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# Callose Formation in Pollen Tubes and Incompatibility

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**Souhrn**

Bylo sledováno množství kallosy v kompatibilních a inkompatibilních pylových láčkách kulturní odrůdy jabloně (*Pirus malus* L.) a tabáku (*Nicotiana alata* LINK et OTTO) po zbarvení resorcinovou modří. Poněvadž je toto barvení specifické na kallosu, je velmi vhodné k selektivnímu barvení pylových láček v čnělkovém pletivu. V inhibovaných láčkách vyrostlých po samosprášení je kallosy relativně k jejich délce nejméně dvakrát tolik než v normálně rostoucích láčkách kompatibilních. Toto je dáno u jabloně především větší délkou kallosových zátek, u tabáku vedle toho větší jejich hustotou. V láčkách kompatibilních se kallosa ukládá pravidelněji než v láčkách inkompatibilních. Intensita hromadění kallosy se v jabloňových láčkách obojího typu zvyšuje s časem. Jejich růst se naopak postupně zpomaluje. Oba tyto jevy jsou patrny především u láček inkompatibilních. Obecně tedy existuje nepřímý vztah mezi množstvím kallosy v pylových láčkách a rychlostí jejich růstu.

Byla vyslovena domněnka, že fysiologická podstata tvorby kallosy spočívá v omezené schopnosti pylových láček využívat v procesu dýchání pyranovou složku metabolisované sacharosy. Glukopyranosa může podle této představy v pylových láčkách sloužit převážně ke stavbě jejich blan, její přebytek, který vzniká zvláště v případě omezeného růstu, se v nich ukládá ve formě polyglukosidu kallosy.

## Summary

1. Attention is called to the possibility of using resorcin blue for the staining of pollen tubes within the style tissue and the method of staining is described.

2. In pollen tubes, the growth of which is inhibited due to incompatibility, there is at least twice the amount of callose relatively to their length as there

is in normally growing compatible tubes. In apple this is primarily due to the greater length of the callose plugs, in tobacco also to their greater density.

3. The regularity of the formation of callose in pollen tubes increases with the rate of their growth.

4. It is suggested that the physiological basis of callose formation consists in the limited capacity of pollen tubes to use the pyran components of metabolized saccharose in the process of respiration.

## Introduction

Callose occurs in pollen tubes primarily in the form of the well-known callose plugs. In addition it forms a component of pollen tube walls, where together with cellulose it composes the inner lamella (MÜLLER-STOLL and LERCH 1957a). Callose plugs in pollen tubes are formed acropetally. During the cultivation of pollen in artificial media no particular system was found to govern their location (MÜLLER-STOLL and LERCH 1957a, 1957b).

Following the hydrolysis of callose from the fungus *Bornetina corium* (MANGIN 1910) and of that contained in the cystoliths of *Ficus elastica* and *Ficus triangularis* (ESCHRICH 1954) and from the sieve plates of the grape vine (FREY-WYSSLING, EPRECHT and KESSLER 1957) D-glucose was found. As a result of a more detailed analysis of callose from the sieve plates of grape vine the presence of a polysaccharide containing chains of 1 : 3—linked $\beta$—D-glucopyranose residues was demonstrated (ASPINALL and KESSLER 1957).

Very little is known as yet about the physiological mechanism of the formation and function of this polysaccharide. Most frequently the conception has been advanced that the callose plugs act as mechanical barriers to the reverse flow of plasma from the growing tips to the older basal parts (MOLISCH 1923, ESCHRICH 1956). MÜLLER-STOLL and LERCH (1957b) put forward a number of objections to this conception and express the opinion that "callose is a necessary product of metabolism in pollen tubes, probably originating after a special irritation in the cytoplasm in contact with the cell wall and which may be locally accumulated under conditions of degeneration". LINSKENS and ESSER (1957) pointed out the connection between callose formation in pollen tubes and incompatibility. After staining with fluorochrome they observed the number and size of callose plugs in the styles of autosterile petunias. They ascertained that in incompatible pollen tubes their number was nearly twice as great as in compatible tubes. The size of the callose plugs was the same in both cases.

