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RESPONSE OF POLLEN TO HEAT STRESS

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SUMMARY

The effect of elevated temperatures on protein synthesis in pollen from *Petunia hybrida* and *Lilium longiflorum* was investigated and compared with their effect in root-tissue tissue of *Petunia*. The results demonstrate that the incorporation of [35-S] methionine into protein does not change in pollen incubated at temperatures up to 40°C, but decreased with 65% at 45°C as compared to incorporation at 27°C.

The qualitative changes that occurred for the proteins synthesized at 27° and 40°C were not identical for pollen from *Petunia* and *Lilium*. The proteins synthesized at 27° and 40°C were very similar within one species. None of the changes in proteins synthesized at 40°C in pollen showed any correlation with heat shock proteins formed in rootlets from *Petunia*.

1. INTRODUCTION

Organisms, plants as well as animals, react to stress by forming and/or activating a defence system, to overcome the non-physiological environment. Many organisms respond to heat stress with a uniform short-term reaction by the synthesis of a specific set so-called heat shock proteins, HSPs (SCHLESINGER et al. 1982, LINDQUIST 1984, MUNRO & PELHAM 1985). This uniform reaction would indicate a crucial role for the heat shock proteins in the response of organisms to heat stress. Though there is so far no conclusive evidence for any specific function of the HSPs (LINDQUIST 1984), there are suggestions that HSPs respond to the need for increased protein catabolism within the cell (ANDERSON et al. 1982).

In contrast to vegetative tissue (SCHLESINGER 1982, MUNRO & PELHAM 1985), the reactions of pollen to heat stress has been rarely investigated (MASCARENHAS & ALTSCHULER 1983, COOPER et al. 1984). This is remarkable since high temperatures during pollen maturation and pollination adversely affect seed yield (MASCARENHAS 1975, 1984).

Pollen of *Tradescantia* respond to heat treatment by adaptation, but do not synthesize HSPs (MASCARENHAS 1984, XIAO & MASCARENHAS 1985). This suggests that pollen do react to high temperatures, however, in a different way than vegetative tissue.

Alterations in qualitative protein synthesis will be caused either by expression of newly formed mRNA or by changes in the physiological state of the pollen. If pollen react to heat stress in a uniform manner, like tissue of many organisms, alterations in expression and/or physiological state may be expressed by the

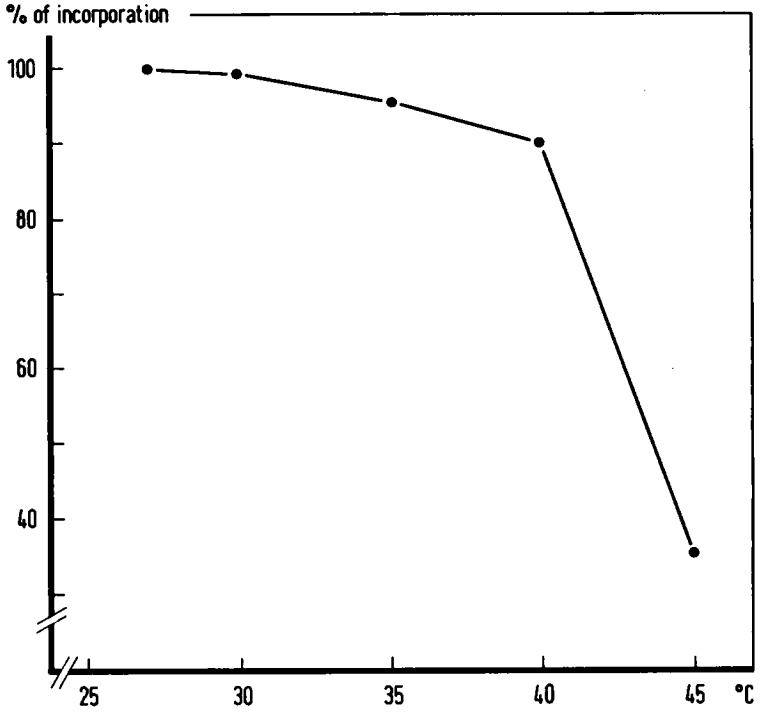

Fig. 1. The incorporation, in percent of uptake, of [35-S] methionine in pollen from *Lilium longiflorum* at different temperature treatments. Pollen were wetted, temperature treated for 10 min and subsequently incubated with 3.7 kBq at 27°C for 30 min. Values are the average of four different experiments. The minimum activity measured was 50 Bq.

