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IN VITRO CULTURE OF ALOE VERA BARBADENSIS MILL.

AHMAD SHARIFKHANI

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IN VITRO CULTURE OF *ALOE VERA BARBADENSIS* MILL.

BY

AHMAD SHARIFKHANI

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

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DEDICATION

To the four pillars of my life: God, my wife, and my parents. Without you, my life would fall apart.
I might not know where the life's road will take me, but walking with you.

God, through this journey has given me strength.

Mother, you have given me so much, thanks for your faith in me, and for teaching me that I should never surrender.

Father, you always told me "move forward" I think I reached to the first step. Thanks for inspiring my love for transportation.

Farzaneh, you are everything for me, without your love and understanding I would not be able to make it.
We made it…

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

IN VITRO CULTURE OF *ALOE VERA BARBADENSIS* MILL.

By

AHMAD SHARIFKHANI

January 2012

Chairman: Associated Prof. Dr. Halimi Mohd Saud, PhD

Faculty: Agriculture

Aloe vera is an important medicinal plant species belonging to the family Liliaceae.The gel of *Aloe vera* makes an excellent treatment for wounds, burns and other skin disorders. Tissue culture technique offers certain advantages over traditional methods of propagation, including making exact copies of the plant; quickly producing mature plants andregeneratingtissue of genetically modified plant. In traditional method of propagation and culture of *Aloe vera*using adventitious shoots and/or buds the frequency of formation of vegetative shoots is very low. Due to increasing industrial demand for *Aloe vera* for production of gel, the development of *in-vitro*regeneration system is necessary to produce unique form and true-to-type plant.

The overall goal of this study was to develop a method of *in-vitro* micropropagation of *Aloe vera* through shoot regeneration from segment disk explants,follow by rooting and acclimatization. In the experiment on detecting the most suitable explant for *in-vitro* propagation, leaf tips, ring cross-section discs, adaxial leaf parts, abaxial leaf parts, segment nodal discs and terminal buds of *Aloe vera* plant were selected for culturing on MS media with and without 6-benzilaminopurine (BAP)and Kinetin

(Kin) as plant growth regulator. Among the parts mentioned above, only segment nodal discs regenerated shootson MS medium supplemented with hormones. In determining the most suitable sterilization treatment for reducing explants contamination, without the use of mercuric chloride, explants were treated with different concentrations of sodium hypochlorite(0.525%, 0.787%, 1.050%, 1.312%, and 1.575%).In this method sodium hypochlorite was an alternative to mercuric chloride. The explants were exposed to each concentration for a period of 20 minutes with vigorous and constant shaking. By Kruskal-Walis test (a nonparametric test), 1.050% sodium hypochlorite gave the highest number (91.7%) of alive and sterilized explants with regeneration potential.Based on theseresults, segment nodal discs of *Aloe vera* were sterilized and cultured on MS media supplemented with different concentrations ofBAP (0, 0.5,1,2,4 and5 mg/l). Data showed that, the number of shoots, increased with the highest mean of shoots per explant on medium supplemented with 4 mg/l BAP and the highest mean percentage of explants producing 100% shootswas obtained on medium with 4 mg/l BAP.The analysis of variance showed significant difference between the treatments. Different concentrations of Kincontrol, 0.5, 1, 2 and 4 mg/l (0, 2.33µM, 4.66µM, 9.30µM and 18.6 µM) were also assessed on shoot multiplication from segment nodal disks.The results reveal that there was no significant difference among different concentrations of Kinetin.Shoot multiplication from segment nodal disks was also studied using different concentrations of Thidiazuron (TDZ) either alone or in combination with Indole-3-butyric acid (IBA). The concentrations of TDZ were 1, 2, 3 and 4 mg/l and five concentrations of IBA were 0, 0.22, 0.57, 1.15 and 2.30 mg/l. Hormone-free MS medium (MSO)was used as control. The highest mean number of shoots proliferated per explant (5.10) was observed on moderate concentration of 2 mg/l TDZ and 1.15

mg/lIBA after eight weeks of culture, which was significantly different with the rest of the treatments. In the study on root induction MS media contain different concentrations of IBA were used. The explants which were previously regenerated using BAP and TDZ - IBA subsequently were cultured on MS media with different concentrations of IBA 0.22, 0.57, 1.15 and 2.30 mg/l. Results showed that the highest mean number of root per explant (5.06) was attained in MS medium supplemented with 1.15 mg/l IBA Highest percentage of root regeneration (100 %) was observed in two concentrations of 1.15 mg/l and 2.30 mg/l IBA followed by 93.33% in 0.57 mg/l IBA. Moreover, highest mean length of roots (14.05 cm) was found in MS medium supplemented with 0.57 mg/l IBA. In the acclimatization study, by applying one-sample Kolmogorove-Smirnove test normality of data was confirmed. On the other hand, the results showed that the plantlets were successfully (96.66 ± 3.33 %) transferred after rooting of microshoots. Using one-sample T-test, no significant difference was revealed between 96.66 % and 100% value of acclimatization of plantlets.

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

BAGI KULTUR TISU *ALOE VERA BARBADENSIS* MILL.