The aim of the present authors' work has been to study callose formation in relation to the growth rate of pollen tubes in styles as determined by the compatibility or incompatibility of pollination. An attempt has also been made to elucidate the physiology of callose formation in the light of present knowledge. The paper further deals with the method of selective staining of pollen tubes in the style tissues.

## Material and Method

Autosterile *Nicotiana alata* LINK et OTTO and fruiting apple trees (*Pirus malus* L.) var. Lord Lambourne on EM IX stocks were used as experimental material. In the case of apple, pollen from the variety Golden Pearmain was used for cross pollination. All experiments were carried out under field conditions. Emasculated, isolated flowers were picked at definite intervals after pollination. They were frozen freely in the air at a temperature of about —20° C and kept in the frozen state until needed. The average day temperatures during the experiments with apple were, for the first day 17·5° C and for the second day 21° C. The experiments with tobacco took place at average day temperatures of 14° C, 14·2° C and 11° C.

For the purpose of following the formation of callose plugs in pollen tubes grown in artificial media, pollen was cultivated in a 10% solution of saccharose containing 0·001% $H_3BO_3$ at a temperature of 25° C. The concentration of pollen was 5 mg. in 0·5 ml. of culture solution over an area of 10 $cm^2$.

Results were worked up by usual statistical methods. In the case of the length of callose plugs $n = 120$, in determining the distances between them 30 measurements were made in each case.

## Results and Discussion

### 1. Method of staining pollen tubes

One of the specific reagents for callose is resorcin blue prepared according to ESCHRICH (1953). It was found that this stain is also admirably suited to the staining of pollen tubes in the style tissue. Only callose in the pollen tubes is stained blue, while the style tissue remains unstained. In view of the fact that resorcin blue does not stain callose following fixation and conservation by the usual methods which involve dehydration of the object, the styles must be treated in the fresh state or else they can be frozen and kept for any length of time until they can be treated. The advantage of this method is that the preparation of the conducting style tissue is easier after thawing. The tubes stain very rapidly and the preparations can be examined immediately. When more pronounced staining is required it is necessary to leave the stained specimens for at least a day in a moist environment. The chief points in favour of this method of staining pollen tubes are its specificity and simplicity.

### 2. The accumulation of callose in compatible and incompatible pollen tubes

Results of the measurement of size of callose plugs and their distances from each other are set out in tab. 1 and 2.

In the case of apple striking differences can be seen here between the amount of callose in pollen tubes from self-pollination and cross-pollination and its progressive accumulation in respect to the growth rate of the tubes. The differences in distances between callose plugs are not significant, but 24 hours after pollination their length in the short incompatible pollen tubes is nearly twice the length of those in the longer compatible pollen tubes (fig. 1 and 2).

Tab. 1. Length of and distance between callose plugs in apple

| | Number of hours after pollination | Approx. length of pollen tubes | Length of callose plugs | Distance between callose plugs |
|---|---|---|---|---|
| | | $\mu$ | $\mu$ | $\mu$ |
| Self-pollination | 24 | 4400 | $13{\cdot}83 \pm 3 \,.\, 0{\cdot}62$ | $428 \pm 3 \,.\, 15{\cdot}65$ |
| | 48 | 4900 | Accumulation of callose along almost entire length of pollen tubes | |
| Cross-pollination | 24 | 5600 | $7{\cdot}44 \pm 3 \,.\, 0{\cdot}18$ | $462 \pm 3 \,.\, 15{\cdot}72$ |
| | 48 | 11000 = style length | $21{\cdot}70 \pm 3 \,.\, 0{\cdot}81$ | $490 \pm 3 \,.\, 17{\cdot}79$ |

Following the second day, when the incompatible tubes had grown through a very short section of the style, they were stained by resorcin blue for almost their entire length (fig. 3). The amount of callose in the incompatible tubes is greater particularly on the second day after pollination, not only in proportion to length of the tubes, but also absolutely.

