

## **Preliminary study of DMSO vitrification technique of Dendrobium sonia 28 using protocorm-like bodies (PLBs) explant**

### **ABSTRACT**

In vitro grown shoot derived from protocorm-like bodies of Dendrobium sonia 28 hybrid were cryopreserved under liquid nitrogen condition, by means of DMSO vitrification method. Prior to the cryopreservation, the shoot were excised into two types of different length of 0.5-1.0 cm and 1.0-1.5 cm. Those entire excised shoot were grown on half-strength Murashige and Skoog (MS) semi solid medium. Upon DMSO vitrification method, the shoots were precultured (24 and 48 hours) at different sucrose concentrations (0.06 M, 0.10 M, 0.25 M, 0.50 M and 0.75 M). Vitrification process proceeded with culturing of the shoots in a loading solution consist of mixture of 2 M glycerol and 0.4 M sucrose for 20 minutes at room temperature (28°C), followed by further dehydration process with DMSO for a different incubation duration (0 minute, 10 minutes, 20 minutes and 30 minutes) at temperature of 0°C and 24°C. The shoots were later plunged into liquid nitrogen. After recovering from the liquid nitrogen storage, the shoots underwent rapid thawing (40°C) and were grown in a regrowth semi solid medium for two days under dark condition. TTC analysis was carried out to determine the viability of the shoots after storage under liquid nitrogen. The highest absorbance value at 540 nm was obtained using 2,3,5, triphenyl tetrazolium chloride (TTC) assay from the treatment of 1.0-1.5 cm shoots precultured for 24 hours in 0.5 M sucrose concentration MS semi-solid medium at 0°C for the 10 minutes incubation time in DMSO solution. The DMSO vitrification method was a crucial step in the orchid cryopreservation of Dendrobium sonia 28 hybrid shoot. Treatment of DMSO solution was proven to be capable of carrying out the dehydration process which was important for the survival rate of Dendrobium sonia 28 hybrid shoot undergoing cryopreservation at ultra low temperature (-196°C).

**Keyword:** Dendrobium sonia 28; DMSO vitrification; TTC assay