UNIVERSITI PUTRA MALAYSIA

DIRECT SHOOT REGENERATION OF Hibiscus rosa-sinensis L. CV. 'BRILLIANT RED'

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FBSB 2012 57

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2012

DIRECT SHOOT REGENERATION OF *Hibiscus rosa-sinensis* L. CV. 'BRILLIANT RED'

By

CHEW TIONG DAR

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

September 2012

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

DIRECT SHOOT REGENERATION OF *Hibiscus rosa-sinensis* L. CV. 'BRILLIANT RED'

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September 2012

Chair: Janna Ong Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Hibiscus rosa-sinensis cv. 'Brilliant Red', a five-petal, single-layered, and bright red perennial herb, is the national flower of Malaysia. For more than 50 years the local people recognise the status and sovereignty of this plant from the perspective of nationality, and even self-esteem. After decades of advancement in plant tissue culture field, propagation of *Hibiscus* spp. via *in vitro* method is starting to pick up its pace. So far, no report has been published to generate *H. rosa-sinensis* with fragrant flowers or flowers with longer life span. Under this context, plant tissue culture is a good means to produce large quantity of *H. rosa-sinensis* rapidly to cater for the market needs and pave ways for genetic manipulation of the plant for desired traits. A medium optimised for 'Brilliant Red' is yet to be formulated. Thus, this study reports on the effects of modifying the Murashige & Skoog (MS) medium in facilitating direct shoot regeneration from nodal explants of *H. rosa-sinensis* L. cv. 'Brilliant Red'. The sterilisation conditions for 6 types of explants (basal leaf, leaf blade, leaf midrib, node,

internode, and shoot tip) harvested from an open field were initially compared. The optimised sterilisation conditions for the explants were found to be 30% Clorox-15min exposure, 15% Clorox-30min exposure, 35% Clorox-15min exposure, 40% Clorox-20min exposure, 10% Clorox-15min exposure, and 5% Clorox-40min exposure for the basal leaf, leaf blade, leaf midrib, node, internode and shoot tip, respectively. Using the optimised sterilisation conditions as mentioned, the survival-contamination percentages for each explant type were as follows: basal leaf: 86%, 0%; leaf blade: 99%, 0%; leaf midrib: 91%, 2%; node: 94.5%, 2%; internode: 97%, 0%; and shoot tip: 60%, 0%. In the direct shoot regeneration study using the nodal explants, MS medium containing 40 g/L sucrose, 0.3% (w/v) activated charcoal, and supplementations with myo-inositol, thiamine and nicotinic acid (Concentration: MS vitamin standard) were found to be suitable. The *in vitro* shoot survival rate was 30% with a mean leaf numbers of 2.7±0.45 produced, and a mean leaf length of 1.71±0.46 cm achieved after 5 weeks of culture on the modified medium. Callus induction from nodal explant could be performed by using 20 μM IBA, with callusing rate at 91.3±1.8% after 5 weeks of culture. Further histological study on the calli revealed that no embryogenic cell was found. Overall, shoot could be induced directly from *H. rosa-sinensis* cv. 'Brilliant Red' nodal explant based on the above formulation. More effort should be put on the indirect shoot regeneration. However, shoots maintenance required further refinement of the formulated MS medium. This study also reveals that callus induction could be an alternative way to produce *H. rosa sinensis* L. cv. 'Brilliant Red' *in vitro* plants.

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

DIRECT SHOOT REGENERATION OF
***Hibiscus rosa-sinensis* L. CV. 'BRILLIANT RED'**

Oleh

CHEW TIONG DAR

September 2012

Pengerusi: Janna Ong Abdullah, PhD

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Hibiscus rosa-sinensis L. cv. 'Brilliant Red' (bunga raya) adalah sejenis tumbuhan herba tahunan di mana bunganya mempunyai lima kelopak berwarna merah. Ia merupakan bunga kebangsaan Malaysia. Sejak 50 tahun dahulu, penduduk tempatan menganggap tumbuhan ini sebagai lambang kedaulatan dan kekuatan jati diri. Kemajuan dalam bidang tisu kultur tumbuhan melalui keadah mikro-pembiakan *Hibiscus* spp telah mula berkembang sejak beberapa dekad dahulu. Setakat ini, masih tiada laporan tentang penghasilan bunga raya wangian dan pokok bunga raya yang berbunga dengan jangka hayat panjang. Dalam konteks ini, kaedah tisu kultur tumbuhan merupakan satu kaedah yang baik untuk menghasilkan bunga raya secara besar-besaran supaya dapat memenuhi keperluan pasaran dan juga membolehkan manipulasi genetiknya untuk mendapatkan sifat-sifat yang diingini. Memandangkan penyediaan media untuk 'Brilliant red' yang optimum masih belum diformulasikan, oleh itu, kajian ini melaporkan kesan pengubahsuaian media Murashige & Skoog (MS) dalam membantu percambahan

