UNIVERSITI PUTRA MALAYSIA

A NOVEL *IN-VITRO* TECHNIQUE FOR SHOOT PROLIFERATION OF *MUSA ACUMINATA* COLLA CV. GRAND NAINE AND ASSESSMENT OF GENETIC STABILITY OF THE MICROPROPAGATED PLANTS

MAHDI SAFARPOUR

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By

MAHDI SAFARPOUR

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

June 2012

DEDICATION

This thesis is dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

It is also dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake.

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

A NOVEL *IN-VITRO* TECHNIQUE FOR SHOOT PROLIFERATION OF *MUSA ACUMINATA* COLLA CV. GRAND NAINE AND ASSESSMENT OF GENETIC STABILITY OF THE MICROPROPAGATED PLANTS

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Chairman: Associate Professor Uma Rani Sinniah, PhD

Faculty: Agriculture

This study was carried out to investigate the efficiency of using horizontal sections of a single shoot-tip as a novel technique for shoot induction and proliferation in *Musa. acuminata* cv. Grand Naine compared to the conventional and routine method of using single shoot-tip culture. The excised single shoot apices were cultured on MS basal medium supplemented with 0, 10, 20 and 30 µM of 6-benzylaminopurine (BAP) in combination with 1.0 µM 1-naphthaleneacetic acid (NAA) for six months with monthly subculture. The results revealed that mean number of proliferated shoots increased with subsequent subculture at all levels of BAP tested, reaching the highest mean of 23.6 shoots on 20 µM BAP at the sixth subculture with an average height of 1.7 cm per explant. In a parallel study, the excised single shoot-tips were initially cultured on MS medium supplemented with 10 µM BAP in combination with 1.0 µM NAA for one

month. After one month, swelled explants were split into three horizontal sections numbered 1 to 3 from top to bottom. Each section was subcultured for two subsequent cycles onto MS media supplemented with the same concentration of BAP. The highest mean number of shoots per section (7.2) was attained on horizontal section number 2. In the next step, the regenerated shoots obtained from section 2, were isolated and cultured on MS medium supplemented with 0, 10, 20 and 30 µM of BAP in combination with 1.0 µM NAA for further shoot proliferation. BAP at the concentration of 10, 20 and 30µM produced an average of 6.2, 8.3 and 7.9 shoots per explant; respectively over four subsequent subcultures. This study showed that it is highly possible to regenerate plantlets from horizontal sections of a single shoot-tip. The results showed that from just one horizontal section of a single shoot-tip of banana cv. Grand Naine, 59.6 shoots can be produced after six months, while only an average of 23.7 shoots is obtained *via* direct regeneration through single shoot-tip culture of the same cultivar. RAPD fingerprinting of randomly selected micropropagated plants and their corresponding mother plants were carried out to assist the genetic stability of micropropagated plants obtained through single shoot-tip culture and horizontal sections of a single shoot-tip culture. The use of 14 decamer oligonucleotides primers resulted in amplification of 98 bands; ranging in size from 210 bp to 2460 bp in plants micropropagated through shoot- tip culture. Out of the 98, 23 bands were polymorphic corresponding to 23.5% polymorphism. Based on RAPD bands pattern, the pair wise values of

similarity coefficients ranged from 0.89 to 0.97 between micropropagated plants and parental plant. However, for plants micropropagated through horizontal sections of single shoot-tip, a total number of 92 scorable bands were amplified with all being monomorphic and similar to the field grown control plants with a similarity index of 1.0. The present study showed that producing plants through micropropagation of a single shoot-tip over six subcultures resulted in 5.3% variation while no genetic variation was observed among micropropagated plants obtained through horizontal sections of a single shoot-tip. In the next step, proliferated shoots obtained through horizontal sections of a single shoot-tip were rooted on full strength MS media and MS medium supplemented with l.0, 3.0 and 6.0 µM IBA. In this study, full-strength MS medium with 6.0 µM IBA produced the highest mean number of roots per explant (14.8) with an average length of 3.5 cm. In the final stage, the rooted plantlets were transplanted to three types of growing medium comprising of sand + perlite (2:1 v/v), sand + perlite + vermiculite (3:2:1 v/v) and peat moss + perlite + vermiculite (3:2:1 v/v). After eight weeks, the highest survival (88.6%) was obtained in potting medium consisting Perlite + Vermiculite + Peat moss. Overall, the results proved the potential of horizontal sections of a single shoot-tip technique to supercede single shoot-tip culture in relation to mass multiplication in banana cv. Grand Naine. Additionally, the high genetic stability of micropropagated plants indicated the reliability of this method to produce large number of true to type plants in a short span of time.

