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## Abstract

Orchids are important ornamental, medicinal, and food plants. Orchids have fascinated people with their extraordinary beauty, their variation in size, color, and shape. Of the world's 25,000 orchid species, some 10% are endangered in their native habitats. There are currently no major international government efforts to conserve orchid germplasm other than by the Ornamental Plant Germplasm Center of the USDA. There is a need to develop procedures to improve orchid seed storage for germplasm conservation in parts of the world where *in situ* conservation is not viable. The goal of this project is to develop a protocol for genebank storage of orchid seed and protocorms. Preliminary studies were conducted on the potential storability of *Brassia* and *Phalaenopsis* seeds. *Brassia* and *Phalaenopsis* seeds were stored at -196°C (liquid nitrogen), -80, -18, 4 or 25°C to determine the optimum conditions for short- and consequent long-term storage. *Phalaenopsis* and *Brassia* seeds adjusted to 45.5% RH over saturated chromium dichromate solution were able to survive 10-day storage. Seeds frozen in liquid nitrogen for 30 min were able to germinate and produce protocorms 19 days after sowing. Liquid nitrogen storage also improved germination and seedling development of some *Phalaenopsis* seed lots from 0 (control) to 37%. Storing *Phalaenopsis* seeds at -80°C and 4°C improved germination similarly, suggesting dormancy was broken by low temperature treatments. On the other hand, seeds stored at 25°C did not germinate. Preliminary results suggest that orchid seeds tolerate freezing even in liquid nitrogen and that cryopreservation may be a viable long-term strategy for orchid germplasm preservation.

## Objectives

- Develop protocols for the cryopreservation of orchid seeds
- Develop standardized seed viability and vigor testing procedures for tropical orchids
- Determine optimum storage temperatures and moisture contents for orchid germplasm preservation

## Comparison of tetrazolium and *in vitro* germination tests

Seeds were collected from the greenhouse and stored briefly at 4°C. Phytamax Orchid Maintenance Medium supplemented with coconut water was used for the *in vitro* germinability testing. *Brassia* seeds had higher germinability and faster protocorm development than the *Phalaenopsis* seeds. Seed metabolic activity was also evaluated using 1% 2,3,5 triphenyl tetrazolium chloride (TZ) by incubating seeds for 24 h at 35°C in the dark and positive staining is shown in Figure 1. There was no correlation between the two tests ( $r^2 = 0.002$ ) possibly due to seed dormancy.



**Figure 1.** Seed viability evaluation using TZ staining. Red coloration shows reduction of the dye to an insoluble formazan by the hydrogenase complexes indicating live embryos.

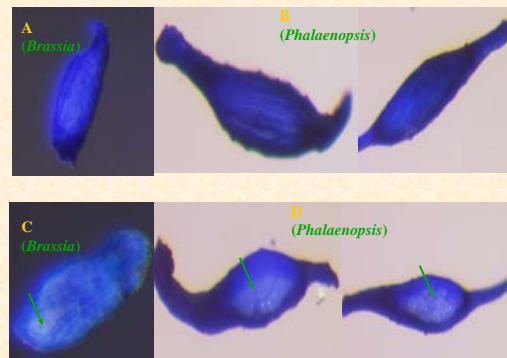
## Seed survival and germinability after 10-day storage at 25, 4, -18, -80 and -196°C

**Table 1.** Protocorm development of seeds stored at 45.5% RH and -80, -18, 4, or 25°C for 10 days or submerged in liquid nitrogen (-196°C) for 30 min before plating on Phytamax Medium.

