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SHORT COMMUNICATION

Pulsatory growth of pollen tubes: investigation of a possible relationship with the periodic distribution of cell wall components

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SUMMARY

Time-lapse video microscopy has revealed that pollen tubes may show pulsatory growth and transient irregularities in the shape of their tip. The question has been addressed whether the earlier reported periodic, band-like distribution of certain cell wall components could be correlated to fluctuations in the growth rate. Pollen tubes of *Nicotiana tabacum, Petunia hybrida* and *Gasteria verrucosa*, known to show regular bands in their cell wall, displayed pulsatory growth, whereas pollen tubes of *Campanula sarmatica* and *Lilium longiflorum*, which have a rather homogeneous cell wall pattern, showed a steady growth. These findings are consistent with the hypothesis that pulsatory growth and band-like cell formation are coupled. However, the idea was not further supported by the observations made on *Canna indica* and *Zea mays*, which showed band-like distribution of pectin but a very steady growth.

Key-words: arabino-galactan proteins, cell wall, pectin, pollen tube growth, video microscopy.

INTRODUCTION

Pollen tube growth is assumed to be regulated by a number of internal factors, including the turgor pressure, the uptake of calcium ions, the production and fusion of Golgi vesicles, and the plasticity of the apical cell wall (Heslop-Harrison 1987; Steer & Steer 1989; Battey & Blackbourn 1993; Mascarenhas 1993; Derksen *et al.* 1995). The growth process requires a high level of coordination between the various factors (see also Cosgrove 1993). It may be questioned whether the entire process is perfectly orchestrated or not. In this view, we first screened pollen tubes on irregularities in the growth

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pattern, and next investigated the possibility that the periodic, band-like distribution of certain wall components in pollen tubes, i.e. arabino-furanosyl residues (Harris *et al.* 1987), arabino-galactan proteins (Li *et al.* 1992; Plyushch *et al.* 1995) and pectin (Li *et al.* 1994), could be related to fluctuations in the growth. This hypothesis was tested by analysing the extension characteristics of single pollen tubes of species presenting either the reported band-like pattern (*Nicotiana tabacum, Petunia hybrida, Zea mays, Canna indica*), or a rather homogenous distribution of their wall components (*Campanula sarmatica* and *Lilium longiflorum*), or a heterogenous pattern (*Gasteria verrucosa*).

MATERIALS AND METHODS

Pollen culture

Fresh pollen of *C. sarmatica* Ker., *L. longiflorum* Thunb., *C. indica* L., *N. tabacum* L., *P. hybrida* Vilm. and *Z. mays* L. was collected and examined at the University of Siena (Italy), whereas pollen of *G. verrucosa* was harvested and studied at the Agricultural University of Wageningen (The Netherlands). Pollen of the first six species was rehydrated for 1–2 h in a moist chamber and then allowed to germinate for 1–2 h in standard Brewbaker & Kwack (1963) liquid medium supplemented with an optimal concentration of sucrose (10% for the first three species, 12% for *N. tabacum* and *P. hybrida*, and 15% for *Z. mays*) as previously described (Li *et al.* 1992 and 1994). In order to accurately screen the growth pattern of single tubes, following germination, the pollen was transferred to a slide chamber, and spread in a thin layer of semi-solid medium (about 6 mg Grade VII low temperature gelling agarose, Sigma, per ml standard medium) that was covered with a drop of liquid medium and maintained open for good oxygen supply (Miller *et al.* 1992). Examination started within 1 h after transfer to this agarose medium and was carried out at stable room temperature under moderate illumination. *G. verrucosa* pollen was cultured in a line mass in agar medium at 21–22°C, exactly as described in Plyushch *et al.* (1995), and examined within the first 3 h of culture.

Growth measurement by means of video microscopy

The pollen tube growth of the six species cultured in Siena, was monitored using an inverted Nikon Diaphot microscope equipped with a 60 × objective and 4 × camera ocular, a Grundig FA76 video camera, and Grundig monitor (final magnification: 2250 ×). Calibrations were made by projection of a Zeiss object micrometer with 10 μm unit divisions. Depending on the species, i.e. tube size (diameter varying between 7 and 20 μm), growth rate and expected distance between cell wall bands, the image of the pollen tube tip was traced at intervals of 25, 50 or 100 s (Table 1) on transparent sheets attached to the surface of the monitor, for a total of 20–40 intervals per tube, in at least 10 tubes per species. The increase in length over each time interval was measured with a calibrated ruler. For *G. verrucosa*, a Hitachi Microcolor camera and a VHS VT M740E video recorder were used to observe the growth.

