

Improving *In Vitro* Propagation of *Cypripedium Parviflorum*

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Cypripedium parviflorum is an orchid that is critically endangered in Vermont and New Hampshire. Our goal is to improve methods of *in vitro* seed culture of *Cypripedium parviflorum* in order to attempt a restoration project of this species in these two states. Our first experiment tested germination rates of *Cyp. Parviflorum* by modifying a standard culture medium we have used in our lab to produce over 50% germination in *Cyp. reginae*. This medium uses a ¼ strength Murishige and Skoog medium but we increased our standard supplementation of 10% sucrose and 10% coconut water to 20% sucrose and 20% coconut water. This experiment yielded a 23% germination rate for *Cyp. parviflorum*, which is a 22% increase from experiments done with the standard 10% sucrose and coconut water *Cyp. reginae* medium. Using the same modified medium, we tested four different seed surface sterilization times: 30, 50, 70, and 90 minutes, which resulted in approximately 4%, 14%, 14%, and 23% germination, respectively. We repeated this experiment, using different seed surface sterilization solutions and pH levels in the medium. One sterilization solution consisted of 10% bleach, and one consisted of 5% bleach combined with 5% hydrogen peroxide. The two pH levels used for the media were 6.0 and 6.6. We found that the groups exposed to the non-peroxide solution of both pH levels for 70 and 90 minutes did substantially better than the hydrogen peroxide group. We are currently experimenting with higher pH levels to achieve higher germination levels and faster developmental rates.