UNIVERSITI PUTRA MALAYSIA

BREAKING OF SEED DORMANCY, PLANTLET REGENERATION AND ANTIOXIDANT ACTIVITIES OF BUNIUM PERSICUM

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BREAKING OF SEED DORMANCY, PLANTLET REGENERATION AND ANTIOXIDANT ACTIVITIES OF *BUNIUM PERSICUM*

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YOUSEF EMAMIPOOR

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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DEDICATION

I wish to dedicate this thesis to my father, for his great spirit, my mother for her merciful heart, my wife, Mahboubeh, for her love and patience, and my dear children, Ladan, Emad and Foad, for their faith in me - to them I am much indebted..

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

BREAKING OF SEED DORMANCY, PLANTLET REGENERATION AND ANTIOXIDANT ACTIVITIES OF *BUNIUM PERSICUM*

by

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August 2012

Chairman: Professor Maziah Mahmood, PhD

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Bunium persicum (Boiss) Fedtsch is a valuable medicinal plant that is facing extinction. A study was conducted to adopt various strategies and techniques to conserve and protect the biodiversity of *B. persicum*. Germination of dormant seeds and also maturation of seedlings under *in vitro* condition to overcome seed dormancy and reduce the juvenile period were studied. Then, the efficiency of direct and indirect shoot regeneration, the capacity of somatic embryogenesis and development of micropropagation methods were investigated. In addition, the antioxidant potential, total phenolic compounds and flavonoid contents of seeds were compared with explants and their related calli.

The seeds were treated at room (25 °C) and chilling (2-5 °C) temperatures with or without plant growth regulators (PGRs). The germination rate of dormant seed was 54.7, 46.7 and 53.3% under moist-chilling, moist-room with 35.2 mg/L gibberellic acid (GA_3)

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and moist-chilling with 1.4 mg/L thidiazuron (TDZ) conditions respectively. In addition, results showed that treatment of seeds with a combination of 1.4 mg/L TDZ with 35.2 mg/L GA_3 under moist-chilling conditions caused maximum seed germination, which was 93.7%. Furthermore, transferring of germinated seeds to Murashige and Skoog (MS), Gamborg (B5), Driver and Kuniyuki (DKW) and MSB (MS minerals with B5 vitamins) for maturation demonstrated that ½ MSB media was the most suitable medium for seedling development.

Successful direct regeneration of *B. persicum* obtained 14.5 ± 1.5 shoots per root explant on MSB medium supplemented with 0.02 mg/L methyl jasmonate (MJ). Application of root, corm and leaf explants from six-month-old seedlings on MSB medium supplemented with various auxins showed that root-derived and corm-derived calli on MSB medium supplemented with 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2, 4-D) by 77.1% and 74.9% respectively induced somatic embryogenesis calli. The somatic embryos transferred to medium supplemented with different concentrations of benzylaminopurine (BAP), kinetin, spermidine, forchlorfenuron (CPPU), chlormequat chloride (CCC), paclobutrazol (PBZ), casein hydrolysate (CH), poly ethylene glycol (PEG) and banana powder, led to maximum plantlet regeneration, which was 65.8 ± 2.6 obtained in ½ MSB medium supplemented with 20 g/L banana powder. Consequently, induction of somatic embryogenesis under 1.0 mg/L 2,4-D was found to be more suitable than other auxins and capacity of banana powder for plantlet development by having indol acetic acid (IAA), cytokinins and gibberellins (GAs) was more than other additives and PGRs.

