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Pollen viability and pollen vigor

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Summary. Investigations were carried out to correlate pollen viability, assessed on the basis of a fluorochromatic reaction (FCR) test, with pollen vigor, assessed on the basis of the time taken for in vitro germination in pollen grains subjected to high humidity (>95% RH) and temperature (38 °C) or storage stress of *Nicotiana tabacum, Agave* sp., *Tradescantia virginiana,* and *Iris* sp. Both high RH and temperature, as well as storage stresses, affected pollen vigor before affecting pollen viability. The results are discussed in the light of available data on the viability and vigor of stressed pollen and of aged seeds. The need for consideration of pollen vigor, particularly in stored pollen, the inadequacy of the methods presently used, and some of the methods suitable to assess pollen vigor are elaborated.

Key words: High temperature and humidity stress – In vitro germination – Pollen grains – Storage – Viability and vigor

Introduction

Pollen viability is generally consididered to indicate the ability of the pollen grain to perform its function of delivering the sperm cells to the embryo sac following compatible pollination. Assessment of pollen viability on the basis of its function is cumbersome, time-consuming, and not always feasible (see Heslop-Harrison et al. 1984). Many short-cut methods that reflect the competence of the pollen to perform its normal function in the pistil have been devised. Of these, the in vitro germination test and the fluorochromatic reaction (FCR) test have been found to be satisfactory. The in vitro germination test cannot be applied to many systems, particularly three-celled systems in which optimal in vitro germination is difficult to achieve. The FCR test works with both two-celled and three-celled systems, and is now being used routinely to assess pollen viability.

Surprisingly, studies on pollen viability have so far not taken into consideration pollen vigor in terms of the time taken for germination and the time necessary for the tube to reach the embryo sac. Extensive studies have been carried out on the viability of seeds in relation to storage (Khan 1982; Priestley 1986). Loss of germinability in seeds is considered to be the final manifestation of the loss of viability.

The loss of vigor generally becomes evident well before the loss of germinability (see Priestley 1986). As pollen grains and seeds are very similar in many physiological manifestations, it is expected that stored pollen, as well as pollen subjected to environmental stresses, may exhibit reduction in vigor before pollen loses its ability to germinate. Consideration of pollen vigor is important in the light of recent exciting studies on the fundamental and applied aspects of pollen competition and selection (see Mulcahy 1979, 1983; Ottaviano and Mulcahy 1989). We have carried out investigations to correlate the FCR test and in vitro germination test with vigor in stressed pollen of *Nicotiana tabacum* (Shivanna and Cresti 1989; Shivanna et al. 1990). Pollen viability was assessed on the basis of the FCR test and vigor was assessed on the basis of the time taken for in vitro germination and pollen tube growth in the pistil. Pollen viability was not affected in pollen grains treated with high RH and high temperature (38/45 °C) stress, but pollen vigor was significantly affected. Pollen samples treated at 38 °C took longer to germinate in vitro and for the pollen tubes to emerge from the cut end of the semivivo implanted styles. Pollen grains

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treated at 45 °C failed to germinate in vitro; they did germinate on the stigma however but the pollen tubes took much longer, when compared to the controls, to reach the ovary.

These studies clearly showed that stressed pollen exhibit loss of vigor before they lose the ability to germinate or to respond to the FCR test. We have now extended these studies to a few other systems to see if such a response is more widespread. This communication presents our results and discusses both the need for taking pollen vigor into consideration, particularly in stored pollen, as well as the inadequacy of the methods presently used to assess pollen vigor.

Materials and methods

Plants of *Nicotiana tabacum* L., *Agave* sp., *Tradescantia virginiana* L., and *Iris* sp. grown in the Botanical Garden of the University of Siena were used as pollen source. *Nicotiana* and *Agave* were grown in the glasshouse, and *Tradescantia* and *Iris* under field conditions. Pollen grains from freshly dehisced anthers were subjected to high RH (>95%) and temperature (38 °C) stress, as described earlier (Shivanna et al. 1990). Pollen grains were uniformly spread on a slide and kept in petridishes lined with moist filter paper. The petridishes were maintained at laboratory temperature (21 ± 2 °C) or at 38 ± 1 °C, in the dark, for up to 4 h. One set of *Agave* pollen was exposed to high RH at 45 °C.

After the treatment, pollen grains were used to test for FCR (Heslop-Harrison and Heslop-Harrison 1970) and in vitro germination. The responses were compared with the fresh pollen.

In vitro germination was carried out in drops of germination medium containing 10% sucrose, 1 m*M* boric acid, and 1 m*M* calcium nitrate (Shivanna et al. 1990). One set of *Nicotiana* and *Agave* pollen were stored under laboratory conditions and monitored for FCR and in vitro germination.