synthesis of specific proteins. Binucleate pollen synthesizes proteins immediately after imbibition (LINSKENS et al. 1970) and continues during the processes of germination and pollen tube growth (KNOX 1984), but have a lag period of 2 hours for pollen tube formation. This enables the study of heat stress during two physiological different periods and makes it possible to test the former hypothesis. A comparison of the synthesis of the individual proteins during these periods in a mono- and dicotyl species may provide indications for the existence of an uniform reaction of pollen to heat stress. In the present study protein synthesis has been investigated for pollen of *Petunia hybrida* and *Lilium longiflorum* during the two periods, the lag phase and growth of pollen tubes, with in vitro incubation at different temperatures. The results are compared with protein synthesis in vegetative tissue of *Petunia hybrida* at the same temperatures.

2. MATERIALS AND METHODS

2.1. Incubation of pollen

Pollen of *Petunia hybrida* and *Lilium longiflorum* were collected from greenhouse

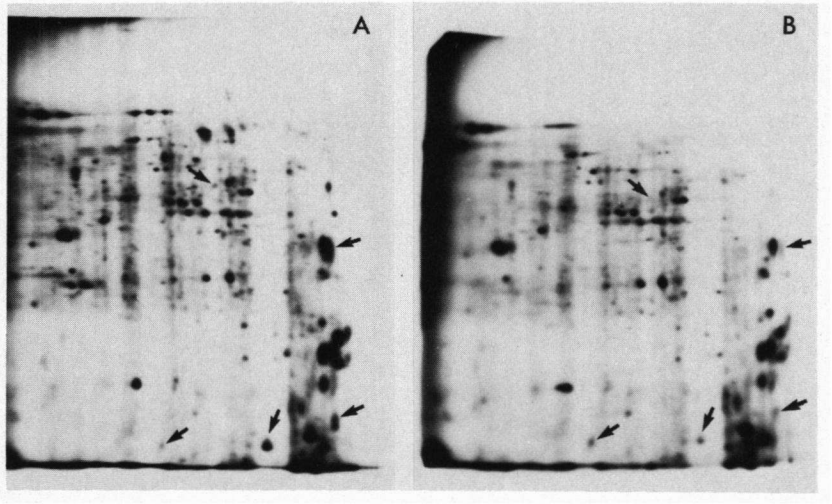

Fig. 2. Fluorogram of proteins synthesized in pollen from *Petunia hybrida* incubated with [35-S] methionine during 30 min immediately after the wetting period. A. Incubation at 27 °C of 2 mg pollen in 30 µl germination medium with 1.1 MBq. B. Incubation at 40 °C with 2.2 MBq. Others as for A. Marked spots indicate reproducible differences in the protein pattern as a result of the temperature treatment.

grown plants (VAN HERPEN 1981) and stored in small quantities at −20 °C in sealed vials. Before any treatment pollen was wetted: 2 mg pollen was consecutively adapted to room temperature, exposed to a relative humidity of 100% for 1 h and swollen in 30 µl germination medium (DICKINSON 1968) for 10 min. The concentrated pollen suspensions were either temperature treated for 10 min, followed by incubation for 30 min at 27 °C, or incubated at different temperatures for 30 min. Immediately before the incubation 300 µl germination medium containing labelled precursor was added. In a second series of experiments pollen was mixed with 300 µl germination medium after the wetting period and incubated at 27 °C for 2 hours. Subsequently this suspension was mixed with labelled precursor and incubated at different temperatures for 30 min. In this way growth of just formed pollen tubes takes place during this half hour period, since pollen of *Petunia* and *Lilium* form pollen tubes after two hours incubation under these conditions.

2.2. Extraction and analysis of proteins

Pollen: To estimate protein synthesis, incorporation of [35-S] methionine (> 30 TBq/mmol, Amersham) into proteins of pollen was measured. After incubation pollen were centrifuged at 50 × g for 30 s and washed 3 times with germination medium in 1 mM methionine. Proteins were solubilised with 60 µl buffer (10 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM PMSF, 2.3% SDS, 50 µg RNase/ml, preheated) (SCHRAUWEN 1985, O'FARREL 1975). Free amino acids (SCHRAUWEN & LINSKENS 1974), radioactivity (SCHRAUWEN 1985), separation and analyses