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Successful indirect shoot regeneration of *B. persicum* was obtained from culture of leaf-derived callus on MSB medium supplemented with 0.4 mg/L 2,4-D. The callus was implanted on ½ MSB medium supplemented with 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L various cytokinins including BAP, kinetin, isopentyl aminopurine (2iP) and zeatin. Results indicated that the most suitable cytokinins for shoot regeneration were 0.6 mg/L kinetin with 34.2 ± 0.6 plantlets per culture. In addition, CPPU, TDZ, spermidine and additives including banana powder, yeast extract and casein hydrolysate influenced callus proliferation more than regeneration. Subsequently, results indicated that efficiency of embryogenesis callus was more than indirect and direct shoot regenerated calli. Also, the effect of different concentrations of sucrose, BAP, PBZ and GA_3 on size of *B. persicum* corms was investigated. Results showed that 90 g/L sucrose with 164.9 ± 2.8 g corm fresh weight (FW) was the most suitable sucrose concentration for growth of corm and shoot numbers.

The antioxidant activity, total phenolic compounds and flavonoids of seed were compared with root, corm, leaf and their related calli. Results of FRAP assay (based on trolox equivalent) showed that leaf segments of six-month-old *B. persicum* with 16.14 mg TE/ g DW had maximum antioxidant activity. Also, result of DPPH assay (based on gallic acid equivalent) for derived calli indicated that corm-derived callus on medium supplemented with 1.0 mg/L NAA (CN) with 12.61 mg GE/g DW showed maximum scavenging of free radicals. However, CN with 18.72 mg GE/g DW had maximum phenolic compounds and root-derived callus under 1.0 mg/L picloram (RP) had maximum flavonoid, which was 7.50 mg RE/g DW.

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The results showed that not only seed but also leaf, corm, root and their related derived callus could be used as natural plant antioxidants. However, *B. persicum* seeds had higher amounts of naringenin (170.3 µg/ g DW), kaempferol (132.7 µg/ g DW) and quercetin +(120.3 µg/ g DW), but leaves had higher amount of naringin (240.6 µg/ g DW), corms had higher amount of naringinin (146.7 µg/ g DW) while roots had higher amounts of rutin (160.6 µg/ g DW).

Results showed that a combination of GA_3 (35.2 mg/L) and TDZ (1.4 mg/L) with 93.7% breaking of seed dormancy and subsequent seedling maturation on ½ MSB medium was the suitable method for cultivation via seed. The result also showed that direct regeneration from root explants with 14.5 ± 1.5, indirect shoot regeneration from leaf-derived callus with 34.2 ± 0.6 and indirect somatic embryodenesis from root-derived callus with 65.8 ± 2.3 plantlets/culture respectively could be reliable methods for *B. persicum* regeneration. Furthermore, leaf and corm-derived callus with 16.1 mg TE/g DW and 11.2 ± 0.1mg GE/g DW antioxidant activity respectively, were found to be suitable as seed replacement. The presence of various flavonoids in seeds rather than leaf, corm and root could be related to more activity of flavonoid biosynthesis enzymes in seeds.

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PEMECAHAN DORMANSI BENIH, PERTUMBUHAN SEMULA DAN AKTIVITI ANTIOKSIDAN BAGI *BUNIUM PERSICUM*

oleh

YOUSEF EMAIPOOR

Ogos 2012

Pengerusi: Profesor Maziah Mahmood, PhD

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Bunium persicum (Boiss.) B. Fedtsch adalah satu tumbuhan ubatan yang berharga dan diancam kepupusan. Suatu kajian telah dijalankan bagi mengamalkan beberapa strategi dan teknik untuk memulihara dan memelihara pertumbuhan *B. persicum*. Pendomestikan *B. persicum* ,pertumbuhan semula secara langsung, jaringan pertumbuhan semula secara somatic embryogenesis telah dijalankan secara jaringan mikropropagas ke atas tumbuhan ini.

Selain daripada itu, potensi antioksidan, jumlah sebatian phenolic dan kanduangan flavonoid benih dibandingkan dengan eksplant yang berkaitan dengan mereka.

