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Dehydration and viability of saffron crocus (*Crocus sativus*)

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Chichiricò, G. 2000. Dehydration and viability of saffron crocus (*Crocus sativus*) pollen. – Grana 39: 275–278. ISSN 0017-3134.

Saffron pollen reaches the shedding stage with a high water content which averages 58% under 18–20°C and RH 55–70%. Because of the precocious incision of anther stomium and the apertures of pollen exine, when the flower opens the pollen is soon exposed to dehydration. As a result, it goes from the highly hydrated stage to 15% water within 2 h from flower opening, and to the minimum 7% within 10 h. The transition to dry state results in a progressive loss of the plasmamembrane integrity, but this is restored after gradual rehydration. On average, 50% of the total pollen grains shows capacity for *semi-vivo* germination regardless of the dehydration degree. Pollen loses the competence for germination within 3 weeks from shedding. The results are discussed in comparison with fertile *Crocus* species.

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Pollen grains at maturity are discharged from the anthers, and through animals, or physical vectors, arrive on stigmas where pollen tubes may extrude which elongate to the ovules for fertilization. During the stages of maturation and dispersal, pollen dehydrates at various extent according to the species (Taylor & Hepler 1997). Dehydration results in the metabolic decay which makes the pollen dormant. Moreover, it causes morphological and structural reorganization of pollen, with plasmamembrane alterations which may impair the pollen viability (Lin & Dickinson 1984, Kerhoas et al. 1987, 1989, Hoekstra et al. 1989, Tiwari et al. 1990, van Bilsen et al. 1994, El-Ghazaly & Grafström 1995); pollen which does not dehydrate before shedding is more vulnerable to post-shedding dehydration (Barnabas 1985, Nepi & Pacini 1993). Thus, if on the one hand the water loss predisposes pollen for the dispersal stage, on the other the extreme dehydration may be fatal for pollen. The pollen viability is usually referred to the plasmamembrane integrity, and evaluated by the fluorochromatic reaction (FCR) test (Heslop-Harrison & Heslop-Harrison 1970).

Previous studies by *in vivo* and *in vitro* methods (Chichiricò & Grill Caiola 1986) on the reproductive biology of the triploid sterile saffron crocus (*Crocus sativus*) suggested both a scarce capacity of pollen for germination, and loss of pollen viability during dispersal. Pollen of fertile *Crocus* species (Chichiricò 2000a) showed high dehydration after shedding, high viability, and high capacity for *semi-vivo* germination; whereas the *in vitro* germination was difficult to accomplish. These observations stimulated us to investigate in saffron pollen the dehydration pattern and the plasmamembrane integrity, as well as the germinability by the *semi-vivo* method, in relation to the ageing of pollen.

MATERIAL AND METHODS

Plants of *C. sativus* (Iridaceae) derived from cultivation of Navelli (L'Aquila, Italy). Anthers were detached at the time

of flower opening, and the pollen for testing was removed at time intervals. Both flowering plants and detached anthers were kept at 18–20°C and RH 55–70%, monitored by a thermohygrograph.

Water content of pollen

The water content was determined by weighing accurately pollen grains before and after heating at 100°C for 24 h. Tests were repeated 5–6 times, by using from 30 mg of hydrated pollen to 100 mg of dehydrated pollen.

Fluorochromatic reaction (FCR) test

FCR was examined by treating pollen with a 10% sucrose solution containing fluorescein diacetate (Heslop-Harrison

