

RESEARCH ARTICLE

Screening of Ole e 1 polymorphism among olive cultivars by peptide mapping and *N*-glycopeptide analysis

Antonio Jesús Castro¹, Audrey Bednarczyk², Christine Schaeffer-Reiss²,
María Isabel Rodríguez-García¹, Alain Van Dorsselaer² and Juan de Dios Alché¹

¹Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (C.S.I.C.), Granada, Spain

²Laboratoire de Spectrométrie de Masse Bio-Organique, IPHC-DSA, ULP, CNRS, Strasbourg, France

In the present paper, we have used 2-DE coupled to MS analysis to examine the molecular variability of the Ole e 1 allergen in three olive cultivars (cvs). Our results confirmed that the predicted polymorphism of Ole e 1 at cDNA level is extended to the expressed protein. The profiles of both the Ole e 1 peptides and the *N*-glycan variants significantly changed among cvs. We observed that Picual and Arbequina cvs presented the highest and lowest degree of Ole e 1 polymorphism, respectively. Some of these peptides and *N*-glycans were distributed in a cv-specific manner. The putative implications of this molecular polymorphism in the development of the allergy symptoms are discussed.

Received: March 24, 2009
Revised: November 12, 2009
Accepted: November 17, 2009

**Keywords:**

N-glycopeptide / Ole e 1 allergen / Olive cultivars / Peptide mapping / Plant proteomics / Polymorphism

1 Introduction

The major olive (*Olea europaea* L.) pollen allergen, called Ole e 1, is an acidic protein that consists of a single 145 amino-acid polypeptide chain and possesses a *N*-linked glycosyl moiety located at Asn¹¹¹, and six conserved Cys that are likely involved in disulfide bonds [1]. Two main variants of Ole e 1, glycosylated (20 kDa) and non-glycosylated (18.5 kDa), together with a minor hyperglycosylated variant (22 kDa) and a dimeric form of the glycosylated protein (40 kDa) are detected on SDS-PAGE gels after staining, and on membranes after immunolabeling with specific antibodies or sera from allergic patients. Ole e 1 nucleotide and

amino acid sequence polymorphism was early reported [2, 3].

The capacity for IgG and IgE binding of Ole e 1 protein largely depends of the integrity of the disulphide bridges and is very sensitive to point changes in the amino acid sequences of the corresponding epitopes [4]. The *N*-glycan structure of Ole e 1 has been elucidated [5, 6]. There is ample evidence that these highly conserved *N*-linked oligosaccharides contribute to the immunogenic and allergenic capacity of Ole e 1 [7, 8], and explain to some extent the cross-reactivity of Ole e 1 with other glycoallergens [9].

We have recently proved that the varietal origin of pollen represents a major source of inconsistency for Ole e 1 sequence [10] in eight olive cultivars (cvs). The substitution of Cys at position 19 in cvs Arbequina (Ar) and Bella de España (Be), the presence of an additional *N*-glycosylation tripeptide in Be, and point changes that might affect the secondary structure and the hydrophilicity/allergenicity profiles of the protein in most of cvs analyzed were the most remarkable effects of this polymorphism. This variability of allergenic molecules with respect to their genetic origin

Correspondence: Dr. Antonio Jesús Castro, Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín (C.S.I.C.), Profesor Albareda 1 E-18008, Granada, Spain

Fax: +34-958129600

E-mail: antoniojesus.castro@eez.csic.es

Abbreviations: Ar, Arbequina; Be, Bella de España; cv, cultivar; Pi, Picual

was first demonstrated for wheat grain allergens after electrophoretic characterization of seven cvs [11]. Besides wheat grains, apple fruit and date palm fruit and pollen from different cvs also differ in their antigenic and allergenic capacity [12–14]. Recently, the genetic basis of the polymorphism of several apple allergens has been established in Prima and Fiesta cvs using a genomic cloning and linkage mapping approach [15–17].

Proteomic techniques in combination with immunological screening have been used to identify allergen isoforms from different sources like pollens [18–20], foods [21–23], dust mites [24], or latex [25]. These techniques are also appropriate to verify whether cDNA variants are present at protein level and to detect isoforms derived from low-abundant cDNA sequences. In addition, they provide information about the quantitative importance of discrete isoforms of these proteins and the existence of PTMs involved in allergy such as glycosylations [26].

Using proteomic techniques, the present work attempts to explore the molecular variability of the Ole e 1 allergen in three olive cvs with regard to: (i) the existence of micro-heterogeneities in the amino acid sequence and (ii) the heterogeneity in the *N*-glycan composition. The putative implications of such polymorphisms in the development of the allergy symptoms are discussed.

2 Materials and methods

2.1 Plant material and sampling

Olive (*Olea europaea* L.) pollen grains of cvs with high (Picual (Pi)) and low (Ar and Be) Ole e 1 content [27] were collected from dehiscent anthers by vigorous shaking of flowering shoots inside large paper bags. Sampling was carried out from discrete trees of the World Collection of Olive Germplasm of the CIFA Alameda del Obispo, in Cordoba (Spain).

2.2 Allergen extracts

Olive pollen samples (0.1 g) were suspended in 2.5 mL extraction buffer consisting of 40 mM Tris-HCl (pH 7.0), 2% v/v Triton X-100, 60 mM DTT, and 10 μ L of a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MI, USA). Proteins were allowed to elute for 2 h at 4°C under continuous stirring. The resulting supernatants were desalted in a PD-10 column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Pollen proteins were then precipitated at –20°C for 1 h in a solution of 20% w/v trichloroacetic acid prepared in acetone. The resulting pellet was resuspended in 0.5 mL solubilization buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 5 mM tributylphosphine, 0.5% v/v Bio-Lyte 3–10 buffer (Bio-Rad, Hercules, CA, USA) and traces of bromophenol blue). Total protein content was estimated using the

2D Quant Kit (Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions.

2.3 2-DE and immunoblotting

Samples containing ~75 μ g of total protein were applied to 18 cm polyacrylamide strips (pH 3–10NL, Bio-Rad) by in-gel rehydration at 30 V for 12 h. Focusing was conducted at 20°C in a Protean IEF Cell (Bio-Rad) as follows: 300 and 1000 V for 1 h each followed by a linear increase from 1000 to 10 000 V, and finally 10 000 V to give a total of 40 kVh. Reduction and alkylation steps were performed as previously described [28]. Protein separation in the second dimension was carried out in a Protean II xi Cell (Bio-Rad) [29]. After completion of SDS-PAGE, the resulting gels were stained with Flamingo fluorescent stain (Bio-Rad) according to the manufacturer's instructions. The apparent molecular mass was calibrated using internal markers (2-D SDS-PAGE Standards, Bio-Rad) after co-electrophoresis. Preparative 2-DE gels for spot excision were performed as above but proteins were visualized by staining with MS compatible silver stain [30].

For immunodetection experiments, 75 μ g of pollen proteins were resolved in 2-D gels as described above. The resulting gels were transferred onto PVDF membranes (Pall, Pensacola, FL, USA) in a Trans-Blot Semi-Dry Transfer Cell (Bio-Rad) according to the manufacturer's instructions. Membranes were probed with 10H1 anti-Ole e 1 mAb [31], diluted 1/1000 in blocking buffer, and the signal was revealed after incubation with a goat anti-mouse IgG Alexa fluor 488-conjugated secondary antibody (Molecular Probes, Eugene, OR, USA) diluted 1/5000.

Images were acquired in a Pharos FX molecular imager (Bio-Rad). The reproducibility of results was confirmed by running each experiment in triplicate.

2.4 Protein identification by nanoLC-MS/MS analysis of peptides and *N*-glycopeptides

2.4.1 Protein tryptic digestion

2-D gel spots of interest in the 20 kDa region were excised and digested in a MassPREP protein digestion station (Waters, Milford, MA, USA). The gel spots were washed twice with 100 μ L of 25 mM NH_4HCO_3 /ACN (1:1). The Cys residues were first reduced by 50 μ L of 10 mM DTT at 57°C for 1 h and then alkylated by 50 μ L of 55 mM iodoacetamide at room temperature for 20 min. After dehydration of the gel bands with ACN, the proteins were digested in gel overnight with 15 μ L of 12.5 ng/ μ L modified porcine trypsin (Promega, Madison, WI, USA) in 25 mM NH_4HCO_3 at room temperature. The generated peptides were extracted with 60% v/v ACN in 5% v/v formic acid followed by removing ACN excess. Each tryptic mixture was split in two parts,

which were analyzed by reverse phase C18 and carbon graphite chromatography, respectively.

