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# Technique for In Vitro Pollen Germination and Short-term Pollen Storage in Caladium

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***Abstract.*** **The sporadic nature of inflorescence production and flower protogyny in caladium (*Caladium* ×*hortulanum* Birdsey) makes it desirable to store pollen and to rapidly assess its viability for cross-pollinations in breeding programs. This study was conducted to develop a procedure to determine caladium pollen viability and to use that procedure to evaluate the effect of short-term storage conditions on pollen viability. The sucrose level in the culture medium was found to have a significant impact on the in vitro germination of caladium pollen; a concentration of 6.8% was determined to be optimal for pollen germination. Caladium pollen lost viability within 1 day under room (24 °C) or freezing (–20 °C) temperatures, but could be stored at 4 °C for 2 to 4 days. Pollen stored at 4 °C produced successful pollinations. Data obtained from large-scale greenhouse pollinations supported use of this in vitro germination assay as a convenient way to evaluate caladium pollen viability (and fertility).**

As tropical ornamental plants, caladiums offer an array of bright leaf colors and intriguing color patterns that are rarely found in other plants. Their excellent performance in summer heat and rains, a condition devastating to many other flowering plants, makes caladiums popular as landscape, container, or hanging basket plants. Commercially available caladiums are primarily propagated from tubers, the plants' underground storage organs. More than 75% of the caladium tubers grown worldwide are produced on muck soil in Lake Placid and Sebring in central Florida (Bell et al., 1998).

Caladiums belong to the aroid family; however, unlike many of its relatives (*Anthurium* Schott, *Spathiphyllum* Schott, and *Zantedeschia* Spreng.), caladium plants develop few blooms (inflorescences) under natural conditions. A flower induction technique was developed by Harbaugh and Wilfret (1979). When tubers (5.4–7.5 cm) were soaked in gibberellic acid ($GA_3$) solution (250–1000 $mg \cdot L^{-1}$), each of the treated plants produced two to four blooms (Harbaugh and Wilfret, 1979). This technique has greatly facilitated cross-breeding in caladiums.

Each caladium bloom is an inflorescence consisting of a spadix and a spathe (Fig. 1). Staminate flowers are borne on the top half to two-thirds of the spadix, while pistillate flowers are on the bottom one-fourth separated by a sterile band. The pistillate flowers are protogynous, becoming receptive several days before the unfurling of the spathe. By the time pollen shedding occurs (anthesis), usually 1 d after the spathe unfurls in the summer, the stigmatic surfaces of pistillate flowers have already lost receptivity and become discolored, softened, and covered with a thick layer of very sticky exudate (Deng and Harbaugh, unpublished).

Caladium plants are responsive to $GA_3$ treatment for flower induction year-round. Nevertheless, substantial differences exist among cultivars in their response to this treatment and in the time it takes from $GA_3$ application to blooming. It is very common that inflorescence production and blooming extend over a period of time as long as 2 or 3 months, and an individual plant may send out zero to two blooms a week during this time. Consequently, it requires many plants to have inflorescences with receptive flowers at the same time others are at anthesis, and cultivars need to be treated and planted on different days. The ability to store pollen, even for a few days, would greatly enhance the chance for successful pollinations.

Pollen of many Araceae species seems to be short-lived and difficult to store. *Aglaonema* pollen lost viability after exposure to 40% to 50% relative humidity (RH) for 4 h (Henny 1985, 1988); *Dieffenbachia* pollen could be stored for only 1–2 d at 90% RH and 5 °C (Henny 1980a, 1980b). In contrast, *Spathiphyllum* pollen remained viable (57% germination) after 24 weeks at 7 °C and 65% RH (Henny, 1978). In the above cases, pollen viability was primarily evaluated by in vivo germination on pistillate flowers. Few studies have been conducted or reported on pollen storage or viability in other Araceae species, although many are important ornamental plants. The objectives of this study were to develop procedures for determination of caladium pollen viability and to define the conditions for short-term storage of caladium pollen for cross-pollination and hybridization breeding.

