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MICROPROPAGATION OF TEA (Camellia sinensis (L.) Kuntze) USING TEMPORARY IMMERSION SYSTEM

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By

REZA AZADI GONBAD

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

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I'd dedicate each of the 214 pages of this thesis to:

My mother who gave me the courage and support to spread my wings and fly

My father who fostered my intellectual development profoundly

My brother who inspired me that difficulties are meant to rouse, not discourage

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

MICROPROPAGATION OF TEA (*Camellia sinensis* (L.) Kuntze) USING TEMPORARY IMMERSION SYSTEM

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Chairman: Associate Professor Uma Rani Sinniah, PhD
Faculty : Agriculture

This research was carried out using tea clone Iran 100 with the aim of producing tea planting materials using temporary immersion system for accelerated mass production of true-to-type plants., Surface sterilization of nodal segment explants (from different positions) was tested using various exposure times to 20% Clorox. The highest survival of 60%was obtained for nodal segment two upon exposure to 20% Clorox for 15 minutes. All following experiments were carried out using nodal segment two. The response of nodal segment to shoot proliferation in solid MS media supplemented with two types of cytokinins namely 6-benzylaminopurine (BAP) at 0, 1, 3, 5 and 7 mg/L and thidiazuron (TDZ) at 0, 0.025, 0.05, 0.075 and 0.1 mg/L was studied. Gibberellic acid (GA_3) at 0, 0.5 and 1 mg/L was included into the media with BAP and TDZ in order obtain multiplication and elongation at the same time. The results demonstrated that BAP was

more effective as it was able to promote induction of shoots by reducing apical dominance in comparison to TDZ. Solid media which comprised of 3 mg/L BAP and 0.5 mg/L GA_3 was the best, resulting in 7.5 shoots with average shoot length of 0.85 cm. Multiple subculture cycles (up to five times) using BAP did not show any adverse effect on shoot multiplication and the presence of BAP was necessary to retain the multiplication rate. In contrast, the effect of constant exposure to low concentration of TDZ stimulated callus formation and induced hyperhydricity. Upon establishment of proliferation in solid media, liquid culture system was tested both under shake and static condition with the use of BAP and TDZ in combination with GA_3 at concentrations similar to those used for solid culture. Optimization of liquid culture system is important for use in temporary immersion system (TIS). Liquid culture with constant shaking gave better results in comparison to that maintained in static state. Liquid culture system also performed better compared to solid culture system with the production of 13.6 shoots per explant on medium containing 3 mg/L BAP + 0.5 mg/L GA_3.

The up-scaling of the mass propagation was carried out using the RITA bioreactor. Various immersion times of 60, 120 and 180 min were tested. RITA with immersion time of 120-minutes produced superior quality plantlets in terms of number of shoots (19) as well as plant morphology. An additional experiment was conducted to introduce the use of polypropylene containers for micropropagation of tea. Experimental results showed that *in*

vitro mass propagation in polypropylene container played a significant role in terms of low cost-high efficiency regeneration system almost comparable to TIS in terms of number of shoot produced.

The shoots produced using RITA was subjected to two rooting treatments namely continue and transfer. The study on *in vitro* rooting concluded that use of solid MS media supplemented with 5 mg/L IBA and subsequent transfer to growth regulator-free MS media resulted in highest percentage of root induction per explant (66.8%) and with a mean of 4.3 roots per explant. Contrastingly, MS media without hormones failed to induce rhizogenesis. Acclimatization was carried out using 4 different types of media; peat moss + vermiculite + perlite (2:2:0 v/v/v), peatmoss + vermiculite + perlite (1:2:1 v/v/v), peatmoss + vermiculite + perlite (1:1:2 v/v/v) and peatmoss + vermiculite + perlite (2:1:1 v/v/v). The best percentage of plant survival (84.5%) as well as best root length, height of plants and number of leaves was obtained in media which consist of peatmoss+ vermiculite+ perlite (2:1:1 v/v). Finally the genetic fidelity of the micropropaged tea plants was assessed using ISSR (Inter-Simple Sequence Repeats). The amplified ISSR locus from 11 markers showed 100% similarity between micropropagated plants (derived in the third subculture of by TIS) and 10 randomly selected acclimatized plantlets with their mother plant DNA of tea clone Iran 100. The present study emphasizes that explants produced in the RITA® does not impede the clonal fidelity of *in vitro* regenerated propagules.

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

PEMBIAKAN MIKRO TEH (*Camellia sinensis* (L.) Kuntze) DENGAN MENGGUNAKAN SISTEM RENDAMAN SEMENTARA

Oleh

REZA AZADI GONBAD

Jun 2012

Pengerusi: Profesor Madya Uma Rani Sinniah, PhD
Fakulti : Petanian

Kajian ini telah dijalankan keatas teh klon Iran 100 untuk menghasilkan anak pokok teh menggunakan teknik kultur tisu melalui sistem rendaman sementara bagi menghasilkan anak benih yang serupa dengan pantas. Pensterilan permukaan eksplan keratan tunas axil (daripada posisi yang berlainan) telah diperolehi dengan mendedahkannya kepada 20% Clorox untuk tempoh masa yang berbeza. Peratus keupayaan hidup tertinggi iaitu sebanyak 60% di perolehi dengan rendaman selama 15 minit bagi keratan tunas posisi nombor dua. Bagi eksperimen seterusnya hanya keratan tunas posisi nombor dua digunakan. Seterusnya, kajian kesan tindak balas keratan tunas axil terhadap proliferasi pucuk pada media MS pejal dengan dua jenis sitokinin iaitu 6-benzylaminopurine (BAP) pada kepekatan 0, 1, 3, 5 dan 7