Pendomestikan berhadapan dengan dua cabaran penting. Malah benih *B. persicum* adalah sangat dorman dan benih hanya boleh di germenasikan pada kawasan bersuhu sejuk semulajadi .Benih yang kering dan lembap di rawat pada kadar suhu yang berbeza

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termasuk pada suhu bilik (25°C) dan suhu sejuk (2-5 °C). Keputusan menunjukkan bahawa terdapat germinasi benih maksimum pada kadar 54.7, 46.7 dan 53.3% di bawah keadaan lembap dan sejuk selepas 60 hari. Keputusan juga menunjukkan perawatan benih dengan 35.2 mg/L GA_3 di bawah keadaan suhu lembap bilik adalah sebanyak 46.7% kadar germinasi dan dengan 1.4 mg/L TDZ di bawah keadaan penyejukan lembap adalah 53.3% dengan dipengaruhi oleh dormansi pematahan benih. Selain daripada itu, keputusan juga menunjukkan perawatan benih dengan kombinasi 1.4 mg/L TDZ dan 35.2 mg/L GA_3 di bawah keadaan sejuk lembap menghasilkan germinasi benih yang maksimum iaitu 93.7%. Tambahan pula, pemindahan benih yang telah digermenasikan kepada MS, B5, DKW dan MSB (mineral MS dengan vitamin B5) untuk proses pematangan menunjukkan bahawa ½ MSB media adalah yang paling sesuai untuk perkembangan anak benih.

Pertumbuhan semula secara langsung yang berjaya bagi *B. persicum* telah diperolehi daripada eksplant dan menunjukkan maksimum perincian 14.5 ± 1.5 bagi setiap eksplant. Medium MSB dengan 0.02 mg/L methyl jasmonate (MJ) digunakan untuk proses pertumbuhan semula. Selain itu, keputusan menunjukkan akar, umbi dan daun daripada eksplant dan juga benih adalah bahan yang tidak sesuai bagi proses pertumbuhan semula bagi MSB, MS, B5 dan DKW yang di tambah dengan 1, 2, 5 dan 6 mg/L BAP atau kinetin.

Pertumbuhan semula secara tidak langsung yang berjaya bagi *B. persicum* bermula dengan percambahan akar, umbi dan daun yang bermula dari anak benih berusia 6 bulan. Eksplant daun pada medium MSB dengan tambahan 0.4 mg/l 2, 4-D digunakan untuk

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induksi kalus. Kalus daun dikulturkan di dalam ½ medium MSB dengan tambahan 0.2, 0.4, 0.6, 0.8, dan 1.0 mg/L perbezaan cytokinins. Medium tersebut bersama dengan 0.6 mg/L kinetin dan 34.2 tumbuhan semula untuk setiap kultur adalah bahan yang paling sesuai untuk pertumbuhan semula bagi kalus daun. Selain daripada itu, kesan daripada perbezaan konsentrasis ukrosa, BAP, PBZ dan GA_3 pada saiz umbi *B. persicum* telah dikaji. Keputusan menunjukkan bahawa pada ½ MSB, hanya konsentrasi sukrosa yang mempengaruhi saiz umbi dan bilangan jaringan untuk tumbuhan baru. Keputusan juga menunjukkan 90 g/ L sukrosa dengan 164.9 ± 2.8 g umbi FW adalah konsentrasi sukrosa paling sesuai untuk perkembangan umbi dan bilangan jaringan.

Embriogenesis somatik yang berjaya diperoleh daripada umbi atau kalus akar di dalam media MSB dengan tambahan 1.0 mg/L 2,4-D. Keputusan menunjukkan kalus akar dengan 77.1% pembentukan embryogenesis somatic mempengaruhi embryogenesis somatik. Dengan itu, embrio somatik di pindahkan ke medium ½ MSB (dengan tambahan konsentrasi berbeza dari BAP, kinetin, spermidine, CPPU, CCC, PBZ, casein hydrolysate, PEG dan tepung pisang) membawa kepada pertumbuhan semula tanaman muda sebanyak 65.8±2.6 dengan perolehan medium ½ MSB pada tambahan 20 g/L tepung pisang.

