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Microsporogenesis *in vivo* and *in vitro*. Autofluorescence of pollen wall of *Lilium* and changes in pollen wall of *Gasteria* in *Lilium* anther

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Abstract

Autofluorescence of the pollen wall of *Lilium* shows a correlative change with its development. When the pollen of *Gasteria* is injected into the anther of *Lilium*, it exhibits almost the same characteristics of wall autofluorescence as those of *Lilium*. Only the last stages of wall development are dissimilar. During this period the pollenkitt sticks to the pollen wall in *Lilium* only. The conclusion is that in the exine of *Gasteria* pollen, in contact with the locular fluid of *Lilium*, polymerization of *Lilium* sporopollenin occurs instead of a structural addition to the wall. This polymerization is a continuous process during the entire development.

INTRODUCTION

During microsporo- and gametogenesis in *Lilium* the volume of the nexine and other structural changes during various stages reveal discontinuous development of the pollen wall (Willemse, Reznickova, 1980; Reznickova, Willemse, 1980). In relation with the tapetum and middle layer, the pollen wall matrix which is partly carbohydrate is impregnated with sporopollenin which polymerises. After this young microspore stage the wall thickens by the addition of globules and membrane-like lamellae.

Pollenkitt also contributes to the pollen wall after mitosis. During development, four phases of increase in volume of nexine are detected. The locular fluid contains products and permits the passage of the products from the surrounding tissues for pollen wall development. A study of autofluorescence of the pollen wall shows many differences. During pollen ontogeny autofluorescence of the wall can be related with some characteristics of development (Willemse, 1972). In this investigation, changes in the autofluorescence of the *Lilium* pollen wall are correlated with its development. By using the developing pollen wall of *Gasteria*

as a system, the influence of the locular fluid of *Lilium* on its wall is detected by changes in autofluorescence; this was achieved by injecting pollen of *Gasteria* into the anther of the lily as well as by culturing pollen in the locular fluid.

MATERIAL AND METHODS

Plants of *Lilium* hybrid 'Enchantment', and of *Gasteria verrucosa* (Haw) Mill.,were grown in the greenhouse. For determination of the various developmental stages as related to the bud length in mm, the same classification was followed as described earlier by Willemse and Reznickova (1980) for lily and for *Gasteria* by Willemse (1972).

The viability of pollen was checked by using a solution of 1% lissamine green (Gurr) in 0.2 M phosphate buffer. Autofluorescence was measured with a Cytophotometer using Leitz microscope with a Ploem incident UV light of 365 nm, a RCA c 31034 Photomultiplier with a Fluke 412 B high voltage power supply and a Goerz RE 541 recorder registered the signals. The measurements were taken at 1.1.50 kV and a Scott interference S 20 filter with motordrive was selected for emission spectrum. Only 10 μm^2 part of pollen was measured using a 50× UV water objective. The mean value and deviation of three measurements were considered as the maximum of the emission spectrum (Emax) — nm wavelength, intensity in mV of this wavelength (I), and change in the intensity during first 30 seconds (—I in %).

For culture, very small drops of locular fluid of lily were put at 20°C in a sterile humid chamber to which *Gasteria* pollen was added. The locular contents of the dry anthers were washed out with sterile water. For injection a glass syringe was used. Fresh *Gasteria* pollen was injected through a small rectangular opening in the bud, by making two holes, about 5-8 mm apart near the connective. The holes and rectangular opening in the bud were sealed with a thin plastic sheet. The transplants were grown in the greenhouse at ± 23° C.

RESULTS

Autofluorescence

Autofluorescence spectra of the pollen wall have one maximum but they are mixed spectra originating from different layers and products composing the pollen wall. Its interpretation should be combined with structural and chemical analysis of the pollen wall. However, with this knowledge the photochemistry becomes very complex. During the developmental process the signals are sometimes related with structural

and chemical changes, hardly with photochemical processes. Therefore a comparative study with only detailed knowledge should be considered very critically.

Autofluorescence of lily pollen wall

Measurements of some stages in pollen wall development in *Lilium:* Fig. 1 shows the first change of blue colour (464 nm) to more yellow colour (527 nm) is followed by a decrease to 517 nm, a stepwise change to 527 nm, and finally to 537 nm. The decrease in intensity shows a fall from 58% at the young microspore to about 8 to 10% in the following stages. The intensity is high in the first free microspores and decreases stepwise; it is partly correlated with the periods in colour changes. Only an increase and gradual decrease is measured at the bud length of 50 mm when the pollen starts to form a lens- like generative cell. Also shown are the periods of pollen wall development, representing addition to the nexine volume and sporopollenin, presence of globules and membrane--like lamellae, and the presence of the pollenkitt, after Willemse and Reznickova (1980) and Reznickova and Willemse (1980).

