**Online suppl. Material and Methods**

*Analytical procedures*

SDS-PAGE was performed in 15% polyacrylamide gels under reducing conditions. Gels were stained with Coomassie Blue R-250 (Sigma-Aldrich) or transferred to nitrocellulose membranes (Amersham Biosciences, Barcelona, Spain) according to Towbin method [1]. Molecular mass determinations were performed with unstained protein markers SM0431 (Fermentas) or with pre-stained protein molecular weight markers (BIO-RAD).

Isoelectric focusing was achieved under reducing conditions in the presence of 3 mM tributylphosphine in a PROTEAN IEF Cell (Bio-Rad) using 7 cm length, pH 3-10 linear ReadyStrip IPG gels (Bio-Rad). After isoelectrofocusing, proteins were separated by SDS-PAGE in 15% polyacrylamide gels under reducing conditions in the presence of 50 mM dithiothreitol and 3.7% iodoacetamide. Proteins were detected by Coomassie Blue R-250 (Sigma-Aldrich) staining or they were transferred to nitrocellulose membranes for their immunological characterization after SDS-PAGE (Sigma-Aldrich).

*Cloning of the allergenic polygalacturonase from O. europaea pollen*

Total RNA from olive pollen was used to synthesize total cDNA using the SMART RACE cDNA amplification kit (BD Bioscience, Clontech, Madrid, Spain), as previously described [2]. Then, an Ole e 14 partial cDNA was obtained by PCR -as previously described [7]- using a sense 5´-ACTCATATGATCCCCCACAATGGTGTCCGT-3´ and antisense 5´-AGAGCGGCCGCCTCGAGTTCACAACCAGGAGGGATAGG-3´ degenerate oligonucleotides, which were designed from the highly conserved amino acid sequence NTDGMHI in most PGs from different sources [7].

Then, the amplification of the whole olive pollen PG-encoding cDNA was amplified by PCR using the partial cDNAs previously obtained using a sense - 5´-actCATATGATCCCCCACAATGGTGTCCGT-3´ - and antisense 5´-AGAGCGGCCGCCTCGAGttcacaaccaggagggatagg-3´ specific oligonucleotide which contained *Nde*I and *Xho*I restriction sites (underlined), respectively. The cDNAs amplified by PCR were purified, cloned into the pCR2.1 vector (Invitrogen, Groning, The Netherlands) and sequenced.

Finally, the complete cDNA-encoding the whole Ole e 14 amino acid sequence without the signal peptide was subcloned into the pET41b plasmid (Novagen, Billerica, MA, USA) previously digested with *Nde*I and *Xho*I restriction enzymes. The obtained pET41b/Ole e 14 construct was used to transform BL21(DE3) *E. coli* cells to produce Ole e 14 as a fusion protein to an 8xHis-tag at the C-terminal end.

*Expression and purification of Ole e 14*

BL21(DE3) cells containing the recombinant constructs were grown overnight at 37ºC in LB medium supplemented with 100 µg/mL kanamycin until reach an optical density at 600 nm of 1.0. Then, cell cultures were induced with 1 mM IPTG and maintained at 16ºC for 48 h. Then, cells were harvested by centrifugation at 5000 rpm during 15 min at 4ºC and inclusion bodies solubilized during 1 hour with 20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 20 mM imidazole and 1 mM 2-mercaptoethanol containing 6 M guanidine hydrochloride. Cultures were clarified by centrifugation at 13000 rpm during 30 min at 4ºC and the supernatant containing Ole e 14 used to purify the recombinant allergen. Briefly, Ole e 14 containing material was applied at 1 mL/min into a His-Trap FF crude (GE Healthcare, Madrid, Spain) for obtaining the protein by refolding on column. To that end, the column was washed with 20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 20 mM imidazole and 1 mM 2-mercaptoethanol containing 6 M urea. Then, a 60 min gradient to 20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 20 mM imidazole and 1 mM 2-mercaptoethanol was performed for the refolding of the protein. Finally, the recombinant protein was eluted by an isocratic gradient with the same buffer containing 0.5 M imidazole.

Fractions containing the protein were analyzed by Coomassie Blue R-250 (Sigma-Aldrich) staining and WB after SDS-PAGE using sera from allergic patients or a pAb raised against Ole e 14, pooled, dialyzed against 50 mM ammonium bicarbonate, and stored at -80ºC until its use. The concentration of the purified recombinant proteins was calculated by spectroscopy in a DU-7 spectrometer (Beckman, Barcelona, Spain) using the theoretical extinction calculated with the ProtParam Software [3] of 0.59 for Ole e 14 or 0.7 for Sal k 6.

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**Suppl. Fig. S1.** Study of the Ole e 14 nucleotide and amino acid sequences.

**A** Nucleotide and amino acid sequences of Ole e 14 are depicted. Aspartic residues described to be involved in the enzymatic activity of PGs are boxed with a dashed line. **B** Confirmation of the existence in olive pollen of the cloned Ole e 14 isoform. Identity and similarity percentages between them were calculated using the cloned Ole e 14 as reference amino acid sequence.

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**Suppl. Fig. S2.** Polygalacturonase sequences observed in the database of olive tree genome.The three identified polygalacturonase isoforms more similar to Sal k 6 than Ole e 14 in olive are shown in the figure in comparison to both allergens. Identity and similarity percentages among them are also shown.

**Supplementary References**

1 Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350-4354.

2 Mas S, Oeo-Santos C, Cuesta-Herranz J, Diaz-Perales A, Colas C, Fernandez J, Barber D, Rodriguez R, de Los Rios V, Barderas R, Villalba M: A relevant ige-reactive 28kda protein identified from *Salsola kali* pollen extract by proteomics is a natural degradation product of an integral 47 kDa polygalaturonase. Biochim Biophys Acta 2017;1865:1067-1076.

3 Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, Appel RD, Hochstrasser DF: Protein identification and analysis tools in the expasy server. Methods Mol Biol 1999;112:531-552.