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ACTIVATION OF PROTEIN SYNTHESIS IN OVARIES FROM PETUNIA HYBRIDA AFTER COMPATIBLE AND INCOMPATIBLE POLLINATION

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SUMMARY

Differences in protein metabolism after cross- or self-pollination are found in Petunia ovaries, before pollentubes reach the ovary. Similarities and differences with another clone from *Petunia hybrida* are discussed, as well as possible. functions of the mentioned phenomena.

1. INTRODUCTION

Several authors already described changes in the metabolism after pollination in flower parts which were not in direct contact with the growing pollentubes (HSUNG & HSIANG 1951, KNAUFT et al. 1970, ROGGEN 1967, HALL & FORSYTH 1967). LINSKENS (1973) mentioned changes in the RNA, amino acid and protein content in the ovaries after pollination. He also found an altered transport of sugars and amino acids in all parts of the *Petunia* flower after cross- or self-pollination (1974).

In a previous report (DEURENBERG 1976) changes in protein metabolism in the ovaries after pollination, which occurred *before* the pollentubes reached the ovary, have been described, whereas also differences in reactivity of the ovary after cross- and self-pollination were observed. In order to establish whether this phenomenon, described for *Petunia hybrida*, clone W166K, was restricted to this special clone or was a more general phenomenon, similar experiments were carried out with clone T2U using the same incompatibility alleles but in a different genetic background.

2. METHODS AND MATERIAL

Buds of *Petunia hybrida*, clone T2U, incompatibility alleles S_3S_3, were emasculated 24 hours before anthesis. One day later, the flowers were pollinated with T2U pollen (selfed) or with W166K pollen (incompatibility alleles S_1S_2, crossed). A third part of the flowers was left unpollinated as a control.

At different times after pollination, the flowers were cut and the ovaries stored in liquid nitrogen. Polysomes were extracted. The content of ribosomal protein was determined and the incorporation capacity of ^{14}C-leucine was measured in an in vitro system containing soluble enzymes and co-factors from rat livers.

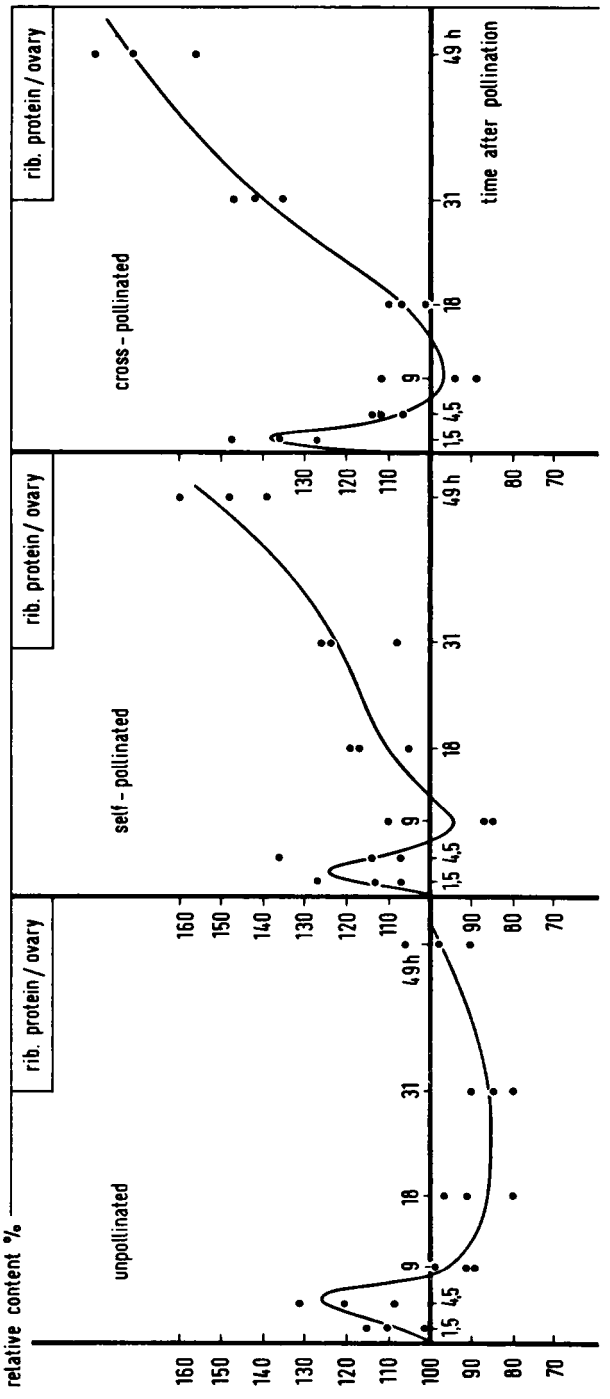

Fig. 1. Relative ribosomal content per ovary in self-, cross- and unpollinated ovaries.