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

TEKNIK NOVEL KULTUR TISU BAGI PROLIFERASI PUCUK UNTUK *MUSA ACUMINATA* COLLA CV. GRAND NAINE DAN PENILAIAN KESTABILAN GENETIK BAGI TUMBUHAN YANG DIBIAKKAN SECARA MIKRO

Oleh

MAHDI SAFARPOUR

Jun 2012

Pengerusi: Profesor Madya Uma Rani Sinniah, PhD

Fakulti : Pertanian

Kajian ini dijalankan untuk mengenalpasti keberkesanan menggunakan bahagian pucuk yang dipotong secara melintang sebagai teknik novel kultur tisu bagi pembentukan dan proliferasi pucuk baru untuk *Musa acuminata* cv. Grand Naine sebagai perbandingan dengan teknik konvensional iaitu dengan menggunakan pucuk tanpa dipotong. Pucuk yang telah diasingkan dikultur pada media asas MS yang mengandungi 0, 10, 20 dan 30 µM 6-benzylaminopurine (BAP) berserta dengan 1.0 µM 1-naphthaleneacetic acid (NAA). Subkultur dilakukan setiap bulan sehingga enam bulan. Hasil kajian menunjukkan bahawa purata bilangan pucuk baru yang terhasil meningkat dengan pertambahan bilangan subkultur untuk setiap kadar BAP yang digunakan. Purata bilangan pucuk yang tertinggi ialah 23.6 pada media

yang mengandungi 20 µM BAP pada subkultur yang keenam dengan purata ketinggian eksplan sebanyak 1.7 cm. Dalam masa yang sama, pucuk yang telah diasingkan dikultur pada media MS yang mengandungi 10 µM BAP berserta dengan 1.0 µM NAA. Selepas sebulan, eksplan yang mula membengkak dipotong secara melintang kepada tiga bahagian daripada atas ke bawah dan dilabel sebagai 1 hingga 3. Setiap bahagian disubkultur sebanyak dua kali pada media MS yang mengandungi BAP pada berkepekatan yang sama. Purata bilangan pucuk baru (7.2) dihasilkan dari bahagian pucuk nombor 2. Seterusnya, pucuk yang terhasil dari bahagian nombor 2 diasingkan dan dikultur pada media MS yang mengandungi 0, 10, 20 and 30 µM of BAP beserta dengan 1.0 µM NAA untuk proliferasi pucuk seterusnya. BAP pada kepekatan 10, 20 dan 30 µM menghasilkan purata pucuk 6.2, 8.3 dan 7.9 setiap eksplan pada subkultur yang keempat. Kajian ini membuktikan bahawa bahagian pucuk yang telah dipotong secara mendatar dari pucuk asal adalah sesuai untuk digunakan bagi proliferasi anak pokok. Keputusan kajian menunjukkan bahawa 59.6 pucuk baru boleh dihasilkan selepas enam bulan dari hanya satu pucuk asal yang dipotong secara melintang berbanding dengan hanya purata sebanyak 23.7 pucuk diperoleh dari kultur pucuk yang tidak dipotong bagi pisang Grand Naine. RAPD telah dijalankan terhadap pokok yang dihasilkan dari pembiakan mikro dan di bandingkan dengan pokok induk bagi menilai kestabilan genetik bagi tumbuhan yang terhasil dari kultur pucuk yang tidak dipotong dan juga bahagian pucuk yang dipotong secara melintang dari pucuk asal.