Di samping itu, pengukuran daripada jumlah flavonoid, sebatian phenolic dan aktiviti antioksi dan umbi, daun, akar dan kalus menunjukkan bahawa kalus akar di bawah 1.0mg/L picloram mempunyai maksimum flavonoid iaitu 7.50 mg rutin bersamaan / g DW. Walau bagaimanapun, kalus umbi di bawah 1.0 mg NAA (C.C., NAA) dengan 18.72 mg asid gallic bersamaan / g DW mempunyai maksimum sebatian fenol.

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Berdasarkan daripada kaedah DPPH, pemerangkapan maksimum radikal bebas dipersembahkan oleh C.C. NAA dengan 12.61 mg asid gallic bersamaan /g DW. Berdasarkan dari ujian FRAP,segmen daun dengan 16.14 mg trolox bersamaan /g DW mempunyai maksimum aktiviti antioksidan.

Kehadiran flavanoid seperti quercetin, kaempferol, rutin, naringenin di dalam ekstrak benih menunjukkan bahawa benih boleh digunakan sebagai tumbuhan antioksidan semula jadi. Walau bagaimanapun, ekstrak benih *B. persicum* dengan jumlah kompaun antioksidan semuladi yang berpatutan boleh digantikan dengan aktioksidan sintetik.

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I certify that a Thesis Examination Committee has met on 10 August 2012 to conduct the final examination of Yousef Emami Poor on his thesis entitled "Breaking of Seed Dormancy, Plantlet Regeneration, and Antioxidant Activities of *Bunium persicum* (Boiss.)" in accordance with the Universities and University College 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 Doctor of Philosophy.

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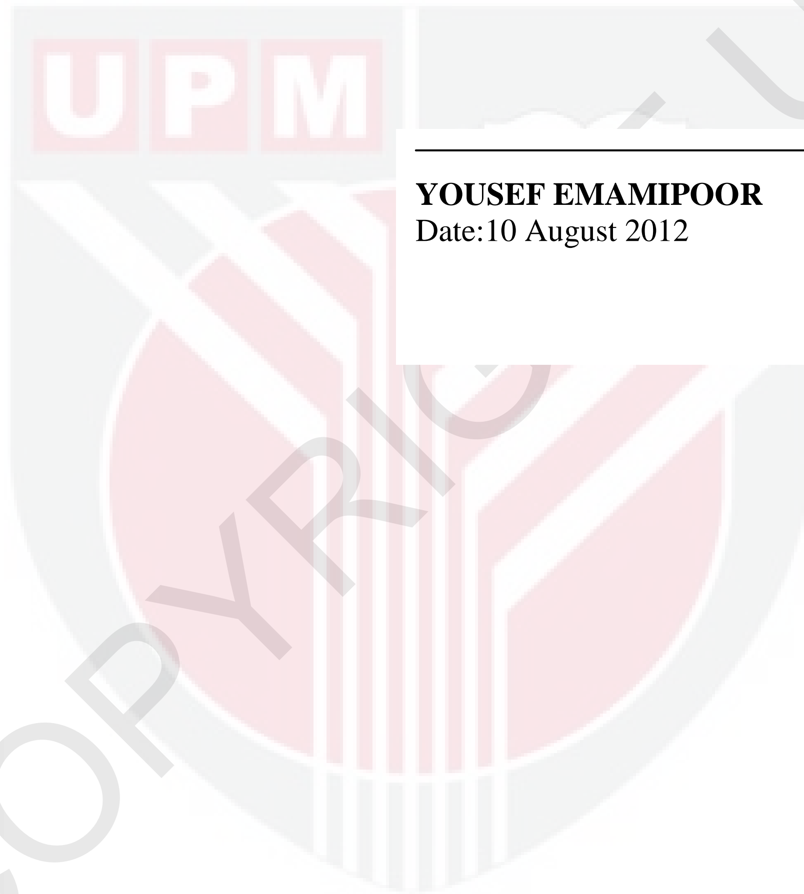
DECLARATION

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.



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YOUSEF EMAMIPOOR
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