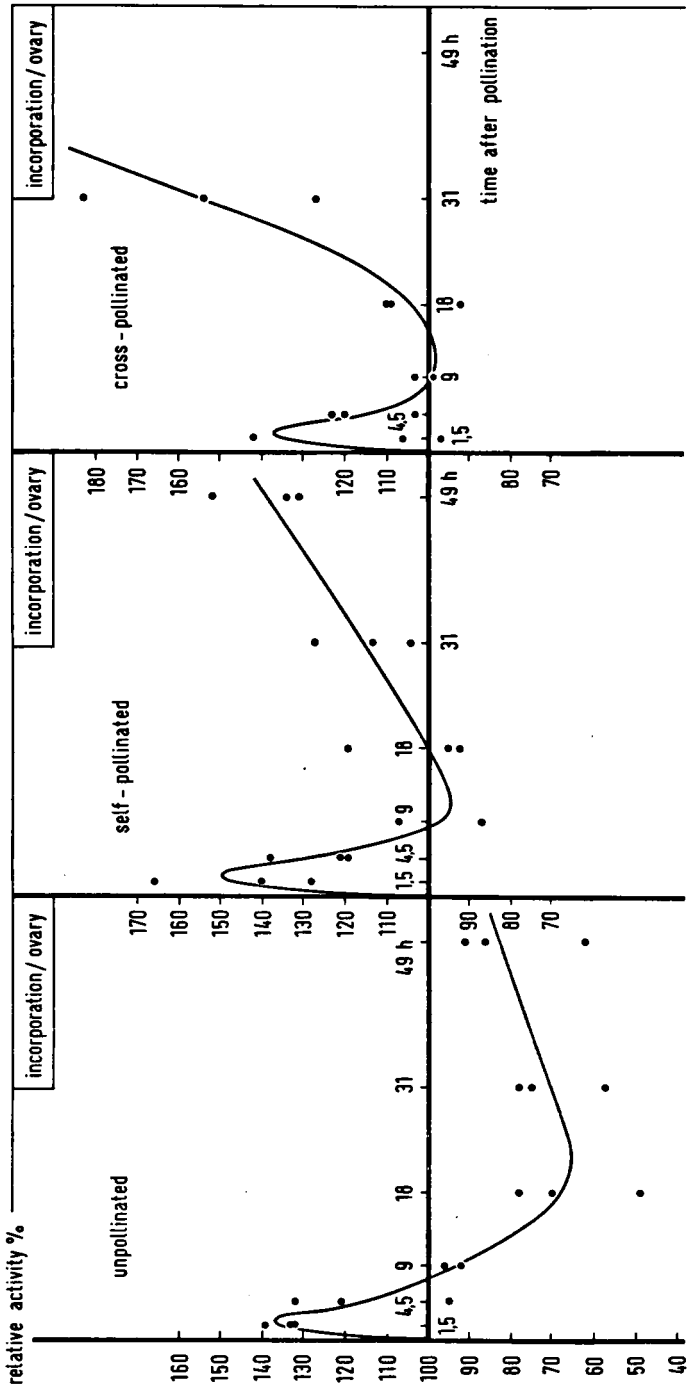

Fig. 2. The relative incorporation activity per ovary in self-, cross- and unpollinated ovaries.

All methods and materials were as described in a previous publication (DEURENBERG 1976). Plants were grown in the greenhouse at 20–25 °C during daytime and 15–18 °C during the night.

RESULTS

3.1 Pollentube growth

After cross-pollination the pollentubes grow through the style into the ovary in about 30 hours under greenhouse conditions. In contrast to that, after selfpollination the pollentubes grow much slower and do not reach the ovary even after 60 hours.

As an additional control on the incompatibility-reaction, some flowers were not cut from the plant. At about two weeks after pollination, the ovaries from cross-pollinated flowers contained many (unripe) seeds. The ovaries after self-pollination, however, were shrunken and dried and contained no or only a few (unripe) seeds.

3.2 Ribosomal proteins content

Fig. 1 shows the content of ribosomal proteins of the ovaries after cross- and self-pollination and of unpollinated flowers. The content of ribosomal proteins at the moment of pollination is set down as 100%. At that moment, the amount of ribosomal proteins was about 8 μg per ovary, as determined according to BLOEMENDAL et al. 1964 and LOWRY et al. 1951).