Menggunakan 14 primer oligonukleotida, sebanyak 98 jalur dihasilkan, dengan julat saiz diantara 210 bp hingga 2460 bp bagi pokok yang terhasil dari kultur pucuk yang tidak dipotong. Daripada 98 jalur ini, 23 daripadanya adalah polimorfik pada tahap 23.5%. Berdasarkan corak jalur RAPD, julat nilai pekali kesamaan berpasangan adalah dari 0.89 kepada 0.97 diantara pokok yang terhasil dari pembiakan mikro dan juga pokok induk. walaubagaimanapun, bagi pokok yang terhasil daripada bahagian pucuk yang dipotong secara melintang dari pucuk asal, 92 jalur telah diskor dan kesemuanya adalah monomorfik dan serupa dengan pokok kawalan dari lapangan dengan indeks kesamaan sebanyak 1.0. Kajian ini menunjukkan bahawa penghasilan pokok melalui pembiakan mikro dari pucuk yang tidak dipotong mengakibatkan 5.3% perbezaan genetik apabila disubkultur sebanyak enam kali manakala tiada perbezaan genetik yang berlaku antara pokok yang terhasil daripada bahagian pucuk yang dipotong secara melintang. Seterusnya, kajian keatas initiasiasi akar dari pucuk baru yang terhasil dari kultur bahagian pucuk yang dipotong secara melintang dengan menggunakan media MS berkepekatan penuh dan media MS yang dibekalkan dengan l.0, 3.0 and 6.0 µM IBA dijalankan. Dalam kajian ini, media dengan 6.0 µM IBA menghasilkan purata bilangan akar yang tertinggi bagi setiap eksplan dengan purata panjangnya adalah 3.5 cm. Akhir sekali, pokok yang mempunyai akar ini kemudiannya dipindahkan ke tiga jenis media pertumbuhan iaitu pasir + perlite (2:1 v/v), pasir + perlite + vermiculite (3:2:1 v/v) dan peat moss + perlite + vermiculite (3:2:1 v/v).

Selepas lapan minggu, keupayaan untuk hidup yang tetinggi (88.6%) diperoleh daripada media yang mengandungi perlite + vermiculite + peat moss. Secara keseluruhannya, hasil kajian ini telah membuktikan kebolehan kaedah kultur bahagian pucuk yang dipotong secara melintang sebagai kaedah pantas bagi menambahkan bilangan pucuk secara besar-besaran berbanding dengan menggunakan pucuk yang tidak dipotong bagi pisang cv. Grand Naine. Di samping itu, kestabilan genetik yang tinggi bagi pokok daripada pembiakan mikro ini telah membuktikan keberkesanan teknik ini untuk digunakan bagi menghasilkan bilangan pokok yang banyak dalam masa yang singkat dan mempunyai sifat yang sama dengan induk.

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Special thanks go to the members of my supervisory committee **Assoc. Prof. Dr. Maheran Abdul Aziz** and **Dr. Sreeramanan Subramaniam** for their suggestions and useful deliberative discussion to make this study more comprehensive and meaningful.

I am immensely and forever grateful to those individuals who have, in one way or another provided me the courage and resilience to pave the way towards the successful completion of this study.

APPROVAL

I certify that a Thesis Examination Committee has met on 15th of June to conduct the final examination of Mahdi Safarpour on his thesis entitled "A Novel *In vitro* Technique for Shoot Proliferation of *Musa acuminata* Cv. Grand Naine and Assessment of the Genetic Stability of Its Micropropagated Plants". In accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Pertanian Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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The thesis was submitted to the senate of Universiti Putra Malaysia has been accepted as the fulfilment requirement for the degree of Master of Science. The members of Supervisor Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for the quotation and citation 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.

MAHDI SAFARPOUR
Date:

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