MICROPROPAGATION AND PHYSIOLOGICAL STUDIES ON SELECTED CITRUS SPECIES

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بسم الله الرحمن الرحيم

Dedicated

to my parents, my spouse,

and to my beloved kids Yahya and Ferdaus

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LIST OF ABBREVIATION

naphthalenacetic acid	NAA
2,4-dichlorophenoxyacetic acid	2,4-D
benzylamino purine	BAP
adenine sulfate	ADS
indolacetic acid	IAA
fluorescein diacetate	FDA
2-isopentyl adenine	2iP
6-furfurylamino purine (kinetin)	K
zeatin	Z
gibberellic acid	GA3
coconut milk	CM
malet extract	ME
orange juice	OJ
yeast extract	YE
casein hydrolysate	CH
Murashige and Skoog medium (1962)	MS
Murashige and Tucker medium(1969)	MT
weight/volume	w/v
volume/volume	v/v
volume/weight	v/w
polyethyleneglycol	PEG
Water use efficiency	WUE

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ABSTRACT

Micropropagation of four citrus species, namely citrumelo *(Poncirus trifoliata* × *C. paradisi*), *C. suhuiensis, C. limonia* and *C. reticulata* was achieved with various explants. Complete plantlet could be obtained within 3 - 4 months. The regeneration capacity of the explants varied within and between species. Multiple shoots were induced from nodal, internodal, leaf, and root explants. The best system was achieved with internodal and leaf explants for citrumelo on MT supplemented with 2 and 3 mg/l BAP. This followed by root explants of *C. reticulata*, nodal explants for *C. suhuiensis* and *C. limonia*. Generally, BAP at 1 to 3 mg/l was required to stimulate shoot regeneration in nodal explants and to induce adventitious shoots in internodal, leaf and root explants.

Morphogenesis of regenerated explants could be maintained by periodical subculture on 2 to 3 mg/l BAP. Generally adding of auxin (NAA) alone to MT basal medium promote root regeneration but did not prohibit shoot regeneration. On the other hand when cytokinin (BAP) was added to the medium either singly or in combination with NAA, callus and shoot formation was observed but, root formation was completely prohibited.

Regenerated shoots from various explants formed roots on MT with or without NAA supplementation. The plantlets obtained were acclimatised for a period of 4 weeks and established in soil. The sequence of changes of leaf anatomy during the process of acclimatisation were studied. It was observed that acclimatisation in citrus involved

changes in leaf anatomy which included development of palisade and spongy parenchyma, increase protoplast density, decrease in the intercellular space and formation of cuticle layer. Isoenzyme analyses showed that there were no somaclonal variations among the regenerated plants.

Protoplasts were isolated from mesophyll and embryo callus in attempts to regenerate plants from protoplast and to induce somatic hybridisation. Citrumelo embryo callus protoplasts were cultured on MT and MS media with or without phytohormone supplementation in liquid and solid medium. First division was obtained but the subsequent division was not sustained due to persistent contamination problems. Further experiment was jeopardized.

The physiological studies namely, photosynthetic rate, light response curve, quantum efficiency and water use efficiency were carried out on tissue cultured seedlings as well as seedlings germinated from seeds. Results indicated that the performance of both types of seedlings were simillar suggesting that the tissue culture plantlets have successfully acclimatised and ready to grow under normal field environment.

ABSTRAK

Mikropropagasi dari berbagai eksplan telah berjaya didapati dari empat species citrus iaitu citrumelo (*Poncirus trifoliata* × *C. paradisi*), *C. suhueinsis*, *C. limonia* dan *C. reticulata*. Regenerasi lengkap ini telah didapati dalam jangkamasa 3-4 bulan. Dari kajian yang telah dijalankan didapati kapasiti regenerasi bagi setiap eksplan adalah berbeza samada intra atau interspesies. Regenerasi pucuk berganda telah berjaya diinduksikan dari eksplan buku, ruas, daun dan akar. Dari kajian yang telah dijalankan didapati regenerasi tumbuhan yang dikultur di atas media MT ditambah dengan 2 dan 3 mg/l BAP adalah terbaik bagi eksplan ruas dan daun (citrumelo) , akar (*C .reticulata*) dan buku (*C. suhueinsis* dan *C. limonia*). Secara amnya, kepekatan BAP (1-3 mg/l) adalah sesuai untuk menginduksikan pucuk dari eksplan buku dan pucuk berganda bagi eksplan ruas, daun dan akar spesies yang dikaji.

Regenerasi pucuk dari eksplan telah disubkultur di atas media MT ditambah dengan BAP (2-3 mg/l). Secara amnya penambahan auksin(NAA) pada media asas MT akan menyebabkan induksi akar tetapi tidak merencat regenerasi pucuk. Sebaliknya di dalam kajian ini apabila BAP sahaja ditambah atau kombinasi BAP dan NAA pembentukan akar didapati telah direncat tetapi pembentukan kalus dan pucuk berlaku.

Tumbuhan lengkap telah didapati apabila pucuk di kultur di atas media MT tanpa hormon atau dengan penambahan NAA. Spesies yang dikaji telah berjaya diaklimatasikan dalam jangkamasa 4 minggu di atas tanah. Kajian anatomi juga dijalankan ke atas perubahan yang berlaku pada daun semasa proses aklimatisasi termasuk pembentukan sel palisad dan parenkima bersepan, peningkatan bilangan protoplast, pengurangan ruang intersellular dan pembentukan lapisan kutikel. Kajian isoenzim yang dijalankan menunjukkan tiada variasi somaklonal berlaku pada regenerasi tumbuhan dari eksplan apabila dibandingkan dengan tumbuhan induk.

Protoplast dari mesofil dan kalus telah berjaya diasingkan. Protoplast dari kalus (embryo) spesies citrumelo telah dikultur di atas media MT dan MS dengan atau tanpa hormon di dalam media cecair dan pepejal. Pembahagian pertama telah diperhatikan di atas media yang dikaji tetapi pembahagian berikutnya tidak diperolehi akibat masalah kontaminasi.

Kajian fisiologi seperti kadar fotosintesis, keluk respons cahaya, keberkesanan kuantum dan keupayaan pengambilan air telah dijalankan ke atas tumbuhan dari **kultur tisu** dan biji benih. Keputusan yang didapati menunjukkan tumbuhan dari **kultur tisu** setelah menjalani proses aklimatisasi mempunyai kebolehan yang sama apabila dibandingkan dengan tumbuhan dari biji benih.

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