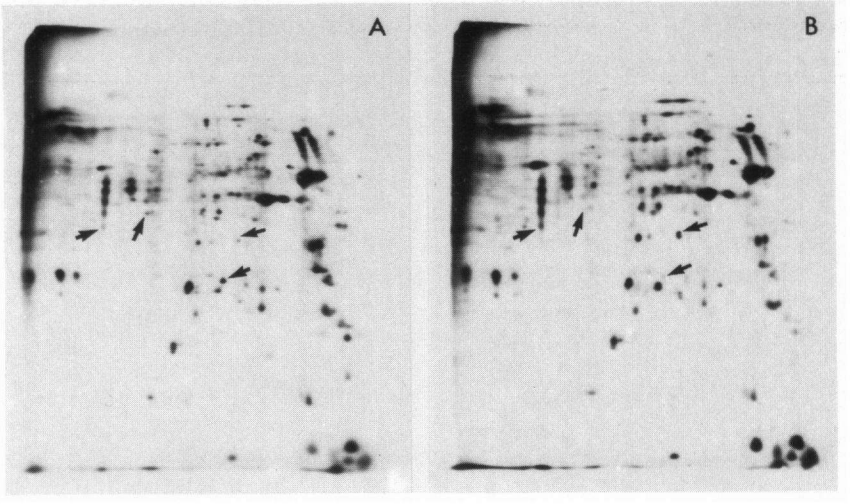

Fig. 3. Fluorogram of proteins of pollen from *Lilium longiflorum*. Conditions are the same as for *figs 2A* and *2B*.

of proteins (O'FARREL 1975, SCHRAUWEN 1985, BONNER & LASKEY 1974) were performed as described previously.

Vegetative tissue: Seeds of *Petunia hybrida* were desinfected in 2.5% NaOCl for 2 min, rinsed with tap water and incubated for 3 days in 0.1 mM $CaCl_2$ at 27 °C. Eighty root tips of 1 cm were cut and incubated in 2 ml MS-medium (COOPER et al. 1984) for 3 hours, then washed and incubated in 1 ml MS-medium at respectively 25 and 40 °C for 1 h. Subsequently 150kBq [35-S] methionine was added and the temperature treatment continued for 3 h (COOPER et al. 1984). The incubation was stopped by the addition of unlabelled methionine, (20 µl 0.1 M). Roots were washed with MS-medium in 1 mM methionine, frozen in liquid nitrogen and sonicated on ice after the addition of 30 µl buffer (20% glycerol, 10% 2-mercaptoethanol, 4% sodium dodecylsulfate, 0.005% bromophenolblue). This suspension was boiled for 3 min and centrifuged (LAEMMLI 1974). Aliquots were used for analyses as for pollen. Proteins from roots of maize were obtained according to COOPER et al. (1984) with adaptations as described above for *Petunia*.

3. RESULTS

3.1. Quantitative protein synthesis in pollen

Concentrated pollen suspensions were treated at either 27°, 35°, 40° or 45 °C for 10 min immediately after the wetting period. Subsequently the suspensions were adjusted to 2 mg pollen per 300 µl germination medium containing 3.7 kBk [35-S] methionine and incubated at 37 °C for 30 min. The uptake and incorporation of the precursor was measured. The incorporation of the label was related to the uptake at 27 °C, set at 100%. *Fig. 1* demontrates a drop at 45 °C. Heat

treatment of 30 min in stead of 10 min showed comparable incorporation of [35-S] methionine into proteins as in *fig. 1.*

3.2. Changes in individual proteins

Wetted pollen of *Petunia* and *Lilium* was mixed with germination medium and labelled precursor and incubated for 30 min at 27° and 40 °C respectively. The results of *figs. 2* and *3* show that within half an hour of protein synthesis a complete set of proteins was synthesized in pollen from both *Petunia* and *Lilium*. The spots on the fluorogram are scattered between molecular weights of 110 and 12 kDalton and isoelectric points of pH 4.5 and 8.6.

The patterns of proteins synthesized in *Petunia* and *Lilium* are different with regard to molecular weights and isoelectrical points (*figs. 2* and *3*). Within one species the pattern of scattered spots at 40 °C (*figs. 2B* and *3B*) was almost similar to the pattern at 27 °C (*figs. 2A* and *2B*). The changes are different for *Petunia* and *Lilium*

Wetted pollen from *Petunia* and *Lilium* mixed with germination medium and incubated for 2 h at 27 °C initiate a pollen tube. Incubation in the following half hour with [35-S] methionine respectively at 27° and 40 °C resulted in incorporation of the precursor. The protein pattern obtained in this pollen tube growth period were similar to those of *figs. 2A* and *2B* for *Petunia* and *figs. 3A* and *3B* for *Lilium*. Accordingly similar changes in these protein patterns could also be demonstrated.

