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TRANSFER OF DEVELOPING BEAN POLLEN IN LILY ANTHERS

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SUMMARY

Developing fertile and sterile microspores and pollen of *Vicia faba* L. are injected into lily anthers during different stages of their development. Changes in autofluorescence of bean microspore or pollen wall are observed and recorded after about 65 hours of incubation. Fertile and sterile tetrads of *Vicia* acquire the autofluorescent characters of lily in all anthers tested. Vacuolated fertile and sterile microspores accept or reject the lily characters. But mature pollen of both types do not accept the lily characters of pollen wall autofluorescence. The results indicate a limited phase in which the pollen wall of the bean accepts products influencing pollen wall autofluorescence. This phase ends when the microspores reach the vacuolated stage.

1. INTRODUCTION

The artificial transfer of developing pollen among members of different plant genera or families is a new method in the study of microsporogenesis (WILLEMSE 1981). It has been shown that developing pollen walls of *Gasteria,* after transfer of *Gasteria* pollen to *Lilium* anthers, acquire the autofluorescence spectrum which is characteristic for lily pollen walls. These changes in autofluorescence occur within 24 hours. Products that cause the autofluorescence of the lily pollen wall are present in the locular fluid. These products are accepted by the *Gasteria* microspore or pollen wall, independent of the viability of the microspore or pollen grain.

To test the influence of the locular fluid of the lily anther on the development of the pollen wall, developing microspores and pollen of *Vicia faba* are injected. Two types of bean plants are available, one with normal pollen development, the other producing sterile pollen. The abortion takes place during the vacuolated stage of the bean microspore. It is caused by a factor in the cytoplasm, called cytoplasma 447 (BOND 1966). Of both plant types the changes in the autofluorescence of the developing pollen wall is known (AUDRAN & WILLEMSE 1981). The present paper deals with the transfer of the developing microspores or the pollen of *Vicia faba* in different stages of the anther of lily.

2. MATERIAL AND METHODS

Tetrads, vacuolated microspores and mature fertile and sterile pollen of *Vicia faba* L. were used for injection in anthers of *Lilium* Hybrid "Enchantment".

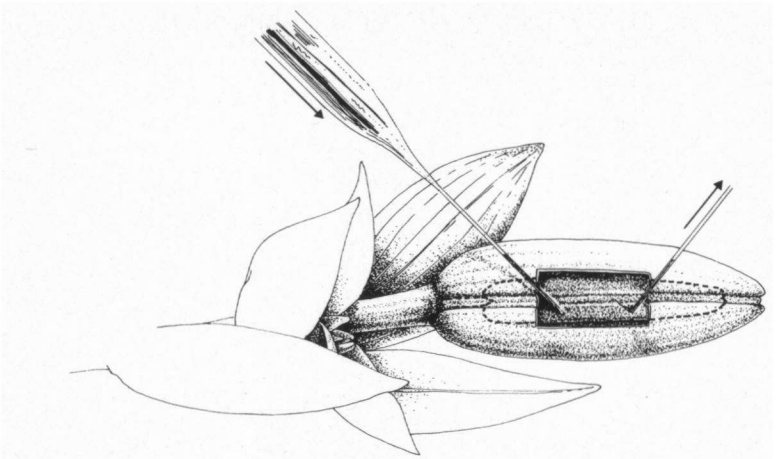

Fig. 1. Injection of lily anther by glass capillars. Whole anther is bordered by a dotted line. The flower bud is opened by making a small window.

The seeds of these bean plants were obtained by courtesy of Dr. G. Duc from the "I.N.R.A." Station of Dijon, France, where the different lines were selected by P. Berthelem, J. Picard and G. Duc. Two plants were used, one with fertile pollen – the maintainer – and one with aborting pollen – the sterile. Tetrads, microspores or pollen transfer was carried out by using capillary needles in which the fluid locular content of *Vicia* anthers was picked up. Mature *Vicia* pollen were sucked up mixed with a very small amount of locular fluid of lily anthers or with water. *Lilium* flower buds and anthers were opened and injected as shown in *fig. 1*. If necessary the contents in the capillaries were pressed slightly during injection. The holes in the anthers were sealed with a small glass ball covered with lanoline. After 65 hours of incubation the contents of the treated anthers were collected and the autofluorescence of the walls of microspores or pollen of bean and lily was determined by a UV-microspectrophotometer as described earlier (AUDRAN & WILLEMSE 1981). A square area of 12 μm^2 microspore or pollen wall was measured. Of the autofluorescence spectra only the maximum of the spectrum was stated in nm wave length. In addition, mature fertile and sterile *Vicia* pollen treated at 40°C for 1 hour with 6% KOH or acetolyzed by acetic acid: H_2SO_4/9:1 at 85°C during 5 minutes were transferred in the lily anther and the autofluorescence was measured. As control a part of the bean microspores or pollen used for transfer was incubated in a drop of lily locular fluid of another anther of the same flower bud during the same period of injection. The viability of pollen was chequed with a buffered solution of 0.5% lissamine green (Gurr). Each experiment consisted of 2 injections and 3 measurements of the autofluorescence of the wall per injection.

