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IN VITRO POLLEN GRAIN GERMINATION AND STARCH CONTENT IN SPECIES WITH DIFFERENT REPRODUCTIVE CYCLE I. LYCOPERSICUM PERUVIANUM MILL.

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SUMMARY

Lycopersicum peruvianum, which has a long flowering period and several reproductive cycles every year, has mature pollen without starch.

During in vitro germination starch is synthesized in June only, when, on the contrary, tubes show a half of the length reached in September. These differences are explained on the basis of environmental factors.

1. INTRODUCTION

Carbohydrates constitute the major fraction of dry matter in most pollen. NIELSEN et al. (1955) report that carbohydrate levels vary with species. Gymnosperm pollen, for instance, is generally low in total carbohydrates (STANLEY & LINSKENS 1974). In angiosperms, trinucleate pollen has a variable amount of carbohydrates: in *Zea mays* the amount is high, while in *Beta* and *Ambrosia* it is reported to be low (LUNDÉN 1954). Binucleate pollen may also be either high or low in carbohydrate content, indicating no correlation between the number of nuclei and the content of carbohydrates (STANLEY & LINSKENS 1974).

Starch content is also reported to be highly variable: from as much as 13% of the dry weight in *Typha latifolia*, down to 1,4% in *Lilium auratum*, 2,6% in *Pinus thunbergii* (HUGEL 1965).

Moreover starch presence in mature pollen is reported to be a characteristic of families (BAKER & BAKER 1979). In this respect these authors report results concerning 133 families among which 20 are starchy, 85 starchless and 28 mixed.

In cultivated species starch may be either present in variable amount or absent in different cultivars of the same species (PACINI et al. 1978; FORINO & AVANZI 1981). In plants with a long flowering period starch presence is also variable with respect to environmental parameters (FRANCHI et al. in press).

The aim of these two joint papers is to analyse starch behaviour during in vitro germination in two species with different characteristics:

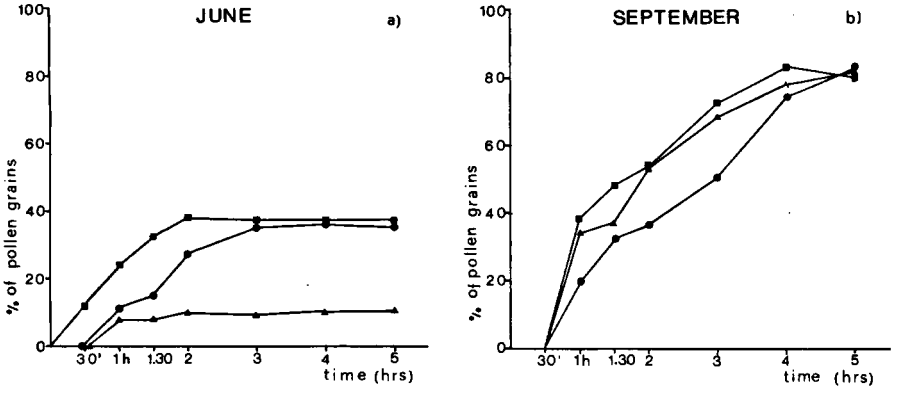


Fig. 1. *Lycopersicum peruvianum* pollen grains: germination percentage at three sucrose concentrations ■—■ 5%, ●—● 10%, ▲—▲ 15%.

