

**STUDIES ON *IN VITRO* PROPAGATION AND
SCREENING OF ATHOCYANIN
AND VOLATILE COMPOUNDS OF
Vanda ORCHIDS**

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ABSTRACT

STUDIES ON *IN VITRO* PROPAGATION AND SCREENING OF ANTHOCYANIN AND VOLATILE COMPOUNDS OF *Vanda* ORCHIDS

Vanda helvola belongs to the Borneo orchid and listed as one of the endangered orchid species. The *in vitro* propagations of *V. helvola* were successfully established through seed germination and leaf segment culture. The effect of basal media (KC, MS, and VW); complex additives (tomato juice, coconut water, yeast extract and peptone); and plant growth regulators (NAA, BAP, 2, 4-D and kinetin) were examined to determine the optimum medium for seed germination, protocorm and seedling growth, protocorm proliferation and PLBs induction from leaf culture. All cultures from the entire studies were maintained under 24 h light conditions at $25 \pm 2^\circ\text{C}$. Seeds of *V. helvola* were aseptically cultured and germinated for over 90% on Knudson C (KC) basal medium supplemented with 10 – 15% (v/v) of tomato juice after 90 days of culture. The germinated seed developed to protocorm and the establishment of *V. helvola* seedling was best performed in medium added with 0.1 – 0.2% (w/v) of peptone. The final seedling stage was achieved with a formation of 4 – 5 leaves and 3 roots. The acclimatization of seedling was then achieved when the well-developed seedling was transferred to pot containing brick pieces and coconut husks (1:2) and mulched with *Sphagnum* sp. The potted plants survived with 55.67% after 12 months, and shifted to Taman Pertanian Lagud Sebrang for further growth. The proliferation of *V. helvola* protocorm was demonstrated on KC basal medium with addition of NAA or BAP. Multiplication of protocorm was high on KC basal medium containing 1 mg/l BAP with a production of 10.57 ± 0.36 new protocorms per responsive explant. The addition of 2.0 mg/l NAA to this treatment has lowered the proliferation rate (7.46 ± 0.36), but increased the shoot/leaf promoting effect of the new protocorms. The PLBs induction from leaf segment culture produced a range of 5 - 9 PLBs on Mitra basal medium supplemented with 0.5 mg/l NAA and 4.0 mg/l BAP after 90 days of culture. Besides the tissue culture study, a preliminary screening on anthocyanin and volatile content has been done on two Borneo *Vanda* species i.e., *V. helvola* and *V. dearei*. The results showed that a high PAL enzyme activity in *V. dearei* flower might concurrent with the present of cinnamaldehyde compound from GCMS analysis, which supported with a strong fragrance emission from this flower. Meanwhile, higher anthocyanin content in *V. helvola* flower extract might be related with the colour of this flower. The successful establishment of the tissue culture techniques on *V. helvola* would be beneficial to the Borneo orchid's conservation, while the preliminary data on anthocyanin pigmentation and volatile content might be useful for future research in understanding the regulation of these compounds.