Polymorphism of the olive pollen allergen Ole e 1 and its biological implications

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The major olive pollen allergen (Ole e 1) consists of a single 145 amino-acid polypeptide chain, which exhibits three glycosylation variants, as well as microheterogeneities at several positions (Villalba *et al*. 1994. J. Biol. Chem. 269: 15217-15222). Synthesis and storage of Ole e 1 takes place in the endoplasmic reticulum of the vegetative cell. The allergen is also localized in the outer layer of the pollen wall (exine) and is released into the culture medium throughout germination (Alché *et al*. 2004. Plant Cell Physiol. 45: 1149-1157). The protein Ole e 1 displays a relevant coverage score with other Ole e 1-like proteins from the *Oleaceae* family, as well as with pollen proteins from maize and tomato among others (Rodríguez *et al*. 2002. Allergy 57: 6-16). Despite Ole e 1 represents up to 20% of total protein of the pollen grain (Castro *et al*. 2003. Int. Arch. Allergy Immunol. 131: 164-173), the biological function of this protein is not completely defined. In this work, we aimed to assess the putative polymorphism of Ole e 1 sequence among olive cultivars.

Using a PCR-based strategy, three Ole e 1 cDNA clones were obtained from pollen of eight different olive (*Olea europaea* L.) cultivars. Multiple alignment of the resulting cDNA sequences showed the presence of a high number of microheterogeneities after comparison with the previously described Ole e 3c and Ole e 5c clones (Accession number= X76395 and X76396, respectively). These microheterogeneities were particularly profuse at the 5´ coding region and the 3´non-coding region. Moreover, a fragment of 38 amino acids was truncated in the 3´ coding region of all the three clones obtained from the “Bella de España” cultivar. The multiple alignment of the 24 predicted amino-acid sequences showed a remarkable polymorphism, notably at the N-terminal region. These changes modified both the hydrophilicity and antigenicity theoretical profiles of the protein, also affecting the predicted secondary structures of the allergen in most of the cultivars studied. Besides, an additional N-glycosilation motif was detected in the “Bella de España” cultivar. Cladistic analysis

demonstrated that the inter-cultivar variability was significantly higher than the intra-cultivar variability, suggesting that this protein might be used as molecular tool to discriminate olive tree cultivars.

Ole e 1 polymorphism was further analyzed by using 2D-electrophoresis in the “Picual” (reference cultivar), “Arbequina” and “Bella de España” cultivars. Resulting 2D protein maps revealed the presence of eight major groups of proteins in the “Picual” cultivar, which were all recognized by an anti-Ole e 1 monoclonal antibody. The existence of multiple isoforms of the same protein strongly suggests the existence of post-translational modifications others than glycosilation, not reported in previous works. Interestingly, major quantitative and qualitative differences concerning Ole e 1 were found in 2-D profiles of both “Arbequina” and “Bella de España” cultivars. The polymorphism described here is in good agreement with the model proposed by Alché and coworkers (2004. Plant Cell Physiol. 45: 1149-1157) in which Ole e 1 might interact with specific style components, thus triggering a signal transduction cascade, which would regulate the initiation and the guidance of pollen tube growth. The relevance of this model within the compatibility system in the olive is discussed.

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