

**PERCAMBAHAN BIJI BENIH DAN PROPAGASI ORKID
ENDEMIK BORNEO, *VANDA DEAREI*
SECARA *IN VITRO***

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**PERPUSTAKAAN
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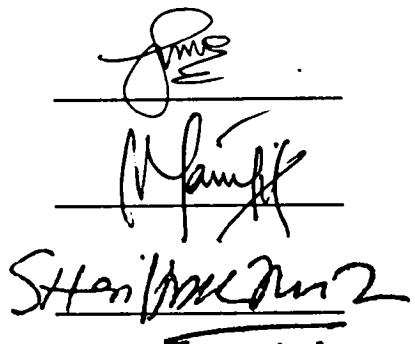


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ABSTRAK

PERCAMBAHAN BIJI BENIH DAN PROPAGASI ORKID ENDEMIK BORNEO, *VANDA DEAREI* SECARA IN VITRO

Vanda dearei merupakan spesies orkid yang endemik dan terancam di kepulauan Borneo. Bunganya berbau harum dan mempunyai bentuk dan warna yang cantik. Ini telah menyebabkan permintaan yang tinggi terhadap spesies ini untuk tujuan pengkomersialan. Akan tetapi, spesies ini mempunyai kitaran pertumbuhan yang lambat dan tidak boleh dibiakkan dengan cepat melalui kaedah propagasi konvensional. Oleh itu, prosedur propagasi kultur tisu telah dibangunkan. Kesan media asas (MS, VW, KC dan Mitra), kompleks aditif (air kelapa, jus tomato, homogenat pisang, ekstrak yis dan pepton), pengawalatur pertumbuhan (BAP, kinetin, NAA, IAA, IBA dan 2,4-D), gula (sukrosa, glukosa dan fruktosa) dan cahaya (24j gelap, 24j cerah dan 16j cerah) terhadap percambahan biji benih, proliferasi protokorm, pertumbuhan dan perkembangan protokorm serta pengaruhan JSP daripada bahagian daun dan akar dilaporkan dalam kajian ini. Hasil kajian menunjukkan bahawa biji benih berjaya bercambah dan membentuk protokorm selepas 25 hari pengkulturan. Percambahan biji benih paling tinggi (45.75%) berlaku di atas media asas KC dan meningkat dengan penambahan masing-masing 0.50% (w/v) ekstrak yis (67.94%) dan 0.1mg/l NAA (80.20%) ke dalam media pengkulturan. Penambahan 1% (w/v) sukrosa mempercepatkan percambahan biji benih (23 hari selepas pengkulturan) dengan 98.39% percambahan. Gula telah meningkatkan pembentukan pucuk. Kultur biji benih yang diletakkan di bawah 24j gelap memamerkan peratusan percambahan yang lebih tinggi (96.33%) berbanding yang dikultur di bawah 24j cerah (94.83%). Di dalam kajian proliferasi, protokorm baru berjaya diaruh selepas 28 hari pengkulturan. Rawatan dengan kombinasi pengawalatur pertumbuhan didapati lebih efektif untuk mengaruh pembentukan protokorm berbanding dengan pengawalatur pertumbuhan tunggal dan dengan penambahan kompleks aditif. Proliferasi protokorm paling tinggi (50.47 ± 10.47 biji protokorm baru) berlaku di atas media yang dibekalkan dengan 0.5mg/l NAA:0.5mg/l BAP. Dalam pencerakinan media asas dan sumber gula, media asas $\frac{1}{4}$ KC dan 1% (w/v) sukrosa memberikan bilangan protokorm baru yang tertinggi: masing-masing dengan 12.41 ± 4.00 dan 11.39 ± 3.35 biji. Media cecair merangsang proliferasi protokorm lebih baik berbanding media pepejal. Akan tetapi protokorm baru yang terbentuk di dalam media cecair cenderung untuk mengalami nekrosis. Sumber eksplan yang berbeza juga mempengaruhi proliferasi protokorm. Selain protokorm, anak pokok dengan dua helai daun juga boleh menghasilkan protokorm baru (21.10 ± 6.61 biji). Proliferasi protokorm adalah lebih tinggi di bawah 16j cerah (50.47 ± 10.47 biji protokorm baru). Dalam kajian pertumbuhan dan perkembangan protokorm, anak pokok berjaya terbentuk selepas 140 hari pengkulturan di atas media asas $\frac{1}{2}$ MS sehingga 73.33% protokorm yang menghasilkan daun (3.21 ± 1.47 helai daun) dan 35% yang menghasilkan akar (1.58 ± 0.50 akar). Penambahan 0.2% (w/v) ekstrak yis meningkatkan peratusan protokorm yang berdaun dan berakar sehingga mencapai 97.78% masing-masing dengan 5.10 ± 2.91 helai daun 1.78 ± 0.73 akar. Kepekatan 4% (w/v) sukrosa merupakan sumber karbon yang terbaik yang berjaya mengaruh sehingga