Clearly there exists here an inverse relation between the growth rate of pollen tubes and the intensity of callose formation or alternatively a direct relation between degree of inhibition of their growth due to incompatibility and the formation of this polysaccharide. The incompatibility of pollen tubes is also marked by the accumulation of callose in the swollen tips (fig. 4), a phenomenon which was never observed in the case of compatible pollen tubes.

Thus the rate of accumulation of callose in pollen tubes can serve as a physiological indication of compatibility or incompatibility in apple. Since the choice of suitable pollinators is also connected with the degree of compatibility, a basis could be provided here for working out a laboratory method for its determination.

Essentially the same results as regards the accumulation of callose in pollen tubes and its relation to incompatibility have been obtained for *Nicotiana* as for apple.

Tab. 2. Length of and distance between callose plugs in *Nicotiana* (60 hours after pollination)

| | Approx. length of pollen tubes | Length of callose plugs | Distance between callose plugs |
|---|---|---|---|
| | $\mu$ | $\mu$ | $\mu$ |
| Self-pollination | 9000 | $12{\cdot}24 \pm 3 \,.\, 0{\cdot}25$ | $562 \pm 3 \,.\, 56{\cdot}80$ |
| Cross-pollination | 41000 | $6{\cdot}51 \pm 3 \,.\, 0{\cdot}02$ | $1015 \pm 3 \,.\, 30{\cdot}10$ |

The relatively greater amount of callose in pollen tubes from self-pollination in relation to their length is not only due to greater size but also to the greater density of the callose plugs. There is an evident regularity in the spacing of callose plugs in compatible pollen tubes in comparison with that in incompatible tubes. As in the case of apple (v. tab. 1), the length of callose plugs in *Nicotiana* is more regular in tubes formed after cross-pollination than in those with inhibited growth. In addition, in the case of incompatible pollen tubes an abnormally increased accumulation of callose in the cell wall sometimes occurs, in others sections of some mm. in length are found to be uniformly stained with resorcin blue. Callose is very frequently accumulated to varying degrees in the expanded tips of this type of tube (fig. 5 and 6). In compatible tubes, on the other hand, callose is laid down exclusively in the form of short, regular plugs (fig. 7) and is never formed in the tips of the tubes (fig. 8). The distance of the first callose plug from the tip of the tube is here about 6 to 7 mm.

The way in which callose collects in incompatible pollen tubes is very similar to the irregular formation of callose described for pollen tubes grown in an artificial medium (Müller-Stoll and Lerch 1957a, 1957b). In this connection a comparison was made for apple between the regularity of plug formation in tubes growing in the style and those growing in an artificial medium. While in natural conditions the median error of the average for values expressing the distance between callose plugs is approximately 16, the median error for the corresponding values for tubes grown in vitro is 29·54. It was further ascertained that a greater amount of callose is formed in apple pollen tubes in articifial culture than under natural conditions of growth in the style (v. seq.). In the light of these facts it can be assumed that the increased accumulation of callose in incompatible pollen tubes is not a primary result of incompatibility, but that it is caused secondarily by inhibition of their growth. In general it can be said that the more optimal the conditions for pollen tube growth the more regular its formation and the smaller its amount.