semula pucuk secara langsung daripada tunas sisi *H. rosa-sinensis* cv. 'Brilliant Red'. Perbandingan dibuat bagi 6 jenis steril eksplan (daun basal, bilah daun, midrib daun, tunas sisi, internod, hujung pucuk) yang dipetik dari tempat terbuka. Didapati bahawa keadaan pensterilan yang optimum untuk eksplan ialah pendedahan 30% Clorox-15min, 15% Clorox-30min, 35% Clorox-15min, 40% Clorox-20min, 10% Clorox-15min, dan 5% Clorox-40min untuk daun basal, bilah daun, midrib daun, tunas sisi, internod, dan hujung pucuk. Melalui kaedah pensterilan optimum yang telah dinyatakan, peratusan kelangsungan hidup-kontaminasi untuk setiap eksplan adalah seperti berikut: daun basal: 86%, 0%; daun bilah: 99%, 0%; daun midrib: 91%, 2%; tunas sisi: 94.5%, 2%; internod: 97%, 0%; dan hujung pucuk: 60%, 0%. Dalam percambahan semula pucuk secara langsung menggunakan tunas sisi, MS media yang mengandungi 40 g/L sukrosa, 0.3% (w/v) arang aktif, dan penambahan dengan myo-inositol, thiamin, dan asid nikotinik adalah sesuai. Peratus hidup bagi pucuk adalah 30% dengan purata bilangan daun sebanyak 2.68±0.45, dan purata panjang daun ialah 1.71±0.46 cm selepas 5 minggu dikultur dalam medium yang telah diubah suai. Induksi kalus boleh dilakukan dengan menggunakan 20 μM IBA, dengan pembentukan kalus sebanyak 91.3±1.8% selepas dikultur selama 5 minggu. Kajian histologi yang lebih mendalam terhadap kalus menunjukkan bahawa tiada embriogenik sel dijumpai. Kesimpulannya, pertumbuhan pucuk boleh dirangsang secara langsung dari tunas sisi *H. rosa-sinensis* L. cv. 'Brilliant Red' berdasarkan formulasi di atas. Walau bagaimanapun, pengekalan pucuk memerlukan optimisasi lagi terhadap formulasi ini. Kajian ini juga menunjukkan bahawa cara induksi kalus berkemampuan untuk menghasilkan *H. rosa-sinensis* L. cv. 'Brilliant Red'.

ACKNOWLEDGEMENT

"A long journey will has its end eventually no matter how arduous it was; a magnificent feast will has its dispersal finally no matter how glorious it was". This sentence is correct for many cases, yet it is just partially true for my project, because the completion of my project is not the end but just one of the milestones along the journey towards new world.

Millions thanks to my supervisor Dr. Janna Ong Abdullah for her invaluable lesson, enthusiastic encouragement, and incessant guidance in helping me to complete this project. Her unceasing endeavor in doing every thing always impresses me greatly. Also, I would like to express my ineffable gratitude to Dr. Parameswari Namasivayam and Dr. Siti Habsah. Their assistance was on time during the most critical period of my work. On the other hands, I would like to convey my gratitude to the lab and department assistants who allowed me to use the research facilities and devices, which had greatly facilitated this project: Encik Hussein Jirangon, Puan Sharipah Samah, Puan Rosna binti Angsor, and Cik Siti Nordiana Abu Bakar.

Besides, I would also like to thank my fellow friends for the assistances they gave especially Miss Kok Sau Yee, Mr. Oii Chai Theam and Puan Siti Nurhadis who repeatedly taught and advised me throughout my work.

Wish all of you be blessed always with good health. Thanks!

APPROVAL

I certify that a Thesis Examination Committee has met on (28th September 2012) to conduct the final examination of (Chew Tiong Dar) on his thesis entitled "Direct Shoot Regeneration of *Hibiscus rosa-sinensis* L. cv. 'Brilliant Red'" 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 degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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APPROVAL

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

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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 previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

CHEW TIONG DAR

Date: 28 September 2012

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