100% protokorm yang berdaun (7.28 ± 0.67 helai daun) dan berakar (3.43 ± 0.42 akar). Dalam kajian pengaruhan JSP, JSP berjaya dihasilkan selepas 35 hari pengkulturan. Rawatan dengan kombinasi 4.0mg/l BAP:1.0mg/l NAA merupakan rawatan yang paling efektif untuk mengaruh pembentukan JSP (12.33 ± 5.46 biji). Selain itu, eksplan pangkal daun menghasilkan lebih banyak JSP (15.13 ± 5.73 biji) berbanding dengan hujung daun (5.40 ± 4.20 biji) dan hujung akar (tiada pembentukan JSP). Keadaan 24j gelap merupakan keadaan yang paling sesuai untuk pengaruhan JSP bagi spesies orkid ini.



ABSTRACT

Vanda dearei is an endemic and endangered orchid species in Borneo. The flower is scented and beautiful in shape and colour. These resulted in a high demand for the species for commercialize purposes. However, this species has a slow growth cycle and cannot be propagated rapidly through conventional propagation methods. Therefore, tissue culture propagation procedures were developed. The effects of basal media (MS, VW, KC and Mitra), complex additives (coconut water, tomato juice, banana homogenate, yeast extract and peptone), plant growth regulators (BAP, kinetin, NAA, IAA, IBA and 2,4-D), sugars (sucrose, glucose and fructose) and light (24h dark, 24h light and 16h light) on seed germination, protocorm proliferation, protocorm growth and development and PLB induction from leaf and root segments are reported in this study. The results showed that seeds were successfully germinated and formed protocorms after 25 days of culture. The highest (45.75%) seeds germination occurred on KC basal medium and increased with the addition of 0.5% (w/v) yeast extract (67.94%) and 0.1mg/l NAA (80.20%), respectively to the basal medium. Addition of 1% (w/v) sucrose accelerates seed germination (23 days of culture) and brought about 98.39% germination. Sugars enhanced shoot production. Seed cultures incubated under 24h dark exhibited a higher germination percentage (96.33%) than the cultures maintained under 24h light (94.83%). In a proliferation study, new protocorms were successfully induced after 28 days of culture. Treatments with combinations of plant growth regulators were more effective in inducing protocorm formation than single plant growth regulators regime and the addition of complex additives. The highest (50.47 ± 10.47 new protocorms) proliferation of protocorms occurred on a medium supplemented with 0.5mg/l NAA: 0.5mg/l BAP. In assays of basal media and sugar sources, $\frac{1}{4}$ KC basal medium and 1% (w/v) sucrose brought about the highest number of new protocorms: 12.41 ± 4.00 and 11.39 ± 3.35 , respectively. Liquid media stimulated protocorm proliferation better than solid media. However new protocorms in the liquid media tend to become necrotic. Different explant sources also affected protocorm proliferation. Other than protocorm, seedling with two leaves can also produce new protocorms (21.10 ± 6.61 new protocorms). Protocorm proliferated to a higher extent under 16h lights (50.47 ± 10.47 new protocorms). In a study of protocorm growth and development, seedlings were successfully produced after 140 days of culture on $\frac{1}{2}$ MS basal medium with up to 73.33% of the protocorms producing leaves (3.21 ± 1.47 leaves) and 35% producing roots (1.58 ± 0.50 roots). Addition of 0.2% (w/v) yeast extract enhanced the percentage of protocorms with leaves and roots up to 97.78% with 5.10 ± 2.91 leaves and 1.78 ± 0.73 roots, respectively. Sucrose concentration of 4% (w/v) was the best carbon sources, which successfully induced up to 100% protocorms with leaves (7.28 ± 0.67 leaves) and roots (3.43 ± 0.42 roots). In a PLB induction study, PLBs were successfully produced after 35 days of culture. Treatment with a combination of 4.0mg/l BAP: 1.0mg/l NAA was the most effective for PLB induction (12.33 ± 5.46 PLBs). In addition, leaf base explants produced more PLBs (15.13 ± 5.73 PLBs) compared to leaf tips (5.40 ± 4.20 PLBs) and root tips (failed to produce PLB). Continuous dark culture conditions were the most suitable for PLB induction in this orchid species.

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