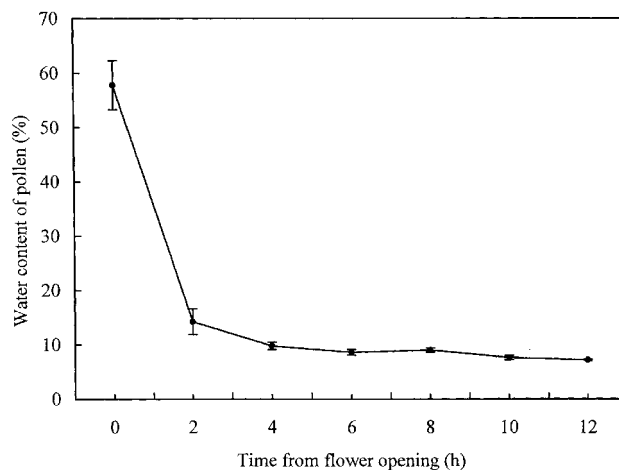
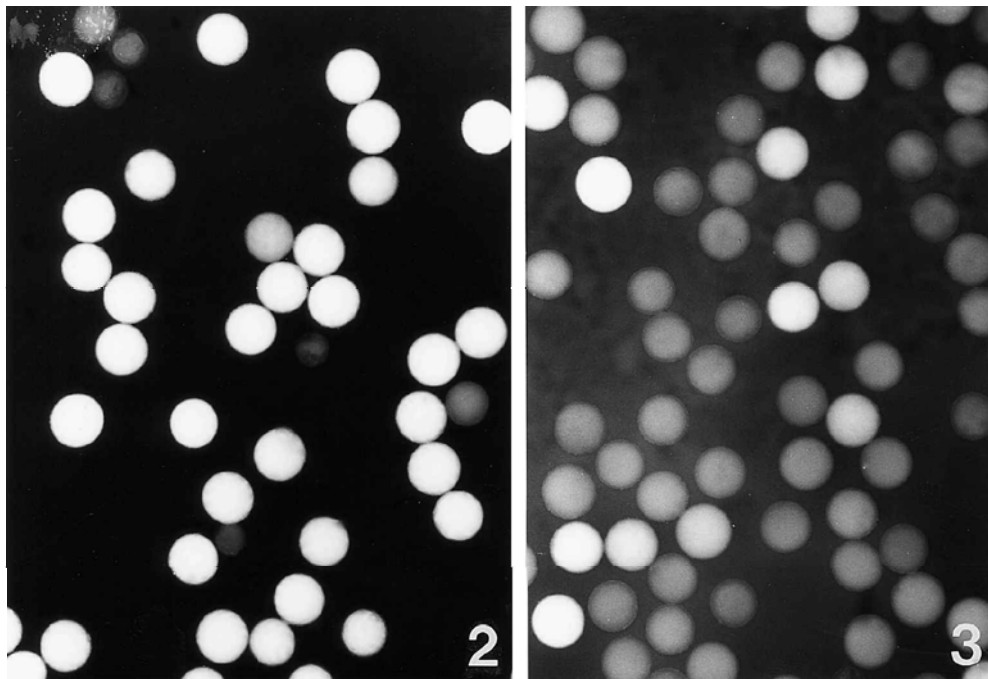


Fig. 1. Pollen dehydration following shedding at 18–20°C and RH 55–70%.



Figs. 2,3. Fluorochromatic reaction in pollen grains at shedding (2) and 4 h after shedding (3). $\times 75$.

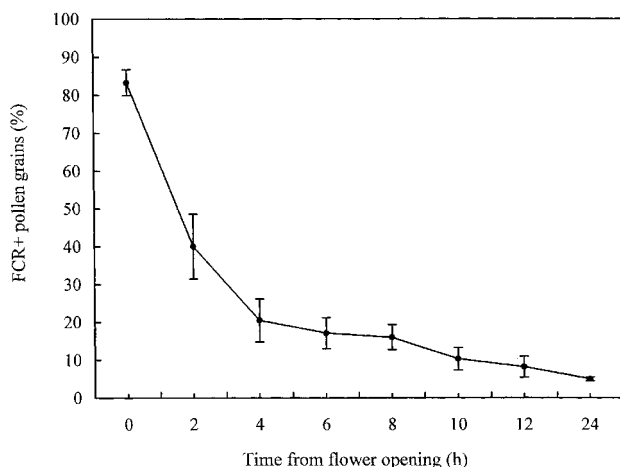


Fig. 4. Decline of FRC positivity in pollen grains following shedding.

