Expression of Ole E 1, the Major Olive Pollen Allergen, during In-Vitro Pollen Germination

M. M'rani-Alaoui, A.J. Castro, J.D. Alché, W. Wang, M.C. Fernández and M.I. Rodríguez-García
Estación Experimental del Zaidín. CSIC
Profesor Albareda 1, E-18008 Granada. Spain

Keywords: *Olea europaea* L., pollen allergen, Ole e 1, in-vitro germination, SDS- PAGE, Northern blot, immunolocalization, endoplasmic reticulum.

Abstract

The presence of the protein Ole e 1 (the olive pollen major allergen) and its transcripts during in-vitro pollen germination have been analyzed. The results obtained showed that: a) a large amount of the protein (with three variants) was found in both the mature and the germinating pollen grain, b) the protein was also secreted to the aqueous germination medium, c) Ole e 1 gene was highly expressed in both the mature and the germinating pollen grain, and d) the allergen was immunolocalized in association with dilated rough endoplasmic reticulum cisternae. These findings clearly support the idea of Ole e 1 playing a role of paramount importance in pollen germination and pollen tube growth.

INTRODUCTION

Olive tree pollen is one of the main causes of allergy by inhalation in Mediterranean countries and some areas of North America (Wheeler, 1992). The major allergen of this pollen (Ole e 1) has been isolated, purified and biochemically characterized (Villalba et al., 1993). Cloning of several cDNAs coding for Ole e 1 have also been recently carried out (GenBank[TM]/EMBL Data Bank S75766 and X76395). Cellular localization of this protein has been studied throughout pollen development (Rodríguez-García et al., 1995). Ole e 1 is located in the cisternae of the rough endoplasmic reticulum.The distribution of Ole e 1 and its transcripts has been investigated during olive pollen development (Alché et al., 1999). The biological function of Ole e 1 is, in spite of the emerging data, still unclear. In order to obtain clues regarding this biological function, we analyze in this paper the temporal and spatial presence of Ole e 1 during in-vitro pollen germination.

MATERIALS AND METHODS

Dehiscent pollen grains were collected from *Olea europaea* L. trees (Var. Picual) in Granada (Spain). Pollen grains kept at -20°C were incubated in a humid chamber at room temperature (RT) for 30min, and then transferred to Petri dishes containing germination medium (10% (w/v) sucrose, 0.03% (w/v) $Ca(NO_3)_2$, 0.01% (w/v) KNO_3, 0.02% (w/v) $MgSO_4$, 0.03% (w/v) boric acid). Petri dishes were maintained at RT in the dark. Samples of culture were taken 8h after the onset of the culture.

Preparation of crude protein extracts, SDS-PAGE separation of polypeptides, and immunoblotting detection of Ole e I bands was essentially carried out as described by Alché et al., (1999). For Northern blot analysis, total RNA was extracted using a RNeasy Plant Total RNA kit (Quiagen) from pollen samples. Denaturing agarose gels were transferred onto a membrane, which was probed with a PCR-generated dig-labelled probe including clone 3c (438 bp) of Ole e 1 (Villalba et al., 1994). Hybridizing bands were detected using an antibody anti-digoxigenin (Fab) conjugated to alkaline phosphatase, diluted 1/10,000 and CSPD (both chemicals from Boehringer Mannheim GmbH, Germany) as the chemiluminescent substrate.

For the immunolocalization of the allergen, germinating pollen was processed as previously described by Rodríguez-García et al. (1995). Immunolocalization was performed using a monoclonal antibody to Ole e 1. Control sections were treated similarly, but omitting

the anti-Ole e 1 monoclonal antibody.

RESULTS

Figure 1a illustrates the Coomassie blue-stained SDS-PAGE polypeptide profiles of pollen crude extracts corresponding to mature and in-vitro germinated olive pollen. The polypeptide of 18 kDa was the most prominent. No major changes neither in the band pattern nor in their relative intensity were detected by this method in the germinated pollen, compared to the mature pollen. Ole e 1 antibody recognized three polypeptides (Fig. 1b) within the range 17-19 kDa in immunoblots corresponding to the same gel shown in Fig. 1a. SDS-PAGE analysis of germination medium samples was unable to detect protein bands in Coomassie-stained gels, although the allergen was detected after immunoblotting experiments using the Ole e 1 antibody (Fig. 2). At the RNA level, Ole e 1 probe hybridized to an abundant single 0.8-kilobase transcript in Northern analysis (Fig. 3).

