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Endoplasmic reticulum as a storage site for allergenic proteins in pollen grains of several Oleaceae

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Summary. The ultrastructure of mature pollen grains of several Oleaceae species (*Olea europaea, Fraxinus excelsior, Syringa vulgaris, Ligustrum vulgare,* and *Forsythia suspensa*) was studied and the immunolocalization of Ole e I, the major allergen of olige pollen, was determined by immunogold labelling. The five Oleaceae pollens studied here showed different intensities of labelling. The Ole e I allergen was localized throughout the rough endoplasmic reticulum. The absence of gold particles in other cell compartments, such as nuclei, pastids, mitochondria, dictyosomes, lipid bodies, and cell wall, as well as the absence of labelling in control preparations, indicate the specificity of immunolocalization. We conclude that endoplasmic reticulum of the mature pollen grain is a storage site for allergenic proteins and is probably also involved in their synthesis.

Keywords: Allergenic protein; Oleaceae; Pollen; Endoplasmic reticulum; Immuno-localization; Ultrastructure.

Introduction

Normal pollen development is well documented (see Shivanna and Johri 1985; Johri et al. 1992). However, few studies have concentrated on the differentiation of the endoplasmic reticulum (ER) during pollen grain development, although information on this process may provide insights into the physiological role of this system in microgametogenesis. Stacks of elongated ER cisternae have been reported from pollen grains of different species (Kroh 1967; Jensen et al. 1974; Cresti et al. 1985, 1988; Pacini and Juniper 1979; Cresti and Keijzer 1985; Ciampolini et al. 1988; Weber 1988; Tiwari et al. 1990; Van Aelst and Van Went 1991; Luegmayr 1993). These stackes have been attributed a role as storage sites for gametophytic proteins or their precursors (Jensen et al. 1974, Pacini and Juniper 1979), and for the materials needed for pollen tube growth (Cresti et al. 1985). However, evidence to support these assumptions is still lacking.

In an earlier study we traced the process of ER differentiation during pollen grain development of *Olea europaea* (Rodríguez-García and Fernández 1990), detailing the ultrastructural changes, distribution and proliferation of ER cisternae from the young microspore to the mature pollen grain stage (anther dehiscence). ER aggregates differ clearly in both quantity and content, while the stacks eventually shrink and disappear, as the cisternae break up into pockets of ER scattered throughout the cytoplasm in the mature pollen grain. Our findings in *Olea* have provided evidence that the rough endoplasmic reticulum (rER) appears to be involved in the storage of an allergenic protein (Rodríguez-García et al. 1995). Here we present further evidence of rER involvement in other Oleaceae. Martín-Orozco et al. (1994) recently reported cross-reactivity between antibodies to the major allergen in olive pollen and other species of Oleaceae (ash, lilac, privet, and forsythia). Searching for possible similarities in the role of the ER in these species, we studied the ultrastructural organization of the ER system in mature pollen and determined the localization of the allergen, Olea antigen I, by immunogold electron microscopy on pollen grains of olive, ash, lilac, privet, and forsythia.

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Material and methods

Plant material

Mature pollen of olive (*Olea europaea*), ash (*Fraxinus excelsior*), privet (*Ligustrum vulgare*), lilac (*Syringa vulgaris*) and forsythia (*Forsythia suspensa*) were obtained from Allergon AB (Välinge, Sweden).

Electron microscopy and immunocytochemistry

Pollen grains were fixed in 3% (v/v) glutaraldehyde in 0.025 M sodium cacodylate buffer for 2 h at room temperature, and postfixed in 1% OsO_4 with 0.025 M cacodylate buffer for 2 h. The samples were dehydrated through a graded series of ethanols and embedded in Epon resin. Ultrathin sections (80 nm) obtained with a Reichert-Jung Ultracut E ultramicrotome (Vienna, Austria) were contrasted with uranyl acetate followed by lead citrate, and were examined with a Zeiss (Oberkochen, Federal Republic of Germany) EM 10C electron microscope at 60 kV.

For immunocytochemistry, the samples were fixed in 3% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 2 h at room temperature without postfixation. The material was then processed as described above for conventional electron microscopy.