& Heslop-Harrison 1970). Some tests were made after rehydrating pollen either by immersing in water, or keeping for 3–4 h in a box at RH 90–95% and 18–20°C. On average 400 pollen grains in triplicate were examined for each test.

Semi-vivo germination

Stigma branches were cut off from fresh flowers of saffron crocus, and pollinated under a stereomicroscope, by depositing 1–2 pollen grains on each stigma papilla up to one hundred grains. 3–4 h after incubation at 18–20°C in moistened Petri dishes, the stigmas were stained with aniline blue (Kho & Baer 1968) which gives fluorescent the pollen tubes when observed under a fluorescence microscope. Data were elaborated by the regression analysis.

RESULTS

Saffron anthers are tetrasporangiate with a longitudinal stomium which slits long before the flower opens. Pollen grains are spheroidal and frequently collapsed. The diameter of shedding pollen grains most frequently ranges from 180 μm to 70 μm .

Water content of pollen

The hydration degree of pollen from the flower opening onwards are reported in Fig. 1. At the flower opening the pollen contained on average 57.8% water, ranging from 63% to 53%; values not significantly different ($p < 0.002$) (not shown) were observed some hours before flowers opened. During the pollen exposure, at 18–20°C and RH 55–70%, the water content fell within some hours, and it decreased to the minimum 7% after 8–10 h, without changing in the following days. The diameter of dehydrated pollen ranged from 84 μm to 55 μm .

Fluorochromatic reaction (FCR)

At the flower opening, 83.3% of the pollen grains treated with fluorescein diacetate shed a bright and persistent fluorescence (FCR+), and the remaining 16.7% grains fluoresced weakly or did not at all (FCR–; Fig. 2). During exposure the pollen lost progressively either the capacity for brightly fluorescing or retaining fluorescence (Fig. 3), and the differences between FCR+ and FCR– grains became less evident, particularly during the first hours. Data on the decay of fluorochromatic reaction are reported in Fig. 4. This shows a sharp decrease of FCR during the first 4 h, followed by a slow decline during which the fluorescence was progressively



Fig. 5. Germinated pollen grains after penetration of pollen tubes in the stigma papille. $\times 80$.

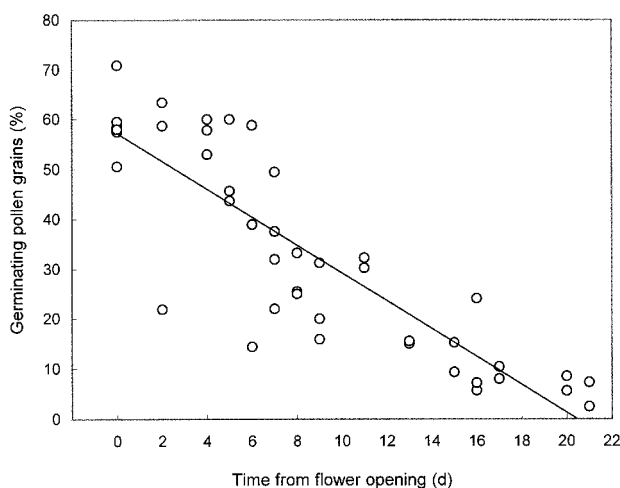


Fig. 6. Decline of pollen germinability during pollen ageing.

released in the medium. Dehydrated pollen recovered the FCR positivity after rehydration into the moistened box; the rehydration by immersing in water was unsuccessful.

***Semi-vivo* germination**

Pollen germinability was examined at time intervals from the flower opening to 21 days after. Pollen tube protruded from exine wall within 50–60 min from pollination, and shortly after penetrated the papilla cuticle to grow downward (Fig. 5). The germinability of shed pollen, including highly collapsed pollen grains (about 18%), averaged 50%; it averaged 59% if such aborted grains were left out. The data of tests without aborted grains were elaborated by the regression analysis, and reported in Fig. 6. This shows that the germinability cycle lasts about 3 weeks, and during this interval the

pollen shows the trend to lose gradually the capacity for germination, with major fluctuations during the first week.

