UNIVERSITI PUTRA MALAYSIA

IN VITRO SELECTION, REGENERATION AND HERBICIDE TOLERANT CALLUS AND SUSPENSIONS CELL CULTURE OF RICE (Oryza sativa L.)

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IN VITRO SELECTION, REGENERATION AND HERBICIDE TOLERANT CALLUS AND SUSPENSIONS CELL CULTURES OF RICE (*Oryza sativa* L.)

BY
KOW CHEONG WEI

Dissertation Submitted in Fulfilment of the Requirements
for the Degree of Doctor of Philosophy
in the Faculty of Science and Environmental Studies
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Dedicated to,

My lovely father, mother, sister and brothers

" Some people may wonder why we must go and climb mountains when it is safer to sleep at home"
Dato' Seri Dr. Mahathir at the launching of Malaysia-Everest Project 1997

"Dream what you dare to dream, Do what you dare to do, And be what you dare to be."
Dr. Walter Doyle Staples

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LIST OF ABBREVIATIONS

AA	Thompson et al. (1986) salts
ACCase	acetyl-coenzyme A carboxylase
ALS	acetolactate sythase
B5	Gamborg et al. (1968) salts
BA	6-benzylaminopurine
2,4-D	2,4-Dichlorophenoxy acetic acid
2,4-DCP	2,4-Dichlorophenol
EPSP	5-enolpyruvyl shikimate-3-phosphate
FAO	Food and Agriculture Organisation
GS	glutamine synthetase
IAA	Indole-3-acetic acid
IRRI	International Rice Research Institute
Kin	Kinetin or 6-furfurylaminopurine
LOX	lipoxygenase
LS	Linsmaier and Skoog (1965) salts
LSC	liquid scintillation counter
MADA	Malaysian Agriculture Development Association
MES	2-(N-Morpholino)-ethanesulfonic
MGT	metribuzin N-glucosyltransferase
MS	Murashige and Skoog (1962) salts

MSD4	Thompson et al.(1986) culture media
NAA	∝-napthalene acetic acid
NBT	nitro blue tetrazolium
OECD	Organisation for Economic Cooperation and Development
PAT	phosphinotricin acetyl transferase
PCA	polycyclic alkanoic acid
pH	hydrogen ion concentration
PO	peroxidase
PPT	L-phosphinothricin
PSI	photosystem I
PQ^+	paraquat monocation radical
PVP	polyvinylpyrrolidonl
SCV	settled cell volume
SOD	superoxide dismutase
TARI	Taiwan Agricultural Research Institute

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

IN VITRO SELECTION, REGENERATION AND HERBICIDE TOLERANT CALLUS AND SUSPENSION CELLS CULTURES OF RICE (*ORYZA SATIVA* L.)

By

KOW CHEONG WEI

SEPTEMBER, 1998

Chairman : Professor Dr. Hajjah Marziah binti Mahmood

Faculty : Faculty of Science and Environmental Studies

The *in vitro* establishment of a rapidly growing, embryogenic and friable callus and suspension cell lines of 27 local *indica* rice (*Oryza sativa*) cultivars were studied. The regeneration capacity of selected cultivars also observed. Differences in culture conditions, growth rate and plant hormone applications were examined to determine the optimal responses. Rice callus was induced from immature seeds on basal MS solid medium supplemented with 10 μM 2,4-D and finely dispersed cell suspension cultures were initiated from the callus using B5 basal liquid medium consisted of 10 μM 2,4-D. These callus and suspensions were maintained in similar medium, respectively.

Basal MS solid medium supplemented with 25 μM BA and 5 μM IAA was most effective for obtaining regenerated plantlets from callus. For cell suspension, regeneration was obtained by using basal MS solid medium with 25 μM BA and 2.5 μM NAA. Out of 27 cultivars investigated, callus of 16 cultivars

and cell suspensions of 11 cultivars were suscessfully regenerated. Cultivar Puteh Perak exhibited good response throughout the experiments.

Radioactivity study was carried out as a confirmation of 2,4-D taken up by the callus and suspension cells. During the stressing and selecting stages, 2,4-D was observed to be taken up by the rice callus and suspension cells.

In the selection and toxicity studies, 2,4-D-tolerant callus cultivar Puteh Perak was selected in MS solid media at 400, 600 and 800 µM 2,4-D concentration while tolerant cell-suspension in basal B5 liquid media containing 200 and 400 µM 2,4-D. Both instantaneous and gradual stressing method were carried out. The tolerant callus and cell-suspension were isolated and maintained.

The selected 2,4-D-treated cultures (cultivar Puteh Perak) were exposed to higher 2,4-D levels (400, 600, and 800 µM). The cultures readily increased in growth but not for the control cultures that were not treated with 2,4-D. Plants were regenerated from the 2,4-D-tolerant cultures. The regenerated 2,4-D-tolerant rice plants were maintained *in vitro* in the laboratory by sub-culturing onto fresh media.