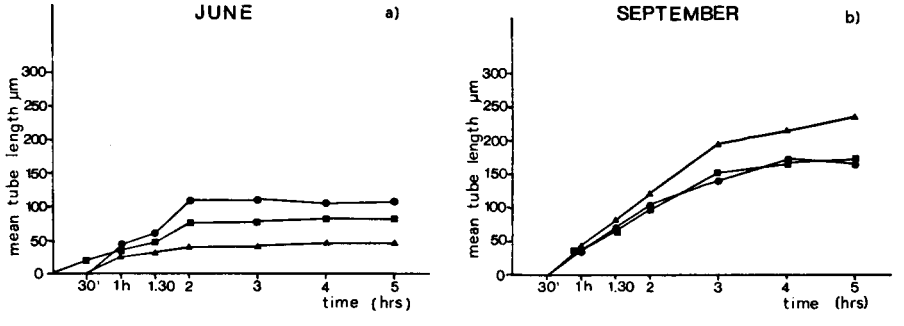
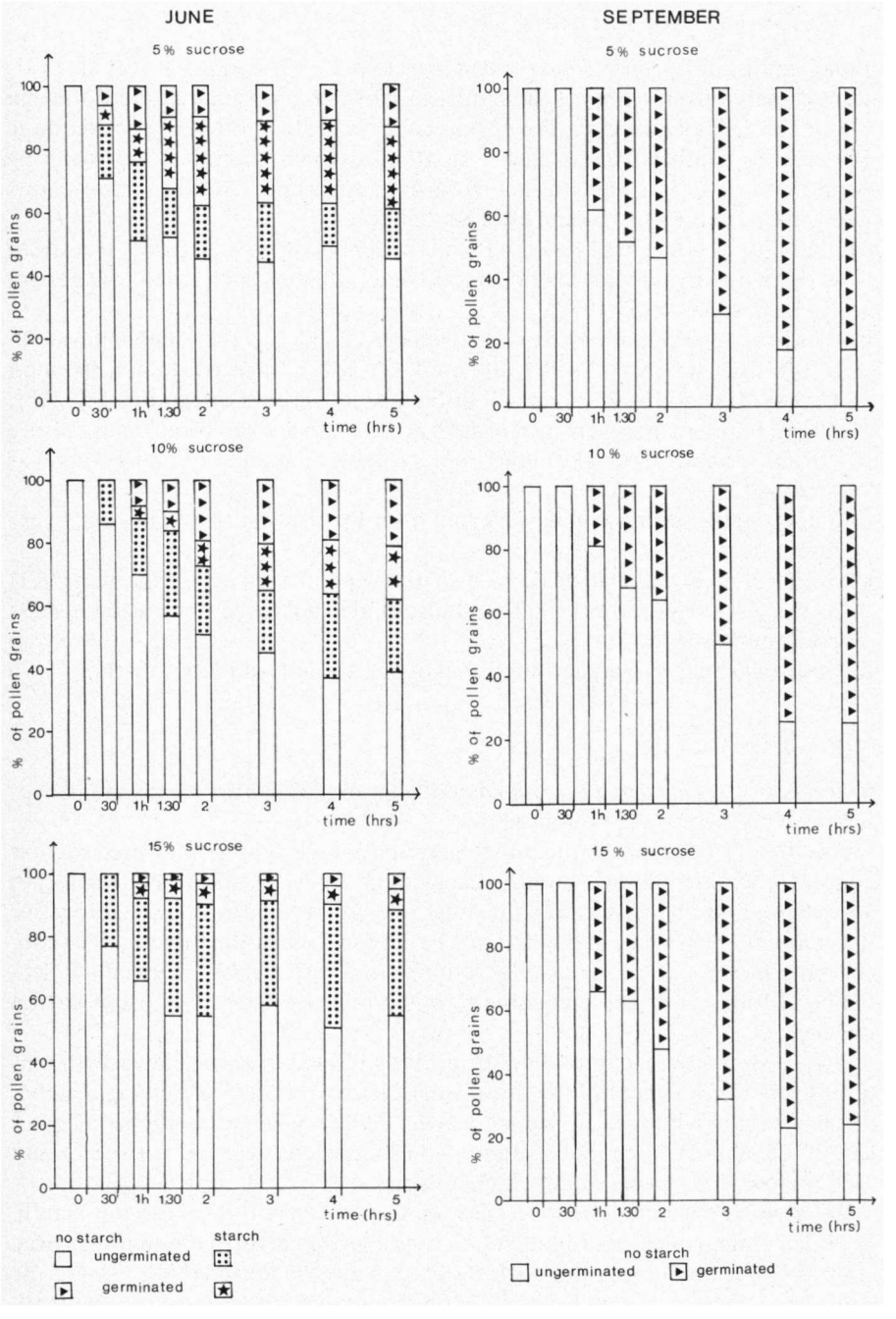


Fig. 2. *Lycopersicum peruvianum* pollen grains: mean tube length at three sucrose concentrations.
a(June: ■—■ 5%, standard error ranging from 1 to 2.7; ●—● 10%, S.E. 0.3 to 3.5; ▲—▲ 15%, S.E. 0.7 to 2.4.
b) September: ■—■ 5%, S.E. 1.2 to 3.1; ●—● 10%, S.E. 1.1 to 2.6; ▲—▲ 15% S.E. 1.4 to 3.0.

a) *Lycopersicum peruvianum* Mill., with no starch at maturity and a long flowering period,
b) *Malus domestica* Borkh. (two cultivars), with starch presence in different amounts in mature pollen grains and a short flowering period.

Lycopersicum peruvianum has no starch at maturity, but starch (stainable in black with an IKI test and birefringent under polarized light) is hydrolized just before pollen grains ripen (PACINI & JUNIPER 1984) with PAS positive substances persisting in mature grains.

A previous analysis of tube elongation in vitro revealed differences in tube growth when sampling was effected at different times during the flowering period. Therefore, it seemed interesting to test germination percentage together with mean tube length and starch content.



Figs. 3, 4. *Lycopersicum peruvianum* pollen grains: histograms of starch presence and distribution in germinated and ungerminated grains. Dots mean starch present. 3) June, 4) September.

