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Pollen Grain Preservation at Low Temperatures in Valuable Commercial Rose Cultivars

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Abstract

Modern rose cultivars require hand pollination for rose hip production and collection of seeds. Breeding programs are often focused on the quality of rose pollen, which is genotype dependent and it is affected by the conditions used for its conservation. In this study *in vitro* pollen germination and shelf life of six commercial rose cultivars were evaluated under different storage conditions in order to establish preservation procedures. Flowers of *Rosa* 'Alba', 'Anastasia', 'Encanto', 'Marvelle', 'Swan' and 'Touch of Class' were collected from plants cultivated in the NIRP International greenhouses. Anthers were removed from flowers and dried on Petri dishes for 24 hours (T= 24°C). Then, they were placed into polyethylene tubes in the freezer (T= -20°C) and in the deep freezer (T= -80°C). Pollen germination was measured soon after 24 hours from flower collection and after 44, 134, and 190 days of storage, respectively. The pollen grains were spread on a culture medium containing agar (0.7%), sucrose (15%), calcium chloride (152 mg L^{-1}) and boric acid (40 mg L^{-1}). Our results confirmed that the viable level in fresh pollen varied among cultivars and also that pollen preservation at low temperatures is cultivar dependent.

INTRODUCTION

Modern rose cultivars are tetraploid roses that require hand pollination for hip production and seed collection. Rose breeders may overcome geographic distances and differences in flowering time by storing pollen of the desired male parents until pollination of the female plants can be performed. Breeding programs are often focused on the quality of rose pollen which is genotype dependent and it is affected by the conditions used for its conservation (Pipino et al., 2011). At room temperature, viability

of rose pollen grains rapidly reduces (Khosh-Khui et al., 1976). Although pollen germination rate tends to decline over storage, Zlesak et al. found that the pollen tube length of pollen stored for two or 52 weeks at -80°C was comparable to fresh pollen and was significantly longer than that of pollen stored at -20°C or 4°C, pointing to -80°C as a favorable temperature for general pollen storage (Zlesak et al., 2009). Pollen degradation during storage conditions could be due to dehydration, which results in loss of pollen colloidal properties. In addition, as reported in seeds, reactive oxygen species (ROS) and reactive nitrogen species (RNS) over-accumulation can inhibit pollen germination (Macovei et al., 2012). In this study, *in vitro* pollen germination and shelf life of commercial cut rose cultivars were evaluated under different storage conditions, in order to establish preservation procedure of rose pollen, which are useful for rose breeding and germplasm resource research.

MATERIALS AND METHODS

Flowers of the commercial cultivars 'Alba', 'Anastasia', 'Encanto', 'Marvelle', 'Swan' and 'Touch of Class' were collected at a stage of bud starting blooming, from plants cultivated in the NIRP greenhouses at Bevera, Ventimiglia (IM, Northwest Italy). The pollen grains were obtained from a bulk of twelve flowers by gathering the anthers during November 2012. Anthers removed from flowers were air dried on Petri dishes for 24 hours (T= 24°C) and weighted. Aliquots of dried anthers were stored in polyethylene tubes in the freezer (T= -20°C) and in the deep freezer (T= -80°C). A germination assay was used to determine the percentage of pollen germination after 24 hours from flower collection (Day 0) and after 44, 134, and 190 days of storage, respectively. The pollen grains were spread on the sterile culture medium (Pipino et al., 2011) containing agar (0.7%), sucrose (15%), calcium chloride (152 mg L^{-1}) and boric acid (40 mg L^{-1}), with a small paint brush (Fig. 1). Germination was evaluated after 24 h of incubation at 24 °C in dark conditions. Pollen was counted germinated when the pollen tube reached a length of at least 1.5 times the pollen grain diameter. The mean pollen germination percentage (mean±SE %) was calculated as the number of germinated pollen over the total number of observed pollen grains (160 each replicate). Four replicates were performed for each treatment of each rose cultivar. Each treatment was represented by 640 normal pollen grains (with a diameter larger than 30 μm, Pipino at al., 2011), to a total of 19.200 pollen grains.

