Kinetics and hydrodynamics of Agapanthus umbellatus pollen-tube growth: A structural and stereological study

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Summary. In vitro pollen germination of Agapanthus umbellatus follows a logistic-type curve. It has a lag phase, which corresponds to pollen grain (PG) hydration, followed by an exponential phase – initial pollen-tube (PT) growth. The lag phase is characterized by an increase of about 40% in the volume of the PG as a result of the hydration process. During the exponential phase the PT emerges, and 40 min later it possesses an ultrastructural organization with a typical two-layer wall and four well-defined zones: the apical, sub-apical, nuclear and vacuolar zones. In this period the material transported by the Golgi vesicles seems to be mostly incorporated into the pollen-tube wall (PTW). Stereological analysis showed that the increase in tube volume is correlated with the increase in the vacuolar compartment at the PG level. The decrease in the relative volume occupied by the mitochondria, generative cell and vegetative nucleus in the PG suggests that these organelles move to the PT. A correlation between the disappearance of lipid droplets in the lag phase and the metabolic reactions that take place during hydration is suggested.

Key words: Pollen tube – Kinetics – Hydrodynamics – Agapanthus sp

Introduction

Pollen germination and PT development have been the subjects of numerous reports, many of them the results of ultrastructural studies (Cresti et al. 1977; Clarke and Steer 1983; Cresti et al. 1988, and references therein). Most of these studies were performed under in vitro conditions, which are very different from those present along the stigma and style in vivo. The artificiality of in vitro systems could be responsible for the changes observed in growth rates, hydrodynamic patterns and ultrastructural features (reviews by Heslop-Harrison 1987 and Steer and Steer 1989). In the particular case of the hydrodynamics of PT growth, the results obtained with in vitro systems must be carefully analyzed because the moisture content of the growth medium is entirely different from that of the stigma and style.

Despite several differences at the physiological level (review by Heslop-Harrison 1987), the ultrastructure of germinating pollen and early tube growth seem to present a common pattern in a wide variety of species (Cresti et al. 1977; Uwate and Lin 1980; Miki-Hirosige and Nakamura 1982; Picton and Steer 1985; Cresti et al. 1985). However, less data are available concerning the kinetics and hydrodynamics of germinating pollen.

In this paper we present a study on the kinetics and hydrodynamics of germinating pollen. The results are discussed taking into account the artificiality of in vitro systems when compared with in vivo conditions and the possible existence of internal and/or external growth conditioning factors.

Materials and methods

Germination conditions

Mature PGs gathered from the anthers of Agapanthus umbellatus L’Her. were sown on agarified (0.7%) medium described by Brewbacker and Kwack (1963) with 1% sucrose (pH 5.5). Germination was performed at 25°C.

Preparation of samples

PGs were collected at 0, 20, 30, 40 and 60 min after sowing. The different samples were fixed for 2 h in 2.5% glutaric dialdehyde and 2.5% freshly depolymerized paraformaldehyde in 0.05 M cacodylate buffer, pH 7.0, at 4°C, post-fixed in 1% buffered osmium tetroxide for 2 h at room temperature and dehydrated by the acetone series. For transmission electron microscopy the material was embedded in Epon-Araldite (Mollenhauer 1964). Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and observed with a JEOL 200CX at 80 kV. Semithin, non-stained sections were observed with a Leitz Dialux optical microscope with DIC optics. For scanning electron microscopy the material was
Fig. 1. Hydrated pollen grain. It presents an oval shape with two possible aperture sites (*). The exine is very thick and porous. Bar: 10 μm. × 3000

Figs. 2 and 3. PT emergence with a detailed view of the pore region. The shape and dimensions of the tube are already defined although the tip slightly changes in the longest tubes. Bar: 10 μm. × 2000 (Fig. 2); × 3200 (Fig. 3)

Fig. 4. Apical region of a PT that is approximately 125 μm long. The paraboloid shape of the apex is evident. Bar: 10 μm. × 5000

freeze-dried, coated with gold and observed with a JEOL JSM T 220.

Stereology and kinetics

The protocols of Williams (1977) and Toth (1982) were used in the stereological study. Five different samples of PGs were analyzed (unhydrated pollen, germinating pollen 20, 30, 40 and 60 min after sowing). Seven blocks were randomly selected from each sample, and an ultrathin section from each block was analyzed. A mean of five photographs at a magnification of × 12000 were randomly taken from each section, which corresponds to an area of approximately 5.77 × 10³ μm². The analysis of the micrographs was performed with a digital counter using a coherent multi-purpose system (Weibel 1969). The cellular structures considered in this study were vacuoles, mitochondria, lipid droplets, vegetative nucleus and generative cell. The values presented correspond to the relative volume (expressed as percentage) of the ratio between the volume of each structure to the total volume of protoplasm.

PG and PT total volumes were determined from SEM microphotographs and semithin sections. The hydrated grain was considered to be an ellipsoid (Fig. 1) and the tube to be a cylinder ending with a paraboloid segment (Fig. 4). Final volumes were calculated according to the formulas:

ellipsoid volume: \( V_1 = \frac{4 \pi abc}{3} \)

cylinder volume: \( V_2 = S_h g \)

paraboloid segment volume: \( V_3 = \frac{\pi r^2 F}{h} \)

where \( a, b \) and \( c \) are the three axis (defined from the center) of PG, \( r \) is the PT radius, \( h \) is the apex length and \( g \) is the PT length (without the apex). Unhydrated PG volume was calculated according to the formula \( V_1 - V_4 \) where \( V_4 \) is the volume increase during