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## Materials and Methods

*Flower induction and pollen collection.* Six *Caladium* ×*hortulanum* cultivars ('Florida Fantasy', 'Florida Red Ruffles', 'Florida Sunrise', 'Florida Sweetheart', 'Red Flash', 'White Christmas') and one breeding line (OB644) were used. Jumbo-sized tubers (6.4–8.9 cm) that were cured and stored at 21 °C and 40% RH for over 2 months were soaked in $GA_3$ solutions (ProGibb T&O, Valent BioSciences, Libertyville, Ill.) at a concentration of 600 $mg \cdot L^{-1}$ for 16 h at room temperature. Tubers were then potted in 20-cm containers (3.5 L in volume) filled with a mix containing 1 Canadian peat : 1 Fafard 2SHL (v/v) (Fafard, Apopka, Fla.) with a starting pH of 5.5–5.8. Tubers sprouted and plants were grown in a shaded glasshouse with 20% to 30% light exclusion under a natural photoperiod at Bradenton, Fla. Glasshouse temperature ranged from 21 to 32 °C. Plants started to bloom 45–65 d after tubers were potted and continued to produce inflorescences for 4 to 8 weeks. Fresh pollen was collected from the staminate flowers by brushing pollen into plastic containers (5.5 cm high × 2.5-cm diameter), using a 5-mm-wide, clean camel-hair brush.

*In vitro germination.* To determine in vitro germination, the hanging drop method (Shivanna and Rangaswamy, 1992) was used with some modifications. About 600 $\mu$L of


Fig. 1. (**A**) A caladium inflorescence with unfurled spathe and the distal portion of its spadix exposed; (**B**) a caladium inflorescence with pollen-shedding staminate flowers, a sterile band, discolored pistillate flowers, and the spathe removed.

culture medium was dropped onto a clean glass slide (7.5 cm × 2.5 cm). The culture medium contained 0.01% boric acid, 1% agar, and 5% sucrose (unless otherwise specified). After the medium became solidified, pollen grains were inoculated onto the medium surface using a 5-mm-wide camel brush. Efforts were made to achieve similar pollen grain densities on different slides. The glass slides were then placed into a staining jar; the open jar was carefully turned to one side so that the medium drops were facing down (or hanging). The jar with the slides was transferred to a humidity chamber where 90% to 100% RH was maintained using water and water-saturated filter paper. The whole assembly was placed in an incubator with temperature maintained at ≈27 °C and light intensity of 30 $\mu$mol·m$^{-2}$·s$^{-1}$. Pollen germination rates were determined after 20–24 h of incubation under a dissecting microscope. Pollen grains were considered to have germinated when their pollen tubes were extended equal to or greater than the pollen diameter.

*Experiment 1: Sucrose concentration*. The effect of sucrose concentrations on pollen germination was evaluated to identify the appropriate rate for in vitro germination of caladium pollen. Sucrose was added to a final concentration of 1%, 3%, 5%, 10%, 15%, or 20% in the culture medium containing 0.01% boric acid and 1% agar. Fresh 'Red Flash' caladium pollen was collected in the morning of the day when pollen shed and used on the same day.

*Experiment 2: Storage conditions*. Several conditions commonly used for short-term storage of pollen in other plant species, including low temperatures (4 or –20 °C) and desiccation, were tested for their potential to extend caladium pollen longevity. Fresh pollen was collected in the morning. Approximately equal amounts of pollen from the five cultivars and one breeding line were mixed together to obtain a sufficient quantity for six storage treatments. The mixed pollen was divided into 18 portions; each portion was put in a plastic container and stored. Six storage conditions (three temperatures × two humidity conditions) were used: 1) room temperature and ambient relative humidity (24 ± 0.5 °C, 50% ± 5% RH); 2) room temperature and desiccation (24 ± 0.5 °C, 22% ± 2% RH); 3) 4 ± 0.5 °C and 80% ± 4% RH in a refrigerator; 4) 4 ± 0.5 °C and desiccation (22% ± 2% RH); 5) –20 °C and ambient humidity in a freezer; and 6) –20 °C and desiccation (22% ± 2% RH). Desiccation was achieved with glass desiccators and silica gel canisters (Fisher Scientific, Pittsburgh) and monitored with hygrometers (Fisher Scientific). In vitro germination rates were determined 1, 2, 4, and 6 d after storage under the above conditions.