The monoclonal antibody (mAb) used, OL-1, was kindly provided by Dr. Lahoz (Departamento de Inmunología, Fundación Jiménez Díaz, Madrid, Spain) and is specific for Ole e I, the major allergen of olive pollen. Its specificity was tested by immunoblotting and crossed radioimmunoelectrophoresis (CRIE) (Lauzurica et al. 1988). Gold grid carrying ultrathin sections were floated on phosphate buffered saline (PBS) containing 5% bovine serum albumin (BSA) for 10 min. This was followed by incubation with the mAb diluted to 1 : 20 in PBS at 37 °C for 1 h. The grids were washed in PBS, and treated with a goat anti-mouse IgG coupled to 10 nm gold particles, diluted 1 : 30 in PBS, for 45 min at 37 °C. They were then washed again in PBS for 15 min, and rinsed in double-distilled water. Finally the sections were stained in 5% uranyl acetate for 10 min. For control treatments mAb was replaced by PBS buffer.

Results

Mature olive pollen grains showed characteristic ultrastructural features. The ER system was usually visualized as abundant pockets or sacs scattered throughout the cytoplasm and only occasionally were stacks of cisternae observed. They contained an electron-dense matrix, darker than the lipid droplets, and were limited by a membrane to which ribosomes adhered. Pockets of rER frequently appeared in close association with lipid droplets, which were abundant in the cytoplasm (Fig. 1 A). In the other Oleaceae species we found no significant differences in the distribution and fragmentation of ER cisternae in the mature pollen grain as Fig. 1 B and C shows for ash and forsythia respectively.

In sections prepared for immunogold electron microscopical localization of Ole e I, the intensity of labelling differed between species. The highest numbers of gold particles were seen in olive pollen grains, which contained many gold particles in the matrix of rER pockets or sacs (Fig. 2 A, B). The initial impression is that the particles are scattered throughout the cytoplasmic matrix, not located in organelles. On closer examination the specific localization of labelling inside the rER became evident. The other cell compartments (i.e., nuclei, pastids mitochondria, dictyosomes, Golgi vesicles, lipid bodies and cell wall) appeared to be free of gold particles (Fig. 2 A, B). Immunogold labelling of Ole e I in lilac, ash and privet pollen also showed gold particles in rER pockets (Figs. 2 B, 2 C), but labelling was much weaker than in olive pollen. Forsythia pollen grains were devoid of labelling, and also control treatments did not show significant gold labelling.

Discussion

Earlier ultrastructural findings on *Olea europaea* allowed the structures that correspond to the ER system in the mature pollen grain to be distinguished (Rodríguez-García and Fernández 1990). The results of the present study show that, when present, the major allergenic protein of olive pollen, Ole e I, is localized in the rER cisternae. The different intensities of labelling between pollen grains of olive, ash, privat and lilac indicate that this protein is most abundant in the first. No labelling was observed in forsythia pollen, probably because the mAb OL-I detected epitope is not present. These results are in accordance with findings by Martin-Orozco et al. (1994) who determined ELISA specific binding for olive, privet, and lilac, but not for forsythia. However, with different mAbs against Ole e I which recognize other epitopes of the protein the authors showed that the Ole e I epitopes are present not only in olive, but also on proteins of the other Oleaceae spp., indicating some cross-reaction between the taxa.

Antigenic proteins have been localized in the pollen of other species such as ryegrass (Vithanage et al. 1982, Staff et al. 1990) and birch (Grote 1991, 1992), but no studies have reported ER as the site of antigenic protein localization, although it has been postulated that ER is responsible for gametophytic protein formation (Knox and Heslop-Harrison 1970, Heslop-Harrison 1975). The presence of labelling inside the rER pockets suggests that immunogold labelling revealed the site of protein storage, rather than synthesis. The large number of gold particles on ER (especially in olive pollen) suggests that under the