2.4.2 NanoLC-MS/MS analysis

Tryptic digests were analyzed by nanoLC-MS/MS using an Agilent 1100 series HPLC-Chip system with Zorbax chip composed of a 40 nL enrichment column and a 75 $\mu\text{m} \times 43 \text{ mm} \times 5 \mu\text{m}$ analytical column (Agilent Technologies, Palo Alto, CA, USA). For analysis of peptides, the column was packed with a C18-SB phase and for *N*-glycopeptides analysis the column was packed with graphitized carbon. The LC system was coupled to an HCTplus ion trap (Bruker Daltonics, Bremen, Germany). The solvent system consisted of 2% v/v ACN and 0.1% v/v formic acid in water (solvent A), and 2% v/v water and 0.1% v/v formic acid in ACN (solvent B). The sample was loaded into the enrichment column at a flow rate set to 3.75 $\mu\text{L}/\text{min}$ with solvent A. Elution was performed at a flow rate of 300 nL/min with a 8–40% linear gradient of solvent B over 7 min (for peptides analysis) or over 37 min (for *N*-glycopeptides analysis) followed by a 70% stage of solvent B over 3 min before the reconditioning of the column at 92% of solvent A. The voltage applied to the capillary cap was optimized to -1800 V . For *N*-glycopeptides analysis, the mass spectrometer ions transmission parameters were optimized for high m/z (m/z 1000) in order to improve the detection of glycopeptide ions. For tandem MS experiments, the system was operated with automatic switching between MS and MS/MS modes. The three most abundant peptides and preferentially doubly charged ions were selected on each MS spectrum for further isolation and fragmentation. The MS/MS scanning was performed in the ultrascan resolution mode at a scan rate of m/z 26 000 per second. A total of six scans were averaged to obtain an MS/MS spectrum. The complete system was fully controlled by ChemStation (Agilent Technologies) and EsquireControl (Bruker Daltonics) software.

2.4.3 Protein identification

Mass data collected during nanoLC-MS/MS analysis were processed, converted into mgf files, and searched against the NCBI nr database (version downloaded in March 2007 containing 4 761 919 sequences) using a local MascotTM server (Matrix Science, London, UK).

2.4.4 *N*-glycopeptides identification

For each nanoLC-MS/MS analysis using the carbon graphite column, typical glycopeptide diagnostic ions corresponding to glycan fragmentation ($[\text{HexNAc} + \text{H}]^+ = 204.1 \text{ Da}$ and $[\text{HexHexNAc} + \text{H}]^+ = 366.2 \text{ Da}$) were extracted from MS/MS

ion current and MS/MS spectra containing diagnostic ions were manually interpreted. Major fragment ions observed allowed us to determine the structures of the *N*-glycans and the masses of the unglycosylated peptides. In order to attribute the *N*-glycan structures identified to a specific *N*-glycosylation site, for each protein identified by RP-HPLC-MS/MS, *in silico* predictions of potential glycopeptides were performed. Masses of the theoretical peptides containing a potential *N*-glycosylation site were compared to those observed in the MS/MS spectra. Results were validated taking into account the interpretation of the three types of fragment ions classically generated for *N*-glycopeptides (sugars neutral loss, non-glycosylated peptide fragments, and glycan backbone internal fragments).

3 Results and discussion

3.1 Reactivity of pollen protein extracts to anti-Ole e 1 antibody in olive cvs

A representative gel pattern of the olive pollen proteome (cv Pi), containing 1474 spots, is shown in Fig. 1. For each cv, the global protein profile was largely reproducible among replicates. The average number of protein spots detected in this study (1452 for Pi, 1521 for Ar, and 1573 for Be) was higher compared with pioneer works in pollen from other

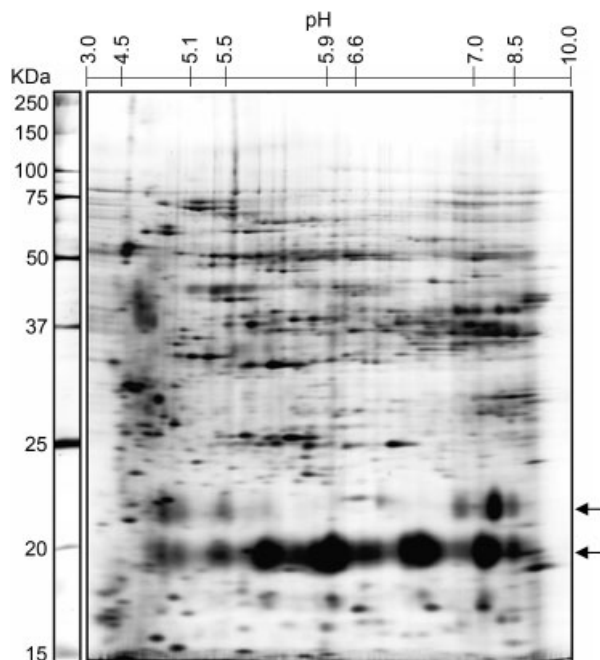


Figure 1. Representative gel of the olive pollen proteome (cv Pi) after 2-DE and silver staining. Approximately 75 μg of total protein per gel was loaded. The numbers at the top of the gel denote the pH gradient in the first dimension, while molecular masses of protein standards are displayed on the left. Major protein “train spots” are indicated by arrows.

plant species such as *Arabidopsis thaliana* [32], rice [33], and tomato [34].

Comparison of 2-D proteins maps from cvs Pi, Ar, and Be showed conspicuous differences at both quantitative and qualitative levels, particularly in the range of 15–20 kDa (Fig. 2). In the cv Pi, a string of prominent spots of about 20 kDa was found, being distributed along the whole pH range. Densitometric studies indicated that the sum of these spots may represent about 15% of the total protein displayed in gels (Fig. 3A). On the other hand, the sum of these spots in Ar and Be represented less than 1% of total protein.

Ole e 1 isoforms distribution in 2-DE maps (Fig. 2) was studied using an anti-Ole e 1 antibody, namely 10H1. The antibody was able to recognize different immunoreactive spots in the molecular mass range of 18–22 kDa, which varied in number and intensity depending on the cv. In agreement with previous data [27, 35], we found conspicuous quantitative differences among cvs with respect to the Ole e 1 content (Fig. 3B). Thus, two groups containing high (cv Pi) and low (cvs Arb and Be) quantities of Ole e 1, respectively, were clearly distinguishable. The content of Ole e 1 in a particular cv can fluctuate in consecutive years and

depending on the geographical locality [36–38] due to variations in certain climatic parameters like rainfall regime or in the degree of exposure to gaseous air pollutants [39]. However, relative differences among cvs remain quite constant among consecutive years and independently of the geographical location of cvs (unpublished, personal observations). In addition, we observed a positive correlation between the Ole e 1 protein and transcript levels in ten olive cvs analyzed [27]. Therefore, the quantitative differences detected here are more likely related to the genetic origin of the pollen than to environmental factors. Interestingly, these quantitative differences might explain to some extent the discrepancies observed in the response of allergic patients to the pollen from different cvs [27, 35, 40].

We also observed qualitative differences among the three cvs with regard to Ole e 1 (Fig. 2). The number of 10H1-reactive spots was significantly different depending on the cv. A “train” of four highly and seven fairly immunoreactive spots was clearly distinguishable in Pi (Fig. 2B). On the contrary, only four and seven spots were present in Ar (Fig. 2D) and Be (Fig. 2F), respectively. All these spots, except Be4, fitted well in their positions with major spots

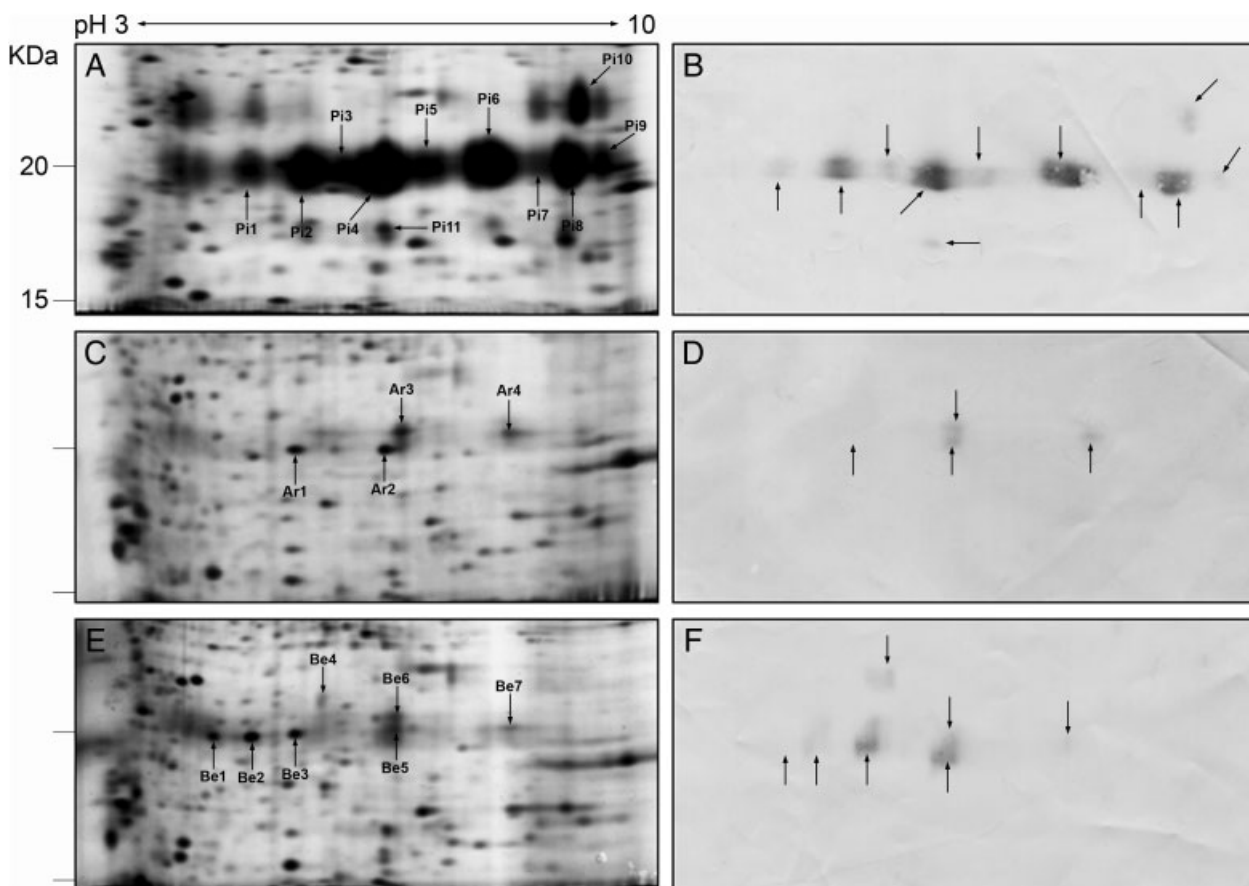


Figure 2. Detailed portions of silver-stained 2-D gels obtained from mature pollen of cvs Pi (A), Ar (C) and Be (E) compared with the corresponding immunoblots (B, D, and F) probed with an anti-Ole e 1 mAb. Marked proteins (arrows) are named in accordance with Table S1 and were identified by nano-LC-MS/MS.

