



UNIVERSITI PUTRA MALAYSIA

***IN VITRO* MUTAGENESIS AND SOMACLONAL VARIATION IN BANANA
TO INCREASE DROUGHT TOLERANCE**

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**IN VITRO MUTAGENESIS AND SOMACLONAL VARIATION IN BANANA
TO INCREASE DROUGHT TOLERANCE**

By

SIAMAK SHIRANI BIDABADI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

June 2011



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DEDICATION

**I wish to dedicate this thesis to my wife Farinaz Shirani Bidabadi who
always understands me and has faith in me.**

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

IN VITRO MUTAGENESIS AND SOMACLONAL VARIATION IN BANANA TO INCREASE DROUGHT TOLERANCE

By

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June 2011

Chairman: Professor Maziah Mahmood, PhD

Faculty: Institute of Tropical Agriculture

Banana production is threatened by several abiotic stresses, such as drought, thus, developing tolerant genotypes towards water stress is needed. Genetic variability in banana is narrow due to low female fertility, therefore, this study aimed to use somaclonal variation caused by ethyl methanesulphonate (EMS) to select drought tolerant mutants of banana. The first step in conducting *in vitro* mutagenesis experiments was to optimize an efficient micropropagation system because of significant loss of many new mutants due to the deficiency of a regeneration system after *in vitro* mutagenesis. Results indicated that the highest rates of proliferation associated with low percentage of abnormality ranging from '3.44 (8% abnormality)', 4.22 (30% abnormality) and 7.67 (24% abnormality) occurred in 'Berangan Intan', 'Berangan' and 'Rastali', respectively on medium containing 22.2 μ M BAP. Although proliferation rate was greater with TDZ

than the other cytokinins, treatments with TDZ produced more abnormality, sometimes as high as 82%. Furthermore, using the RAPD markers, the somaclones regenerated by TDZ exhibited higher percentage of polymorphism than BAP. Consequently, supplementation of 22.2 μM BAP in MS medium was assumed the most suitable for commercial micropropagation system with low frequency of abnormal shoot production.

Different ethyl methanesulphonate (EMS) treatments were applied to investigate their effects on proliferating shoot tips and to evaluate the potential of EMS to create variability among banana cultivars. The percentage of surviving shoot tips ranged from 88.71, 81.10 and 90.62 with 150 mM EMS for 30 min to 37.78, 34.44 and 31.03 with 250 mM EMS for 60 min in 'Berangan Intan', 'Berangan' and 'Rastali' respectively. The average number of shoots per explant declined significantly from the controls to the highest dose and duration of EMS. The recommended treatments were adjudged to be 60 min/200 mM and 30 min/250 mM of EMS for all cultivars tested in this study, which resulted in phenotypic variations of 10.74% and 9.65% respectively for 'Berangan Intan', 12.42% and 7.20% respectively for 'Berangan', 13.17% and 14.78% respectively for 'Rastali'.

In vitro selection involving 14 EMS –induced mutant somaclones of banana through screening of shoot tips on media stressed with different levels of polyethylene glycol (10, 20 and 30 g L⁻¹) based on morphological, physiological and molecular markers was attempted to develop drought-tolerant lines. Data recorded at each stress level, showed that mutants L₂₋₅

and L₁₋₅, followed by L₂₋₄, L₁₋₄ and then L₁₋₃ and L₂₋₆ demonstrating stronger tolerance against water stress, exhibited higher shoot vigour, fresh weight increase, proliferation rate, proline, relative water and chlorophyll content than the rest and control parental clones. In spite of the relatively large number of polymorphic bands found in the 14 clones of *Musa* cultivars 'Berangan Intan' and 'Berangan', only primers opc01, opa11, opa20 and opc04 generated bands that could be considered as potential markers to identify drought resistant somaclones. The amplified fragments observed to be specific for the somaclones L₂₋₅ and L₁₋₅ as more drought tolerant and somaclones L₂₋₃, L₁₋₄, L₂₋₆ and L₁₋₆ as moderately drought tolerant could be markers for initial estimation and selection for water stress tolerance in banana cultivars.

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

**MUTAGENESIS DAN VARIASI SOMAKOLONAL *IN VITRO* DALAM
PISANG UNTUK MENINGKATKAN TOLERANSI TERHADAP
TEKANAN KEMARAU**

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Pengeluaran pisang diancam oleh beberapa tekanan abiotik seperti kemarau, dengan demikian, penghasilan kultivar yang toleran terhadap kemarau diperlukan. Kepelbagaian genetik pada pisang adalah sempit kerana kesuburan bunga betina rendah, oleh kerana itu, kajian ini bertujuan untuk menggunakan variasi somaklonal disebabkan oleh etil metanasulfonat (EMS) untuk memilih mutan toleran terhadap kemarau dikalangan pisang. Langkah pertama dalam eksperimen *in vitro* mutagenesis adalah untuk mengoptimumkan sistem mikropropagasi yang cekap kerana kehilangan yang signifikan dari mutan baru disebabkan oleh kekurangan sistem regenerasi setelah *in vitro* mutagenasi. Keputusan kajian menunjukkan bahawa tahap proliferasi tertinggi yang berkaitan dengan peratusan abnormaliti rendah bermula dari 3.44 (8% abnormal), 4.22 (30% abnormal)

dan 7,67 (24% abnormal) berlaku pada, 'Berangan Intan', 'Berangan' dan 'Rastali', masing-masing di dalam medium yang mengandungi 22,2 pM BAP. Walaupun tahap proliferasi lebih tinggi dengan TDZ daripada sitokinin lain, rawatan dengan TDZ menghasilkan lebih abnormaliti, hingga setinggi 82%. Selanjutnya, dengan menggunakan penanda RAPD, somaklon yang diregenerasi oleh TDZ menunjukkan peratusan polimorfisme yang lebih tinggi daripada BAP. Akibatnya, suplementasi 22,2 pM BAP pada medium MS paling sesuai untuk sistem propagasi mikro komersil dengan frekuensi abnormaliti yang rendah dalam pengeluaran pucuk.