3. RESULTS AND CONCLUSIONS

The autofluorescent signal is mainly caused by the presence of a pollen wall, which develops from the tetrad stage. The change of this signal can be related to the development of the pollen wall (WILLEMSE 1972). The results of the injections expressed as changes in the autofluorescent signal are presented in *fig. 2.*

It appears that the tetrad stages of both types of *Vicia* pollen accept the autofluorescent characters of lily pollen wall in all the different stages of the development of the lily anther. At a bud length of 26–28 mm the lily microspore is in its late vacuolated stage, at about 32 mm near the first mitosis, at about 38 mm at the stage of the young pollen grain and at about 43 mm in the stage of the formation of the lens-like generative cell (REZNICKOVA & WILLEMSE 1980). Pollenkitt formation takes place in anthers with a length of 34–41 mm.

The double maximum of the fertile tetrad of the emission spectrum is dominated by the autofluorescent signal of lily.

After the incubation in the locular fluid the same results are stated. The viability of the tetrads is lost in both conditions.

The vacuolated microspores of the maintainer accept the autofluorescent signal of lily when injected in an anther of about 21 mm. But after injection in an anther of about 32 mm, the population of vacuolated bean microspores of the maintainer type does not accept the autofluorescent signal of lily. In the

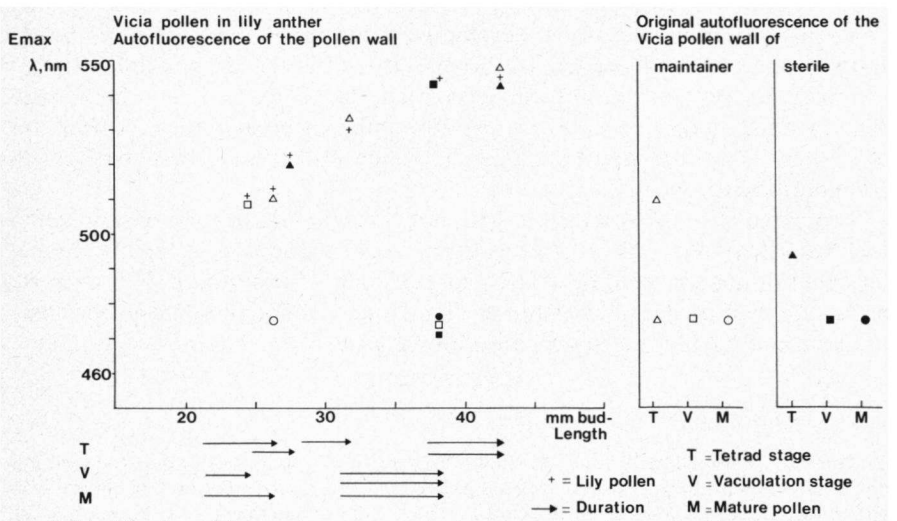

Fig. 2. Modification of the autofluorescence of microspore and pollen wall of fertile, the maintainer, and sterile *Vicia* plants after transfer of tetrads, vacuolated microspores and mature pollen in the lily anther. The autofluorescence expressed in the spectral maximum of lily pollen is indicated by a + sign. The autofluorescence of the wall of microspores or pollen of the maintainer type is presented by open symbols, different for each stage of development. The closed symbols are indicating the same for the sterile type. The duration of the treatment, about 65 hrs, is represented by a line. Only the main value of the maximum of the emission spectrum E_{max} in nm is noted.

population of vacuolated microspores of the sterile type partly the wall accepts the signal of lily, partly its own signal remains unaltered. This difference in acceptation can probably be an interference with the Pollenkitt formation in the lily anther, or it is a result of the difference in microspore wall compositon or a difference in the developmental stage between the maintainer and the sterile type.

After incubation in the locular fluid a comparable result is observed. All microspores loose their viability.

The walls of mature *Vicia* pollen do not accept the autofluorescent characters of lily pollen. The same occurs in the locular fluid. Also acetolyzed or with KOH treated mature bean pollen, which treatments change the autofluorescent characters of the pollen wall, remain unaltered after injection or incubation in locular fluid.

These results indicate that the autofluorescent products present in the developing pollen wall of lily are accepted in the microspore wall of *Vicia* in the tetrad stage. These products remain still available in the lily anther with a length of about 40 mm. Vacuolated microspores may accept the lily autofluorescence. Mature pollen do not accept this signal. So it can be concluded that acceptation of fluorescent lily pollen wall material depends on the stage of development of the pollen wall of the bean.

During the vacuolated stage of the *Vicia* microspore, the endexine formation takes place (AUDRAN & WILLEMSE 1981). This formation is partly a result of the activity of the cell of the microspore. Because of the loss of cellular viability in all stages of bean microspore development during the treatments, the acceptation seems to be independent of the viability of the cell. Although the endexine and probably ectexine are still developing, the ability to accept the fluorescence producing products may be lost during the vacuolated stage of bean microspores. This is an indication that the pollen wall formation is partly completed during the vacuolated stage.

Compared with the results in transfer of *Gasteria* microspores and pollen in the lily anther (WILLEMSE 1981) this type of pollen accepts also the lily character of autofluorescence till the late phase of pollen formation in lily. *Gasteria* shows a longer period of acceptation than *Vicia,* but both can accept the characteristics of the lily pollen wall autofluorescence.

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