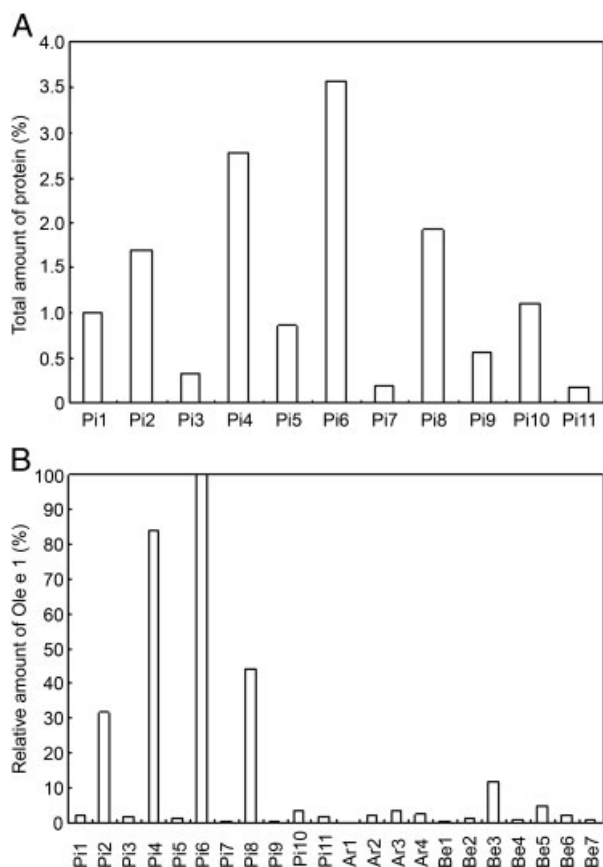


Figure 3. (A), Chart representing densitometric data corresponding to Ole e 1-containing spots from the gel in Fig. 1. Values are represented as percentages of the total amount of protein in the gel. (B), Densitometric data corresponding to Ole e 1 protein spots from blots in Fig. 2B, D and F. Values are displayed as relative percentages referred to the spot Pi6, which showed the higher optical density (100%).

detected in cv Pi. Most of protein spots showed different pIs, which covered the pH range between 5.0 and 8.5. This shift in pI in either the acidic or basic direction is likely caused by differences in the primary structure (polymorphism in the amino acid sequence) and/or PTMs such as different levels of glycosylation, as we further discuss.

3.2 Peptide mapping of Ole e 1 protein in olive cvs

Protein spots that bound to the anti-Ole e 1 antibody were subjected to MS analysis. Despite the lack of olive protein sequence data, all the spots analyzed matched positively with Ole e 1 sequences present in the NCBI database. Spots Be1, Be2, Be3, and Be4 also gave significant scores with a thioredoxin-dependent peroxidase, whereas Be7 displayed a significant homology with a cyclophilin, indicating the presence of co-migrating proteins in these spots (Tables S1 and S2, Supporting Information). Certain

peptides were uniquely identified after C18 chromatography (e.g. DGNGSITFTEVG YTR), while some others (e.g. glycopeptides) were only detected when a carbon graphite stationary phase was used. In addition, the identification of Ole e 1 in spots Be1, Be4, Be5, Be6, Ar1, Ar2, and Ar3 was only achieved after using the carbon graphite column. These results suggest that the combination of the results obtained by LC-MS/MS analysis of tryptic peptides mixtures using two different chromatographic columns allows a better characterization of glycoproteins. An example of the quality of MS/MS spectra when only a single amino acid was changed is shown in Fig. S1 (Supporting Information).

Each spot analyzed consists of a complex mixture of Ole e 1 isoforms, independently of the cv considered. This is supported by the fact that most of the peptides identified displayed a number of variants within a single spot. According to this, each spot analyzed would consist of at least five to nine different Ole e 1 isoforms (see Table S1, Supporting Information), resulting from the combination of these peptides. However, the final number of isoforms for a given spot or cv could not be determined from our data due to incomplete coverage of the tryptic fragments. Therefore, our results here confirm that the cv (i.e. the genetic origin of the pollen studied) is also a major source of polymorphism at protein level, as stated for cDNAs [10].

The degree of Ole e 1 polymorphism, defined as the number and type of amino acid substitutions detected as well as the number of positions affected in the protein sequence, varied among spots in a given cv, and also depended on the cv considered (Tables 1 and 2). Consequently, Ole e 1 polymorphism values in Ar were lower compared with Be and Pi cvs. Despite amino acid substitutions being spread over the whole Ole e 1 protein sequence, those peptides containing amino acids at positions 45–60 and 87–104 were shown to be the most variable (six to seven different substitutions and four to six positions involved), whereas amino acids comprised between positions 61 and 71 displayed a single at position 69. Most of variable amino acid positions registered only two amino acid variants, but some positions (50, 95, and 99) showed up to three different substitutions. A total of 21 positions out of

Table 1. Analysis of Ole e 1 polymorphism among three olive cvs: number of proteins and peptides identified, amino acids substitutions, and positions affected in the peptides containing amino acids at positions 24–40, 45–60, 61–71, 72–87, 88–104, and 105–115

Cv	No. of proteins	No. of peptides	No. of aa substitutions	No. of positions affected
Pi	33	28	24	21
Be	31	20	18	18
Ar	21	15 ^{a)}	12 ^{a)}	12 ^{a)}

a) Values of Ar are underestimated since the peptide containing amino acids at positions 88–104 could not be identified in the MS/MS analysis.

Table 2. Distribution of Ole e 1 peptides identified by nanoLC–MS/MS in each spot analyzed of three olive cvs

Peptides ^{a)}	Position/ variant	P ^{b)}											Be ^{b)}						Ar ^{b)}					
		Pi1	Pi2	Pi3	Pi4	Pi5	Pi6	Pi7	Pi8	Pi9	Pi10	Pi11	Be1	Be2	Be3	Be4	Be5	Be6	Be7	Ar1	Ar2	Ar3	Ar4	
AGFITELSEFIPGASVR	28↔28/T↔N	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	2	1,2	2	2	2	2	2	2	2	2	2	2	2	1,2
	30↔30/L↔F	1,2	1,2	2	1,2	1,2	1,2	1,2	1,2	1,2	–	1,2	2	2	2	–	2	2	2	–	–	–	2	1
	38↔38/S↔G	2	2	2	2	1,2	1,2	2	1,2	1,2	2	2	–	–	–	–	2	2	2	–	–	–	–	–
	39↔39/V↔L	–	–	–	–	–	–	1	2	2	1,2	2	–	–	–	–	–	–	–	–	–	–	–	–
FFITELSEFIPGAGVR		1,2	2	–	–	2	2	–	–	2	–	–	2	–	2	–	2	2	–	–	–	–	–	1,2
		2	2	2	2	2	2	–	–	2	–	2	2	–	–	2	2	–	–	–	–	–	–	
		1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		–	–	–	–	–	–	–	–	–	–	–	–	–	2	2	2	–	–	–	–	–	–	
DKENGDVTFTEVG YTR		1	1,2	1,2	1,2	1,2	1,2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1,2
		–	–	–	1	2	2	2	2	2	2	1	2	2	2	2	2	2	–	2	2	2	2	2
		1	1	1,2	1,2	1,2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		–	–	–	–	–	1,2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
KNGDITFTEVG YTR		–	1	1	1	1	1,2	1,2	1,2	1,2	2	–	–	–	–	–	–	–	1	–	–	–	–	1
		1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2	2	1,2	2	2	2	2	2	2	2	2	2	2	2	1,2
		1,2	1,2	1,2	1,2	1,2	1,2	2	1,2	2	1,2	2	2	1,2	2	2	2	2	2	–	–	–	2	1,2
		?	1	1	1,2	1,2	1,2	1,2	1,2	1,2	2	2	2	2	2	2	2	2	2	–	–	–	2	2
DHKNEFCEITLSSSR		?	–	2	1,2	2	2	2	2	–	–	2	2	2	2	2	2	2	–	–	–	2	2	2
		?	–	–	–	–	–	–	2	–	–	–	2	2	2	2	2	–	–	–	–	–	–	–
		?	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		?	–	–	–	–	–	–	–	–	–	–	–	?	?	?	?	–	?	?	?	?	?	?
KDCDEIPIEGWAKPSLK		1	2	–	–	–	–	–	–	–	?	–	?	?	?	?	2	–	?	?	?	?	?	?
		2	1,2	1,2	1,2	2	2	–	–	–	?	–	?	?	?	?	–	2	?	?	?	?	?	?
		–	1	–	1	1	–	–	–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
		2	1,2	2	1,2	1	1	2	1,2	2	?	2	?	?	?	?	–	–	?	?	?	?	?	?
KDCNEIPIEGWAKPSLK		–	1,2	–	1,2	1	–	–	–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
DCNEIPIEGWAKPSLK							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
							2		–	–	?	–	?	?	?	?	–	–	?	?	?	?	?	?
FKLNTVNGTTR ^{c)}	106↔106/M↔K↔I	2	2	2	2	2	2	2	2	2	–	–	–	–	–	2	2	2	2	–	–	2	2	2
		2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2
		2	2	2	2	2	2	2	2	2	–	1	2	–	–	–	2	2	–	–	–	2	–	–
		2	2	–	–	–	–	1,2	1,2	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–