In the first hours after pollination, until about 9 hours, there were no differences in the amount of ribosomal proteins between pollinated (crossed or selfed) and unpollinated ovaries. In all three cases a first maximum was found between 1.5 and 4.5 hours after pollination. Subsequently the protein content declined until about 9 hours.

Whereas in the unpollinated ovaries the amount of ribosomal proteins decreased farther till 20 hours, an increase was found in the pollinated ovaries from 9 hours after pollination and on. After cross-pollination the increase was greater than after self-pollination.

3.3 Incorporation activity of ^{14}C-leucine

As shown in *fig. 2*, an increase in incorporation capacity was found with polysomes from pollinated as well as from unpollinated ovaries. This increase reached a first maximum between 1.5 and 4.5 hours after pollination. Then, a decrease occurred, which showed a minimum at about 20 hours with polysomes from unpollinated ovaries. However, after pollination, crossed or selfed, the minimum was reached at about 10–12 hours. With polysomes from unpollinated ovaries a slight increase was found from 18 till 49 hours. After pollination, the increase after about 12 hours was a very strong one, especially after cross-pollination.

Between 18 and 30 hours after pollination, a difference in incorporation capacity appeared with polysomes from cross-pollinated flowers, and with those after self-pollination. Whereas the incorporation activity at 31 hours

after self-pollination was about 120% polysomes from cross-pollinated ovaries showed an increase of the incorporation activity of about 150–160%. At 49 hours, the incorporation activity with polysomes after cross-pollination was about 250–300%, whereas after self-pollination this amounted to 140%.

4. DISCUSSION

As *fig. 1* shows, a difference in reaction of the ovary from pollinated and unpollinated flowers appeared not earlier than about 9 hours after pollination. After cross- or self-pollination, a sudden increase was observed in the ribosomal protein content, whereas in the untreated flowers the ribosomal protein content in the ovaries remained fairly constantly between 9 and 31 hours.

The incorporation activity increased after 9 hours after pollination, crossed or selfed, whereas in unpollinated flowers the incorporation decreased farther. Since the pollentubes only grow into the style for about 1/3 of the style length at 9 hours after pollination. some kind of signal has to be derived from the stigma or the style. This signal translocates to the ovary in order to induce the activation of the protein metabolism.

From *fig. 2* it is clear, that there does not only exist a difference in reaction between pollinated and unpollinated ovaries, but also between cross- and self-pollinated ones. The increase in incorporation activity after cross-pollination is much stronger than after self-pollination. This greater increase has been found as early as between 18 and 31 hours and also was found in a later stage. This means, that the ovary differently reacts after self- or cross-pollination at a moment that no pollentube could have reached the ovary. So, is seems justified to conclude that the above mentioned signal also contained information about what kind of pollination has taken place.

Whether there occur two signals, one having information about pollination or not, and another about the *kind* of pollination, selfed or crossed, one only can speculate. The results here presented, are in good agreement with those described in a previous paper (DEURENBERG 1976), where similar experiments were carried out with clone W166K. So, the above mentioned phenomena of reactivity of the protein metabolism of the ovary before a direct contact of the pollentubes with the ovary seem to be a general one in Petunia.

The fact, that recognition of pollination and possibly also the kind of pollination has occurred before 9 hours after pollination, is in agreement with results mentioned by VAN DER DONK (1974). He established the recognition of cross- or self-pollination in a very early stage of the pollentube/style interaction. GILISSEN (in the press) showed, that changes in the wilting processes of the flower after pollination only can take place in case pollen has germinated and grown into the style. About 3–4 hours after pollination the first pollentubes are found in the style.

The profiles for the incorporation of ^{14}C-leucine and for the amount of ribosomal proteins from ovaries of clone T2U differ in two respects from those of W166K (DEURENBERG 1976). First, there is a difference at the very beginning. In

T2U, all profiles start with an increase, whereas in W166K there is a decrease. When one starts the experiments 3–4 hours later and at the same time assumes that before the moment of emasculating there was an increase and subsequently a decrease in incorporation activity and content of ribosomal proteins, in all experiments the profiles would start with a decrease.

The difference at the end of the described experiments, e.g. the decline of the profiles of W166K in contrast with the experiments with T2U, is more difficult to explain. One can speculate about a process of "conditioning" of the tissues of the ovaries necessary for a good reception of the male gametes. In the case of W166K the ovary has to wait after this conditioning for the pollentubes which arrive a little later because of the longer style in W166K. However, in T2U the processes of conditioning and fertilization are better coordinated with regard to timing. Perhaps this process of conditioning could be a process for overcoming a second barrier for the pollentubes. This second barrier for self-pollentubes supposedly only can be broken down after a signal arriving from the stigma or the style after cross-pollination.

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