In rootlets from *Petunia* the protein synthesis is completely different at 27° and 40 °C (*fig. 4*), synthesis of protein at 40 °C being comparable to HSPs in maize. Proteins synthesized at 40 °C in roots from *Petunia* with molecular weights and isoelectrical point identical to those in maize for the 18 kDalton area are marked with an arrow.

4. DISCUSSION

The viability of pollen from *Petunia* and *Lilium* was adversely affected by temperatures of 45 °C. Temperatures up to 40 °C did not cause significant changes in quantitative protein synthesis. For this reason the influence of temperature treatment on the synthesis of individual proteins was tested at 40 °C.

Comparison of the fluorograms of *figs. 2* and *3* demonstrate that the synthesized proteins in pollen from *Petunia* and *Lilium* are different with regard to molecular weights and isoelectric points. These differences have to be attributed to the genetical background of these species.

In roots from *Petunia* proteins synthesized at respectively 27° and 40 °C are entirely different. However, the change is uniformly directed towards a specific group of proteins, HSPs, as for other organisms (SCHLESINGER et al. 1982, LINDQUIST 1984, MUNRO & PELHAM 1985). Even in two different species, i.e. roots of *Petunia* and maize, the formed HSPs have identical molecular weights and isoelectric points as shown in *fig. 4* for the proteins of the 18 kDalton area by arrows.

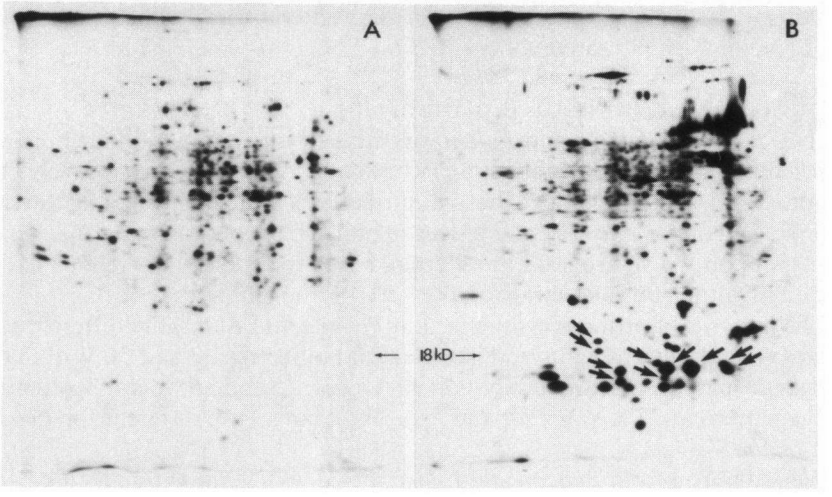

Fig. 4. Fluorogram of proteins of roots from *Petunia hybrida*. After preincubation of 3 h at 25°C and incubation 1 h at 25°C (A) resp. 1 h at 40°C (B); 150 kBq [35-S] methionine was added. The incubation was continued for 3 h.

The changes in the proteins synthesized in pollen, which appeared as a result of the heat treatment, are variable. In response to heat treatment some proteins are increased in quantity or are even newly synthesized, while others diminish or disappear (*figs. 2* and *3*), as for pollen from maize (COOPER et al. 1984). However, the changes which could be demonstrated are different for pollen from *Petunia* and *Lilium*. As a result of pollen treatment at elevated temperatures neither a drastic change in the total protein pattern appeared nor the synthesis of HSPs could be shown (*figs. 2, 3* and *4*). This is in agreement with the reaction of pollen from *Tradescantia* to heat stress (MASCARENHAS & ALTSCHULER 1983, XIAO & MASCARENHAS 1985) but in contrast to vegetative tissue (SCHLESINGER et al. 1982).

This indicates that in mature pollen no mRNA synthesis takes place and that the protein machinery is present in a complete form which newly has to be activated before pollen germination. The latter hypothesis is supported by the fact that the proteins synthesized during the two periods of physiological development were similar, for 27 ° as well as for 40 °C. This hypothesis is furthermore supported by the absence of influence of transcription inhibitors (TUPY 1982) at the stage of pollen tube growth. Therefore, the change in the synthesized proteins in pollen at elevated temperatures are presumably not encoded from new mRNA, as in vegetative tissue, but may be caused in the final stages of the proteins synthesis.

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