2. MATERIALS AND METHODS

Pollen grains of *Lycopersicum peruvianum* clone $F_{13}(S_{12}S_{13})$ (CRESTI et al. 1977) were gathered from plants of the same age growing in open air at the Botanical Garden of Siena University. Pollen was collected at two different periods, June 1983 and September 1983, namely a month after the beginning of the flowering period and before its end. In fact, from our experience, we know that plants living in open air do not bloom after September.

a) Viability of pollen was tested with lactophenol cotton blue 0.08% (FRANCHI et al. in press) and with the fluorescein diacetate method (HESLOP-HARRISON & HESLOP HARRISON 1970).

b) The in vivo germination test was effected up to 5 hrs in the following 3 mediums H_3BO_3 100 mg/l, $MgSO_4$ 200 mg/l, $Ca(NO_3)_2$ 300 mg/l, KNO_3 300 mg/l (SARFATTI et al. 1974) added to 3 different sucrose concentrations (5%, 10%, 15%). Experiments were performed in Petri dishes, at room temperature (18–20 °C) and under the same indirect light. A sample of 500 grains was analysed.
Tube length was measured every half hour for the first two hours and every hour after.

c) Starch content was evidenced with an iodine potassium iodide solution (IKI) as suggested by JENSEN (1962) and also under polarized light using a Zeiss photomicroscope.

d) Statistical analysis was accomplished by the "Standard Error" method.

3. RESULTS

Viability of *Lycopersicum peruvianum* pollen was 100% both in June and in September with the two methods used.

Fig. 1a and *1b* compare the pollen germination percentages at three sucrose concentrations in the two months considered. In *fig. 1a* the curve related to 15% sucrose is much lower than the other two. On the contrary, no significative difference in the pattern and in the rate of germination at the three sucrose concentrations seems to be detectable from *fig. 1b* where results are given for September. Moreover, in September the germination percentage is almost twice that in June.

Fig. 2a shows tube length of pollen grains collected in June. The growth pattern is very much similar at the three sugar concentrations, 10% giving a higher value in length. However, in September (*fig. 2b*) the pollen tube reaches a higher length, i.e. almost twice that in June. No difference is detected between 5 and 10% sucrose, 15% giving slightly higher values.

As far as starch content is concerned, mature pollen grains never show starch. In pollen grains collected in June, starch is synthesized before germination starts. A class of germinating grains with starch can also be detected (*fig. 3*). On the contrary, pollen grains collected in September never show starch in the cytoplasm, neither in ungerminated grains, nor in germinating and germinated grains (*fig. 4*).

4. DISCUSSION

Nowadays there is a consensus of opinion that pollen tube growth "in vivo" consists of two phases, the first autotrophic and the second heterotrophic. During the latter in compatible pollination, polysaccharidic reserves in the pistil are mobilized (HERRERO & DICKINSON 1979).

ROGGEN (1967) also demonstrated the induction of enzymes for carbohydrate metabolism in the vicinity of the growing pollen tubes. Vice versa, in the case of incompatible pollination, reserves are not mobilized (HERRERO & DICKINSON 1979).

Moreover, CRESTI et al. (1980) reported that, for binucleate pollen grains of *Lycopersicum peruvianum*, both germination "in vivo" and pollen tube organization are similar to what has been observed "in vitro". The only exception is that "in vivo" the generative cell divides, forming the gametes, as soon as it penetrates into the tubes, while "in vitro" the generative cell moves into the tube, but its division has never been observed. Because up to now no definitive data are available on the "in vitro" growth phases of *Lycopersicum peruvianum* pollen, this lack of the second haploid mitosis can not be clearly explained (on this topic see also MULCAHY & MULCAHY 1983).

It is also remarkable that the patterns of germination and of tube growth are the same when observed at the three sucrose concentrations in both periods. The exception observed at 15% sucrose, for the pollen collected in June, can be explained on the basis of the osmotic inhibition of a high concentration of sugar in the growth medium (MATHIEU et al. 1983).

The differences detected between tests effected in June and September can be explained on the basis of environmental factors. Pollen tube growth depends on the temperature regime during the flower development, high temperature causing a greater length of the tubes than the low temperature regime (VAN HERPEN & LINSKENS 1981). This would explain that in our results pollen grains collected in September from flowers developed in late August have a longer tube during in vitro germination.

Temperature during maturation also affects carbohydrate content (STANLEY & LINSKENS 1974). *Pelargonium* pollen, matured at higher temperature, contained less starch than that maturing at lower temperature (SEARS & METCALF 1926). On the other hand low light intensities during microsporogenesis can reduce sugar pools in anthers (GOSS 1971). Although mature *Lycopersicum peruvianum* pollen never shows starch, grains collected in June, under a lower temperature regime, and a higher light intensity, might have a higher carbohydrate content to explain starch synthesis which occurs just before and during germination (*fig. 3*).

The lack of starch formation detected in September could mean a faster metabolism which may also justify the higher germination percentage and the longer tube growth.

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