a) Amino acid microheterogeneities present in Ole e 1 peptides are shown in bold.
b) The presence (number) or absence (–) of each Ole e 1 peptide is indicated for each spot analyzed. The symbol “?” means that none of the variants of the corresponding peptide was identified. 1: C18 column; 2: carbon graphite column.
c) The molecular masses of peptides FKLNTVNGTTR and ,FILNTLNGTTR were very similar and could not be distinguished in the MS/MS spectra.

145 amino acids of Ole e 1 were replaced, covering approximately 15% of the whole sequence.

We found that four amino acid substitutions (at positions 91, 95, 99, and 110) were placed in immunodominant T-cell epitopes [58], and two others (at positions 56 and 110) corresponded to residues involved in IgG and IgE binding [41]. All these moieties are relevant for the allergenic response [41, 42]. These point changes might also affect the final 3-D folding of the Ole e 1 protein, which is another factor affecting the reactivity of the protein to IgG and IgE antibodies [4]. Therefore, together with the conspicuous variations in the amount of Ole e 1 found among these cvs [27], both quantitative and qualitative differences in Ole e 1 polymorphism could be also important in determining why pollen from certain cvs are more allergenic than others [27, 35]. Additionally, this polymorphism linked to the varietal origin of the pollen released to the atmosphere could also explain why allergic patients from different geographic locations exhibit differential reactivity to Ole e 1 [8, 43], as olive varieties are not cultivated homogeneously over large regions. However, the concomitances of other parameters (*i.e.* other major and secondary allergens) cannot be discarded and should be also evaluated.

Peptide composition significantly changed among varieties and among spots within a given cv (Table 2). The relative importance of each isoform could be estimated by analyzing their presence in the different spots and cvs. Thus, the peptide AGFITELSEFIPGASVR was only detected in spot Pi1 while AGFITELSEFIPGASVR was common to all spots analyzed. Similarly, the peptide DGENGKITFTEVGYTR was exclusively identified in Be. The numerical analysis of peptide sequences showed that Pi presented the highest variability, while Ar displayed the lowest proportion of peptide variants (Table 1). The olive tree has been cultivated for several millennia, leading to the appearance of an extensive germplasm that is currently made of at least 1250 cvs reported in 54 countries and conserved in 100 collections ([44], <http://www.oleadb.it/olivodb.html>). Therefore, it is conceivable that the polymorphism reported for the Ole e 1 protein until present is just the tip of the iceberg.

3.3 Study of *N*-glycan variability in olive cvs by performing LC-MS/MS analysis of *N*-glycopeptides

It is now evident that the genetic origin (*i.e.* cv) of pollen represents a major source of inconsistency for Ole e 1 at both nucleotidic and amino acid level ([10], the present work). However, nothing is known about the contribution of the *N*-glycan moiety to the polymorphism of Ole e 1 in different cvs. The alignment of the 33 Ole e 1 sequences identified in our study showed the existence of four isoforms of a peptide (positions 105–115 in the amino acid sequence) containing a putative *N*-glycosylation site. These glycopeptides were identified by nano-LC MS/MS using a carbon

graphite column coupled to an ion trap (Table 2). The peptides FKLNTVNGTTR and FILNTLNGTTR displayed very similar monoisotopic masses (1249.68 and 1248.68, respectively) and could not be discriminated in the MS/MS spectra. Indeed, CID MS/MS of *N*-glycopeptides performed on an ESI-IT generates preferentially fragment ions corresponding to the neutral loss of sugars on the peptide backbone. Thus, the MS/MS spectra were not informative enough to fully characterize the amino acid sequence of the glycopeptide. Moreover, all peptides except FILNTLNGTTR were present in several Ole e 1 sequences, while the FKLNTVNGTTR isoform was absent in Be and Ar.

The primary structure of the *N*-glycans of Ole e 1 has been elucidated in two independent works, in which the parameter “cultivar” was not considered [5, 6]. Thus, a high-mannose component (8% Man₇GlcNAc₂) and two complex type moieties containing β (1,2)-xylose and one (54%) or two (23%) terminal GlcNAc residues were identified as the main oligosaccharides. In these studies, fucosylated structures only accounted for 9% of the glycan pool.

Table 3 presents the distribution of *N*-glycans identified by nano-LC MS/MS in each protein spot from the three cvs. The individual spot picking from 2-D maps combined with good quality MS/MS spectra (Fig. S2, Supporting Information) allowed us the identification of a wide range of *N*-glycan moieties, including six different oligomannosidic compounds and eight biantennary complex type chains, mostly containing fucose and/or xylose residues linked to the core. The number of structures detected was higher compared with these two earlier studies described above, likely because of the different strategy followed here. Using an analytical approach based on glycopeptides analysis, the glycan composition can be readily correlated to the attachment site, due to the peptide moiety identification.

In most of the spots analyzed we found a pool of different *N*-glycan moieties. Nonetheless, we could assign some *N*-glycan moieties to specific Ole e 1 isoforms and confirm their presence in some spots after connecting our results of peptide mapping and *N*-glycan analysis. For instance, gi1362134 and gi14424429 Ole e 1 isoforms were present in the spot Pi1. They showed four different *N*-glycan moieties (*i.e.* Man₆GlcNAc₂, Man₇GlcNAc₂, GlcNAcMan₃XylGlcNAc₂, and GlcNAcMan₃XylGlcNAcFucGlcNAc) attached to the Asn residue of FILNTLNGTTR and FKLNTVNGTTR peptides, respectively. Interestingly, we found that the *N*-glycan profile differed considerably among spots as well as among cvs (Table 3). Thus, depending on the spot, the pool of identified glycans varied from 0 to 9 in Pi, from 1 to 9 in Be and from 1 to 6 in Ar. This variability makes the final glycoform profile to change depending on the cv considered. In agreement with earlier data, Man₇GlcNAc and GlcNAcMan₃XylGlcNAc₂ were the most widely distributed *N*-glycans in the three cvs analyzed. On the contrary, the *N*-glycans Man₉GlcNAc₂, Man₃XylGlcNAcFucGlcNAc, and Hex₂GlcNAcMan₃XylGlcNAc₂ were restricted to spots Pi7, Be5, and Be6, respectively. In addition, some glycans (*e.g.* Man₄GlcNAc₂)

Table 3. Distribution of *N*-glycans identified by nanoLC-MS/MS in each spot analyzed of three olive cvs

<i>N</i> -glycans ^{a)}	P ⁱ ^{b)}											Be ^{b)}							Ar ^{b)}				Total spots ^{c)}	
	Pi1	Pi2	Pi3	Pi4	Pi5	Pi6	Pi7	Pi8	Pi9	Pi10	Pi11	Be1	Be2	Be3	Be4	Be5	Be6	Be7	Ar1	Ar2	Ar3	Ar4		
High-mannose type																								
Man ₄ GlcNAc ₂	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	2	
Man ₅ GlcNAc ₂	-	-	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	5	
Man ₆ GlcNAc ₂	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	13	
Man ₇ GlcNAc ₂	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	18	
Man ₈ GlcNAc ₂	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	11	
Man ₉ GlcNAc ₂	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
Complex type																								
GlcNAcMan ₃ GlcNAc ₂	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	
HexGlcNAcMan ₃ GlcNAc ₂	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	7	
Hex ₂ GlcNAcMan ₃ XylGlcNAc ₂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1	
GlcNAcMan ₃ XylGlcNAc ₂	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	20	
GlcNAc ₂ Man ₃ XylGlcNAc ₂	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	10	
GlcNAcMan ₃ XylGlcNAcFucGlcNAc	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-	+	+	13	
GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	11	
Man ₃ XylGlcNAcFucGlcNAc	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1	
Total <i>N</i> -glycans <i>per spot</i>	8	9	9	8	8	7	7	8	7	4	0	1	2	1	1	8	9	6	2	1	6	3		

a) Hex indicates that a mannose or galactose residue is present in the *N*-glycan moiety.
b) The presence (+) or absence (-) of each *N*-glycan is indicated for each spot analyzed.
c) Number of spots containing the corresponding *N*-glycan moiety.

were found to be distributed in a cv-specific manner. Further analysis of released glycans will allow us to confirm the precise structure of these N-glycans.

