soil + sand+ organic matter+ vermiculite mixture (2:2:1:1) which gave the highest number of leaves (6.5), plant height (3.5cm) and percentage of plant survival (80%). As for callus induction, induction from seed and leaves was 90% on medium with 0.5 $mgL^{-1}$ (w/v) NAA, while induction from shoot tip and stem was 90% and 83% respectively on medium with 0.2 $mgL^{-1}$ (w/v) KIN. Our study has successfully developed a plant regeneration system from easily available stem and green juvenile leaf explants, as compared to the seasonally available and limited seed and young leaf explants that are generally reported by other researches. Furthermore, callus induction was successful from various mangosteen explants for future utilization in the development of a plant regeneration protocol from callus, as callus also has the potential of producing improved varieties via various genetic modification techniques today.

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

# KESAN HORMON PENGAWAL ATUR PERTUMBUHAN POKOK TERPILIH KE ATAS REGENERASI MANGGIS (*Garcinia mangostana* L.) DENGAN MENGUNAKAN TEKNIK KULTUR TISU

**Oleh**

**MOHAMMAD HOSSEIN TORABI SIRCHI**

**April 2009**

**Pengerusi: Prof. Madya Dr. Mihdzar Abdul Kadir, Ph. D**

**Fakulti: Pertanian**

Sistem regenerasi anak pokok *in-vitro* melalui organogenesis dan pembentukan kalus telah berjaya dihasilkan dari pelbagai eksplan manggis (biji benih, pucuk, batang dan daun hijau) di bawah pengaruh pelbagai kombinasi hormon pengawal atur pertumbuhan pokok (PGR). Kajian regenerasi pucuk baru dari eksplan biji, daun dan batang telah mencapai kadar regenerasi yang memuaskan iaitu sebanyak 60% dari jumlah eksplan yang dikulturkan (70%, 60% dan 60% masing-masing) dalam medium asas MS yang mengandungi pelbagai kombinasi auksin dan sitokinin [4.0 $mgL^{-1}$ (w/v) BAP dengan kombinasi 0.2 $mgL^{-1}$ (w/v) NAA untuk eksplan biji; 1.0 $mgL^{-1}$ (w/v) BAP dengan kombinasi 0.05 $mgL^{-1}$ (w/v) KIN

untuk eksplan batang; 2 $mgL^{-1}$ (w/v) BAP untuk eksplan daun]. induksi pucuk dari eksplan pucuk manggis tidak berjaya. Namun begitu, di antara tiga eksplan yang berjaya menghasilkan regenerasi tersebut, regenerasi dari eksplan daun menghasilkan min bilangan pucuk yang tertinggi iaitu 46.5 pucuk (eksplan biji dan batang masing-masing hasilkan sebanyak 9.3 dan 8.9 bilangan pucuk). Min tinggi pucuk yang terbentuk dari eksplan daun mencapai 0.48 cm, manakala min tinggi pucuk dari eksplan batang dan biji benih adalah lebih tinggi (0.7 cm and 0.8 cm masing-masing). Seterusnya, pucuk-pucuk *in-vitro* tersebut berjaya melengkapkan pembentukan akar dalam medium MS pada kekuatan penuh tanpa pengaruh hormon IBA, dan mencatatkan peratus pembentukan akar (87%) serta bilangan akar (3.8) yang tinggi (selepas tujuh bulan pengkulturan). Berikutan pembentukan anak benih (lengkap dengan pucuk dan akar), anak-anak benih berjaya dipasukan dan diaklimatasi dalam medium yang mengandungi campuran tanah + pasir + bahan organik + vermiculite (pada kadar 2: 2: 1: 1), dengan menghasilkan pembentukan min bilangan daun (6.5), min tinggi pokok (3.5cm) dan peratus kehidupan pokok (80%). Kajian pembentukan kalus pula mencatatkan peratus pembentukan sebanyak 90% dari eksplan biji dan daun dalam medium yang mengandungi 0.5 $mgL^{-1}$ (w/v) NAA, manakala eksplan pucuk dan batang mencatatkan 90% dan 83% masing-masing dalam medium yang mengandungi 0.2 $mgL^{-1}$ (w/v) KIN. Kesimpulannya, kajian ini telah berjaya menghasilkan protokol regenerasi pokok dengan menggunakan pelbagai organ

pokok manggis yang sedia ada dan mudah didapati seperti batang dan daun hijau, juvena, berbanding dengan penggunaan biji benih bermusim dan daun merah muda yang terhad atau bermusim, sepertimana yang diamalkan oleh kebanyakan penyelidik. Tambahan pula, kejayaan pembentukan kalus dari pelbagai eksplan manggis ini merupakan langkah pertama penjanaan kajian regenerasi pokok dari kalus, kerana protokol ini berpotensi diterapkan dalam penghasillan baka-baka manggis yang lebih berkualiti melalui pelbagai teknik modifikasi genetik.

