DEVELOPMENT OF IN VITRO PROPAGATION OF *Dimorphorchis lowii* (ORCHIDACEAE) THROUGH SEMI-SOLID AND LIQUID CULTURE SYSTEMS

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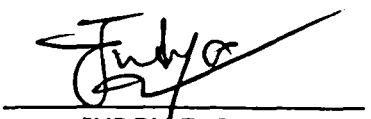

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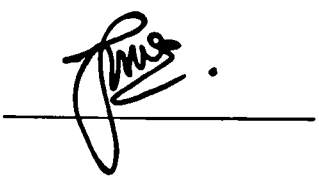

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ABSTRACT

Dimorphorchis lowii with its spectacular dimorphic flowers is well known to orchid enthusiast. This species belongs to Vandaeae tribe and Aeridinae subtribe. Like most of the other endemic orchids in Borneo island, this species is facing combined threats from habitat loss, habitat degradation, and problems in viable seed production and inefficient conventional vegetative propagation. Therefore *in vitro* propagation offers an important measure for multiplication and conservation of this species. In this study, protocols for *in vitro* propagation of *D. lowii* through semi-solid and liquid systems were developed. The effects of basal media (½MS, MS, ½KC, KC, VW, and Mitra), plant growth regulators (KIN, BAP, TDZ, 2,4-D, NAA, IBA, and IAA), complex additives (banana homogenate, coconut water, tomato juice, peptone, and yeast extract), carbon sources (fructose, glucose, and sucrose), light and dark photoperiods (16-hr light, 24-hr dark, and 24-hr light), and type of leaf segments (leaf tip, middle, and leaf base) on callus induction from leaf explant; protocorm-like-bodies (PLBs) proliferation from callus and shoot development; PLBs proliferation in liquid shake flask culture; shoot multiplication and rooting were investigated. The effect of immersion time on PLB proliferation in temporary immersion bioreactor systems (RITA® and twin-flask (BIT®) systems was also investigated. Plantlets were subjected to photoautotrophic and photoheterotrophic conditions prior acclimatization process to study their effects on the survival percentage. Callus induction from leaf tip explant was triggered when half-strength MS medium was used as basal medium and cultures were incubated under 24-hr dark photoperiod. However, the percentage of callus formation was very low at only 2.00%±1.47. TDZ at 3.0 mg/L with NAA at 0.046 mg/L could increase percentage of callus formation. Sucrose at 2.0% (w/v) showed more favourable result with the callus formation at 42.00%±9.60. Leaf tip segment exhibited highest potential in callus formation with the percentage of 50.00%±20.50. KC medium was the most suitable for PLB proliferation and development, yielding the highest percentage of survival at 36.00%±16.52 and 5.83±1.95 new PLBs per proliferated explant. Number of new PLBs was increased to 8.38±2.45 when 2.0 mg/L TDZ was supplemented in KC medium with 60.00%±24.47 PLBs produced shoots. NAA with TDZ at 2.0 mg/L in combination enhanced the number of new PLBs to 10.53±4.50 with 76.00%±23.14 PLBs developed shoots. Addition of 15% (v/v) coconut water in KC medium increased the number of new PLBs to 16.88±6.52 and 70.00%±42.02 developed shoots. Sucrose at 2.0% (w/v) presented the best results producing 11.55±5.63 new PLBs with 72.00%±16.20 produced 10.22±6.17 shoots. The most favourable liquid medium for PLB proliferation in liquid culture system was KC medium with 52.00%±25.88 survival. The percentage of survived explants was increased to 96.00%±5.48 producing 5.13±1.63 new PLBs when 3.0 mg/L TDZ was supplemented in the medium. Auxin was not suitable for PLBs proliferation when applied alone. Complex additive peptone at 0.2% (w/v) was found to be the best in promoting the formation of new PLB with 11.70±5.01. PLB proliferation at 86.00%±10.35 was obtained when sucrose at 1.0% (w/v) added to the medium. Further study to compare the use of complex additive peptone at 0.2% (w/v) and PGR TDZ at 3.0 mg/L to optimize PLB proliferation under light and dark photoperiod was carried out. Peptone was found to stimulate PLBs proliferation with 84.00%±37.03 under 16-hr light photoperiod