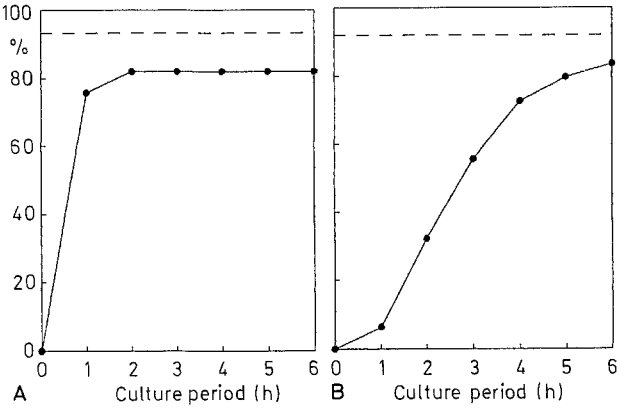

Fig. 1 A and B. *Nicotiana:* FCR and in vitro germination responses of fresh pollen (**A**) and pollen stored for 20 days under laboratory conditions (**B**)

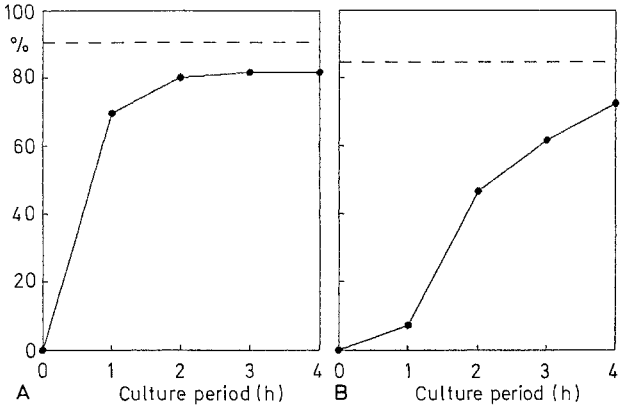

Fig. 2 A and B. FCR and in vitro germination responses of pollen grains of *Agave* treated for 4 h with high RH at laboratory temperature (**A**) and at 38 °C (**B**)

Results

Nicotiana tabacum

Pollen grains were stored under laboratory conditions and tested for FCR and in vitro germination at 10-day intervals. Fresh pollen showed over 90% FCR and over 80% germination whithin 1 h (Fig. 1 A). Pollen grains stored for 10 days did not show any decline in FCR or in the speed of germination. Even in pollen samples stored for 20 days, there was no decline in FCR value or in the final percent germination. However, these took much longer time to germinate (Fig. 1 B); unlike fresh pollen, in which most of the pollen grains germinated in the first hour, only about 6% of the stored pollen germinated in the first hour, and maximum germination was reached only by 6 h.

Agave sp.

There is considerable pollen sterility in this species. Only about 40% of the pollen grains are healthy and FCR-positive; the remaining pollen grains are empty and shrivelled. The fertile pollen can easily be distinguished from the sterile pollen under the microscope. Only fertile pollen grains were considered for scoring FCR and in vitro germination. Two types of stress were applied – high Red. Hum. at 38 °C and storage at laboratory conditions. Figure 2 presents the responses of pollen to Red. Hum. and temperature stress. The responses of pollen grains subjected to high Red. Hum. laboratory temperature were very much similar to fresh pollen and showed over 90% FCR. The majority of pollen grains germinated in 1 h and of the remainder, no grains germinated beyond 2 h. Pollen grains exposed to high Red. Hum. at 38 °C showed about 85% FCR. However, only about 10% of the pollen grains germinated within 1 h; pollen grains continued to germinate for up to 4 h and reached just over 70%. There was no more germination beyond 4 h. Pollen grains subjected to high RH at 45 °C showed over 80% FCR, but failed to germinate in vitro.

The responses of pollen grains stored for 5 days was also very similar to those treated with high RH at 38 °C

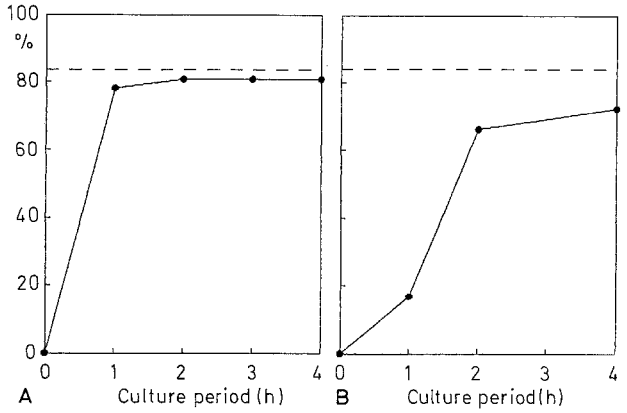

Fig. 3A and B. *Agave:* FCR and germination responses of fresh pollen (**A**) and pollen stored for 5 days under laboratory conditions (**B**)