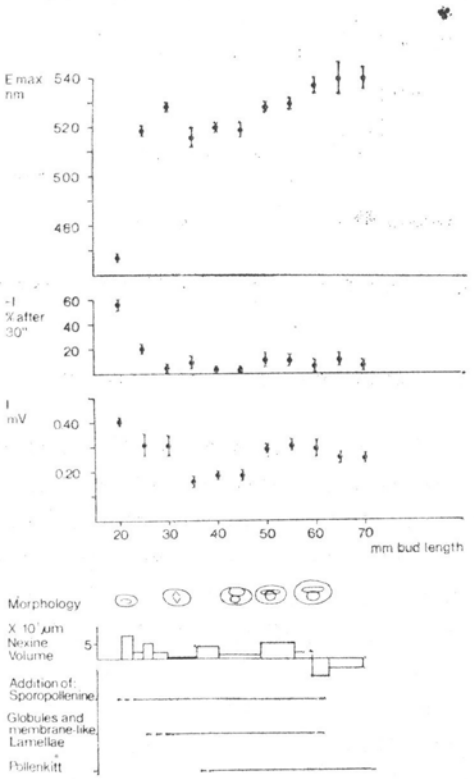

Fig. 1. Autofluorescence of lily exine

Autofluorescence of *Gasteria* pollen wall

Fig. 2 represents the autofluorescence of the pollen wall of *Gasteria*, considering mainly the selected stages for injection. Compared with the lily, the colour is more blueish (497 nm) and the intensity has a higher level, the values of the decrease in intensity are in a comparable range. This difference is due to the species characteristics of the development of the pollen wall, its composition and structure.

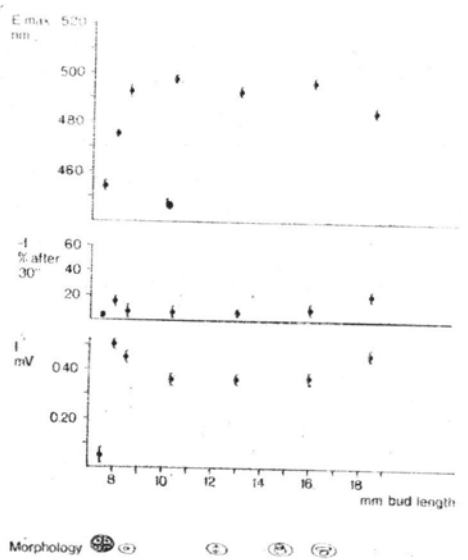

Fig. 2. Autofluorescence of *Gasteria* exine

Changes in autofluorescence of *Gasteria* pollen wall

Autofluorescence of the young and old *Gasteria* pollen wall during periods of increase of nexine volume and the intervals when pollen remained for 24 hours in lily anthers, is given in Fig. 3. The pollen wall shows no structural changes under the light microscope. Most of the pollen were still alive. In all stages the *Gasteria* pollen wall takes over the signal of the *Lilium* pollen wall. The colour, the intensity and decrease in intensity are comparable with the lily pattern. Also at the young microspore stage the transition to yellow colour (500-520 nm) shows the lily pattern. However, at bud length of 50 nm, the *Gasteria* pollen wall does not follow the colour and intensity of lily. Differences related to the changes in the nexine volume are also not detected. After

four days of transfer all *Gasteria* pollen died, except a few which remained alive at the bud length of 15 nm. The autofluorescence is represented in Fig. 4.

The tendency to follow the lily pattern is observed again. Although some differences are clear. After the bud length 50 mm the *Gasteria*

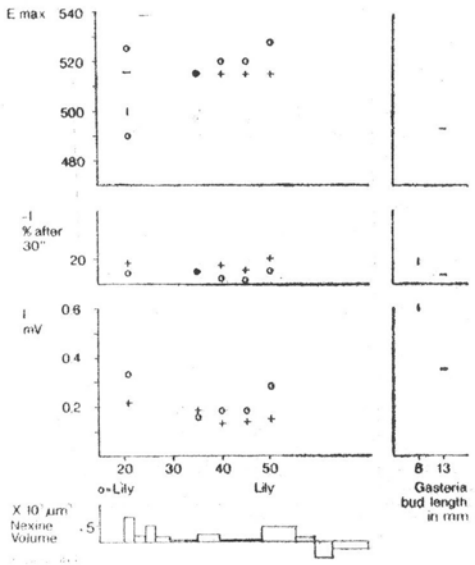

Fig. 3. Autofluorescence of *Gasteria* exine after 24 h in lily anther

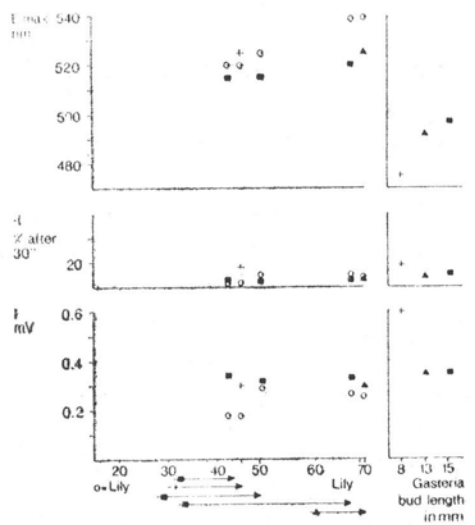

Fig. 4. Autofluorescence of *Gasteria* exine after 4- to 9-day transfer in lily anther