but 24-hr light photoperiod was favourable in terms of number of new PLBs produced with 6.92±3.08 per explant. In the temporary immersion bioreactor systems, the RITA® system was favourable than the twin-flask (BIT®) system by producing 66.00%±38.15 of PLB proliferation with 12.56±3.46 new PLBs per proliferated explant when cultures were immersed for 30 min per 6-hr per day. Shoot multiplication was enhanced when 2.0 mg/L KIN was supplemented in the medium, producing 5.05±2.01 shoots per explant. The use of 15% (v/v) coconut water as complex additive promoted multiplication by two-fold producing 13.83±6.12 shoots per explant. Sucrose at 2.0% (w/v) showed favourable result generating 4.03±3.16 shoots per explant. KC medium was found to be the best medium, yielding 4.69±3.93 shoots per explant. Root formation was improved when 1.0 mg/L IAA combined with 0.5 mg/L IBA were supplemented in the medium, producing 1.93±0.96 roots per explant. The use of 15% (v/v) coconut water as complex additive promoted root formation by almost two-fold yielding 3.00±1.44 roots per explant. Glucose at 2.0% (w/v) showed favourable result, producing 1.90±1.14 roots per explant and KC medium was better used as basal medium generating 3.00±1.44 roots per explant. In acclimatization, well-developed rooted plantlets were subjected to photoautotrophic and photoheterotrophic in *in vitro* conditions. After 60 days, plantlets were transferred to the potting mixture containing coco peat, sphagnum moss and charcoal in the ratio of 1:1:1. Higher survival rate of plantlets was obtained in plantlets that were subjected to photoheterotrophic condition with 78.00%±41.48 survival percentage after 60 days of acclimatization. It can be concluded that methods for *in vitro* propagation using various explants were successfully developed *via* semi-solid medium. Liquid medium also provided an alternative for PLB proliferation especially in TIBS using RITA system. Therefore *in vitro* propagation can be used as an alternative to propagate *D. lowii* for mass propagation.