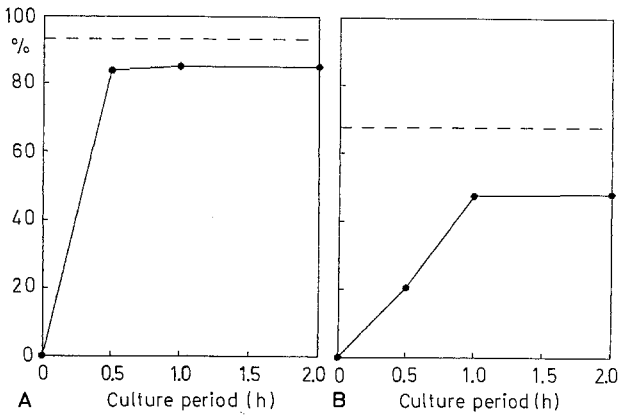

Fig. 4A and B. FCR and in vitro germination responses of pollen grains of *Tradescantia* treated for 30 min with high RH at laboratory temperature (**A**) and at 38 °C (**B**)

(Fig. 3). Storage did not affect FCR score but significantly delayed germination.

Tradescantia virginiana

Pollen grains of *Tradescantia* are more sensitive to heat stress than those of *Nicotiana* and *Agave*. The FCR value was drastically reduced when they were subjected to high RH at 38 °C for 1 h or more. Therefore, the treatment was confined to 30 min. The results are presented in Fig. 4. Even 30-min exposure considerably reduced the FCR value. Most of the pollen grains treated with high RH at laboratory temperature germinated within 30 min, and the percent germination was close to the FCR value. About 10% of the pollen grains treated at 38 °C showed protrusion of the tube during the treatment (before culture). Such pollen grains did not show FCR and the tubes did not continue growth after culture. Only about 50% of the pollen grains germinated even after 2 h, which was considerably lower than the FCR value.

Unlike *Nicotiana* pollen, which showed a 30–50 min lag phase before germination, *Tradescantia* pollen had a very short lag phase; germination was initiated within a few minutes after culture. In one experiment, pollen grains were cultured in drops of medium in petri dishes; a field was immediately focused under an inverted microscope, and pollen grains were continuously monitored for the time of pollen tube emergence (Fig. 5). In control cultures, germination was initiated within 3 min, and most of the pollen grains had germinated within 10 min. The average time for germination for the control pollen was 5.5 min.

Pollen grains treated at 38 °C showed marked variation in the time taken for pollen tube emergence. Although a few pollen grains showed tube emergence within 3–5 min, a majority of them took more than 10 min, and in some it was delayed for up to 35 min. The average

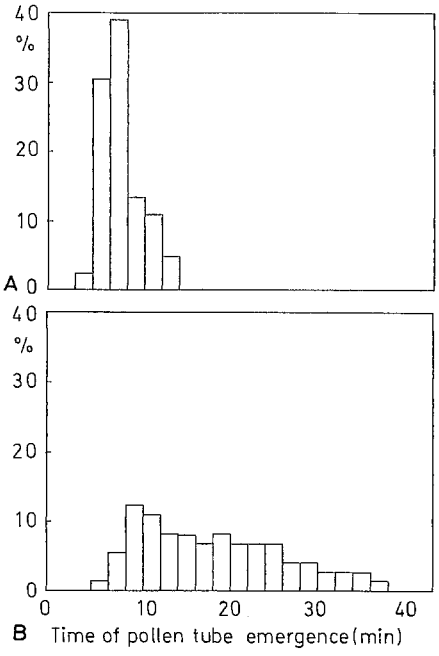

Fig. 5A and B. Frequency distribution of pollen samples of *Tradescantia* treated with high RH at laboratory temperature (**A**) and at 38 °C (**B**) for the time taken for pollen tube emergence. Each bar represents 2-min intervals

time for pollen tube emergence of this pollen sample was 16.2 min.

Iris sp.

Pollen grains of *Iris* were subjected to high RH and temperature stress for up to 4 h. Exposure of pollen grains to high RH at 38 °C for 4 h drastically affected both FCR and germination; only about 10% of the pollen showed FCR and there was no germination. However, pollen grains subjected to 2 h stress showed only marginal re-

duction in FCR but significant delay in germination, as in other systems.