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| | |
|---|---|
| ANOVA | Analysis of Variance |
| BAP | 6-Benzylaminopurine |
| cm | centimeter |
| ° | Centigrade Degrees |
| 2, 4-D | 2, 4-Dichlorophenoxyacetic acid |
| DNMRT | Duncan's New Multiple Range Test |
| g | Gram |
| H | Hour (s) |
| ½ | half |
| ¼ | Quarter |
| IAA | Indol-3-Acetic acid |
| IBA | Indole-3-Butyric acid |
| KIN | Kinetin, (6-furfurylaminopurine) |

| | |
|---|---|
| MS | Murashige and Skoog |
| NAA | α-Naphthalene Acetic Acid |
| % | Percentage |
| PGR | Plant Growth Regulator |
| pH | -log (H+) |
| L | liter |
| M | Molar |
| μM | micromolar, $10^{-3}$ mM |
| mg | milligram |
| ml | milliliter |

# CHAPTER 1

# INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) with high promising economic value is one of the tropical fruit trees belonging to the family of *Guttiferae*, order of *Malpighiales*, and class of *Magnoliophyta* (Almeyda and Martin, 1976; Felipe and Dela, 2001; Verheij and Coronel, 1991). The sweet and creamy fruits are popularly consumed fresh, and used as a source for candy, nut, preserve production and topping for ice-cream and sherbets. The leaves, rind and bark of the tree are used as herbal remedies for thrush, dysentery, diarrhea, cystitis, gonorrhoea, eczema and as wound astringents. Root decoctions are also used for regulation of the female menstruation cycle. Fruit rinds contain tannins used for tanning leather and as natural black dye. The wood is used to manufacture spear handles, rice pounders, wooden shelter and cabinets (Felipe and Dela, 2001; Suci *et al.*, 2007). In 1997, Malaysia exported 1,802 tonnes of fruits (mostly to Singapore and Hong Kong) valued at RM 1.7 million which continued to increase to about 2,447 tonnes and valued at RM4.7 million by the year 2006 (FAMA, 2007; Suci *et al.*, 2007). However, there is a need for greater production potential as mangosteen trees are very slow in growth, taking 10-12 years before fruiting

(Felipe and Dela, 2001; Normah *et al.*, 1995). Furthermore, the fruits are produced seasonally and each fruit normally contains two seeds which are recalcitrant, meaning they are sensitive to desiccation (Normah *et al.*, 1995; Schmidt, 2000). Recalcitrant seeds have low vitality and short viability (Normah *et al.*, 1995; Suci *et al.*, 2007). Generally, the planting materials which were used by farmers were from seedlings or conventional vegetative propagation which are slow growing and non homogenous (Felipe and Dela, 2001; Normah *et al.*, 1995; Te-Chato and Aengyong, 1988). Plant regeneration through tissue culture is an alternative approach for mass propagation of mangosteen planting materials (Felipe and Dela, 2001; Normah *et al.*, 1995; Te-Chato and Aengyong, 1988; Te-Chato and Lim, 1999). Tissue culture as a new technology has been used for propagating various horticultural and forest species successfully (Pierik, 1987). Large number of planting materials can be produced by this technique within a short period of time (Pierik, 1987; Razdan, 2005). Woody plants are slow growing and with poor root induction (Almeyda and Martin, 1976; Meera *et al.*, 2006). Rooting is still the main problem that has not been satisfactorily overcome in mangosteen (Normah *et al.*, 1995; Te-Chato and Aengyong, 1988; Te-Chato *et al.*, 1992). Some researchers have reported on the low success of regeneration and rooting in mangosteen (Felipe and Dela, 2001; Normah *et al.*, 1995; Te-Chato and Aengyong, 1988; Te-Chato *et al.*, 1992). Due to this, more studies on the regeneration and rooting of mangosteen need to be conducted (Normah *et al.*,

1995). In Malaysia, several papers have reported on regeneration of mangosteen using seeds (Normah *et al.*, 1992, Rashid *et al.*, 1995). This study was carried out with the following objectives: (1) to determine the most suitable concentration and combination of plant growth regulators for *in vitro* shoot regeneration and callus production from seed segment, (2) to determine the most suitable concentration and combination of plant growth regulators for *in vitro* shoot regeneration and callus production from shoot tip and stem, (3) to determine the most suitable concentration and combination of plant growth regulators for *in vitro* shoot regeneration and callus production from leaf segments, (4) to determine the most suitable medium salt strength and IBA concentrations for rooting of *in vitro* shoots, and finally (5) to determine the most suitable potting medium for plantlet performance during acclimatization.