The localization of Ole e 1 within the pollen grain coincided with results previously published by our group (Rodríguez-García et al., 1995; Alché et al., 1999). Briefly, Ole e 1 was located in association with dilated RER cisternae (Fig. 4a), and in the pollen wall of mature olive pollen (not shown). Cytoplasmic organelles were totally devoid of the allergen. Within the pollen tube, the allergenic protein was mainly located in the lumen of RER cisternae, scattered through the citoplasm, and in the pollen tube wall (Fig. 4b), whereas nuclei showed no gold particles (not shown). Negative controls prepared by omitting the primary antibody showed no significant labeling (Fig. 4c).

DISCUSSION

Experiments of immunolocalization and Western blotting confirm that the major allergen Ole e 1 is extensively present in both the pollen grain and the pollen tube during in-vitro germination. This allergen is found in the form of three variants. (non-glycosylated, mono and diglycosylated), as has been extensively described (Batanero et al., 1994; Alché et al., 1999). No changes in the proportion of these three variants have been described, from which the mono-glycosylated variant is the most abundant, and therefore the only one detected in the stages or localizations where Ole e 1 was scarce (as is the case of culture medium). A high degree of polymorphism is also a characteristic for many pollen allergens (Johnson and Marsh, 1965; Swoboda et al., 1995). Ole e 1 transcripts have been massively found in dehiscent pollen as well as during in-vitro pollen germination, coincidentally with the high levels of the allergen, thus confirming the suggestion of Ole e 1 expression being controlled at the transcriptional level (Alché et al., 1999). Ole e 1 biological function is up to date completely speculative. Antisense repression experiments carried out by Muschietti et al. (1994), using *LAT52* complementary transcripts produced abnormal function in maize pollen, including hydration and/or germination deficiencies. We have previously suggested (Alché et al., 1999) a putative role for this allergen (representing up to a 20% of the total protein weight) in the creation of the osmotic gradient needed for pollen hydration and germination. The results obtained in this paper show a high level of Ole e 1 expression throughout in-vitro pollen germination. This expression may contribute to the maintenance of such osmotic gradient.

ACKNOWLEDGEMENTS

This work was supported by Spanish Research Projects MCT Ref. BMC2000-1484 and INIA, Ref. CA 099-003

Literature Cited

Alché, J.D., Castro, A.J., Olmedilla, A., Fernández, M.C., Rodríguez, R., Villalba, M. and Rodríguez-García, M.I. 1999. The major olive pollen allergen (Ole e I) shows both gametophytic and sporophytic expression during anther development, and its synthesis and storage takes place in the RER. J. Cell Sci. 112(15): 2501-2509.

Batanero, E., Villalba, M. and Rodríguez, R. 1994. Glycosylation site of the major

allergen from olive tree pollen. Allergenic implications of the carbohydrate moiety. Mol. Immunol. 31: 31-37.

Johnson, P. and Marsh, D.G. 1965. "Isoallergens" from rye grass pollen. Nature 206: 935-937.

Muschietti, J., Dircks, L., Vancanney, G. and McCormick, Sh. 1994. LAT52 protein is essential for tomato pollen development: pollen expressing antisense *LAT52* RNA hydrates and germinates abnormally and cannot achieve fertilization. Plant J. 6: 321-338.

Rodríguez-García, M.I., Fernández, M.C. and Alché, J.D. 1995a. Immunocytochemical localization of allergenic protein (*Ole e* I) in the endoplasmic reticulum of the developing pollen grain of olive (*Olea europaea* L.). Planta 196: 558-563.

Swoboda, I., Jilek, A., Ferreira, F., Engel, E., Hoffmann-Sommergruber, K., Scheiner, O., Kraft, D., Breiteneder, H., Pittenauer, E., Schmid, E., Vicente, O., Heberle-Bors, E., Ahorn, H. and Breitenbach, M. 1995. Isoforms of Bet v 1, the Major Birch Pollen Allergen, Analyzed by Liquid Chromatography, Mass Spectrometry, and cDNA Cloning. J. Biol. Chem. 270: 2607-2613.

Villalba, M., Batanero, E., López-Otín, C., Sánchez, L.M., Monsalve, R.I., González de la Peña, M.A., LaHoz, C. and Rodríguez, R. 1993. The amino acid sequence of *Ole e* I, the major allergen from olive tree (*Olea europaea*) pollen. Eur. J. Biochem. 216: 863-869.