The activities of selected enzymes IAA-oxidase, lipogenases, peroxidase, catalase and superoxide dimutase were determined in the control and 2,4-D-treated cultures in both callus and cell suspension cultivar Puteh Perak. The IAA-

oxidase and peroxidase specific activity increased with the treatment in 800-1000 μM and 200-1000 μM 2,4-D respectively. However, reduction of lipoxygenases specific activity was obtained with the application of 2,4-D at 200 μM and above. Treament with 2,4-D exhibited a gradual reduction in both catalase and superoxide dimutase specific activity.

Abstrak dissertasi dikemukakan kepada Senat Universiti Putra Malaysia untuk memenuhi keperluan untuk Ijazah Kedoktoran Falsafah

PEMENCILAN, REGENERASI DAN KAJIAN KEKANGAN TERHADAP RACUN RUMPAI BAGI KULTUR KALUS DAN SEL AMPAIAN PADI *(ORYZA SATIVA* L.) SECARA *IN VITRO*

oleh

KOW CHEONG WEI

SEPTEMBER 1998

Pengerusi : Profesor Dr. Hajjah Marziah binti Mahmood

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Penjanaan kulturan kalus dan sel ampaian dengan kadar pertumbuhan yang tinggi, embriogenik dan sihat bagi 27 kultivar padi (*Oryza sativa*) kumpulan padi *indica* yang sedang ditanam di Malaysia telah dijalankan secara *in vitro*. Kapasiti regenerasi bagi kultivar-kultivar terpilih juga cerap. Faktor-faktor termasuk keadaan kultur, kadar pertumbuhan dan rawatan hormon tumbuhan telah dijalankan untuk mencari satu keadaan optimum pertumbuhan kalus dan sel ampaian.

Kalus padi telah diaruhkan daripada biji benih padi belum matang di atas suatu medium pepejal (agar) MS asas yang mengandungi 10 μM 2,4-D manakala sel ampaian diaruhkan daripada kalus dalam medium cair (tanpa agar) B5 asas yang juga mengandungi 10 μM 2,4-D. Kalus dan sel ampaian yang teraruh masing-masing dikultur dan diselenggara dalam medium yang sama.

Medium pepejal MS asas yang dibekalkan dengan kombinasi hormon 25 μM BA dan 5 μM IAA didapati paling berkesan bagi tujuan regenerasi anak pokok padi daripada kalus. Bagi sel ampaian pula, medium cecair B5 yang mengandungi kombinasi hormon 25 μM BA dan 2.5 μM NAA telah merupakan medium yang paling sesuai. Melalui eksperimen ini, 27 kultivar padi telah dikaji, kalus yang teraruh daripada 16 kultivar dan sel ampaian yang teraruh daripada 11 kultivar telah berjaya dalam proses regenerasi. Secara perbandingan antara 27 kultivar yang terlibat, kultivar Puteh Perak memperlihatkan respons yang baik sepanjang masa eksperimen dijalankan.

Kajian radioaktiviti telah dijadikan sebagai suatu ujian untuk mempastikan 2,4-D yang terkandung dalam medium rawatan kekangan dan pemencilan telah diserap oleh kalus dan sel ampaian padi.

Dalam eksperimen pemencilan dan kajian kekangan terhadap 2,4-D, kalus kultivar Puteh Perak yang terkekang terhadap 2,4-D telah diasingkan dalam medium yang berkepekatan 2,4-D pada 400, 600 dan 800 μM, manakala sel-ampaian yang terkekang 2,4-D diasing dalam medium berkepekatan 2,4-D pada 200 dan 400 μM. Kedua-dua cara pemencilan iaitu 'serta-merta' dan 'berperingkat' dijalankan. Kalus dan sel ampaian yang kekang kepada 2,4-D berjaya diasing dan diselenggarakan.

Kalus dan sel-sel ampaian kultivar Puteh Perak yang terasing dalam kajian kekang 2,4-D telah didedahkan kepada medium yang berkepekatan 2,4-D

lebih tinggi, didapati kalus dan sel-ampaian ini masih berupaya menahan dan tumbuh tetapi kalus dan sel-ampaian kawalan gagal dalam ujian. Anak-anak pokok padi boleh diperolehi dalam proses regenerasi yang diselenggara dan dikultur dengan pertukaran medium baru dapat dijalakan dalam makmal secara *in vitro*.

Kajian aktiviti-aktiviti enzim terpilih termasuk IAA-oksidase, lipogenase, perosidase, katalase and superosik dimutase telah dijalankan terhadap kultur kawalan dan kultur kekangan 2,4-D. Aktiviti spesifik enzim IAA-oksidase dam perosidase menurun dalam rawatan 2,4-D, iaitu pada 800 ke 1000 μM bagi IAA-oksidase dan 200-1000 μM bagi perosidase. Tetapi aktiviti spesifik lipogenase bertambah pada sukatan 200 μM kandungan 2,4-D dan ke atas. Rawatan 2,4-D dalam kajian aktiviti enzim katalase dan superosik dimutase berkeputusan di mana aktiviti mulai turun ketika sukatan 2,4-D bertambah.