RESULTS AND DISCUSSION

About one gram of anthers was recovered from each cultivar ('Alba' 0.914 g, 'Anastasia' 1.115 g, 'Encanto' 1.353 g, 'Marvelle' 1.079 g 'Swan' 0.774 g and 'Touch of Class' 1.031 g). A cultivar-dependent behavior in fresh pollen germination efficiency was observed. The cultivars 'Anastasia' and 'Marvelle' showed low germination rates (0.985±0.47 and 0.875±0.38 **%** *in vitro* germination, respectively), soon after flower collection (Day 0). Their pollen grains were not further used in the conservation trials. The best performing cultivars at Day 0 were 'Alba' and 'Encanto' with 57.1±3.04 and

55.1±1.34 % pollen germination, respectively (Table 1). After 44 days of storage at low temperatures pollen germination was reduced in the cultivars 'Swan' and 'Touch of Class', while no significant differences were observed in 'Encanto'. After 134 days of storage pollen germination capacity was also reduced in the cultivar 'Alba'. The cultivar 'Encanto' increased pollen germination after 134 and 190 days of storage both in the freezer and in the deep freezer (Fig. 2). The protracted storage time increased the portion of pollen grains able to germinate, as reported by Wrońska-Pilarek and Tomlik-Wyremblewska for nine wild rose species (with the exception of *R. rubiginosa*), that showed viability higher or similar to that before storage at -25°C for six months (Wrońska-Pilarek and Tomlik-Wyremblewska, 2010). The effect of the two storage temperatures was compared for each cultivar and few differences were observed (Fig. 3). According to González-Benito et al. (2004), cryopreservation is a valid and economical technique for the conservation of genetic resources of vegetatively propagated plants, such as cut flower roses. The results in this study also indicated that the rapid thawing process allowed the maintenance of the pollen grain germination capacity, as reported for kiwi pollen (Borghezan et al., 2011).

CONCLUSIONS

Our results confirm that the viable level in fresh pollen of commercial rose cultivars varies among cultivars and also that pollen preservation at -20°C and -80°C is cultivar dependent. The 'Encanto' rose pollen could be stored for more than six months at low temperatures without reducing pollen grain germination capacity. Our results are also in agreement with the statement that pollen shelf life depends to a large extent on the degree to which vital activity can be reduced, without reducing the pollen grain germination power. The procedure for flower collection and handling were appropriate for valuable commercial rose cultivars pollen grain conservation. However, more experiments on effective fruiting using preserved pollen grains still need to be performed.

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Tables

Table 1. *In vitro* pollen germination percentages in modern rose commercial cultivars stored at low temperatures up to 190 days.

Rose cultivar	Storage conditions						
Days	0	44		134		190	
T	24°C	-20°C	-80°C	-20°C	-80°C	-20°C	-80°C
'Alba'	57.1 a[1]	52.2 a	43.1 b	27.8 c	24.1 cd	19.7 d	22.0 cd
'Encanto'	55.1 b	53.5 b	55.5 b	86.7 a	96.3 a	92.2 a	89.4 a
'Swan'	47.6 a	33.6 b	24.1 cd	20.9 d	26.6 cd	28.4 bc	32.3 b

'Touch of Class'	21.8 a	11.1 c	16.3 b	14.1 b	15.5 b	16.1 b	20.2 a

[1]For each cultivar means followed by different letters indicate significant differences by one way ANOVA at 5% probability.

Figures

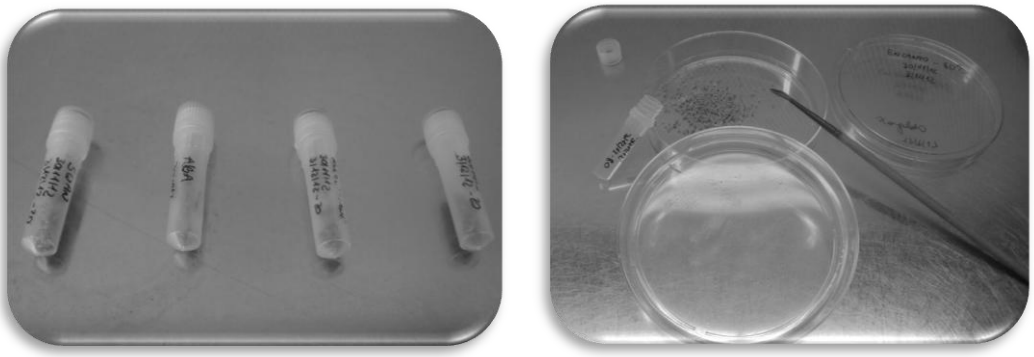

Fig. 1 Air dried anthers were stored in polyethylene tubes in the freezer (T= -20°C) and in the deep freezer (T= -80°C) for conservation trials. Over the storage period, germination readings were made by immediately spreading pollen on the culture medium, with a small paint brush.