Villalba, M., Batanero, E., Monsalve, R.I., González de la Peña, M.A., LaHoz, C. and Rodríguez, R. 1994. Cloning and Expression of Ole e I, the major allergen from olive tree pollen. J. Biol. Chem. 269: 15217-15222.

Wheeler, A.W. 1992. Hypersensitivity to the allergens of the pollen from the olive tree (*Olea europaea*). Clin. Exp. Allergy 22: 1052-1057.

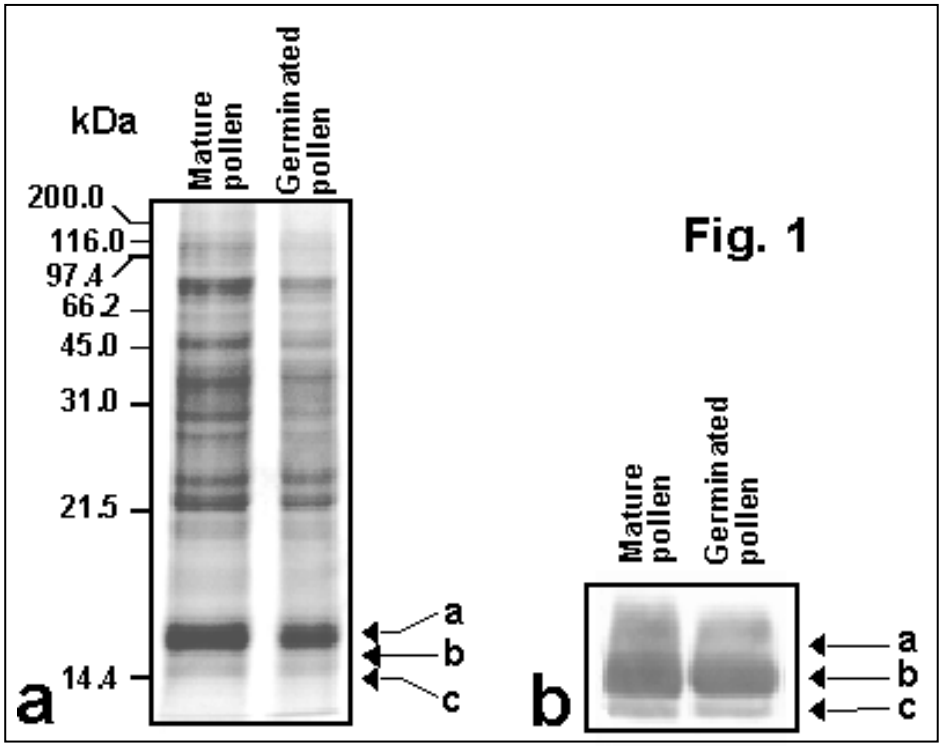

Fig. 1

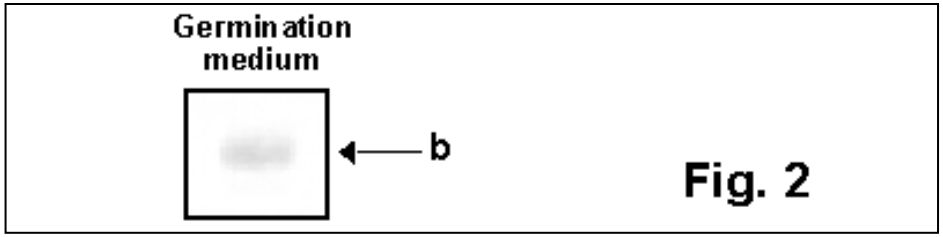

Fig. 2

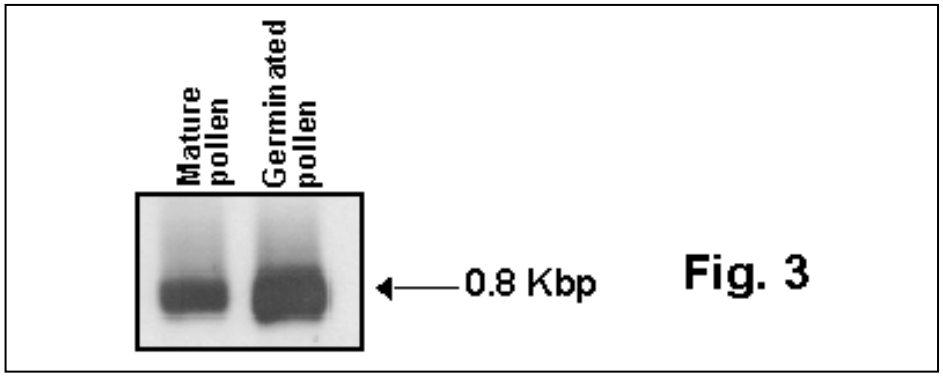

Fig. 3

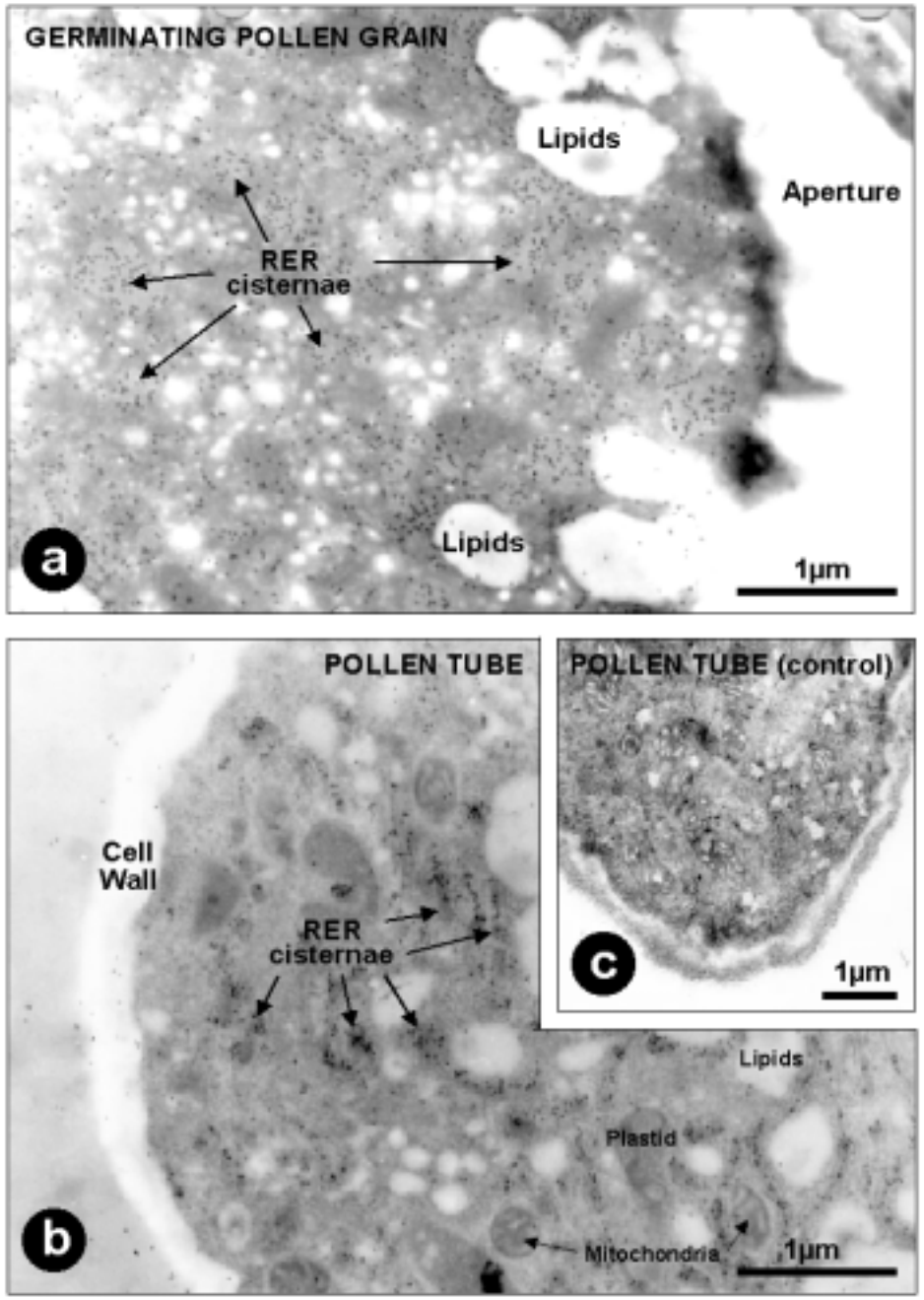

Fig. 4