*Statistical analysis*. Treatments were replicated three times in each experiment, and all the treatments were placed in the humidity chamber in a completely randomized fashion. In Expt. 1, data were subjected to regression analysis using SAS (PROC REG, SAS Institute, Cary, N.C., 2003) to determine the relationship of sucrose levels to percent pollen germination.

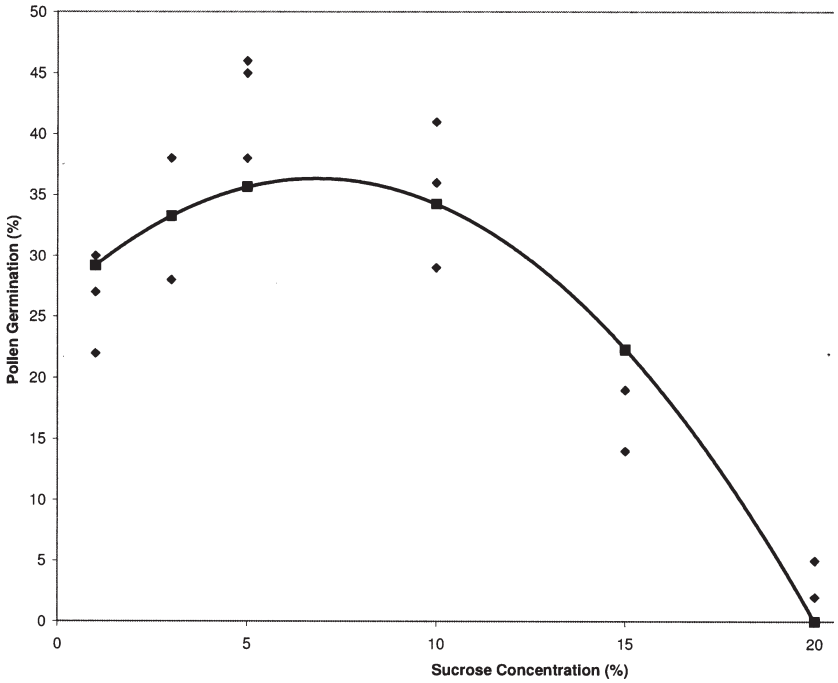


Fig. 2. In vitro germination rates of fresh 'Red Flash' caladium pollen on culture media containing different concentrations of sucrose. Symbols in the figure (♦) show the observed pollen germination rates, while (— ■ —) indicates the predicted values using the quadratic equation: $y = 26.509 + 2.8853x - 0.2108x^2$, where y = percent pollen germination and x = percent sucrose concentration.

Table 1. Change of in vitro germination rates of caladium pollen stored under different conditions over a period of 6 d.

| | In vitro pollen germination rates (%) ---------- Duration of storage (days) --------- | | | | |
|---|---|---|---|---|---|
| Storage conditions | 0[z] | 1 | 2 | 4 | 6 |
| Room | 35.8 | 4.9 | 0.1 | 0.3 | 0.2 |
| Room + desiccation | 35.8 | 3.0 | 3.5 | 1.3 | 1.6 |
| 4 °C | 35.8 | 32.5 | 16.6 | 3.9 | 1.1 |
| 4 °C + desiccation | 35.8 | 19.5 | 17.9 | 5.2 | 1.8 |
| –20 °C | 35.8 | 0.7 | 0.8 | 0.4 | 0.2 |
| –20 °C + desiccation | 35.8 | 0.7 | 0.1 | 0.1 | 0.1 |
| LSD (=0.05) | | 4.4 | 3.4 | 2.8 | 1.1 |

[z]Data were taken from fresh pollen (a single control) before subjecting to storage treatments.

In Expt. 2, statistical analyses were performed on data using the general linear model provided in SAS (PROC GLM, SAS Institute, 2003) and treatment means were separated where appropriate.