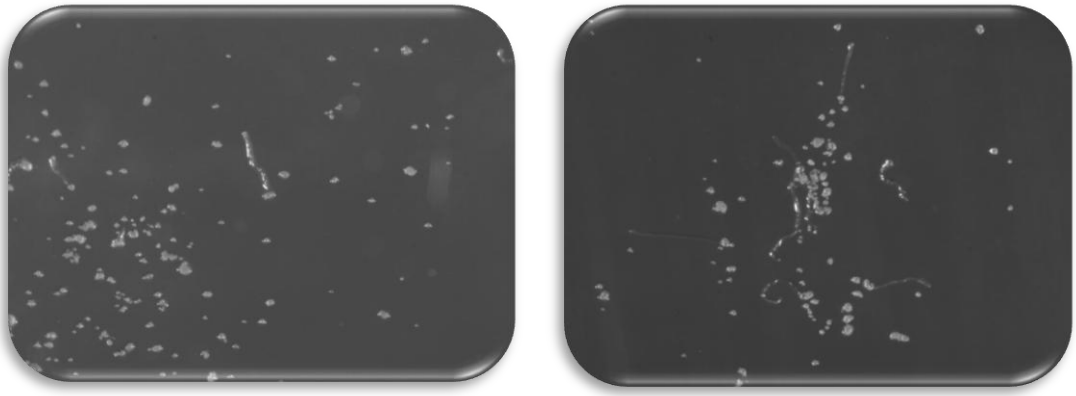

Fig. 2 'Alba' (left) and 'Encanto' (right) rose pollen spread on the culture medium, after 190 days of storage in the deep freezer. Pollen tubes developed from the germinated pollen grains.

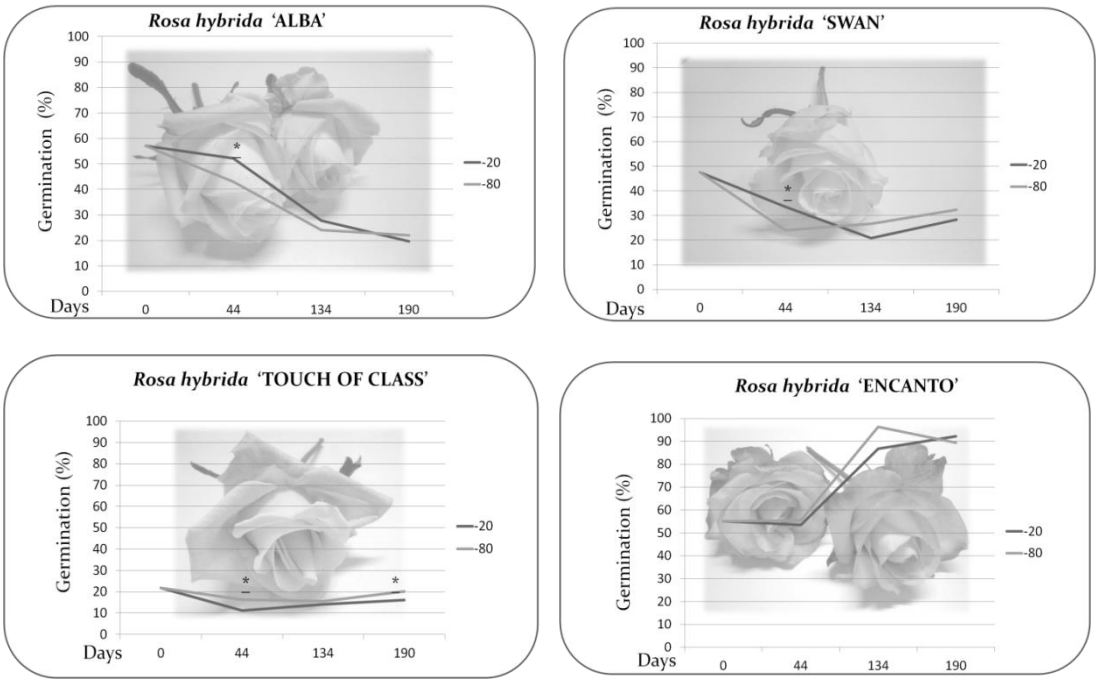

Fig. 3 'Alba', 'Swan', 'Touch of Class' and 'Encanto' rose pollen germination percentages at Day 0 (fresh pollen) and after 44, 134 and 190 days of storage at -20°C and -80°C. For each experimental point the asterisk indicates significant differences by one way ANOVA at 5% probability.