Oleh

AHMAD SHARIFKHANI

Januari 2012

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Aloe vera adalah tumbuhan ubatan berasal dari famili Liliaceae. Gel *Aloe vera* amat berkesan untuk merawat luka, kulit terbakar dan juga masalah kulit yang lain. Penggunaan teknik kultur tisu mempunyai kelebihan yang tersendiri berbanding kaedah pembiakan secara tradisional, ini termasuklah penghasilan tumbuhan yang seragam dengan tumbuhan induk, penghasilan tumbuhan matang yang lebih pantas dan pembiakan tisu tumbuhan yang diubahsuai genetiknya. Berdasarkan permintaan gel *Aloe vera* yang meningkat saban hari, pembangunan sistem pembiakan *in-vitro* adalah diperlukan untuk menghasilkan tumbuhan unik dan sekata dengan tumbuhan induk. Matlamat keseluruhan kajian ini adalah untuk membangunkan teknik mikropropagasi *in-vitro Aloe vera* melalui regenerasi dari bahagian cakera eksplan, disusuli dengan pengakaran dan aklimatisasi.

Didalam kajian untuk mengenal pasti bahagian eksplan yang paling sesuai untuk tujuan pembiakan *in-vitro,* hujung daun, cakera-silang cincin, adaksial daun, abaksial daun, cakera bernodal dan tunas hujung *Aloe vera* telah dipilih untuk pengkulturan pada media MS samada bersama atau tanpa hormon 6-benzilaminopurin (BAP) dan Kinetin (kin) sebagai pengawalatur tumbesaran tumbuhan. Walau bagaimanapun hanya segmen cakera bernodal beregenerasi dengan penggunaan medium MS

mengandungi hormon. Didalam eksperimen menentukan rawatan pensterilan yang paling sesuai untuk mengurangkan kontaminasi eksplan, kepekatan berbeza natrium hipoklorit (0.525%, 0.787%, 1.050%, 1.312%, 1.575%) telah digunakan ke atas eksplan. Eksplan didedahkan kepada setiap kepekatan selama 20 minit dengan goncangan yang kuat dan sekata. Ujian Kruskal-Walis menunjukkan bahawa natrium hipoklorit berkepekatan 1.050% menghasilkan peratusan tertinggi (91.7%) eksplan disteril yang hidup. Berdasarkan keputusan terdahulu, bahagian cakera bernodal *Aloe vera* telah disterilkan dan dikulturkan pada medium MS dengan kepekatan 6-benzilaminopurin (BAP) (2.22 µM, 4.44 µM, 8.88µM, 4 mg/l, 22.20 µM). Analisis data menunjukkan bahawa bilangan pucuk per eksplan paling tinggi apabila menggunakan BAP berkepekatan 4 mg/l dan peratus tertinggi eksplan yang menghasilkan 100% pucuk pada media yang sama. Analisis varians menunjukkan bahawa terdapat perbezaan signifikan antara rawatan-rawatan BAP yang digunakan. Kepekatan Kin yang berlainan 0, 0.5, 1.0, 2.0 dan 4.0 mg/l telah digunakan untuk menilai penggandaan pucuk dari cakera nodal. Keputusan ini menunjukkan tiada perbezaan signifikan pada kepekatan Kin yang berlainan dari segi bilangan pucuk yang digunakan. Kajian peningkatan cambahan pucuk dari cakera nodal juga dikaji menggunakan Thidiazuron (TDZ) sahaja atau gabungan bersama asid indole-butirik (IBA). Kepekatan TDZ ialah 1, 2, 3 dan 4 mg/l dan kepekatan IBA adalah 0, 0.22, 0.57, 1.15 dan 2.30 mg/l. Media MS tanpa hormon digunakan sebagai kawalan. Purata tertinggi percambahan pucuk setiap eksplan diperolehi dari kepekatan sederhana 2 mg/l TDZ dan 1.15 mg/l IBA selepas pengkulturan selama lapan minggu; perbezaan bererti berbanding rawatan yang lain. Dalam kajian pengaruhan akar, media MS dengan kepekatan IBA yang berbeza telah dilaksanakan. Eksplan yang biak menggunakan BAP dan TDZ-IBA seterusnya dikultur dalam media MS

mengandungi kepekatan IBA sebanyak 0.22, 0.57, 1.15 dan 2.30 mg/l. Keputusan menunjukkan purata tertinggi akar setiap eksplan (5.06) diperolehi dari media MS mengandungi 1.15 mg/l IBA. Peratus tertinggi (100%) percambahan akar didapati pada kepekatan 1.15 dan 2.30 mg/l IBA diikuti dengan 93.33% pada 0.57mg/l IBA. Tambahan pula, purata panjang akar tertinggi (14.05 cm) didapati dalam media MS dengan 0.57 mg/l IBA. Dalam kajian aklimatisasi, keputusan menunjukkan peratus (96.66 ± 3.33%) berjaya dipindahkan selepas pengakaran pucuk mikro. Tiada perbezaan ketara antara 96.66% dan 100% nilai aklimatisasi apabila ujian-T satu sampel digunakan.

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APPROVAL

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Master of Science. The members of the Supervisory Committee were as follow:

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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.

AHMAD SHARIFKHANI

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