

## Propagation of asymbiotically germinated seedlings with liquid culture for endangered lady's slipper orchid (*Cypripedium macranthos* Sw.)

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We had already reported the successful germination for green pods of purple lady's slipper orchid (*Cypripedium macranthos* Sw.). The green pod methods is to take immature seeds in green capsules, sterilize the capsule, and take out the sterile seeds. This method, however, needs very critical time of harvest. The critical time of seed harvest changes depending upon the species, condition of the specimen, and climatic influence, and the right time lies between 5 and 12 weeks after fertilization.

In this study, the mature seeds were collected after 120-130 days with hand-pollination of lady's slipper orchids. Mature seeds are usually dormant and it has to be overcome, either with hormone or storing the seeds near freezing for two or three months to break dormancy. The seeds were first surface sterilized with 70% ethanol and then transferred 1% NaOCl for 10-15 minutes, followed by rinses 3 times with sterilized distilled water. The cypripedium seeds consists of an embryo within a seed coat known as a testa. The testa is water repellent and the seed has a large air space between the embryo and testa so the seed tends to float on water. We had resolved the problems with vacuum pump to soak water into the testa before sterilization. The seeds were placed on liquid or agar solidified germination media. Cultures were incubated at 24±1°C in dark. The seeds were germinated in 6-8 weeks in liquid suspension culture (germination percentage over 18%); however, the seeds on agar solidified media took more than 5 months to germinate and the germination percentage less than 5%. The most effective media for liquid culture was 1/4 strength Murashige and Skoog (MS) medium with 50 ml/l coconut water (4 brix°) at pH 5.8.

**Key words:** Lady's slipper orchid, *C. macranthos*, Liquid media

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