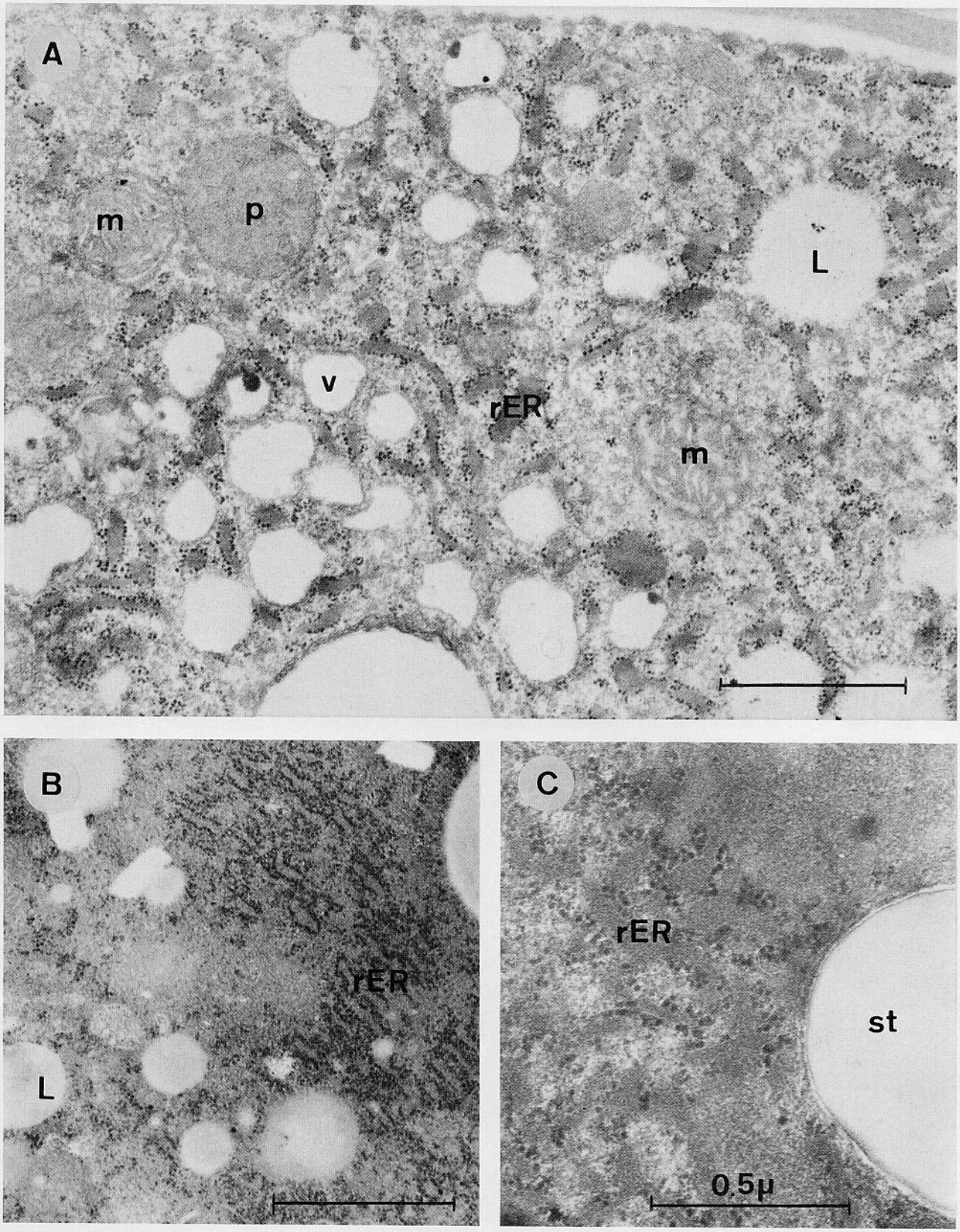

Fig. 1 A–C. Cytoplasm of mature pollen grains. **A** Olive pollen. Note rER pockets scattered throughout the cytoplasm. **B** Ash pollen. Stacks of short rER cisternae. **C** Forsythia pollen. Detail of rER pockets. Note the high density of the rER matrix, similar to the matrix of olive pollen. Bars: A and B, 1 μm; C, 0.5 μm. *L* Lipid body; *m* mitochondria; *p* plastid; *rER* rough endoplasmic reticulum; *st* starch; *v* vesicles