pollen does not follow the colour change of *Lilium* and the intensity is less changed. In a six day culture in the locular fluid, or diluted locular fluid, nearly all *Gasteria* pollen died. The autofluorescence is depicted in Fig. 5. In this experiment the characteristics of the lily are also taken over, except in the intensity of the locular fluid from bud length of 40 and 50 mm, which is mixed with water. The decrease in intensity is more changed in the locular fluid of bud length of 30 mm.

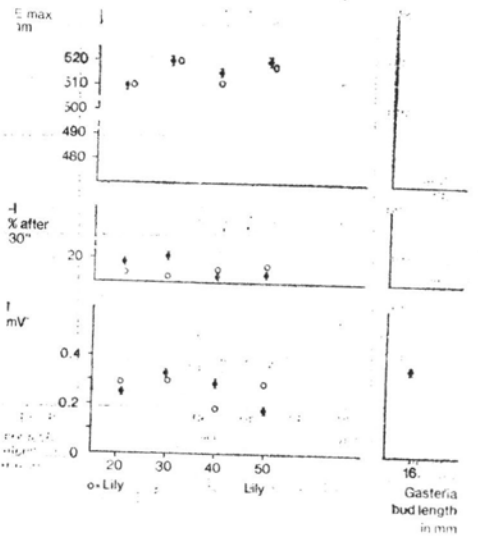

Fig. 5. Exine autofluorescence of *Gasteria* pollen after 6 days in locular fluid of lily

DISCUSSION AND CONCLUSIONS

Autofluorescence of developing pollen wall of lily

Five periods of colour shift of the developing pollen wall of lily can be distinguished. In combination with these, four periods of change in intensity and two of decreases are observed. The first period in which the carbohydrate matrix gets the sporopollenin is characterized by a shift to a yellow colour and a decrease in intensity and an increased fading. During this moment the polymerization of sporopollenin starts and is a feature of the young microspore. The nexine volume increases too. In the following period, until pollen mitosis, the colour shifts to 517 nm. During this time sporopollenin is polymerizing and globules and membrane-like lamellae are added to the pollen wall. The intensity is low at this time. During following period, just after microspore mitosis, the pollenkitt, which has a spectral maximum of 565 $\pm$ 2 nm and the nexine

volume increase. This is added to the pollen wall, causes a shift to 527 nm and an increase in intensity. The last period probably is characterized by diminishing sporopollenin polymerization as well as addition of globules and membrane-like lamellae and continuation of pollenkitt addition to the pollen wall. The colour is more yellow but with low intensity. The values of autofluorescence are somewhat higher than in *Lilium* hybrid "Bright Star" but show the same pattern (Willemse, 1972). From this pattern of autofluorescence a periodical pollen wall building can be determined. Its relationship with the different processes need to be ascertained experimentally.

Change in autofluorescence of *Gasteria* pollen wall

In the anther and in locular fluid the autofluorescence of the pollen wall of *Gasteria* resembles with that of the lily. The colour shift to about 517 nm is the most characteristic phenomenon. Correlating with development, it can be suggested that polymerization of lily sporopollenin as well as addition of globules and membrane-like lamellae is taken over. The pollenkitt does not influence the *Gasteria* pollen wall. After a bud length of 50 mm the autofluorescence colour of *Gasteria* does not follow the colour change of *Lilium*. Under light microscope none or little pollenkitt material of lily was seen on the *Gasteria* pollen wall. This can be explained by the activity of the pollenkitt in pollen wall formation in *Lilium*, which is not operative on the *Gasteria* pollen wall, as is concluded by Reznickova and Willemse (1980). The pollen wall of *Gasteria* does not recognize the pollenkitt of lily. The formation of nexine with its discontinuous increase in volume is not expressed in the autofluorescence of *Gasteria* pollen. So, at the bud length of 50 mm, an increase occurs in the nexine volume, but the *Gasteria* pollen wall does not change to 530 nm colour and has no increase in intensity.

In the cultures of locular fluid, there is a steady state in development, the pollen wall of *Gasteria* takes up the present precursors of lily sporopollenin. This indicates that uptake and polymerization of the sporopollenin is the main cause of change in autofluorescence. The conditions for polymerization are probably present in the pollen wall of *Gasteria*. The differences observed in the intensity of fluorescence of *Gasteria* pollen wall after 24 hours are better comparable with those of lily than those which stay for more days. This probably indicates that the influence is dependent on the living state of the cell. Such a suggestion requires that the experiments need more study. Nevertheless, on comparing the results it is clear that the autofluorescence of the pollen wall of *Gasteria* is positively influenced by lily characteristics.

Acknowledgments

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