Short-term Storage of Almond Pollen

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Abstract. Almond [*Prunus dulcis* (Mill.) D.A. Webb] breeding programs require successful techniques for pollen storage. We studied the pollen viability of two almond cultivars, 'Ramillete' and 'Desmayo Largueta', during 8 weeks of storage, in conditions that simulated standard situations including storage at 4, 22, and 4 °C alternating with 22 °C (4 °C/22 °C). Viability remained at 60% or more for 2 weeks under all three conditions. After the second week, germination capacity decreased rapidly at 22 °C, but remained above 50% for as long as 8 weeks at 4 °C or 4 °C/22 °C.

Most almond cultivars are self-incompatible and late-flowering cultivars may bloom as much as 6 weeks after early ones (Dicenta, 1991). Usually, the pollen is collected and dried before pollination of the female parent and the differences among cultivars in flowering time often require pollen storage. Adequate dehydration and low storage temperatures are crucial for maintaining viability (Cobo, 1980; Visser, 1955).

Pollen viability can be assessed by germination and growth in a sugar and agar medium. This technique can determine the influence of external conditions on germination and subsequent pollen tube growth (García, 1978; García and Egea, 1979; Hill et al., 1985; Parfitt and Almehdi, 1984). The results of such in vitro germination assays agree with those obtained in vivo by recording fruit set (Klungness et al., 1983; Loreti et al., 1979).

Our objective was to determine the viability of the pollen of two almond cultivars stored under three different temperature conditions.

Materials and Methods

Cultivars. The pollen of two Spanish almond cultivars, 'Ramillete' and 'Desmayo Largueta', which previously showed a high in vitro germination capacity (García, 1978; García and Egea, 1979), was evaluated.

Storage conditions. The pollen was collected and dehydrated for 48 h at 22 °C in a desiccator with calcium chloride [approximate relative humidity (RH) of 20%]. Samples

of ≈0.5 g were stored for 8 weeks in hermetically sealed 25-mL glass vials (RH ≈30%) at 4 °C, 22 °C, or at 4 °C for 18 h and 22 °C for 6 h (4 °C/22 °C) each day.

In vitro germination of pollen. Pollen samples were taken weekly during 8 weeks of storage. A fine paint brush was used to deposit the pollen on the surface of the agar in a petri

dish containing 25 mL of culture medium (15% sucrose, 1.2% agar) (García, 1978; García and Egea, 1979; Parfitt and Almehdi, 1984; Remy, 1953) using one dish per treatment. The petri dishes were incubated at 22 °C, the optimum temperature for the in vivo growth of almond pollen tubes (Socias, 1974) for 6 h to obtain maximum germination (García and Egea, 1979). Pollen germination was observed using an optical microscope at 40× and was considered to have occurred when the length of a pollen tube exceeded its diameter (Ducon, 1968). Longer incubation periods produced more growth of pollen tubes but not higher percentages of germination (García, 1978).

Statistical analysis. Eight different fields of vision, each with an area of 12.5 mm², and ≈250–300 pollen grains per field were examined per dish. Each count was considered as one replicate. A two-way analysis of variance was performed on the percentage germination using the SAS statistical analysis package (SAS Institute, 1989). Means were separated by Tukey's multiple range test at *P* ≤ 0.05.

Results and Discussion

No significant differences were observed between 'Ramillete' and 'Desmayo Largueta' under the different pollen storage conditions. However, significant differences were ob-

Fig. 1. Effect of temperature on time course of pollen viability of almond cultivars Ramillete and Desmayo Largueta during 8 weeks of storage. Bars show standard deviation about mean.

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served among storage conditions for both genotypes (Fig. 1). Pollen viability at the beginning of storage (just after drying) was 88.3% in 'Ramillete' and 86.2% in 'Desmayo Langueta', which is similar to previous results (García and Egea, 1979). These percentages can be considered high with respect to the in vitro germination rates of typical almond cultivars, which ranged from 12% to 95% in other assays (García and Egea, 1979; Hill et al., 1985; Klungness et al., 1983; Loreti et al., 1979; Parfitt and Almekhdi, 1984). For both cultivars, the change in pollen viability with time was similar for all three conditions assayed (Fig. 1). Beginning at ≈ 2 weeks, the viability of the pollen stored at 4 °C and at 4/22 °C decreased gradually until week 8, when germination at 4 °C and 4/22 °C was 63.2% and 57.5% ('Ramillete') and 57.2% and 47.8% ('Desmayo Langueta'), respectively. Viability during storage at 22 °C fell sharply after the second week to become almost nil by week 5 (Fig. 1). This strong influence of temperature on stored pollen viability has been mentioned by several earlier authors (Cobo, 1980; Parfitt and Almekhdi, 1984; Visser, 1955). Not only did the germination percentage of pollen stored at 22 °C drop sharply, but the rate of pollen tube

growth was also reduced; this became more pronounced with time. The color of the pollen also faded after week 2 (data not shown).

In conclusion, pollen stored at 4 °C was viable for 8 weeks or longer. Exposure to 22 °C for 6 h per day did not significantly reduce viability during 8 weeks of storage. We conclude that pollen can be sent to other research centers at room temperature and remain viable provided transport takes no more than 2 weeks.

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