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OBSERVATIONS ON MICROTUBULES AND NUCLEI MOTILITY IN THE POLLEN TUBE OF OLIVE (*OLEA EUROPAEA* L.)

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In order to study microtubule organization during pollen germination and pollen tube growth in *Olea europaea*, we applied immunofluorescence microscopy using mouse monoclonal antibody against α-tubulin as primary antibody and FITC-conjugated goat antimouse IgG as secondary antibody. DAPI enabled observation of the vegetative nucleus entering the emerging pollen tube before the generative cell. The latter then overtakes the vegetative nucleus once both are inside the pollen tube. The generative cell remains ahead of the vegetative nucleus until it is finally divided into two gametes. This cell division occurs when the generative cell is close to the tip of the pollen tube. Possible connections between microtubules and nuclear migration in the pollen tube are discussed.

Key words: *Olea europea* L., pollen tube, microtubules, movement, generative cell, vegetative cell nucleus.

INTRODUCTION

The cytoskeleton is the molecular machinery providing the force that drives organelle movement in the pollen tube (Cai et al., 2000). Actin filaments play an essential role in pollen tube growth (Heslop-Harrison and Heslop-Harrison, 1988), and they have been implicated in the movement of the vegetative cell nucleus and cytoplasmic organelles (Tiwari and Polito, 1988; Heslop-Harrison and Heslop-Harrison 1989a,b). Microtubules (MTs) seem to be involved in the translocation of organelles and nuclei, although cytological, biochemical and molecular data supporting this hypothesis are scarce (Aström et al., 1995; Cai et al., 2000). Pollen germination is a dynamic process, and the cytoskeletal organization changes with the events that occur during pollen tube growth. Most of the cytoplasm and organelles of the vegetative cell move into the emerging pollen tube after pollen germination. The vegetative cell nucleus (VN) and the generative cell (GC) also enter the growing pollen tube. The question is which of the two nuclei is the first to enter.

The aim of this study is to determine the behavior of the vegetative cell nucleus and the generative cell inside the growing pollen tube of olive pollen grain and to contribute to our understanding of the role of MTs in the displacement of nuclei within the pollen tube.

MATERIALS AND METHODS

Pollen grains of *Olea europaea* L. trees (Var. Manzanilla and Picual) were collected in Granada (Spain) during the months of May or June. Immunofluorescence microscopy was used to observe the arrangement of MTs following pollen germination in vitro (Brewbaker and Kwack, 1963). Germinated pollen grains were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.025 M cacodylate buffer, pH 7.5, for 2 h at room temperature. For immunolocalization of MTs, the primary

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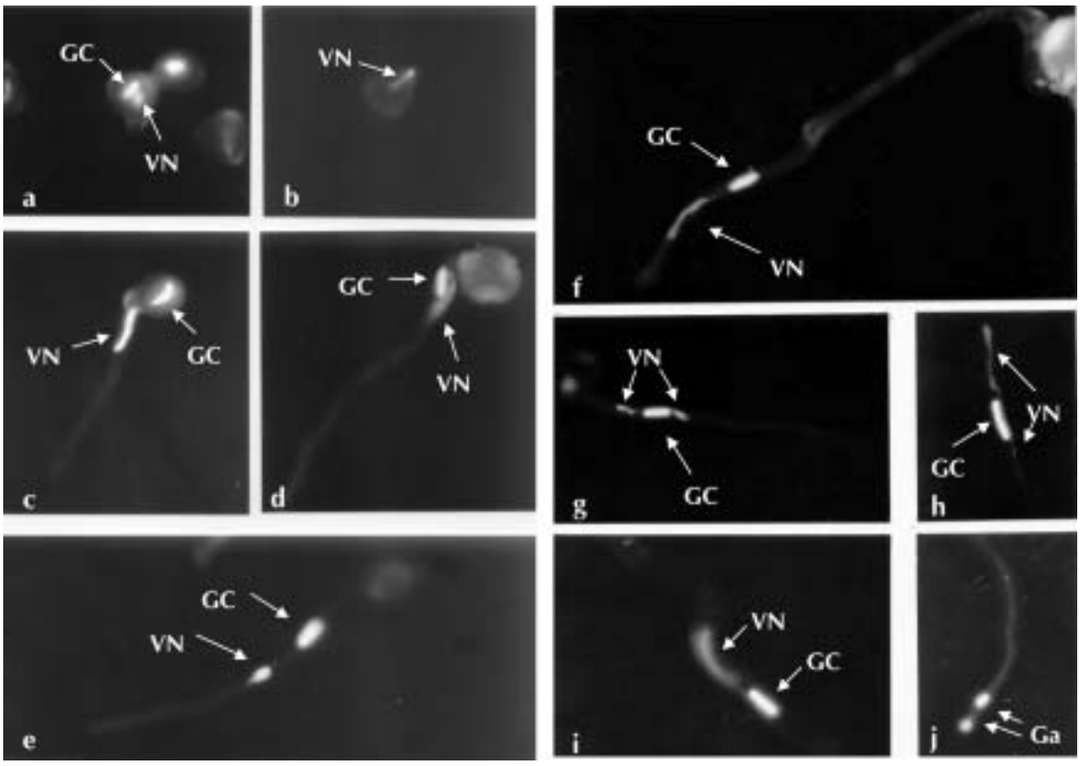