As an approach to express the relative growth fluctuation, the following values were determined for all single pollen tubes except those of *G. verrucosa*: (i) the average increase in length per interval, also determined over the first 10 intervals of measurement ($\bar{x}_{\text{average}}$; unshown); (ii) the standard deviation corresponding to the increase in length per interval (as defined here above) over the first 10 intervals of measurement (SD_i,

Table 1. Growth rate, growth pattern and the periodic deposition of certain cell wall components in pollen tubes

Species	Interval (s)	Average growth rate[a] ± SD (number of tubes) (μm min^{-1})	Growth pattern in *n* tubes[b]			Periodic deposition of wall components	
			Steady	Weakly Pulsatory	Pulsatory	Pectin (1,2)	Arabinogalactan protein (3,4)
C. sarmatica	50	2·5 ± 0·9 (14)	10	0	0	−	
L. longiflorum	50	4·3 ± 0·6 (11)	13	0	0	−	
C. indica	25	14·4 ± 3·6 (10)	10	0	0	+	
N. tabacum	50	1·8 ± 0·6 (16)	12	4	1	+	+
P. hybrida	100	0·6 ± 0·2 (11)	0	4	7	+	
Z. mays	50	4·3 ± 0·6 (11)	12	0	0	+	
G. verrucosa	50	2·3 ± 0·9 (20)	10	0	10		+

[a]Measured over the entire period of observation.
[b]Based on 20–40 interval measurements in each pollen tube.
+present; − absent.
(1) Li *et al.* 1994.
(2) Li *et al.*, unpublished results.
(3) Li *et al.* 1992.
(4) Plyushch *et al.* 1995.

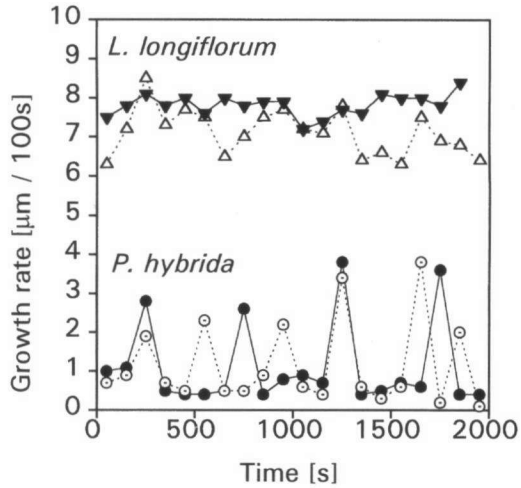

Fig. 1. Growth rate in two pollen tubes of *Lilium longiflorum* (△ and ▼), and two pollen tubes of *Petunia hybrida* (○ and ●) measured at regular interval. The lily pollen tubes show a rather steady growth with some slight fluctuations or minor amplitude with respect to the average growth rate. The *Petunia* pollen tubes show a pulsatory growth. The growth rate during the pulses is several times higher than that during periods of slow growth.

unshown); (iii) the ratio of the two above values times a factor 100, called here coefficient of variation (CV), summarized as $CV = 100\ SD_i/\bar{x}_{average}$ (shown in Fig. 3).

Fine fluctuations in growth rate in *L. longiflorum* and *P. hybrida* were further visualized by recording a narrow longitudinal section through the apex of a number of pollen tubes, at time-lapses of 5 and 15 s, respectively, according to the principal described by Mineyuki & Gunning (1988), using either the kymograph function of the image processing system Image-1/AT (Universal Imaging Corporation, Media) or the image processing software of a MRC-500 Bio-Rad Confocal Laser Scanning Microscope.

RESULTS

There was a large variation in the growth rate of single pollen tubes of different species, and among tubes of the same group (Table 1; Fig. 1), which is common for materials with different genotypes (Johnson *et al.* 1976; Walsh & Charlesworth 1992; Mascarenhas 1993). In particular, pollen tubes of *C. indica* were fast growing, showing an average growth rate of 14 µm min^{-1}.

