UNIVERSITI PUTRA MALAYSIA

# ORGANOGENESIS, SOMATIC EMBRYOGENESIS AND REGENERATION OF IMMATURE MALE FLOWERS OF BANANA CULTIVARS

KEYNOOSH KASHEFI

# ORGANOGENESIS, SOMATIC EMBRYOGENESIS AND REGENERATION OF IMMATURE MALE FLOWERS OF BANANA CULTIVARS

**By**

**KEYNOOSH KASHEFI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Agricultural Science**

**May 2008**

**Dedicated to:**

**My kind parents, whom I am indebted with all love**
**My beloved spouse**
**My sweet daughter: Nikta**

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

**ORGANOGENESIS, SOMATIC EMBRYOGENESIS AND REGENERATION OF IMMATURE MALE FLOWERS OF BANANA CULTIVARS**

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**Chairman: Associate Professor Maheran Abdul Aziz, PhD**

**Faculty: Agriculture**

This study was carried out to establish a reliable and practical protocol of plant regeneration through organogenesis and somatic embryogenesis of immature male flowers of four cultivars of *Musa* spp. cvs. Berangan, Rastali, Mas and Raja as well as micromorphological studies and optimization of plant transformation protocol via microprojectile bombardment of immature male flowers of *Musa* spp. cv. Rastali.

Immature male flowers are highly proliferated meristems which are ideal materials for organogenesis and embryogenesis in *Musa* spp. Immature male flowers of four cultivars of *Musa* spp. cvs. Berangan, Rastali, Mas and Raja were cultured on a modified MS medium containing 0, 9, 18 and 36 μM BAP. The study showed a reasonably high percentage of adventitious bud induction on MS medium supplemented with 9 μM BAP for cultivars Rastali and Raja and 18 μM BAP for cultivars Berangan and Mas respectively within 4 to 8 weeks from the initiation of culture. Regeneration of shoots from the adventitious buds derived from immature male flowers of cultivars Berangan, Rastali, Mas and Raja were investigated on MS medium supplemented with 4.5, 9, 18

and 36 μM BAP after four weeks of culture with a weekly subculture interval. It was observed that BAP at 4.5 μM produced the highest number of shoots and BAP at 36 μM produced the highest shoot-length for all cultivars tested after four subcultures. Subculturing significantly affected the mean number of shoots produced with the highest mean number of 40.33, 5.66, 23.66 and 19.00 shoots produced in cvs. Rastali, Raja, Berangan and Mas respectively in the third subculture (out of four subcultures). The mean shoot height also increased over subculture cycles in all cultivars.

Different coconut water preparations (filter-sterilized and autoclaved) in combination with the best BAP concentration for adventitious bud induction determined earlier for each cultivar were investigated. Highest mean number of adventitious buds after three subcultures (17.30) was attained on medium containing 50 $mlL^{-1}$ filter-sterilized coconut water combined with BAP at 9 μM for cvs. Rastali and Raja while 18 μM for cvs. Berangan and Mas.

Shoots obtained from the immature male flowers of cvs. Rastali, Raja, Berangan and Mas were rooted on half-strength MS medium supplemented with 1.0 μM IBA and the plantlets produced were successfully acclimatized in the growth chamber.

Histological and Scanning Electron Microscopy (SEM) studies were carried out on male flowers of *Musa* spp. cv. Rastali placed on 9 μM BAP treatment at the initial stage of culture and the first, second and fourth subculture. Sequential changes were observed

starting from globular mass like structures (bulges) to adventitious buds and finally producing multiple shoots.

Somatic embryogenesis of *Musa* spp. cv. Raja was established using immature male flower hands. Highest percentage of embryogenic callus formation (41.97%) was obtained on 13.5 μM 2,4-D for all flower hand positions assessed. Flower hand position 8 produced the highest percentage (48.25%) of embryogenic callus formation for all levels of 2,4-D tested. The study revealed that the embryogenic cell suspensions initiated from the embryogenic callus/complex had high potential towards somatic embryogenesis. The highest percentage of somatic embryos germination (62.36%) was attained on medium with 0.17 μM BAP after two weeks of culture.

Transformation study showed that the target distance of 9 cm along with helium pressure of 1350 and 1550 psi were the most efficient combinations for particle gun bombardment of immature male flower buds of cv. Rastali whereby 58.26% and 57.63% of the bombarded plates showed a high GFP gene expression.

Overall, this study indicated that immature male flower buds of *Musa* spp. cultivars Berangan, Rastali, Mas and Raja can be the appropriate materials for *in vitro* regeneration via organogenesis and somatic embryogenesis as well as for gene transformation via particle bombardment.

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

**ORGANOGENESIS, EMBRIOGENESIS SOMA DAN REGENERASI BUNGA JANTAN TIDAK MATANG BEBERAPA KULTIVAR PISANG**

Oleh

**KEYNOOSH KASHEFI**

**May 2008**

**Pengerusi: Profesor Madya Maheran Abdul Aziz, PhD**

**Fakulti: Pertanian**

Kajian ini dilakukan untuk mendapatkan protokol yang sesuai dan praktikal bagi regenerasi menerusi organogenesis dan embriogenesis soma daripada bunga jantan tidak matang empat kultivar *Musa* spp. iaitu. Berangan, Rastali, Mas dan Raja serta mengoptimakan protokol transformasi gen menerusi peledakan mikroprojektil pada bunga jantan tidak matang *Musa* spp. cv. Rastali.

