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Short communication

Low temperature storage and in vitro germination of cherimoya (*Annona cherimola* Mill.) pollen

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Abstract

Due to the protogynous dichogamy of cherimoya and to the absence of proper pollinating vectors, hand-pollination with fresh pollen is a common practice for cherimoya commercial production. In order to optimize the process of hand-pollination, in this work we have studied the conservation of cherimoya pollen at −20, −80 and −196 °C for up to 3 months. In vitro pollen germination of fresh pollen was 57.1% and it was progressively reduced with conservation time at the three temperatures studied reaching a minimum after 3 months of storage of 10.4%, 14.2% and 13.6% at −20, −80 and −196 °C, respectively. Differences in germination among temperatures were only significant during the first 2 weeks of storage. Field pollinations with pollen stored for up to 3 months at the three temperatures show no yield differences compared to pollinations performed with fresh pollen. The results indicate that pollen collected and stored at sub-zero temperatures at the beginning of the cherimoya blooming season can be used along the whole blooming season avoiding the need of collecting fresh pollen daily.

Keywords: Annonaceae; Cryopreservation; In vitro pollen germination; Pollen viability

1. Introduction

Cherimoya (*Annona cherimola* Mill.) belongs to the Annonaceae, one of the largest tropical and subtropical families of trees, shrubs and lianas that include about 130 genera and 2300 species with a worldwide distribution, 900 of which are found in the Neotropics (Chatrou et al., 2004). Three genera (*Annona*, *Rollinia* and *Asimina*) contain species with edible fruits, such as cherimoya (*A. cherimola*), sugar apple (*A. squamosa*), atemoya (a hybrid between *A. cherimola* and *A. squamosa*), soursop (*A. muricata*), custard apple (*A. reticulata*), ilama (*A. macroprophyllata*), soncoya (*A. purpurea*), rollinia (*Rollinia mucosa*) or pawpaw (*Asimina triloba*). Cherimoya and atemoya are the most important worldwide both commercially and for local consumption. Cherimoya seems to be originated in the interandean valleys of southern Ecuador and northern Peru (Van Damme et al., 2000) although Spain is currently the major world producer of cherimoyas with about 3000 ha followed by Chile with about 1000 ha. Cherimoyas are also produced at a limited commercial scale in other Latin American countries where most of the cherimoya fruits sold or consumed are collected from the wild or from backyard trees (Van Damme et al., 2000).

Cherimoya has hermaphroditic flowers with a gynoecium and an androecium of pyramidal form surrounded by two whorls of three petals, the inner small and scale-like and the outer green-like and fleshy. The gynoecium is located in the centre of the conic receptacle and is composed of up to 300 carpels, each carpel containing a single ovule that will form a syncarp after fertilization. The androecium is found below the gynoecium forming a helicoidal structure with up to 200 stamens. Cherimoya presents protogynous dichogamy, with hermaphroditic flowers where female and male structures do not mature simultaneously generally preventing self-fertilization in the same flower (Schroeder, 1971). Moreover, flowers of the same genotype are synchronized and, consequently, transfer of pollen between different flowers of the same genotype is also prevented. At anthesis, usually at midday under our environmental conditions, the flowers are in female stage with receptive stigmas. The following day the flower switches to the male stage around 4–5 p.m., depending on environmental conditions, when the anthers start to dehisce and the stigmas are no longer receptive (Farré et al., 1976). Due to the lack of overlap between male and female stages in cherimoya and the

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lack of proper pollinating agents outside its native range, hand pollination with pollen and stamens together is a common practice for commercial production (Schroeder, 1971; Soria et al., 1990).

The cherimoya blooming season in Spain starts in late May and lasts through July whereas the regular harvesting season extends from October to December. However, under appropriate cultural practices (mainly pruning before the blooming season), it is possible to induce flowering in August and early September; the fruits set by these flowers are harvested in March–April. Thus, in order to optimize the pollination for both fall and spring harvesting it could be interesting to conserve pollen collected during June for pollination in June, July and August. Cherimoya pollen is tricellular and is dispersed from the anthers in tetrads. The individual pollen grains are released after contact with a proper germination medium (Rosell et al., 1999). Similarly to other tricellular pollen (Brewbaker, 1967; Hanna and Towill, 1995; Yamaguchi and Ishikawa, 2000; Towill and Walters, 2000), cherimoya pollen has a short viability and a high sensitivity to desiccation and, consequently, conservation is problematic. In this sense, no previous reports are available on the conservation of cherimoya pollen at sub-zero temperatures. Thus, the main objective of this work is to develop a method for mid-term storage of cherimoya pollen (up to 3 months) comparing different sub-zero temperatures in order to optimize the current process of hand-pollination in this species.