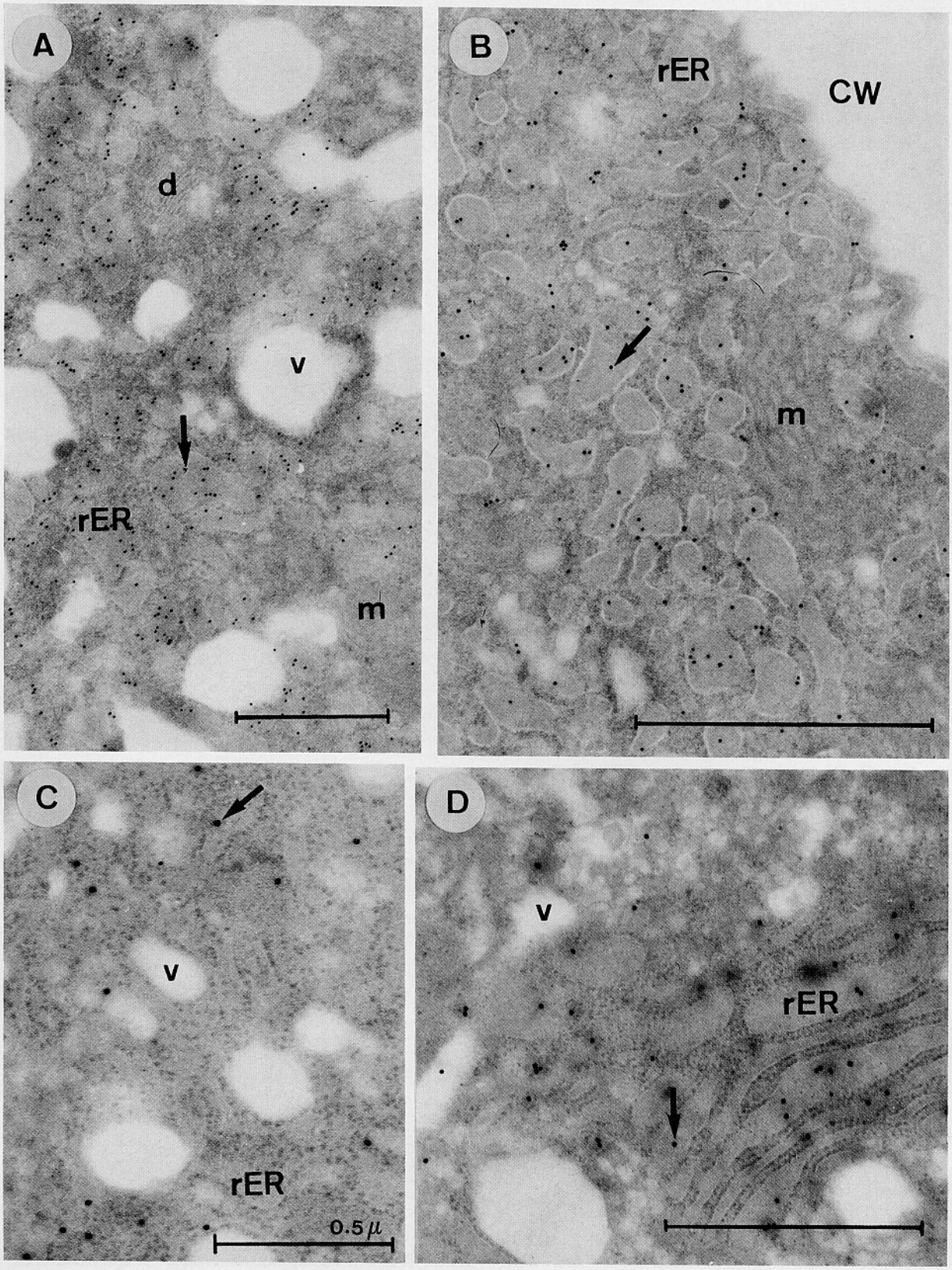

Fig. 2 A–D. Immunogold localization of Ole e I protein. Gold particles inside rER pocket (arrows). **A** and **B** Olive pollen, in which labelling was most intense. **C** Lilac pollen. **D** Privet pollen. Bars: A, B, and D, 1 μm; C, 0.5 μm. *CW* Cell wall; *d* dictyosome; *m* mitochondria; *rER* rough endoplasmic reticulum; *v* vesicles

conditions of sample processing there was apparantly no diffusion of this protein from ER.

The pollen wall has usually been identified as the principal site of enzymatic activity (Knox and Heslop-Harrison 1969, 1970), and the presumptive site allergenic proteins. The absence of the major allergenic protein of olive in the pollen wall may have been due to studying the mature pollen grain prior to the rehydration stage. Rehydration may facilitate passive protein leakage through the cell membrane along a concentration gradient (Simon 1974). Thus, the protein may be able to move to the cell wall only after rehydration. Another possibility is that this protein does not cross the cell membranes and therefore would not be detectable in the cell wall. The protein may be released only when the pollen grain explodes due to rapid hydration upon contact with the mucosa. In this case it could produce an allergic reaction. In this scenario, the function of the protein could be linked to pollen tube growth, rather than pollen-stigma interaction. Finally, because several allergens have been identified in olive pollen (Blanca et al. 1983, Vela et al. 1982), proteins other than that detected by the mAb we used may be localized on the pollen wall.

In conclusion, the ER system of the mature pollen grain is a storage site of allergenic proteins, and is probably also involved in their synthesis.

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