UNIVERSITI PUTRA MALAYSIA

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

KOW CHEONG WEI

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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 Universiti Putra Malaysia

1998
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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Characteristic of rice suspension cells (cv. Puteh Perak) (a) before stressing and selection and (b) after stressing with 400 μM 2,4-D in B5 basal media (40x).
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<table>
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<tr>
<td>AA</td>
<td>Thompson et al. (1986) salts</td>
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<tr>
<td>ACCase</td>
<td>acetyl-coenzyme A carboxylase</td>
</tr>
<tr>
<td>ALS</td>
<td>acetolactate synthase</td>
</tr>
<tr>
<td>B5</td>
<td>Gamborg et al. (1968) salts</td>
</tr>
<tr>
<td>BA</td>
<td>6-benzylaminopurine</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2,4-Dichlorophenoxy acetic acid</td>
</tr>
<tr>
<td>2,4-DCP</td>
<td>2,4-Dichlorophenol</td>
</tr>
<tr>
<td>EPSP</td>
<td>5-enolpyruvyl shikimate-3-phosphate</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
</tr>
<tr>
<td>GS</td>
<td>glutamine synthetase</td>
</tr>
<tr>
<td>IAA</td>
<td>Indole-3-acetic acid</td>
</tr>
<tr>
<td>IRRI</td>
<td>International Rice Research Institute</td>
</tr>
<tr>
<td>Kin</td>
<td>Kinetin or 6-furfurylaminopurine</td>
</tr>
<tr>
<td>LOX</td>
<td>lipoxygenase</td>
</tr>
<tr>
<td>LS</td>
<td>Linsmaier and Skoog (1965) salts</td>
</tr>
<tr>
<td>LSC</td>
<td>liquid scintillation counter</td>
</tr>
<tr>
<td>MADA</td>
<td>Malaysian Agriculture Development Association</td>
</tr>
<tr>
<td>MES</td>
<td>2-(N-Morpholino)-ethanesulfonic</td>
</tr>
<tr>
<td>MGT</td>
<td>metribuzin N-glucosyltransferase</td>
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<tr>
<td>MS</td>
<td>Murashige and Skoog (1962) salts</td>
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<tr>
<td>MSD4</td>
<td>Thompson et al. (1986) culture media</td>
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<tr>
<td>NAA</td>
<td>α-naphthalene acetic acid</td>
</tr>
<tr>
<td>NBT</td>
<td>nitro blue tetrazolium</td>
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<tr>
<td>OECD</td>
<td>Organisation for Economic Cooperation and Development</td>
</tr>
<tr>
<td>PAT</td>
<td>phosphinotricin acetyl transferase</td>
</tr>
<tr>
<td>PCA</td>
<td>polycyclic alkanoic acid</td>
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<td>pH</td>
<td>hydrogen ion concentration</td>
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<td>PO</td>
<td>peroxidase</td>
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<td>PPT</td>
<td>L-phosphinothricin</td>
</tr>
<tr>
<td>PSI</td>
<td>photosystem I</td>
</tr>
<tr>
<td>PQ⁺</td>
<td>paraquat monocation radical</td>
</tr>
<tr>
<td>PVP</td>
<td>polyvinylpyrrolidone</td>
</tr>
<tr>
<td>SCV</td>
<td>settled cell volume</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>TARI</td>
<td>Taiwan Agricultural Research Institute</td>
</tr>
</tbody>
</table>
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 successfully 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.

Treatment with 2,4-D exhibited a gradual reduction in both catalase and superoxide dismutase 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

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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 teraruli daripada 16 kultivar dan sel ampaian yang teraruh daripada 11 kultivar telah berjaya dalam proses regenerasi. Secara perbandingan antara 27 kultivar yang terlibat, kultivar Putih 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 Putih 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 Putih 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 dijalankan dalam makmal secara
\textit{in vitro}.

Kajian aktiviti-aktiviti enzim terpilih termasuk IAA-oksidase, lipogenase,
perosidase, katalase dan superosik dimutase telah dijalankan terhadap kultur
kawalan dan kultur kekangan 2,4-D. Aktiviti spesifik enzim IAA-oksidase dan
perosidase menurun dalam rawatan 2,4-D, iaitu pada 800 ke 1000 \( \mu \text{M} \) bagi IAA-
oksidase dan 200-1000 \( \mu \text{M} \) bagi perosidase. Tetapi aktiviti spesifik lipogenase
bertambah pada sukatan 200 \( \mu \text{M} \) 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.