Fig. 1. Behavior of the generative cell (GC) and vegetative cell nucleus (VN) during pollen germination and pollen tube growth. DAPI staining. Ga – gametes. × 2500.

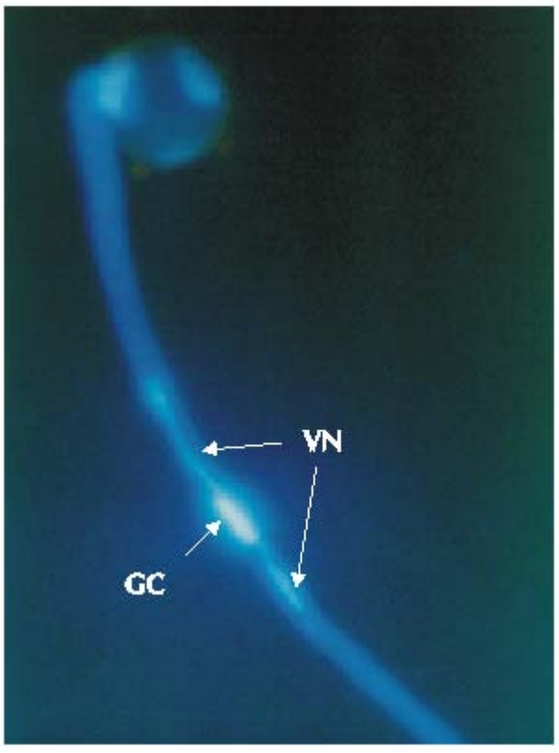
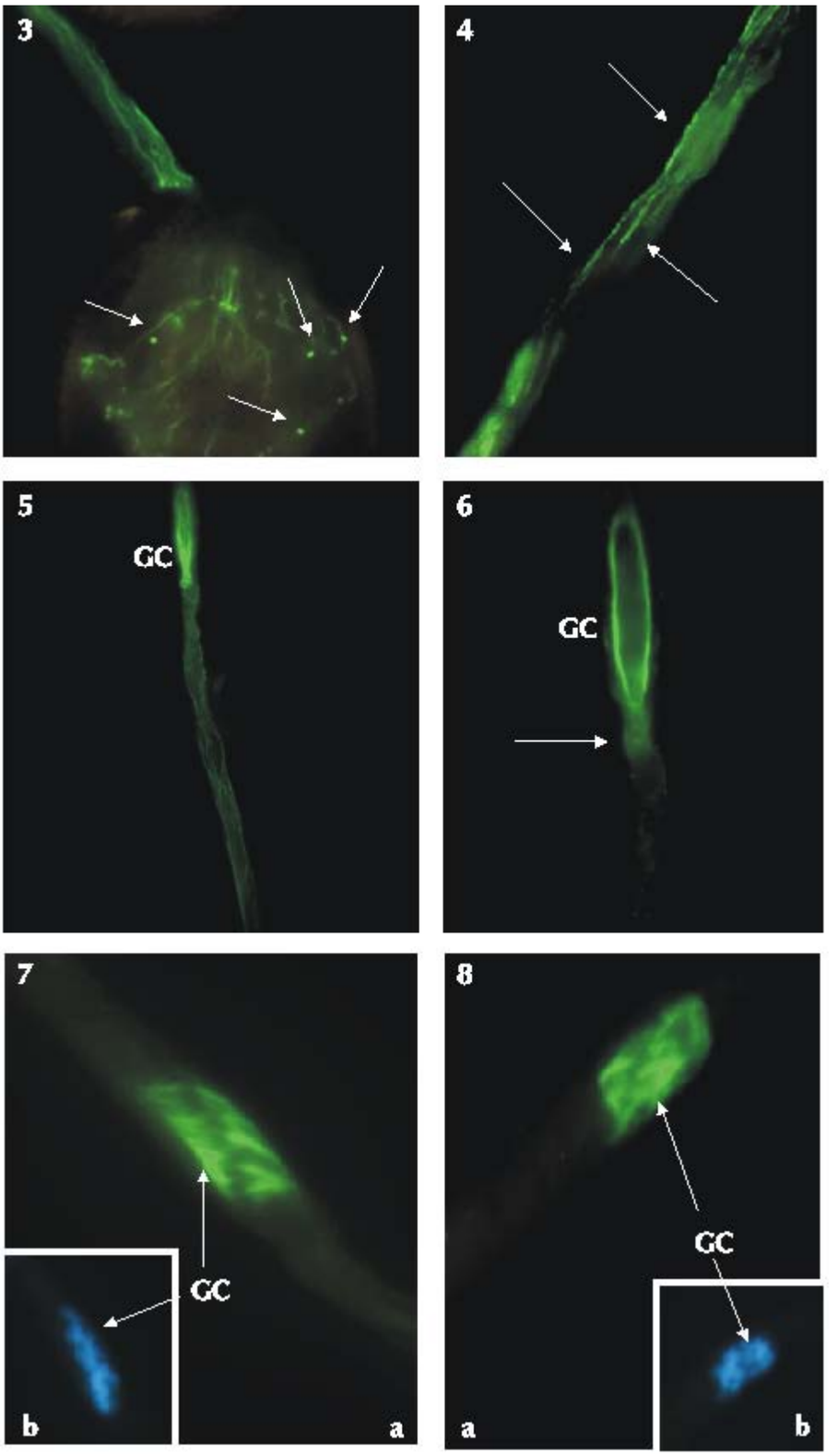


Fig. 2. DAPI staining of germinated pollen grain. Generative cell (GC) catching up with the vegetative cell nucleus (VN). × 3500.

Figs. 3–8. Growing pollen tube: (**a**) FITC anti α-tubulin staining, (**b**) DAPI staining. **Fig. 3.** MT-network within the pollen grain. Arrows show patches of tubulin. × 4620. **Fig. 4.** Bundles extending down the basal pollen tube more or less parallel to the long axis. × 4620. **Fig. 5.** MT bundle along the tube and stronger fluorescence of the spindle-shaped GC. × 3000. **Fig. 6.** Spindle-shaped GC with bright fluorescence elongated with a "tail" (arrow). × 4620. **Fig. 7.** Microtubular organization (**a**) of the metaphase stage during GC division (**b**). × 4620. **Fig. 8.** Microtubular system of the mitotic spindle (**a**) during GN anaphase (**b**). × 4620. MT – microtubule; GC – generative cell; GN – generative cell nucleus.


3
4
5
GC
6
GC
7
GC
b
a
8
GC
a
b

antibody was mouse monoclonal antibody against α-tubulin and the secondary antibody was FITC-conjugated goat antimouse IgG. DAPI staining allowed us to monitor the nuclei.