## Results and Discussion

*Experiment 1*. The sucrose concentration in the medium showed a significant effect on in vitro pollen germination (Fig. 2). The regression analysis indicated a quadratic relationship for the effect of sucrose levels on percent pollen germination:

$$y = 26.509 + 2.8853x - 0.2108x^2$$
$$R^2 = 0.8222^{**}$$

where y = pollen germination in percentage, and x = sucrose concentration in percentage. When the sucrose concentration was raised from 1% to 5%, the germination rate increased from 26.3% to 43.0%. Sucrose concentrations at 10% or greater seemed to inhibit pollen germination, and only 3.0% of the pollen grains germinated at 20% sucrose. The highest percentage germination of the six tested sucrose concentrations was observed when 5% sucrose was used in the medium. The most optimal concentration for in vitro germination of caladium pollen was estimated at 6.8% using the quadratic equation. Compared to many other plant species, the in vitro germinability of caladium pollen seemed low (Boyle 2001; Henny 1978; Martinez-Gomez et al., 2002).

*Experiment 2*. Caladium pollen lost viability very quickly during storage (Table 1). Only ≈5% of the pollen grains stored at the ambient temperature and humidity for 1 d remained viable. Freezing temperatures (–20 °C) seemed even more detrimental to caladium pollen. More than 99% of the pollen grains lost the ability to germinate in vitro after being stored at –20 °C for 1 d. In contrast, a low temperature of 4 °C seemed to slow down, to some extent, the decline of caladium pollen germinability during storage. Under this condition, ≈4% of the pollen grains stored for 4 d germinated and produced normal pollen tubes in vitro. Nevertheless, sharp drops in germination rates occurred after 2 d of storage at 4 °C. Regard-

less of temperatures used, desiccation at 22% RH failed to improve pollen viability during storage (Table 1).

The 4 °C low temperature storage condition was used in our hybridization breeding program to maximize the number of crosses for hybrid population development. Data on the rate of successful pollinations and seed development were pooled from more than 500 inflorescences pollinated from 18 Apr. to 18 June 2003, and seemed to support the results from the in vitro germination experiments (Table 2). When fresh pollen was used, 64.8% of the pollinated inflorescences developed seeds. The success rate (percentage of successful pollinations) dropped when cold-stored pollen was used, as was expected from our pollen storage tests. The longer the pollen was stored at 4 °C, the less the pollinations were successful, and fewer seeds were obtained from each inflorescence. However, pollen storage allowed for pollinations that could not have been made otherwise due to unavailable receptive flowers when pollen was ready.

Caladiums are tropical plants and very sensitive to temperatures below 15 °C (Marousky and Raulston, 1974). Nevertheless, their pollen could be stored at 4 °C for several days and used for pollination. In vitro germinability of caladium pollen grains seemed to be in agreement with their ability to produce seed from pollinations conducted in greenhouses; thus, in vitro germination may serve as a convenient way to evaluate caladium pollen fertility. This information may be of use in caladium pollen storage studies and for genetic improvement and breeding of caladiums and other aroid species.

Table 2. Caladium seed production from inflorescences pollinated with fresh pollen or pollen stored at 4 °C.

| Pollen sources[z] | Developmental stages of inflorescences pollinated: 2 d before spathe unfurling | 1 d before spathe unfurling | Unfurling | Total no. of successful pollinations/ attempted | Relative seed no. produced per inflorescence |
|---|---|---|---|---|---|
| Fresh | 73/113[y] (65)[x] | 183/270 (68) | 50/89 (56) | 306/472 (65) | +++++ |
| 4 °C, 1 d | 2/6 (33) | 9/17 (53) | 4/5 (80) | 15/28 (54) | +++ |
| 4 °C, 2 d | 2/4 (50) | 3/8 (38) | 1/5 (20) | 6/17 (35) | ++ |
| 4 °C, 3 d | 0/8 (0) | 3/6 (50) | 2/3 (67) | 5/17 (29) | + |

[z]Pollen freshly collected or stored at 4 °C and 80% RH in a refrigerator for 1–3 d.
[y]Numbers of successful and attempted pollinations (i.e., pollinated inflorescences), respectively.
[x]Percent successful pollinations.

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