Bunga jantan tidak matang adalah meristem berpotensi tinggi dan bahan yang ideal untuk organogenesis dan embriogenesis soma pada *Musa* spp. Bunga jantan tidak matang bagi empat kultivar *Musa* spp. iaitu. Berangan, Rastali, Mas dan Raja dikultur pada medium MS dimodifikasi yang mengandungi 0, 9, 18 dan 36 µM BAP. Kajian ini menunjukkan peratus induksi meristem tunas adventitius yang tinggi pada medium MS dengan 9 µM BAP bagi kultivar Rastali dan Raja manakala 18 µM BAP bagi kultivar Berangan dan Mas dalam tempoh 4 hingga 8 minggu selepas inisiasi kultur. Regenerasi

pucuk daripada tunas adventitius yang diperoleh daripada bunga jantan tidak matang *Musa* spp. *cv.* Berangan, Rastali, Mas dan Raja dikaji pada medium MS mengandungi BAP berkepekatan 4.5, 9, 18 dan 36 µM selepas empat minggu dikultur dengan pensubkulturan pada setiap minggu. Melalui pemerhatian didapati bahawa 4.5 µM BAP menghasilkan bilangan pucuk tertinggi manakala 36 µM BAP menghasilkan panjang pucuk tertinggi pada semua kultivar yang dikaji selapas empat subkultur. Didapati bahawa subkultur mempengaruhi bilangan min pucuk yang terhasil dengan min bilangan pucuk mencapai 40.33, 5.66, 23.66 dan 19.00 bagi kultivar Rastali, Raja, Berangan dan Mas pada subkultur ke-3 (dari empat subkultur). Min ketinggian pucuk turut meningkat pada setiap kitaran subkultur bagi setiap kultivar.

Gabungan air kelapa (pensterilan turas dan diautoklaf) dengan kepekatan BAP yang telah dikenalpasti paling sesuai untuk induksi tunas adventitius bagi setiap kultivar dari kajian sebelumnya juga telah dikaji. Bilangan min tunas adventitius tertinggi selepas tiga subkultur (17.30) diperoleh pada medium yang mengandungi 50 $mlL^{-1}$ air kelapa (pensterilan turas) dengan kombinasi BAP pada 9 µM bagi cv. Rastali dan Raja manakala 18 µM bagi cv. Berangan dan Mas. Pucuk yang diperoleh daripada bunga jantan tidak matang cv. Berangan, Rastali, Mas dan Raja diakarkan pada medium MS separa kepekatan yang mengandungi 1.0 µM IBA dan plantlet yang terhasil diaklimatisasi di dalam kebuk tumbesaran.

Kajian histologi dan mikroskopi pengimbas elektron (SEM) telah dijalankan ke atas bunga jantan *Musa* spp. cv. Rastali, yang dikulturkan di dalam rawatan 9 µM BAP,

pada peringkat permulaan kultur dan pada subkultur pertama, kedua dan keempat. Perubahan yang berturutan jelas diperoleh bermula daripada struktur globul (benjolan) sehingga pembentukan tunas adventitius dan akhirnya pengeluaran tunas berganda.

Embriogenesis soma bagi *Musa* spp. cv. Raja telah diperoleh dengan menggunakan bunga jantan tidak matang. Peratus tertinggi pembentukan kalus embriogenik (41.97%) diperoleh pada 13.5 μM 2,4-D untuk semua posisi kluster bunga yang diuji. Kajian ini menunjukkan sel ampaian embriogenik mempunyai potensi yang tinggi terhadap pembentukan embrio soma. Peratus percambahan embrio soma tertinggi (62.36%) diperoleh pada 0.17 μM BAP selepas dua minggu dikultur.

Kajian transformasi menunjukkan jarak sasaran 9 cm dan tekanan helium 1350 dan 1550 psi adalah kombinasi yang baik untuk transformasi bunga jantan tidak matang cv. Rastali melalui teknik peledakan mikroprojektil di mana 58.26% dan 57.63% kultur yang bagi setiap kombinasi menunjukkan ekspresi gen GFP yang tinggi.

Keseluruhannya kajian ini menunjukkan bunga jantan tidak matang *Musa* spp. cv. Berangan, Rastali, Mas and Raja sebagai bahan yang paling sesuai untuk regenerasi *in vitro* melalui organogenesis dan embriogenesis soma dan juga untuk transformasi gen menerusi kaedah peledakan mikroprojektil.

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I certify that an Examination Committee met on 20th May 2008 to conduct the final examination of Keynoosh Kashefi on her Master of Agriculture Science thesis entitled "Organogenesis, Somatic Embryogenesis and Regeneration of Immature Male Flowers of Banana Cultivars" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Act 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 was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Agricultural Science. The members of the Supervisory Committee were as follows;

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Date: 10 July 2008

## 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 UPM or at any other institution.

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**KEYNOOSH KASHEFI**

Date:

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