The clinical relevance of such variability will be determined by the contribution of the carbohydrate to the total allergenic capacity of the molecule, which is still a matter of controversy. The IgE-binding and *in vitro* histamine-release effects of the Ole e 1 glycan have been demonstrated [8]. However, experiments with recombinant forms and a non-glycosylated mutant of Ole e 1 suggest that the allergenic capability of the polypeptide core could be dominant in the allergen [44, 45].

4 Concluding remarks

In the present work, we have confirmed that the predicted polymorphism of Ole e 1 at cDNA level is extended to the expressed protein (*i.e.* actual allergen exposure). We have also shown for the first time the existence of an important inter-cv variability in the peptide and N-glycan profiles. Since the olive germplasm is extensive, we can even expect a higher variability for this molecule as this study is extended to other cvs. This molecular complexity might explain to some extent why Ole e 1 displays such a high allergenic capacity in the olive pollen, and makes it difficult to determine the final number of Ole e 1 isoforms present in a particular cv. Interestingly, we noticed that the lower the amount of Ole e 1, the poorer is the pool of isoforms present in the pollen grain. Therefore, both parameters (quantity and quality) might be important in determining why some olive cvs are more allergenic than others. In our opinion, this factor should be considered for optimal design of allergen formulations, including the design of recombinant allergens for immunotherapy.

This work was supported by projects BFU2004-00601/BFI and P06-AGR-01791 from the MEC and the Junta de Andalucía, respectively.

The authors have declared no conflict of interest.

5 References

- [1] Villalba, M., Batanero, E., López-Otín, C., Sánchez, L. M. *et al.*, The amino acid sequence of Ole e I, the major allergen from olive tree (*Olea europaea*) pollen. *Eur. J. Biochem.* 1993, **216**, 863–869.
- [2] Lombardero, M., Barbas, J. A., Moscoso del Prado, J., Carreira, J., cDNA sequence analysis of the main olive allergen, Ole e I. *Clin. Exp. Allergy* 1994, **24**, 765–770.
- [3] Villalba, M., Batanero, E., Monsalve, R. I., González de la Peña, M. A. *et al.*, Cloning and expression of Ole e I, the major allergen from olive tree pollen. Polymorphism analysis and tissue specificity. *J. Biol. Chem.* 1994, **269**, 15217–15222.
- [4] González, E., Villalba, M., Lombardero, M., Aalbers, M. *et al.*, Influence of the 3D-conformation, glycan component and microheterogeneity on the epitope structure of Ole e 1, the major olive allergen: use of recombinant isoforms and specific monoclonal antibodies as immunological tools. *Mol. Immunol.* 2002, **39**, 93–101.
- [5] Kolarich, D., Altmann, F., N-glycan analysis by matrix-assisted laser desorption/ionization mass spectrometry of electrophoretically separated non-mammalian proteins: application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. *Anal. Biochem.* 2000, **285**, 64–75.
- [6] van Ree, R., Cabanes-Macheteau, M., Akkerdaas, J., Milazzo, J. P. *et al.*, (1, 2)-Xylose and (1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J. Biol. Chem.* 2000, **275**, 11451–11458.
- [7] Batanero, E., Villalba, M., Rodríguez, R., Glycosylation site of the major allergen from olive tree pollen. Allergenic implications of the carbohydrate moiety. *Mol. Immunol.* 1994, **31**, 31–37.
- [8] Batanero, E., Crespo, J. F., Monsalve, R. I., Martín-Esteban, M. I. *et al.*, IgE-binding and histamine-release capabilities of the main carbohydrate component isolated from the major allergen of olive tree pollen, Ole e 1. *J. Allergy Clin. Immunol.* 1999, **103**, 147–153.
- [9] Batanero, E., Villalba, M., Monsalve, R. I., Rodríguez, R., Cross-reactivity between the major allergen from olive pollen and unrelated glycoproteins: evidence of an epitope in the glycan moiety of the allergen. *J. Allergy Clin. Immunol.* 1996, **97**, 1264–1271.
- [10] Hamman-Khalifa, A. M., Castro, A. J., Jiménez-López, J. C., Rodríguez-García, M. I. *et al.*, Olive cultivar origin is a major cause of polymorphism for Ole e 1 pollen allergen. *BMC Plant Biol.* 2008, **8**, 10.
- [11] Weiss, W., Vogelmeier, C., Görg, A., Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis* 1993, **14**, 805–816.
- [12] Kwaasi, A. A. A., Parhar, R. S., Tipimeni, P., Harfi, H. A. *et al.*, Cultivar-specific epitopes in date palm (*Phoenix dactylifera* L.) pollenosis. Differential antigenic and allergenic properties of pollen from ten cultivars. *Int. Arch. Allergy Immunol.* 1994, **104**, 281–290.
- [13] Vieths, S., Jankiewicz, A., Schöning, B., Aulepp, H., Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen. *Allergy* 1994, **49**, 262–271.
- [14] Kwaasi, A. A. A., Harfi, H. A., Parhar, R. S., Collison, K. S. *et al.*, Cultivar-specific IgE-epitopes in date (*Phoenix dactylifera* L.) fruit allergy. *Int. Arch. Allergy Immunol.* 2000, **123**, 137–144.
- [15] Gao, Z. S., van de Weg, W. E., Schaart, J. G., van der Meer, I. M. *et al.*, Linkage map positions and allelic diversity of two Mal d 3 (non-specific lipid transfer protein) genes in the cultivated apple (*Malus domestica*). *Theor. Appl. Genet.* 2005, **110**, 479–491.
- [16] Gao, Z. S., van de Weg, W. E., Schaart, J. G., Schouten, H. J. *et al.*, Genomic cloning and linkage mapping of the Mal d 1 (PR-10) gene family in apple (*Malus domestica*). *Theor. Appl. Genet.* 2005, **111**, 171–183.