mg/L dan thidiazuron (TDZ) pada kepekatan 0, 0.025, 0.05, 0.075 dan 0.1 mg/L telah dijalankan. Acid giberelik (GA_3) pada kepekatan 0, 0.5 dan 1 mg/L (BAP) juga di masukkan kedalam media bersama BAP dan TDZ untuk memperolehi proliferasi dan pemanjangan pada masa yang sama. Daripada kajian ini, didapati bahawa penambahan BAP adalah lebih sesuai berbanding TDZ kerana ianya terbukti sebagai penggalak yeng lebih sesuai untuk induksi pucuk melalui pengurangan dormansi apikal. Media pejal yang mengandungi 3 mg/L BAP dan 0.5 mg/L GA_3 adalah media yang terbaik bagi menghasilkan sebanyak 7.5 pucuk dengan purata kepanjangan 0.85 cm. Subkultur berterusan, sehingga lima kali menggunakan kepekatan BAP yang optimum tidak mangakibatkan sebarang kesan buruk terhadap proses penggandaan pucuk, malah BAP diperlukan secara berterusan untuk berlakunya proliferasi. Sebaliknya penggunaan TDZ walalupun pada kadar yang rendah menghasilkan pucuk yang kurang baik, merangsang pembentukan kalus dan mendorong hiperhidrisiti. Setelah menjalankan kajian keatas media pejal, penggunaan media cair yang mengandungi BAP dan TDZ berserta GA_3, telah di uji menggunakan dua keadah iaitu kultur cair bergoncang dan tanpa goncang (statik) untuk mendapatkan kepekatan optima bagi di gunakan dalam sistem TIS. Kultur cecair yang digoncang secara berterusan memberikan keputusan yang lebih baik berbanding dengan yang berada dalam keadaan statik. Berdasarkan kepada perbandingan secara keseluruhannya, sistem kultur cair adalah lebih baik berbanding sistem kultur pejal kerana ia menghasilkan 13.6 pucuk bagi

setiap ekplan yang dikultur dengan media yang mengandungi BAP 3 mg/L + GA_3 0.5 mg/L.

Penghasilan pucuk pada kadar yng tinggi telah diperolehi dengan menggunakan sistem bioreaktor. Pelbagai jangkamasa rendaman iaitu 60, 120 dan 180 minit telah di uji. Klon Iran 100 telah digandakan menggunakan bioreaktor rendaman sementara RITA®. Didapat bahawa TIS dapat menghasilkan pucuk berkualiti tinggi dari segi bilangan pucuk (19) dan juga ciri morfologi pucuk tanpa hiperhidrisiti, apabila tempoh rendaman telah ditetapkan sehingga 120 minit. Kajian tambahan telah dijalankan untuk memperkenalkan bekas polypropalina untuk di gunakan dalam kultur tisu teh bagi mengurangkan kos. Keputusan eksperimen menunjukkan bahawa bekas plastik (polypropalina) boleh menghasilkan pucuk dengan baik pada kos yang rendah sejajar dengan bilangan pucuk yang di hasilkan oleh sistem TIS.

Dua teknik pengakaran telah di gunakan untuk anak pokok yang dihasilkan menggunakan RITA iaitu menggunakan sistem berterusan dan sistem pindah. Kajian terhadap pengakaran in-vitro menunjukkan bahawa penggunaan MS media pejal ditambahkan dengan 5 mg/L IBA dan seterusnya dipindahkan kepada media MS tanpa penggalak pertumbuhan mencatat peratus pengakaran yang tertinggi bagi setiap eksplan (66.8 %) dan purata bilangan akar tertinggi bagi setiap eksplan (4.3). Sebaliknya, media

MS tanpa hormon penggalak gagal mendorong rhizogenesis. Proses aklimatisasi dijalankan dengan menggunakan empat jenis media iaitu peat moss + vermiculite + perlite (2:2:0 v/v/v), peatmoss + vermiculite + perlite (1:2:1 v/v/v), peatmoss + vermiculite + perlite (1:1:2 v/v/v) and peatmoss + vermiculite + perlite (2:1:1 v/v/v). Peratus kemandirian tumbuhan (84.5%), panjang akar, ketinggian tumbuhan dan bilangan daun yang tertinggi telah dihasilkan oleh media campuran peatmoss+vermiculite+perlite (2:1:1 v/v). Pada akhirnya, ujian penanda ISSR (Inter-Simple Sequence Repeats) telah dgunakan untuk mengesahkan kestabilan genetik bagi tumbuhan teh yang dihasilkan dari kaedah TIS memandangkan ianya penting untuk industri. Penanda ISSR dari 11 primer yang di gunakan menunjukkan 100% persamaan antara tumbuhan yang dibiakkan secara mikro dan 10 anak pokok yang telah disesuaikan dan dipilih secara rawak dengan DNA pokok induk teh klon Iran 100. Kajian ini menunjukkan bahawa eksplan yang dihasilkan menggunakan RITA® tidak mengakibatkan perubahan genetik bagi bahan yang dibiakkan secara *in vitro*.

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I certify that a Thesis Examination Committee has met on 14 June 2012 to conduct the final examination of Reza Azadi Gonbad on his thesis entitled "**MICROPROPAGATION OF TEA (*Camellia sinensis* (L.) Kuntze) USING TEMPORARY IMMERSION SYSTEM**" 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 relevant degree of Doctor of Philosophy.

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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.

REZA AZADI GONBAD

Date: 14. JUNE. 2012

TABLE OF CONTENTS