DISCUSSION

Pollen hydration

The life cycle of pollen comprises two major developmental stages, the first occurring within the anther and the second on compatible stigmas, separated by a quiescency stage. Fluctuations in water content play a major role in the transition, so the quiescency follows the pollen dehydration which usually begins inside the anther, and the revival follows the pollen rehydration. The first developmental stage in saffron pollen ends without involving dehydration; at the flower opening saffron pollen is hydrated at about 58%. The highest hydration values so far reported for shedding pollen (Taylor & Hepler 1997) do not go beyond 60%. In fertile *Crocus* species (Chichiricò: unpubl.), the pollen is either partially or, most frequently, not hydrated at shedding, with highest values averaging 52.8%. Thus, saffron pollen is programmed for reaching the shedding stage in highly hydrated form, and this implies that both pollen and anther are structurally equipped. Pollen has a thick intine (Grilli Caiola et al. 1985, Chichiricò 1989) which allows to hold water, and it is adequately waterproofed by the pollenkit covering (Chichiricò 1999 *a*). The anther wall is lined with tapetal membrane and covered with a cuticular layer. However, in conformity with the dehiscence program of *Crocus* anther (Chichiricò: 2000 *b*), saffron anther undergoes slitting of stomium long before the flower opening. Evidently, the anther incision get pollen ready for dehydration, but this is strictly controlled inside the flower by the sheathing leaves and tepals.

Saffron pollen dehydrates immediately after the flower opening. Major dehydration occurs during the first hours,

and the equilibrium with the environment at 18–20°C and RH 55–70% is reached within 8–10 h, with about 7% water content. Similar extreme dehydration, but by degrees, we observed in other *Crocus* species in relation to morphological and cytological pollen features (Chichiricò: unpubl.). In the present case, the dehydration pattern may be strictly related with the exine apertures (Chichiricò 1999 *a*) which are 35–40% of the entire pollen surface. Further, a relationship may be with the developmental abnormalities due to the plant triploidy (see Chichiricò 1999 *b*); the incomplete development of sporoderm and/or protoplast may facilitate the water loss.

Pollen viability

Pollen plasmamembranes, according to the species, have a diverse endurance to dehydration, which is usually higher for entomophilous species (see Bassani et al. 1994). Pollen which is vulnerable to dehydration may revive if its plasmamembranes are restored during rehydration (see Taylor & Hepler 1997). In saffron pollen the plasmamembrane is continuous in most grains (83.3% FCR+), and it does not suffer the water shifts. Its continuity is interrupted during dehydration, the graph lines relative to FCR and dehydration tests are overlapping, and it is restored after controlled rehydration. This accounts for the capacity of pollen for germination and viability, both previously underestimated (Chichiricò & Grilli Caiola 1986) by using either *in vitro* or *in vivo* methods. Underestimation of pollen germinability by the *in vivo* method (Chichiricò & Grilli Caiola 1986), in comparison with the *semi-vivo* method which is more feasible and more reliable, we observed also in fertile *Crocus* species (Chichiricò 2000 *a*). The FCR and germinability data are comparable with cytological data (Chichiricò 1989) which distinguish saffron pollen in: 1) densely cytoplasmic and starchless grains (about 62%), and 2) sparsely cytoplasmic and starchy grains (about 38%). Pollen grains of the first group may support germination. Pollen grains of the second group failing in protoplast development, evidently fail also in producing pollen tube, regardless of the plasmamembrane integrity. The pollen of fertile *Crocus* species (Chichiricò 1999 *a*, 2000 *a*) is densely cytoplasmic and starchless, and the FCR positivity (about 100%) is higher than the germinability (about 87%).

In summary, saffron pollen, despite its genetic unbalance (see Chichiricò 1999 *b*), is fairly competent for germination and rather longevous, supported by the plasmamembrane adaptability to extreme dehydration which follows pollen shedding. However, it is lacking in vigour to bring to an end pollen tube growth (Chichiricò & Grilli Caiola 1984, 1986), and in this saffron pollen is comparable to senescent pollen of fertile *Crocus* species (Chichiricò 2000 *a*).

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