- [17] Gao, Z. S., van de Weg, W. E., Schaart, J. G., van Arkel, G. *et al.*, Genomic characterization and linkage mapping of the apple allergen genes Mal d 2 (thaumatin-like protein) and Mal d 4 (profilin). *Theor. Appl. Genet.* 2005, **111**, 1087–1097.
- [18] Fujimura, T., Shigeta, S., Kawamoto, S., Aki, T. *et al.*, Two-dimensional IgE-binding spectrum of Japanese cedar (*Cryptomeria japonica*) pollen allergens. *Int. Arch. Allergy Immunol.* 2004, **133**, 125–135.
- [19] Corti, V., Cattaneo, A., Bachi, A., Rossi, R. E. *et al.*, Identification of grass pollen allergens by two-dimensional gel electrophoresis and serological screening. *Proteomics* 2005, **5**, 729–736.
- [20] Petersen, A., Dresselhaus, T., Grobe, K., Becker, W. M., Proteome analysis of maize pollen for allergy-relevant components. *Proteomics* 2006, **6**, 6317–6325.
- [21] Beyer, K., Grishina, G., Bardina, L., Grishin, A. *et al.*, Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions. *J. Allergy Clin. Immunol.* 2002, **110**, 517–523.
- [22] Yu, C. J., Lin, Y. F., Chiang, B. L., Chow, L. P., Proteomics and immunological analysis of a novel shrimp allergen, Pen m 2. *J. Immunol.* 2003, **170**, 445–453.
- [23] Natale, M., Bisson, C., Monti, G., Peltran, A. *et al.*, Cow's milk allergens identification by two-dimensional immunoblotting and mass spectrometry. *Mol. Nutr. Food Res.* 2004, **48**, 363–369.
- [24] Batard, T., Hrabina, A., Bi, X. Z., Chabre, H. *et al.*, Production and proteomic characterization of pharmaceutical-grade *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* extracts for allergy vaccines. *Int. Arch. Allergy Immunol.* 2006, **140**, 295–305.
- [25] Yagami, T., Haishima, Y., Tsuchiya, T., Tomitaka-Yagami, A. *et al.*, Proteomic analysis of putative latex allergens. *Int. Arch. Allergy Immunol.* 2004, **135**, 3–11.
- [26] Aalberse, R. C., Clinical relevance of carbohydrate allergen epitopes. *Allergy* 1998, **53**, 54–57.
- [27] Castro, A. J., Alché, J. D., Cuevas, J., Romero, P. J. *et al.*, Pollen from different olive tree cultivars contains varying amounts of the major allergen Ole e 1. *Int. Arch. Allergy Immunol.* 2003, **131**, 164–173.
- [28] Görg, A., Postel, W., Günther, S., Two-dimensional electrophoresis. The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 1988, **9**, 531–546.
- [29] Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, **227**, 680–685.
- [30] Richert, S., Luche, S., Chevallet, M., van Dorselaer, A. *et al.*, About the mechanism of interference of silver staining with peptide mass spectrometry. *Proteomics* 2004, **4**, 909–916.
- [31] Lauzurica, P., Gurbindo, C., Maruri, N., Galocha, B. *et al.*, Olive (*Olea europea*) pollen allergens. I: immunochemical characterization by immunoblotting, CRIE and immunodetection by a monoclonal antibody. *Mol. Immunol.* 1988, **25**, 329–335.
- [32] Holmes-Davis, D., Tanaka, C. K., Vensel, W. H., Hurkman, W. J. *et al.*, Proteome mapping of mature pollen of *Arabidopsis thaliana*. *Proteomics* 2005, **5**, 4864–4884.
- [33] Dai, S. J., Lei, L., Chen, T. T., Chong, K. *et al.*, Proteomic analyses of *Oryza sativa* mature pollen reveal novel proteins associated with pollen germination and tube growth. *Proteomics* 2006, **6**, 2504–2529.
- [34] Sheoran, I. S., Ross, A. R. S., Olson, D. J. H., Sawhney, V. K., Proteomic analysis of tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) pollen. *J. Exp. Bot.* 2007, **58**, 3525–3535.
- [35] Carnés-Sánchez, J., Iraola, V. M., Sastre, J., Florido, F. *et al.*, Allergenicity and immunochemical characterization of six varieties of *Olea europaea*. *Allergy* 2002, **57**, 313–318.
- [36] Jiménez-López, J. C., Hammam-Khalifa, A. M., Romero, P. J., Alché, J. D. *et al.*, Intra and intercultivar variability of Ole e 1 in olive pollen. Preliminary analysis of patient's response to different cultivars extracts. *Polen* 2004, **14**, 386–387.
- [37] Fernández-Caldas, E., Carnés, J., Iraola, V., Casanovas, M., Comparison of the allergenicity and Ole e 1 content of 6 varieties of *Olea europaea* pollen collected during 5 consecutive years. *Ann. Allergy Asthma Immunol.* 2007, **98**, 464–470.
- [38] Buters, J. T., Kasche, A., Weichenmeier, I., Schober, W. *et al.*, Year-to-year variation in release of Bet v 1 allergen from birch pollen: evidence for geographical differences between West and South Germany. *Int. Arch. Allergy Immunol.* 2008, **145**, 122–130.
- [39] Rogerieux, F., Godfrin, D., Sénéchal, H., Motta, A. C. *et al.*, Modifications of *Phleum pratense* grass pollen allergens following artificial exposure to gaseous air pollutants (O₃, NO₂, SO₂). *Int. Arch. Allergy Immunol.* 2007, **143**, 127–134.
- [40] Waisel, Y., Geller-Bernstein, C., Keynan, N., Arad, G., Antigenicity of the pollen proteins of various cultivars of *Olea europaea*. *Allergy* 1996, **51**, 819–825.
- [41] Cárdbaba, B., Del Pozo, V., Jurado, A., Gallardo, S. *et al.*, Olive pollen allergy: searching for immunodominant T-cell epitopes on the Ole e 1 molecule. *Clin. Exp. Allergy* 1998, **28**, 413–422.
- [42] González, E., Villalba, M., Quiralte, J., Batanero, E. *et al.*, Analysis of IgE and IgG B-cell immunodominant regions of Ole e 1, the main allergen from olive pollen. *Mol. Immunol.* 2006, **43**, 570–578.
- [43] Rodríguez, R., Villalba, M., Monsalve, R. I., Batanero, E. *et al.*, Perfiles de reconocimiento alérgico del polen de olivo. Implicación en la diagnosis e inmunoterapia. *Rev. Real Acad. Cienc. Exactas Fís. Natural.* 1998, **92**, 253–258.
- [44] Quiralte, J., González, E., Arias de Saavedra, J. M., Villalba, M. *et al.*, Immunological activity of recombinant Ole e 1 in patients with *Olea europaea* pollinosis. *Int. Arch. Allergy Immunol.* 2000, **122**, 101–107.
- [45] Villalba, M., Huecas, S., González, E., Batanero, E. *et al.*, Expression in *Pichia pastoris* and characterization of a non-glycosylated mutant of Ole e 1. *J. Allergy Clin. Immunol.* 1999, **103**, S88.

PROTEOMICS

Supporting Information for Proteomics

DOI 10.1002/pmic.200900192

Antonio Jesús Castro, Audrey Bednarczyk,
Christine Schaeffer-Reiss, María Isabel Rodríguez-García,
Alain van Dorsselaer and Juan de Dios Alché

**Screening of Ole e 1 polymorphism among olive cultivars by peptide mapping
and N-glycopeptide analysis**

Additional files

Figure S1. Comparison of the MS/MS spectra of two peptides allowing to identify the variant L $\leftrightarrow$ F in position 30 of Ole e1 amino acids sequence with (A) MS/MS spectrum of the doubly charged parent ion at m/z 818.4 corresponding to the peptide FITEELSEFIPGAGVR and (B) MS/MS spectrum of the doubly charged parent ion at m/z 835.4 corresponding to the peptide FITEFSEFIPGAGVR.

Figure S2. MS/MS spectra of the glycopeptide LNTVN¹¹¹GTTR, glycosylated in Asn¹¹¹. (A), MS/MS spectrum from parent ion at m/z 1036.5 corresponding to the peptide LNTVNGTTR with the N-glycosylation HexNAcMan₃GlcNAc₂ in position Asn¹¹¹. (B), MS/MS spectrum from parent ion at m/z 1102.5 corresponding to the peptide LNTVNGTTR with the N-glycosylation HexNAcMan₃XylGlcNAc₂ in position Asn¹¹¹. N-glycans are represented using the symbol nomenclature outlined by the consortium for functional glycomics (<http://www.functionalglycomics.org>). (■)N-acetylhexosamine, HexNAc; (●) hexose, Hex; (★) xylose, Xyl.

Table S1. List of olive Ole e 1 proteins identified after IEF/SDS-PAGE. A C18 reversed phase column was used for the analysis of tryptic digests by nano-LC-MS/MS.

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi1	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLIER (89) FITESEFIPGASVR (73) DGENGSIITFEVGYTR (88) KDCDEIPVEGWVKPSLK (56)	FILNTLNGTTR	32
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLVER (80) AGFITESEFIPGASLR (55) DKENGDTVTFTEVGYTR (116)	FMLNTVNGTTR	40
	Ole e 1 (<i>Olea europaea</i>)	15491/6.34	gi 1362129 gi 2465131	AEGLYSMLVER (80) FITESEFIPGASVR (73) EKENGDIITFEIGYTR (90)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16454/5.91	gi 1362132	ITFEVGYTR (76) AEGLYSMLIER (89) FITESEFIPGAGVR (61)	FMLNTVNGTTR	24
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 13195753 gi 1362133	AEGLYSMLVER (80) NGDITFEVGYTR (79) AGFITESEFIPGASVR (59)	FILNTVNGTTR	30
Pi2	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 37724593 gi 37724597	AEGLYSMLVER (83) AGFITESEFIPGASLR (77) DKENGDTVTFTEVGYTR (133) KDCNEIPTEGWVKPSLK (68)	FILNTLNGTTR	56
	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITESEFIPGASLR (77) DKENGDTVTFTEVGYTR (133) DHKNEFCEITLISSGR (93) KDCNEIPTEGWAKPSLK (67)	FKLNTVNGTTR	53
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (83) FITESEFIPGASVR (50) EKENGDIITFEIGYTR (85) DHKNEFCEITLISSGR (93) KDCDEIPIEGWAKPSLK (60)	FILNTVNGTTR	51
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) NGDITFEVGYTR (85) KNGDITFEVGYTR (79) DHKNEFCEITLISSGR (93)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER (83) NGDITFEVGYTR (85) KNGDITFEVGYTR (79) AGFITESEFIPGASVR (87)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFEVGYTR (66) AEGLYSMLIER (75) KDCDEIPTEGWVKPSVK (55)	FILNTVNGTTR	26
Pi3	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (72) FITESEFIPGASVR (76) EKENGDIITFEIGYTR (88) DHKNEFCEITLISSGR (69) KDCDEIPIEGWAKPSLK (57)	FILNTVNGTTR	51
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (72) KNGDITFEVGYTR (55) DHKNEFCEITLISSGR (69)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429	AEGLYSMLVER(ox) (72) DKENGDTVTFTEVGYTR (94) DHKNEFCEITLISSGR (69)	FILNTLNGTTR	31

1
2
3

Table S1. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi3	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129 gi 33323443 gi 33325111 gi 33329730 gi 33329734 gi 33329744 gi 33329750 gi 37548749	AEGLYSMLVER (ox) (72) AGFTELSEFIPGASVR (68) DHKNEFCEITLISSGR (69)	FILSTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLIER (73) FITELSEFIPGASVR (76)	FMLNTVNGTTR	17
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (65) AEGLYSMLIER (73)	FILNTVNGTTR	14
Pi4	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (83) FITELSEFIPGASVR (94) EKENGDTTFTEIGYTR (130) DHKNEFCEITLISSGR (87) KDCDEIPIEGWAKPSLK (60)	FILNTVNGTTR	51
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 37724593 gi 37724597	AEGLYSMLVER (83) AGFTELSEFIPGASLR (64) DKENGDTVTFTEVGYTR (130) DHKNEFCEITLISSGR (87) KDCNEIPTEGWVKPSLK (77)	FILNTLNGTTR	56
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (83) AGFTELSEFIPGASVR (79) DKENGDTVTFTEIGYTR (120) DHKNEFCEITLISSGR (87) KDCNEIPTEGWAKPSLK (66)	FILNTVNGTTR	52
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVGYTR (130) DHKNEFCEITLISSGR (87)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (82) AEGLYSMLIER (75) DHKNEFCEITLLSSSR (72) KDCDEIPTEGWVKPSVK (68)	FILNTVNGTTR	37
Pi5	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 37724593 gi 37724597	AEGLYSMLVER (83) AGFTELSEFIPGASLR (65) DKENGDTVTFTEVGYTR (120) DHKNEFCEITLISSGR (80) KDCNEIPTEGWVKPSLK (68)	FILNTLNGTTR	56
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVGYTR (99) AGFINELSEFIPGASVR (75) DHKNEFCEITLISSGR (83)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (83) FITELSEFIPGASVR (91) EKENGDTTFTEIGYTR (84) DHKNEFCEITLISSGR (82)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER (83) KNGDITFTEVGYTR (99) AGFTELSEFIPGASVR (81)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLIER (79) FITELSEFIPGASVR (91)	FMLNTVNGTTR	17
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	AEGLYSMLIER (79) KDCDEIPTEGWVKPSVK (57)	FILNTVNGTTR	19