## 3. An attempt to elucidate the physiology of callose formation in pollen tubes

It is well known that the best medium for the cultivation of most pollen in vitro is one containing saccharose. This fact could be explained by Lvov's hypothesis (1950) on the significance of saccharose as an active carbohydrate for respiration. It is supported by the observation that of three sugars used, glucose, fructose and saccharose, germinating pollen takes the greatest amount of saccharose from the environment for its respiration (O'Kelley 1955). Similarly, in styles through which pollen tubes have grown primarily a decrease in saccharose can be observed (Linskens 1955). According to Lvov (1950) saccharose is of importance as a substrate for respiration mainly in older organs, where the enzymatic systems which activate hexoses and make them available for the respiratory process, are ceasing to act. He further assumes that the significance of saccharose consists in its active fructofuranose content and that respiration in older organs takes place exclusively at the expense of this active furan component. Glucopyranose then remains as a secondary, unconsumed component of the respiratory process. If it is assumed that there exists a weakly active enzymatic system for the utilization of pyran forms of sugars in pollen tubes, this would serve as an explanation of the formation of the polyglucoside callose in them, the function of which is as yet unknown. According to this hypothesis the glucose part of metabolized saccharose in pollen tubes is mainly used as a component in the formation of their walls, while the residue, as a result of the limitations of its use in respiration, is laid down in the form of polysaccharide callose.

In this manner it is possible to explain the increased accumulation of callose in pollen tubes which are inhibited by the incompatibility reaction. Where growth is inhibited the use of glucopyranose for the formation of the tube walls is not possible to such an extent as in rapidly growing pollen tubes. If at the same time sugar consumption in incompatible pollen tubes is approximately the same as in compatible ones (LINSKENS 1955), much more unconsumed glucose will remain in the short inhibited tubes, particularly in proportion to their length, with the result that callose will accumulate to a greater extent. Similarly, in artificial medium, where optimal conditions for the growth of pollen tube are not present, callose accumulates more than under natural conditions of compatible pollination. Thus for example, in the case of pollen from the apple variety Golden Pearmain the length of callose plugs in cultures in vitro was $33{\cdot}37\ \mu \pm 3 \,.\, 3{\cdot}19$ after 24 hours, the distance between them was $241\ \mu \pm 3 \,.\, 29{\cdot}54$; in pollen tubes of the same variety growing in styles these values the day after pollination were, as has already been stated, $7{\cdot}44\ \mu \pm 3 \,.\, 0{\cdot}18$ and $462\ \mu \pm 3 \,.\, 15{\cdot}72$.

In the same way it is possible to explain the fact that the amount of callose in pollen tubes increases with the concentration of saccharose in the nutrient environment not only absolutely but also in proportion to length (MÜLLER-STOLL and LERCH 1957a); where there is a sufficiency of saccharose in the environment a pollen tube consumes the easily metabolized furan component primarily in respiration, while pyranose is deposited to an increased extent as its polymer callose.

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## Образование каллозы в пыльцевых трубках и явление несовместимости

ЯРОСЛАВ ТУПЫ

### Резюме

Мы изучали количество каллозы в совместимых и несовместимых трубках пыльцы культурных сортов яблони (*Pirus malus* L.) и табака (*Nicotiana alata* LINK et OTTO) после окраски их резорциновой синей. Так как окраска является специфической по отношению к каллозе, то она весьма удобна для селективного окрашивания пыльцевых трубок в тканях пестика. В ингибированных трубках, развившихся после самоопыления, количество каллозы по отношению к их длине не менее, чем в два раза завышено по сравнению с нормально растущими совместимыми трубками. Это явление у яблони вызвано, прежде всего, большей длиной каллозных затычек, у табака, кроме того, еще и большей их густотой. В совместимых трубках каллоза откладывается более регулярно, чем в несовместимых. Интенсивность накопления каллозы обоих типов пыльцевых трубок яблони повышается со временем. Наоборот, рост их постепенно замедляется. Оба эти явления заметны, прежде всего, у несовместимых трубок. Следовательно, общим явлением является косвенное отношение между количеством каллозы в пыльцевых трубках и скоростью их роста.

Высказано предположение, что физиологическая сущность образования каллозы заключается в ограниченной способности пыльцевых трубок использовать в процессе дыхания пирановую часть метаболизированной сахарозы. Глюкопираноза, следуя этому представлению, служит в пыльцевых трубках преимущественно для постройки их стенок. Ее избыток, возникающий особенно в случае ограниченного роста, откладывается в форме полиглюкозида каллозы.