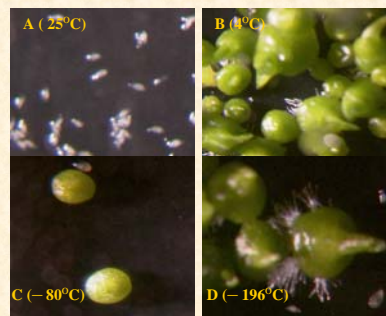
Seed Type	Protocorm Development at 40 days after sowing (%)					
	Storage Temperature (°C)					
	Control	-196	-80	-18	4	25
<i>Brassia</i> LSD=50	69 <sup>a*</sup>	44 <sup>ab</sup>	36 <sup>ab</sup>	39 <sup>ab</sup>	58 <sup>a</sup>	0 <sup>b</sup>
<i>Phalaenopsis spp</i> (9450) LSD=28	3 <sup>b</sup>	--	17 <sup>b</sup>	9 <sup>b</sup>	46 <sup>a</sup>	0 <sup>b</sup>
<i>Phalaenopsis sogo</i> 'Little Angel' x 'Golden Treasure' LSD = 21	0 <sup>c</sup>	37 <sup>a</sup>	17 <sup>abc</sup>	2 <sup>bc</sup>	23 <sup>ab</sup>	2 <sup>c</sup>

\*Means followed by the same letter within a row are not significantly different.

-- Cultures were contaminated and no data is available

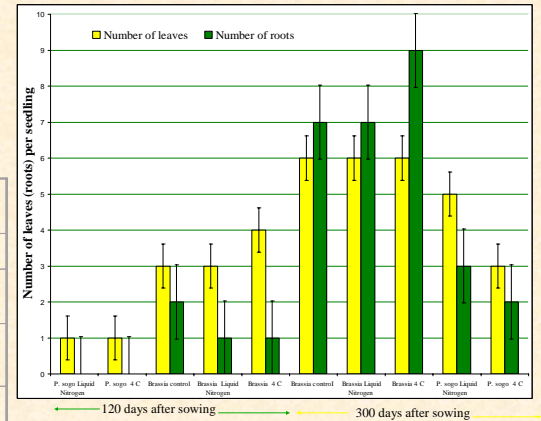


**Figure 2.** Effect of liquid nitrogen treatment on the testa of *Brassia* and *Phalaenopsis* seeds. A & B are untreated controls; C & D, seeds treated with liquid nitrogen. Seeds were stained with Toluidine O Blue. Ruptured testa may have encouraged embryo expansion, resulting in greater germination.



**Figure 3.** Protocorm development on Orchid Medium at 70 days after sowing of *Brassia* seeds stored at different temperatures. A: imbibed ungerminated seeds stored at 25°C. B: protocorms with single leaves developing from seeds stored at 4°C. C: protocorms without leaves developing at a slower rate from seeds stored at -80°C. D: protocorms with single leaves developing from seeds treated with liquid nitrogen (-196°C).

## Seedling development following storage



**Figure 3.** Seedling development at 120 and 300 days after sowing (DAS) from *Brassia* and *Phalaenopsis* seeds stored at -196 or 4°C. Freezing in liquid nitrogen encouraged greater leaf and root development in *Phalaenopsis* compared with storage at 4°C. *Brassia* did not show the same response to freezing in liquid nitrogen.

## Discussion

Initial seed germinability and seedling development differed among species. Seed germination and embryo staining (TZ) were not highly correlated ( $r^2 = 0.002$ ) possibly because of:

- Some form of dormancy which was later broken by cold storage
- False positives associated with the TZ test caused by microbial respiration seems unlikely but may be involved (Sawma and Mohler, 2002)
- TZ test is not always consistent due to the variation in the permeability of the seed coat, but in our study TZ over estimated potential germination (Vujanovic et al., 2000)

Increased seed germination in the *Phalaenopsis* species after cold storage may be attributed to:

- Metabolic systems that are responsible for organogenesis in the seedlings were activated by the cold treatment (Rasmussen, 1995)
- Cold treatment may have alleviated dormancy (Rasmussen, 1995)
- Treatment with liquid nitrogen may have ruptured the testa making it easier for embryo expansion (Fig 2) explaining why there was a higher percentage of protocorm development after liquid nitrogen treatment

## Conclusion

Cryopreservation of *Brassia* and *Phalaenopsis* seeds maybe a viable technique for long-term germplasm conservation but more long-term storage experiments are required.

## References

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- Vujanovic, V., M. St-Arnaud, D. Barabé, and G. Thibeault. 2000. Viability testing of orchid seed and the promotion of coloration and germination. Annals of Botany. 86: 79-86.