

## **Effect of basal media and carbon sources on callus culture maintenance of *Vanda dearei***

### **Abstract**

*Vanda dearei* is an endemic orchid of Borneo and has been listed as an endangered orchid in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). *Vanda dearei* has beautiful pale yellow-flowers, large petals and strongly scented. Therefore, in vitro micropropagation has been applied in order to develop a novel micropropagation method to mass produce this species. Through callus culture techniques, orchids with limited resources can be mass propagated in a shorter period. However, callus culture in orchid is hardly maintain due somatic embryogenesis properties and easily regenerated to plantlets. Thus, this study aims to develop an efficient protocol for callus cultures of *V. dearei* by manipulating basal media strengths and carbon sources. Callus induced from the leaf segments of *V. dearei* were used as explants and were cultured on KC, Mitra, MS and VW basal medium at different nutrient strengths (1/4, 1/2, 1 and 2x) added with 1.0:0.1 mg/l TDZ:NAA and 1 to 4% (w/v) of sucrose, glucose or fructose, respectively. All cultures were incubated in the dark with temperature of  $25\pm 2^{\circ}\text{C}$ . Results showed that callus growth has improved with decreased nutrients strength of basal media. Quarter strength of Mitra medium promotes the best condition for callus maintenance to approximately  $8.00\pm 17.89\%$  at 8 weeks of culture. This is followed by the 1/2 strength of MS and 1/4 strength of VW with  $8.00\pm 10.95\%$  and  $5.00\pm 10.00\%$ , respectively. Callus grown on the other basal strengths are mainly differentiated and developed into protocorm like bodies (PLBs), especially at double strengths (100±0% explants turn into PLBs). In addition, low percentage of necrosis (less than 28%) was also observed on Mitra basal medium compared to the other media (more than 36%). Sucrose has been identified as the best carbon source to support callus growth followed by glucose and fructose. Addition of 1% (w/v) sucrose increased callus maintenance up to  $32\pm 17.9\%$ , promote cell differentiation and increased average size of callus ( $1.52\pm 0.63$  callus score). This treatment also support the longest retention time of explant

maintained in callus for 5 weeks and has the lowest percentage of callus necrosis ( $20 \pm 24.5\%$ ).