Rawatan etil metanasulfonat (EMS) yang berbeza dilaksanakan untuk menyiasat pengaruhnya terhadap perkembangan pucuk dan untuk menilai potensi EMS untuk menghasilkan variasi antara kultivar pisang. Peratus apeks hidup berada di antara 88.71, 81.10, dan 90.62 pada 150 mM EMS selama 30 minit sehingga 37.78, 34.44 dan 31.03 pada 250 mM EMS selama 60 minit dalam 'Berangan Intan', 'Berangan' dan 'Rastali' masing-masing. Purata jumlah pucuk per eksplan menurun secara signifikan dari kawalan ke dos dan tempoh masa EMS tertinggi. Rawatan yang diputuskan adalah 60min/ 200 mM dan 30min/ 250 mM EMS untuk semua kultivar yang diuji dalam kajian ini, mengakibatkan peratusan variasi fenotipik 10.74% dan 9.65% untuk 'Berangan Intan', 12.42% dan 7.20% masing-masing untuk 'Berangan', 13.17% dan 14.78% untuk 'Rastali'.

Pemilihan *in vitro* yang melibatkan 14 EMS-mutan somaklon pisang diinduksi melalui pemilihan pucuk pada media dengan pelbagai peringkat polietilena

glikol (10, 20 dan 30 g L⁻¹) berdasarkan morfologi, fisiologi dan penanda molekular untuk menghasilkan pokok yang toleran terhadap kemarau. Data menunjukkan bahawa mutan menunjukkan toleransi kemarau yang lebih tinggi di kalangan L₂₋₅ dan L₁₋₅, diikuti oleh L₂₋₄, L₁₋₄ dan kemudian L₁₋₃, dan L₂₋₆, pada setiap peringkat tekanan, seperti kesegaran pucuk yang tinggi, pertambahan berat basah, tahap proliferasi, prolin, relatif air dan kandungan klorofil berbanding yang lain dan klon kawalan parental. Walaupun jumlah besar relatif band polimorfik ditemui di dalam 14 klon *Musa* spp., kultivar 'Berangan Intan' dan 'Berangan', hanya primer opc01, opa11, opa20 dan opc04 yang menghasilkan band yang boleh dianggap sebagai penanda yang berpotensi untuk mengenalpasti somaklon tahan kemarau. Fragmen diamplifikasi, yang diketahui khusus untuk somaklon L₂₋₅ dan L₁₋₅ sebagai lebih toleran kemarau dan somaklon L₂₋₃, L₁₋₄, L₂₋₆ dan L₁₋₆ sebagai toleran kemarau tahap sederhana boleh dijadikan penanda untuk jangkaan awal dan pemilihan terhadap toleransi kemarau di dalam kultivar pisang.

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APPROVAL

I certify that an Examination Committee has met on 29th June 2011. to conduct the final examination of Siamak Shirani Bidabadi on his Doctor of Philosophy thesis entitled "*In vitro* mutagenesis and somaclonal variation in banana to increase drought tolerance" 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.

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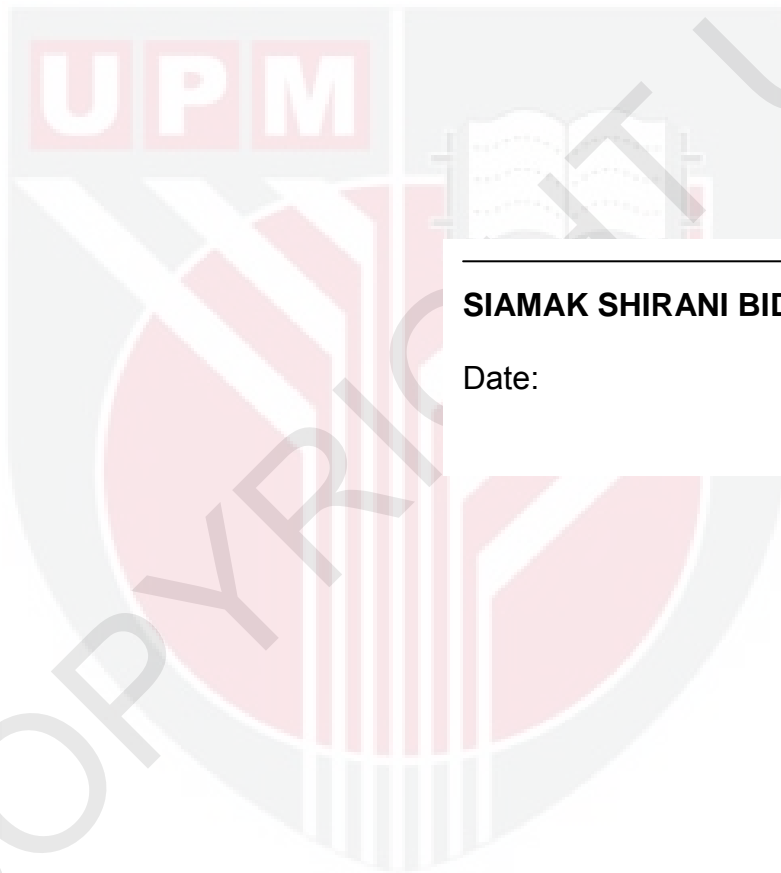
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DECLARATION

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 other institutions.



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