All 35 examined pollen tubes of *C. indica, L. longiflorum*, and *Z. mays* presented, as individuals, a rather steady extension rate over the period of examination (Table 1; Fig. 2A). For these species the average CV among 25 or 50 s intervals was lower than 15 (Fig. 3). Some variations in growth rate occurred, but they were minor with respect to the average growth rate (Fig. 1), as was also evident from the kymograph images (Fig. 2A). Conversely, pollen tubes of *C. sarmatica* showed a less regular growth pattern with progressive fluctuations (Table 1), reflected in the average CV of 21 (Fig. 3). Pulsatory growth (Figs 1 and 2B) was revealed most explicitly in pollen of *P. hybrida* (the mean CV was 50) and *G. verrucosa*, and less prominently in *N. tabacum* (Table 1). In

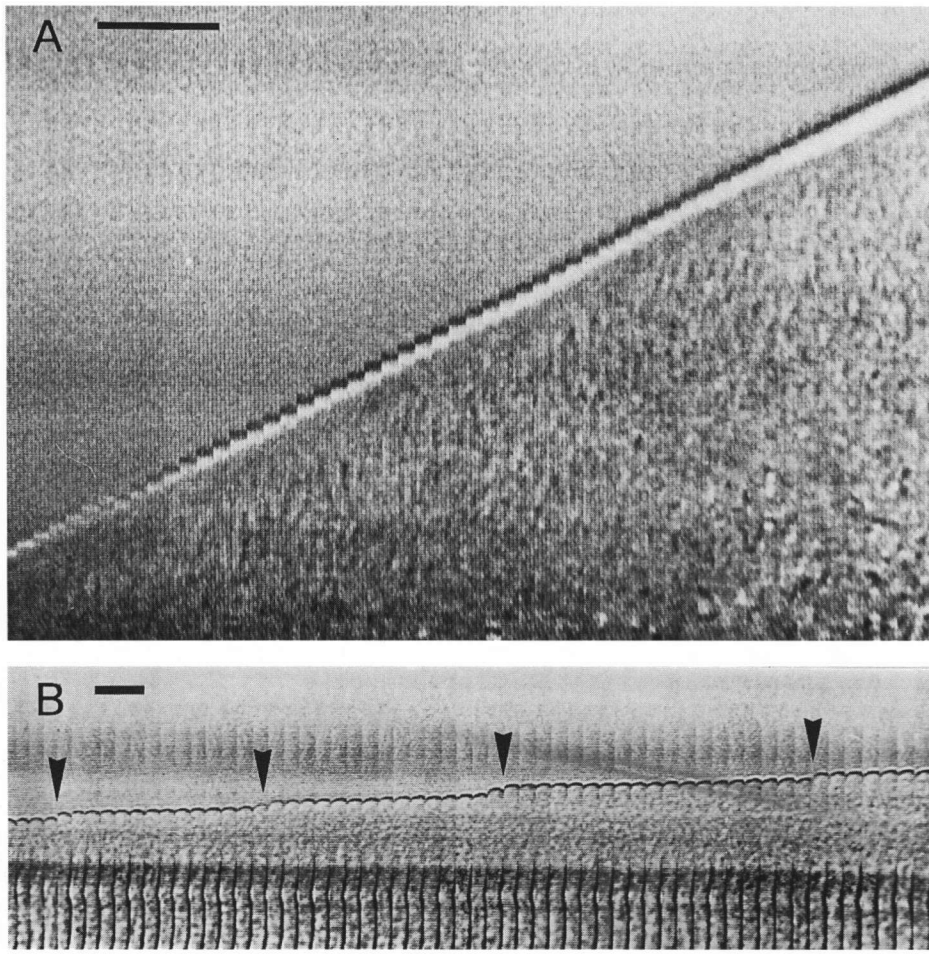

Fig. 2. Kymograph time-lapse video imaging of growing pollen tubes of *Lilium longiflorum* (A) and *Petunia hybrida* (B). The tube shown in (A) had a steady growth (the time-lapse between two images was 5 s; bar = 5 μm), whereas the tube shown in (B) showed pulsatory growth (the arrows indicate the pulses of growth; the time-lapse between two images was 15 s; bar = 20 μm).

P. hybrida, alternations were measured between a state of very slow and apparently steady growth of less than 1 μm min^{-1}, which extended for several minutes, and a phase of sudden rapid growth that lasted for about 20 s (corresponding to an extension rate of up to 12 μm min^{-1}). In this species, the distance between two areas related to subsequent periods of pulsatory growth was about 4–6 μm. In *G. verrucosa*, pulsatory growth occurred with a higher frequency (about each 25 s) than in *P. hybrida*. Periods of slow growth were correlated with the formation of translucent ring-like constrictions in the tube.