1

2

Table S1. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi6	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVG YTR (120) AGFINELSEFIPGASVR (51) DHKNEFCEITLISSGR (78)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITELSEFIPGASLR (87) DKENGDTVTFTEVG YTR (112) DHKNEFCEITLISSGR (78) KDCNEIPTEGWAKPSLK (52)	FKLNTVNGTTR	53
	Ole e 1 (<i>Olea europaea</i>)	14873/7.74	gi 33323443 gi 33325111 gi 37548749	AEGLYSMLVER (83) ENGDITFTEVG YTR (97) AGFITELSEFIPGASVR (65) DHKNEFCEITLISSGR (78) KDCNEIPTEGWAKPSLK (52)	FILNTVNGTTR	56
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (83) FITELSEFIPGASVR (72) DHKNEFCEITLISSGR (78)	FILNTVNGTTR	28
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLIER (70) FITELSEFIPGASVR (72)	FMLNTVNGTTR	17
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVG YTR (68) AEGLYSMLIER (70)	FILNTVNGTTR	14
Pi7	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (80) KNGDITFTEVG YTR (122) DHKNEFCEITLISSGR (106)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	14873/7.74	gi 33323443 gi 33325111 gi 33329730 gi 33329734 gi 33329742 gi 33329744 gi 33329750 gi 37548749	AEGLYSMLVER (80) ENGDITFTEVG YTR (51) AGFITELSEFIPGASVR (84) DHKNEFCEITLISSGR (106)	FILNTVNGTTR	43
	Ole e 1 (<i>Olea europaea</i>)	14432/5.85	gi 33329740	AEGLYSMLVER (80) AGFITELSEFIPGAGVR (79) DHKNEFCEITLISSGR (106)	FILNTVNGTTR	34
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER (80) AGFITELSEFIPGASLR (74) DHKNEFCEITLISSGR (106)	FILNTLNGTTR	32
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136 gi 3256212 gi 33329754 gi 33329756 gi 33329758	FMLNTVNGTTR (53)	FMLNTVNGTTR	7
Pi8	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVG YTR (111) AGFINELSEFIPGASVR (80) DHKNEFCEITLISSGR (56)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129 gi 33323443 gi 33325111 gi 37548749	AEGLYSMLVER (83) AGFITELSEFIPGASVR (84) DHKNEFCEITLISSGR (56) KDCNEIPTEGWAKPSLK (63)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITELSEFIPGASLR (81) DHKNEFCEITLISSGR (56) KDCNEIPTEGWAKPSLK (63)	FKLNTVNGTTR	42

Table S1. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi8	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136 gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER (62) FMLNTVNGTTR (76)	FMLNTVNGTTR	15
Pi9	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFEVGYTR (82) AGFINELSEFIPGASVR (94) DHKNEFCEITLISSGR (83)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129 gi 33323443 gi 33325111 gi 33329730 gi 33329734 gi 33329742 gi 33329744 gi 33329750 gi 37548749	AEGLYSMLVER (83) AGFITELSEFIPGASVR (76) DHKNEFCEITLISSGR (83)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER (83) AGFITELSEFIPGASLR (66) DHKNEFCEITLISSGR (83)	FILNTLNGTTR	32
Pi10	Ole e 1 (<i>Olea europaea</i>)	14432/5.85	gi 33329740	AGFITELSEFIPGAGVR (76)	FILNTVNGTTR	13
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362131 gi 1362132 gi 1362136 gi 1362137 gi 3256212 gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER (72)	FKLNTVNGTTR	8
Pi11	Ole e 1 (<i>Olea europaea</i>)	16536	gi 2465127	FILNTVNGTTR (71) AEGLYSMLVER (79) AGFITELSEFIPGASVR (59) DKENGDTVTFTEIGYTR (102)	FILNTVNGTTR	37
	Ole e 1 (<i>Olea europaea</i>)	16318	gi 1362135 gi 37724593 gi 37724597	FILNTVNGTTR (71) AEGLYSMLVER (79) AGFITELSEFIPGASLR (67)	FILNTVNGTTR	26
	Ole e 1 (<i>Olea europaea</i>)	15384	gi 1362134	FILNTLNGTTR (66) AEGLYSMLVER (79) AGFITELSEFIPGASLR (67)	FILNTLNGTTR	28
	Ole e 1 (<i>Olea europaea</i>)	16474	gi 1362131	ITFTEVGYTR (57) FILNTVNGTTR (71) AEGLYSMLIER (72)	FILNTVNGTTR	22
Be1	Thioredoxin- dependent peroxidase (<i>Plantago major</i>)	17381/5.34	gi 52851172	FALLVEDLK (54) GVDEILCISVNDPFVMK (105)	None	16
Be2	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (66) AEGLYSMLIER(ox) (68)	FILNTVNGTTR	14
	Thioredoxin- dependent peroxidase (<i>Plantago major</i>)	17381/5.34	gi 52851172	FALLVEDLK (52) VILFAVPGAFTPTCSMK (56) GVDEILCISVNDPFVMK (104)	None	26

1

Table S1. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Be3	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130 gi 1362133 gi 13195753	NGDITFTEVGYTR (100)	FILNTVNGTTR	9
	Thioredoxin-dependent peroxidase (<i>Plantago major</i>)	17381/5.34	gi 52851172	FALLVEDLK (58) VILFAVPGAFTPTCSMK (60) GVDEILCISVNDPFVVMK (102)	None	26
Be7	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130 gi 1362133 gi 13195753	KNGDITFTEVGYTR (78)	FILNTVNGTTR	10
Ar4	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (66) NEFCEITLISSGR (103) KNGDITFTEVGYTR (60)	FILNTVNGTTR	27
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (66) KNGDITFTEVGYTR (60) AGFITEELSEFIPGASVR (76)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (66) NEFCEITLISSGR (103) AGFITEELSEFIPGASLR (55) DKENGDVTFTEVGYTR (58)	FILNTLNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362132 gi 1362137	AEGLYSMLIER (79) GSITFTEVGYTR (68) FITEELSEFIPGAGVR (70)	FKLNTVNGTTR	27

a) Spots are named accordingly to Fig. 1.

b) Theoretical molecular mass (Da)/Theoretical isoelectric point.

c) Accession number in NCBI database after BLAST homology search. All accession numbers corresponding to Ole e 1 proteins identified with the same set of peptides are grouped (for each group the first accession number is in bold).

d) Percentage of amino acids in reference proteins covered by matching peptides from nano-LC-MS/MS analysis.

2

3

Table S2. List of olive *Olea e 1* proteins identified after IEF/SDS-PAGE. A carbon graphite column was used for the analysis of tryptic digests by nano-LC-MS/MS.

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi1	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129 gi 33323443 gi 33325111 gi 37548749	AEGLYSMLVER (83) AGFITESEFIPGASVR (55) KDCNEIPTEGWAKPSLK (55)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITESEFIPGASLR (68) KDCNEIPTEGWAKPSLK (55)	FKLNTVNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (65) AEGLYSMLIER(ox) (76) FITEFSEFIPGAGVR (83)	FILNTVNGTTR	24
	Ole e 1 (<i>Olea europaea</i>)	15491/6.34	gi 1362129 gi 2465131	AEGLYSMLVER (83) FITESEFIPGASVR (83) KDCDEIPIEGWAKPSLK (56)	FILNTVNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) AGFINESEFIPGASVR (73)	FILNTVNGTTR	20
Pi2	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 37724593 gi 37724597	AEGLYSMLVER (83) AGFITESEFIPGASLR (76) DKENGDTVTFTEVGYTR (63) KDCNEIPTEGWVKPSLK (58)	FILNTLNGTTR	44
	Ole e 1 (<i>Olea europaea</i>)	15491/6.34	gi 1362129 gi 2465131	AEGLYSMLVER (83) FITESEFIPGASVR (78) KDCDEIPIEGWAKPSLK (63)	FILNTVNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITESEFIPGASLR (76) DKENGDTVTFTEVGYTR (63) KDCNEIPTEGWAKPSLK (59)	FKLNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	15522/7.75	gi 1362132	CAPQVFNK (51) AEGLYSMLIER (77) FITESEFIPGAGVR (72) KDCDEIPVEGWVKPSLK (50)	FKLNTVNGTTR	37
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER (83) NGDITFTEVGYTR (81) AGFITESEFIPGASVR (75)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) NGDITFTEVGYTR (81) AGFINESEFIPGASVR (69)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (65) AEGLYSMLIER (77) FITEFSEFIPGAGVR (76)	FILNTVNGTTR	24
	Ole e 1 (<i>Olea europaea</i>)	15491/6.34	gi 1362129 gi 2465131	AEGLYSMLVER (83) FITESEFIPGASVR (83) EKENGDTFTEIGYTR (104) KDCDEIPIEGWAKPSLK (57)	FILNTVNGTTR	43
Pi3	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (65) AEGLYSMLIER(ox) (75) FITEFSEFIPGAGVR (64) DHKNEFCEITLLSSSR (91)	FILNTVNGTTR	35
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER (83) NGDITFTEVGYTR (103) AGFITESEFIPGASVR (79)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	15491/6.34	gi 1362129 gi 2465131	AEGLYSMLVER (83) FITESEFIPGASVR (83) EKENGDTFTEIGYTR (104) KDCDEIPIEGWAKPSLK (57)	FILNTVNGTTR	43