The microscope was an Axiophot MPM 650 (Zeiss) connected to a computer. Measurements employed a 63× oil immersion Plan Apo objective (numerical aperture = 1.4), a 0.8 μm measurement diaphragm and a 40× Plan condenser (numerical aperture = 0.6). The FITC signal was observed with a blue filter and 510 nm dichroic mirror in the microscope. Image acquisition was performed with a monochrome video camera (Sony Hi Resolution ssc-m 370ce CCD-iris) and digital images (708×561 pixels, 8 bits) were stored in bmp format.

RESULTS

The vegetative cell nucleus (VN) was observed to enter the emerging pollen tube before the generative cell (GC) (Fig. 1). The next observation was of the GC catching up with the VN (Figs. 1, 2) and then moving ahead of it. Finally the GC divided into two gametes (g) when close to the tip of the growing pollen tube (Fig. 1j). Meanwhile, the VN, now behind the generative/gametic cells, was hardly visible.

Tubulin labelling in the germinated pollen grain appeared as a meshwork inside the pollen grain extending into the elongated pollen tube through the pollen grain aperture (Fig. 3). Fluorescent patches of tubulin were interposed and located preferentially in the cortical network of the pollen grain. Bundles of MTs extended down the basal pollen tube. They were oriented preferentially parallel to the long axis of the tubulin fluorescence meshwork, or in arrays that seemed to be predominantly cortical, running more or less parallel to the direction of elongation (Fig. 4). MTs were not observable at the tube apex, as occurs in other species (Pierson and Cresti, 1992) with some exceptions (Del Casino et al., 1993). The strong fluorescence of the GC was noteworthy and corresponded to the cytoskeletal apparatus of this cell (Fig. 5). MTs were arranged as bundles and located around the generative nucleus in the peripheral part of the GC cytoplasm. GCs were spindle-shaped and their distal ends were elongated in the form of "tails" (Fig. 6). We also observed reorganization of interphase MTs into the mitotic spindle during generative cell division (Figs. 7, 8).

DISCUSSION

In *Olea europaea*, as in other binucleate pollen grains, the GC is tightly associated with the VN inside the pollen tube. When the VN-GC complex is close enough to the tip of the growing pollen tube, the GC tends to be ahead of the VN and the GC divides into two gametes. However, the debate as to which enters the pollen tube first – the VN or the GC – still remains open. Three possibilities have been considered: (a) the VN enters first; (b) the GC precedes the VN; and (c) the GC overtakes the VN once both are inside the pollen tube (Raghavan, 1997). Few studies on nuclear movement and the growing pollen tube have been published to date (Venema and Koopmans, 1962; Laitiainen et al., 2002) and no conclusive evidence exists in support of any one of the above-mentioned hypotheses, but our results in olive pollen support the possibility that the GC reaches and passes the VN once both are inside the pollen tube.

That the actin cytoskeleton plays a relevant role in the movement of organelles and nuclei into the pollen grain is now widely accepted (Heslop-Harrison and Heslop-Harrison, 1988, 1989c; Lin et al., 1996). Based on inhibitor experiments, actin filaments appear to be one of the main driving elements in the cytoplasmic streaming of pollen tubes. However, pollen tube MTs have also been related with the movement of both the GC and the VN (Cresti et al., 1990; Aström et al., 1995; Laitiainen et al., 2002). Our observations of the distribution of MT bundles in the olive pollen tube support this idea.

On the other hand, observations of the displacement of the GC are rare in the existing literature. The only well-known function proposed for the MT system in GCs has been that of maintaining the characteristic spindle shape of the latter (Heslop-Harrison et al., 1988). However, no evidence presently excludes the possibility that microtubules are also instrumental in the migration of the GC through the pollen tube. Furthermore, the idea that the GC is passively displaced by actin filaments and cytoplasmic streaming of the pollen tube does not appear to be sufficient. The finding that the GC actively reaches and overtakes the VN suggests that the generative cytoskeleton could also be involved in this movement. The well-represented MT system in GCs, as well as the characteristic shape and programmed movement of the latter, support the hypothesis that GC and sperm cells partially promote their own movement inside the pollen tube.

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