Except for *G. verrucosa*, the shape of the expanding pollen tube tip was in the other species commonly uniform and symmetrical. However, the following modulations in the growth mode were sometimes observed in all species: (a) a continuous asymmetrical expansion of the tip resulting in the curvature of the tube; (b) a well-balanced alternate tip expansion on different flanks, ultimately resulting in the formation of a straight tube;

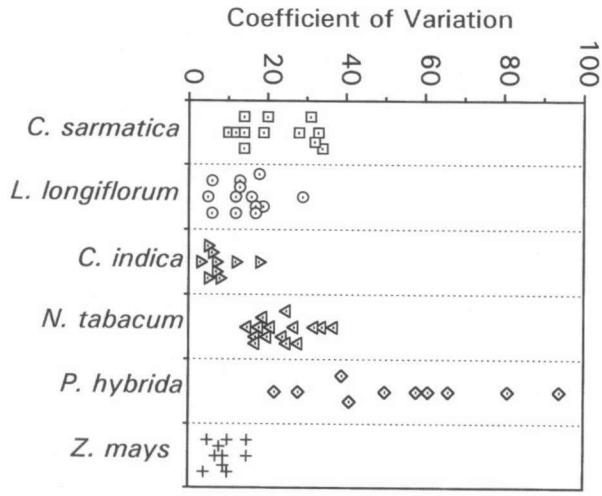

Fig. 3. Coefficient of variation (CV) of the increase in length of pollen tubes from six species, determined over the first 10 intervals of measurement. $CV=(100)\ (SD)\div(\bar{x}_{\text{average per interval}})$. Note that some data points in the plot have been spread out over the width of the column for a clearer display.

(c) tip expansion focused in the middle of the dome followed by the gradual broadening of the tube tip, eventually giving rise to a straight tube.

DISCUSSION

The main conclusions that can be drawn from this study are that single pollen tubes may show important fluctuations in their growth rate over time, and that the shape of the pollen tube tip may show transient irregularities. Fluctuations in growth rate were most obvious in *P. hybrida, N. tabacum* and *G. verrucosa*, where pulsatory growth was observed: a phase of steady slow growth lasting for a half minute to a few minutes alternated with a relatively short period (about 20 s) in which the extension rate was several times higher than during the phase of slow growth. With the exception of the intermediate periods of steady growth, the pulsatory growth observed in this study resembles the pattern consisting of continuous oscillations which Tang *et al.* (1992) measured in *Aloe zebrina*. Regarding pulsatory growth, we postulate that the turgor pressure within the pollen tube gradually increases during the minutes of slow growth, and that when the force applied on the apical cell wall surpasses a certain threshold, the tip region rapidly expends. This process in turn leads to the decrease of turgor pressure and the next period of slow growth starts. Other factors that are expected to contribute to pulsatory growth might be a periodic relaxation of the cell wall (Cosgrove 1993) or temporal variations in the production and fusion of vesicles.

In the other species examined in this study, the growth process was rather steady, although small fluctuations could be noticed too, suggesting the presence of not exactly coordinated underlying feedback mechanisms.

Pulsatory growth was only observed in the three species (*P. hybrida, N. tabacum* and *G. verrrucosa*) which also show periodicity in the distribution of certain cell wall components (Li *et al.* 1992; Li *et al.* 1994; Plyushch *et al.* 1995). In *P. hybrida* the distance between two adjacent bands of pectin sheath labelled by monoclonal antibody

JIM 5 directed against polygalacturonic acid (Li *et al.* 1994 and unpublished data), was of the same order of magnitude as the distance in the tube corresponding to two subsequent periods of pulsatory growth (4–8 μm vs. 4–6 μm). In *G. verrucosa*, fluctuations in growth were correlated with the appearance of constrictions and translucent bands in the wall. These structures colocalize with a band-like labelling pattern of monoclonal antibody (JIM 8) directed against arabinogalactans (Plyushch *et al.* 1995). In the above three species, the hypothesis that growth fluctuations can contribute to the formation of band-like wall patterns is plausible. Also, the findings ir *L. longiflorum* and *C. sarmatica*, which both show a homogeneous distribution of pectin (Li *et al.* 1994) and a steady or slowly fluctuating growth, are compatible with the concept that growth behaviour and the pattern of cell wall deposition may be related. However, the measurement of very steady growth in *C. indica* and *Z. mays*, where a band-like distribution of pectin has been found (Li *et al.* 1994; Li *et al.*, unpublished data) demonstrates that not all species show growth characteristics that conform to the proposed model.

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