2. Materials and methods

Pollen from the cherimoya cultivar Campas was used in this work. Flowers in female stage were collected from the field at 4:00 p.m., approximately half an hour before they switch to the male stage, and carried to the laboratory. Immediately after anther dehiscence, pollen was subjected to different treatments. A first experiment consisted in evaluating pollen longevity at 4 °C; for this, pollen was stored at 4 °C and in vitro pollen germination evaluated daily as described below. The other experiments involved desiccation of pollen prior to the storage.

In order to optimize desiccation time we first checked the effect of four different moisture contents (obtained by increasing the desiccation time) at a fixed storage temperature (−20 °C). Afterwards, dehisced anthers with pollen from 25 flowers were dehydrated on glass Petri dishes in a silica gel desiccator during 90 min since previous observations showed a decrease in pollen germination after longer desiccation times. The moisture content of the pollen was determined by drying the pollen in an oven at 85 °C for 2 days. After the desiccation step, pollen was placed in 1.5 mL cryovials and stored at −20, −80 and −196 °C in liquid nitrogen. Pollen was thawed at 1, 7, 15, 30, 60 and 90 days after storage by keeping the samples at room temperature for 5 min. After thawing pollen was hydrated in a covered tray with wet filter paper for 200 min at room temperature.

For in vitro pollen germination, approximately 0.02 g of pollen with stigmas (Rosell et al., 1999) were placed on a 35 mm Petri dish with 1–2 mL of liquid germination medium at room temperature. A slightly modified pollen germination medium based on those described by Rosell et al. (1999) was used. This medium consisted of 8% sucrose, 200 mg/L $MgSO_4 7H_2O$, 250 mg/L $Ca(NO_3)_2 4H_2O$, 100 mg/L KNO_3 and 100 mg/L H_3BO_3. Pollen germination was observed under a Leica DML microscope. Data were collected on four Petri dishes with at least 200 pollen grains in each one. Pollen was considered as germinated when the length of the tube was longer than the grain diameter. Data were subject to arcsine root square transformation and an ANOVA analysis was carried out. Duncan's multiple range tests were used for means separation ($p \leq 0.05$). Statistical analyses were performed with SPSS 12.0. statistical software (SPSS Inc. Chicago, USA).

Field pollinations with fresh pollen and pollen stored at sub-zero temperatures were performed by applying the pollen with the anthers with a paintbrush on the stigmas just before flower opening; the pollinated flowers were covered by a cotton plug to avoid additional pollination with unwanted pollen.

3. Results and discussion

Pollen germination of freshly collected pollen was 57.1% (Table 1) a similar value to tricellular pollen from other species such as *Zea mays* (Inagaki, 2000) or *Brassica campestris* (Mulcahy and Mulcahy, 1988). However, this is a higher value than that reported previously for cherimoya (Rosell et al., 1999) probably due to the fact that in the later work a storage step during 20 h at 7–8 °C is performed before germination. Our results indicate that fresh cherimoya pollen stored at 4 °C showed a short longevity and no in vitro pollen germination was obtained 6 days after the pollen is released from the anthers (Table 1).

The moisture content of pollen after desiccation for 90 min was 49.7% compared to 85.7% of fresh pollen just after anther dehiscence. Although this can be considered a high moisture content for pollen storage compared to the results in other species (Hanna and Towill, 1995), tricellular pollen shows a high metabolic activity and moisture content compared to bicellular pollen (Barnabás and Kovács, 1997) and, consequently, a common feature of tricellular pollen is that desiccation below some critical level can induce injury.

Table 1
Cherimoya pollen germination (mean ± S.D.) after storage at 4 °C during six consecutive days

	Time (days)						
	0	1	2	3	4	5	6
% Pollen germination	57.1 ± 11.5 a	42.7 ± 12.4 b	39.4 ± 10.9 b	20.1 ± 13.9 c	11.7 ± 6.3 cd	4.8 ± 2.8 d	0 e

Means followed by the same letter in a row are not significantly different ($p \leq 0.05$) by Duncan's multiple range test.