ABSTRAK

PEMBANGUNAN PROPAGASI IN VITRO Dimorphorchis lowii (ORCHIDACEAE) MELALUI SISTEM MEDIA SEPARA-PEPEJAL DAN MEDIA CECAIR

Dimorphorchis lowii dengan dua jenis bunga yang luar biasa cantiknya digemari ramai oleh para pengemar orchid. Orkid ini termasuk dalam 'tribe' Vandaeae dan 'subtribe' Aeridinae. Sama seperti orkid endemik yang lain yang terdapat di kepulauan Borneo, spesis ini mengalami pelbagai ancaman daripada kehilangan habitat, penyusutan habitat dan masalah di dalam penghasilan biji benih yang berkeupayaan dan pembiakan melalui kaedah propagasi konvensional adalah tidak efisien. Oleh itu, propagasi in vitro menawarkan teknik yang penting untuk penggandaan dan konservasi orkid ini. Di dalam kajian ini, protokol untuk propagasi in vitro orkid Dimorphorchis lowii melalui sistem media separa-pepejal dan cecair telah dibangunkan. Kesan media (½MS, MS , ½KC, KC, V&W, dan Mitra), pengawalatur pertumbuhan (KIN, BAP, TDZ, 2,4-D, NAA, IBA, dan IAA), komplek aditif (air kelapa, jus tomato, homogenat pisang, pepton, dan extrak yis), sumber karbon (fruktosa, glukosa, sukrosa), cahaya (16 jam cerah, 24 jam gelap, dan 24 jam cerah), dan jenis segmen eksplan daun (hujung daun, bahagian tengah, bahagian pangkal) terhadap pengaruhan kalus dari eksplan daun; proliferasi jasad seperti protokom (JSP) daripada kalus dan perkembangan pucuk; proliferasi JSP di dalam media cecair, penggandaan pucuk, dan pengakaran telah dikaji. Kesan masa rendaman terhadap proliferasi JSP di dalam sistem rendaman sementara (RITA® dan flask berkembar atau BIT®) juga telah dikaji. Anak pokok dirawat secara fotoautropik dan fotoheterotropik sebelum proses aklimatisasi untuk mengkaji kesan rawatan in terhadap peratus anak pokok yang hidup. Pengaruhan kalus dari daun didapati terbaik dengan penggunaan media MS dengan kepekatan separuh dan kultur di letakkan di dalam gelap 24 jam. Tetapi peratus pengaruhan kalus adalah rendah iaitu hanya 2.00%±1.47. TDZ dengan kepekatan 3.0 mg/L dan NAA dengan kepekatan 0.046 mg/L, boleh meningkatkan peratus pengaruhan kalus. Sukrosa pada kepekatan 2.0% (w/v) didapati lebih berkesan dengan menghasilkan pengaruhan kalus sebanyak 42.00%±9.60. Hujung daun didapati mempunyai potensi yang tinggi dengan menghasilkan peratus pengaruhan sebanyak 50.00%±20.50. Media KC didapati paling sesuai untuk proliferasi and perkembangan JSP dengan menghasilkan 36.00%±16.52 JSP baharu yang hidup dan *5.83±1.95 JSP baharu per eksplan yang berproliferasi. Bilangan JSP baharu meningkat ke 8.38±2.45 bila 2.0 mg/L TDZ ditambah di dalam media KC dan 60.00%±24.47 JSP menghasilkan pucuk. NAA dan TDZ dengan kepekatan 2.0 mg/L didapati dapat mempertingkatkan bilangan JSP baharu sebanyak 10.53±4.50 dengan 76.00%±23.14 JSP menghasilkan pucuk. Penambahan 15% (v/v) air kelapa di dalam media juga didapati meningkatkan bilangan JSP baharu sebanyak 16.88±6.52 dengan 70.00%±42.02 tumbuh menjadi pucuk. Sukrosa dengan 2.0% (w/v) didapati menghasilkan 11.55±5.63 JSP baharu dengan 72.00%±16.20 tumbuh menghasilkan 10.22±6.17 pucuk. Media cecair yang terbaik untuk proliferasi JSP ialah media KC dengan menghasilkan 52.00%±25.88 eksplan yang hidup. Peratus eksplan yang hidup ditingkatkan ke 96.00%±5.48 dengan penghasilan 5.13±1.63 JSP baharu dengan penambahan 3.0 mg/L TDZ di dalam*