Discussion

Our earlier investigations on *Nicotiana tabacum* (Shivanna and Cresti 1989; Shivanna et al. 1990) and the results of the present investigation clearly show that high RH and temperature stress, as well as storage stress, affect pollen vigor before they affect pollen viability or germinability. Although there were differences between species as to the extent of stress at which full viability is maintained, in all the species tested, the loss of vigor manifested itself before the loss of viability. Pollen of *Nicotiana* and *Agave* maintained full viability even when exposed to high RH at 38 °C for up to 4 h, while pollen of *Tradescantia* lost some viability even when the treatment was confined to 30 min. Nevertheless, the stressed pollen invariably took longer to germinate. In *Nicotiana,* in which the studies have been extended to in vivo (Shivanna et al. 1990), the delay in germination was observed in the pistil also; pollen tubes took much longer to grow through the style.

There have been a few other reports in recent years on the responses of stored pollen, which show the loss of vigor without the loss of germinability (see Shivanna and Johri 1984; Kumar et al. 1988). In *Malus domestica* (Bellani et al. 1984), pollen grains stored for 1 year did not show any loss of in vitro germinability. However, their ability to incorporate nucleic acid precursors (^{3}H thymidine and ^{3}H uridine) into the nucleus as well as the cytoplasm was significantly reduced. In *Crotalaria retusa* (Jain and Shivanna 1990), in vitro germination of pollen grains stored in hexane under laboratory conditions was as good as fresh pollen after 30 days; however, pollen tube length was drastically reduced in stored pollen. Thus, as in seeds, loss of vigor seems to be a general response of stressed pollen whether due to high RH and temperature or storage.

In the literature on seeds, seed vigor has been considered different from seed viability or germinability (see Ching 1982). The speed of germination is considered to be an indicator of seed vigor. In a range of crop plants it has been shown that aged seeds produce inferior plants and lower yields, even though the levels of their germinability are still relatively high (see Priestley 1986). Stored seeds with reduced vigor are also less able to withstand a wide variety of environmental stresses.

Seed vigor, therefore, is considered to be a more reliable index for predicting plant growth rate and crop production than a simple assessment of the capacity to germinate (see Perry 1978). In recent years, it has been recommended that seed deterioration be assessed as the reciprocal of vigor (Association of Official Seed Analysts, Seed Vigor Text Committee, 1983) rather than of germination.

Although extensive studies have been carried out on pollen viability, particularly in connection with storage, there is hardly any information on the loss of vigor.

The ultimate aim of pollen storage has been the establishment of 'pollen banks', through which the pollen of a desired species/variety can be routinely obtained at any time of the year and at any place in the world. If such pollen banks are to become a reality, it will be important to consider the vigor of the stored pollen, in addition to the viability determined on the basis of FCR or in vitro germination. Storage conditions will be optimized, not only for the maintenance of viability but also for the maintenance of vigor. Studies must be extended to explain the causes for the loss of vigor and its consequences on pollen-pistil interaction, on fertilization, and on the subsequent progeny.

A few recent studies have shown the potential of using pollen storage as a selection pressure (Pfahler 1986; Pallais et al. 1986). For an effective and rational use of pollen storage as a means of selection pressure, it would be necessary to have details of the viability as well as the vigor of the pollen samples.

Our investigations (Shivanna and Cresti 1989; Shivanna et al. 1990; present studies), as well as many other studies (see Shivanna and Heslop-Harrison 1981; Heslop-Harrison et al. 1984), have shown that the FCR test reflects the ability of the pollen to germinate. Our earlier studies on *Nicotiana* pollen (Shivanna et al. 1990) indicated that the FCR test, to a large extent, reflects the ability of pollen grains to effect fertilization. For example, in one of the experiments with *Nicotiana,* stressed pollen grains that failed to germinate in vitro did germinate on the stigma and effected fertilization, although pollen tubes reached the ovary about 40 h later than in controls. However, the FCR test does not reflect the vigor of the pollen. The in vitro germination test can be used to assess the vigor of the pollen, provided the rate of germination is monitored over a period of time and then compared with the responses of the fresh pollen. However, most of the studies in which in vitro germination has been used to assesses pollen viability tend to give the final germination percentage reached after a fixed time, generally much longer than the time required for germination of fresh pollen. Such studies therefore do not indicate the vigor of the pollen. The semivivo technique is very effective in studying pollen vigor (Shivanna et al. 1990). It should be possible to standardize semi vivo technique even for those systems that do not show satisfactory in vitro germination. Alternatively, pollen vigor can be studied by carrying out in vivo pollinations and monitoring pollen tube growth in the pistil through aniline blue fluorescence.

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