# Cryopreservation of the North Borneo Phalaenopsis gigantea J.J.Sm. Using a Vitrification Approach


## ABSTRACT

Phalaenopsis gigantea is an endangered orchid found in Borneo and Kalimantan. A cryopreservation protocol using vitrification was developed using seeds as explants. Seeds from green unburst capsules were precultured on New Dogashima Medium supplemented with 0-0.5 M sucrose prior to treatment with loading solution and vitrification with PVS2. Loading durations from 0-60 min and PVS2 dehydration time from 0-7 hours were tested. Following vitrification, seeds were stored in liquid nitrogen for one week before being recovered and subsequently cultured on a germination medium. The viability of the seeds post cryopreservation was evaluated based on 2,3,5-triphenyltetrazlium chloride reduction assay (TTC) by the seeds and germination. Seeds survived the cryopreservation treatments and germinated. Seeds precultured on the medium containing 0.3 M sucrose exhibited the highest germination (8.3% ± 2.3), while a 10-min loading time yielded the maximum germination (13.4% ± 2.9). Seeds dehydrated in PVS2 for 7 hours had the highest germination percentage (13.9% ± 2) after liquid nitrogen storage. The TTC and the germination test did not give similar results for the viability of seeds. Cryopreserved seeds developed into seedlings and showed normal morphology. Given that the seeds of Phalaenopsis lost viability at room temperature very fast, this protocol can potentially be used for its long-term storage which can assist in the conservation programme of the species.