media pengkulturan. Auxin secara individu didapati tidak mempengaruhi proliferasi JSP sebaik sitokinin. Komplex aditif pepton pada kepekatan 0.2% (w/v) pula didapati dapat merangsangkan proliferasi JSP dengan jumlah JSP baharu sebanyak 11.70±5.01. *Proliferasi JSP sebanyak 86.00%±10.35 terhasil apabila 1.0% sukrosa (w/v) ditambah di dalam media. Kajian selanjutnya dijalankan untuk membandingkan penggunaan komplex aditif 0.2% (w/v) pepton dengan pengawalatur tumbuhan 3.0 mg/L TDZ dengan cara mengoptimasikan proliferasi JSP di dalam gelap dan cerah. Pepton didapati dapat merangsangkan proliferasi JSP dengan peratusan setinggi 84.00%±37.03 di bawah 16 jam cerah sementara itu 24 jam cerah didapati meningkatkan bilangan JSP baharu sebanyak 6.92±3.08 per eksplan. Di dalam kajian sistem rendaman sementara, Sistem RITA® adalah lebih baik berbanding dengan sistem flask kembar (BIT®). Dengan sistem RITA®, peratusan proliferasi JSP menjangkau 66.00%±38.15 dengan penghasilan 12.56±3.46 JSP baharu per eksplan yang berproliferasi dengan perendaman selama 30 min setiap 6 jam setiap hari. Pengandaan pucuk dapat ditingkatkan dengan penggunaan 2.0 mg/L KIN dengan menghasilkan 5.05±2.01 pucuk per eksplan. Seterusnya dengan penggunaan air kelapa sebanyak 15% (v/v) sebagai komplex aditif, ianya meningkatkan penggandaan pucuk dua kali ganda dengan penghasilan 13.83±6.12 pucuk per eksplan. Sukrosa dengan 2.0% (w/v) adalah lebih berkesan dengan penghasilan 4.03±3.16 pucuk per eksplan. Media KC didapati adalah yang terbaik dengan menghasilkan 4.69±3.93 pucuk per eksplan. Pembentukan akar telah ditingkatkan dengan penambahan 1.0 mg/L IAA dengan kombinasi 0.5 mg/L IBA di dalam media pengkulturan dan menghasilkan 1.93±0.96 akar per eskplan. Penggunaan air kelapa sebanyak 15% (w/v) sebagai komplex aditif juga dapat menambahbaik penghasilan akar dua kali ganda dengan menghasilkan 3.00±1.44 akar per ekplan. Glukosa dengan 2% (w/v) didapati lebih baik dengan penghasilan 1.90±1.14 akar per eksplan. Media KC didapati lebih baik sebagai media asas dengan menghasilkan 3.00±1.44 akar per eksplan. Dalam proses aklimatisasi, pucuk yang lengkap dengan akar dirawat dalam keadaan fotoautotropik dan fotoheterotropik selama 60 hari dan kemudian dipindahkan di dalam pasu plastik yang mengandungi tanah gambut, lumut sphagnum dan serpihan arang dengan nisbah 1:1:1. Peratusan anak pokok hidup yang dirawat dalam keadaan fotoheterotropik didapati lebih tinggi iaitu sebanyak 78.00%±41.48 dibandingkan dengan anak pokok yang dirawat dalam keadaan fotoautotropik. Sebagai kesimpulannya, kaedah propagasi in vitro dengan menggunakan pelbagai eksplan telah berjaya dibangunkan melalui media separa-pepejal. Media cecair juga boleh digunakan sebagai alternatif untuk proliferasi JSP terutamanya di dalam sistem rendaman sementara dengan menggunakan sistem RITA. Oleh itu, propagasi in vitro boleh digunakan sebagai alternatif untuk propagasi penggandaan orkid D. lowii.*

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<	Less than
±	Plus minus
½ X	Half concentration
°C	Degree celcius
µM	Micromolar
$\mu molm^{-2}s^{-1}$	Micromol per meter square per second
2, 4-D	2,4-dichlorophenoxyacetic acid
ANOVA	Analysis of variance
AP2/ERF	Apetala 2/ethylene-responsive element binding factor
AUX1	Auxin influx
BAP	6-benzylaminopurine
Ca	Calcium
CKX	Cytokinin oxidase gene
Co	Cobalt
CRD	Complete randomized design
Cu	Copper
DAC	Day after culture
DMRT	Duncan Multiple Range Test
DNA	Deoxyribonucleic acid
DNSO	De novo shoot organogenesis
DSCKX1	*Dendrobium* sonia cytokinin oxidase gene
Fe	Ferum (Iron)
GA	Gibberellic acid
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
K	Potassium
KC	Knudson C medium
KIN	6-furfurylaminopurine
Lux	Lumen per meter squared
Mg	Magnesium
Mn	Manganese
Mo	Molybdate
Mpa	MegaPascal
MS	Murashige and Skoog medium
M	Molar
mm	Millimeter
mg/l	Milligram per liter
N	Nitrogen
NAA	α-Naphtaleneacetic acid
NaOH	Sodium hydroxide
P	Phosphorus
Psi	Pounds/square inch
PFD	Photon flux densities
PGRs	Plant growth regulators
PIN	Pin-formed protein
PLBs	Protocorm like bodies

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