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi3	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITELSEFIPGASLR (73) DKENGDTVTFTEVGYTR (50) KDCNEIPTEGWAKPSLK (59)	FKLNTVNGTTR	42
Pi4	Ole e 1 (<i>Olea europaea</i>)	16318/5.73	gi 1362135	AEGLYSMLVER (83) AGFITELSEFIPGASLR (67) DKENGDTVTFTEVGYTR (96) DHKNEFCEITLISSGR (53) KDCNEIPTEGWVWKPSLK (70) EDVPQPPVSQFHIQGVYCDTCR (63)	FILNTVNGTTR	68
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131	AEGLYSMLVER (83) FITELSEFIPGASVR (78) EKENGDTTFTEIGYTR (59) DHKNEFCEITLISSGR (53) KDCDEIPIEGWAKPSLK (56) EDVPQPPVSQFHIQGVYCDTCR (63)	FILNTVNGTTR	67
	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITELSEFIPGASLR (67) DKENGDTVTFTEVGYTR (96) DHKNEFCEITLISSGR (53) KDCNEIPTEGWAKPSLK (58)	FKLNTVNGTTR	53
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (83) AGFITELSEFIPGASVR (80) DHKNEFCEITLISSGR (53) KDCNEIPTEGWAKPSLK (58) EDVPQPPVSQFHIQGVYCDTCR (63)	FILNTVNGTTR	57
	Ole e 1 (<i>Olea europaea</i>)	14575/7.74	gi 13195753	AEGLYSMLVER (83) NGDITFTEVGYTR (103) AGFITELSEFIPGASVR (80) DCNEIPTEGWAKPSLK (66)	FILNTVNGTTR	43
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (71) AEGLYSMLIER (76) FITEFSEFIPGAGVR (90) DHKNEFCEITLLSSSR (87)	FILNTVNGTTR	35
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) NGDITFTEVGYTR (103) AGFINELSEFIPGASVR (60) DHKNEFCEITLISSGR (53)	FILNTVNGTTR	41
Pi5	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131	AEGLYSMLVER (80) EKENGDTTFTEIGYTR (116) DHKNEFCEITLISSGR (75) KDCDEIPIEGWAKPSLK (68) EDVPQPPVSQFHIQGVYCDTCR (92)	FILNTVNGTTR	56
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (80) AGFITELSEFIPGASVR (79) DKENGDTVTFTEIGYTR (119) DHKNEFCEITLISSGR (75) EDVPQPPVSQFHIQGVYCDTCR (92)	FILNTVNGTTR	56
	Ole e 1 (<i>Olea europaea</i>)	16318/5.73	gi 1362135	AEGLYSMLVER (80) AGFITELSEFIPGASLR (69) DKENGDTVTFTEVGYTR (110) DHKNEFCEITLISSGR (75) EDVPQPPVSQFHIQGVYCDTCR (92)	FILNTVNGTTR	57

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi5	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (80) NGDITFTEVG YTR (91) AGFINELSEFIPGASVR (75) DHKNEFCEITLISSGR (75)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVG YTR (65) AEGLYSMLIER (77) FITEFSEFIPGAGVR (84) DHKNEFCEITLLSSSR (78)	FILNTVNGTTR	35
Pi6	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITELSEFIPGASLR (65) DKENG DVT FTEVG YTR (117) DCNEIPTEGWAKPSLK (58) DHKNEFCEITLISSGR (89) EDIPQPPVSQFHIQQVYCDTCR (109)	FKLNTVNGTTR	68
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131	AEGLYSMLVER (83) FITELSEFIPGASVR (85) EKENG DIT FTEIG YTR (109) DHKNEFCEITLISSGR (89) KDCDEIPIEGWAKPSLK (54) EDVPQPPVSQFHIQQVYCDTCR (77)	FILNTVNGTTR	67
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVG YTR (125) AGFINELSEFIPGASVR (83) DHKNEFCEITLISSGR (89)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (83) AGFITELSEFIPGASVR (83) DCNEIPTEGWAKPSLK (58) DKENG DVT FTEIG YTR (53) DHKNEFCEITLISSGR (89) EDVPQPPVSQFHIQQVYCDTCR (77)	FILNTVNGTTR	67
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133	AEGLYSMLVER (83) KNGDITFTEVG YTR (125) AGFITELSEFIPGASVR (83) DCNEIPTEGWGKPSLK (67)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	TINPLGFFK (50) AEGLYSMLIER(ox) (73) FITELSEFIPGASVR (85) EDVPQPPVSQFHIQQVYCDTCR (77)	FMLNTVNGTTR	40
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	TINPLGFFK (50) ITFTEVG YTR (62) AEGLYSMLIER(ox) (73) FITEFSEFIPGAGVR (99)	FILNTVNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	14759/5.89	gi 33329750	ENG DIT FTEVG YTR (104)	FILNTVNGTTR	11
Pi7	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (83) AGFITELSEFIPGASVR (84) DKENG DVT FTEIG YTR (109) DHKNEFCEITLISSGR (90) KDCNEIPTEGWAKPSLK (55) EDVPQPPVSQFHIQQVYCDTCR (83)	FILNTVNGTTR	68

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi7	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (83) AGFITELSEFIPGASLR (58) DKENGDTVTFTEVG YTR (120) DHKNEFCEITLISSGR (90) KDCNEIPTEGWAKPSLK (55) EDIPQPPVSQFHIQGQVYCDTCR (74)	FKLNTVNGTTR	68
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVG YTR (104) AGFINELSEFIPGASVR (81) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131	AEGLYSMLVER (83) FITELSEFIPGASVR (73) EKENG DITFTEIG YTR (106) DHKNEFCEITLISSGR (90) EDVPQPPVSQFHIQGQVYCDTCR (83)	FILNTVNGTTR	55
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	FMLNTVNGTTR(ox) (51) AEGLYSMLIER (75) FITELSEFIPGASVR (73) DHKDEFCEITLISSSR (50) EDVPQPPVSQFHIQGQVYCDTCR (83)	FMLNTVNGTTR	52
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362132 gi 1362137	AEGLYSMLIER (75) GSITFTEVG YTR (72)	FKLNTVNGTTR	16
Pi8	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (79) AGFITELSEFIPGASLR (73) DKENGDTVTFTEVG YTR (67) DHKNEFCEITLISSGR (82) KDCNEIPTEGWAKPSLK (69) EDIPQPPVSQFHIQGQVYCDTCR (73)	FKLNTVNGTTR	68
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (79) AGFITELSEFIPGASVR (75) DKENGDTVTFTEIG YTR (102) DHKNEFCEITLISSGR (82) KDCNEIPTEGWAKPSLK (69)	FILNTVNGTTR	52
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (79) KNGDITFTEVG YTR (103) AGFINELSEFIPGASVR (120) DHKNEFCEITLISSGR (80)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (79) FITELSEFIPGASVR (73) EKENG DITFTEIG YTR (106) DHKNEFCEITLISSGR (82)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	14432/5.85	gi 33329740	AEGLYSMLVER (79) AGFITELSEFIPGAGVR (88) DHKNEFCEITLISSGR (82)	FILNTVNGTTR	34
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136 gi 33329754 gi 33329756 gi 33329758	FMLNTVNGTTR (77) AEGLYSMLIER(ox) (75) FITELSEFIPGASVR (73)	FMLNTVNGTTR	25

1
2
3
4
5
6
7

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi9	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	AEGLYSMLVER (79) AGFITELSEFIPGASLR (59) DKENGDTVTFTEVG YTR (127) DHKNEFCEITLISSGR (94) KDCNEIPTEGWAKPSLK (60) EDIPQPPVSQFHIQQVYCDTCR (97)	FKLNTVNGTTR	68
	Ole e 1 (<i>Olea europaea</i>)	14575/7.74	gi 13195753	AEGLYSMLVER (79) KNGDITFTEVG YTR (125) AGFITELSEFIPGASVR (73) DCNEIPTEGWAKPSLK (65) DHKNEFCEITLISSGSK (51)	FILNTVNGTTR	57
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (79) KNGDITFTEVG YTR (125) AGFINELSEFIPGASVR (82) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (79) AGFITELSEFIPGASVR (73) DKENGDTVTFTEIG YTR (117) DHKNEFCEITLISSGR (94) KDCNEIPTEGWAKPSLK (60)	FILNTVNGTTR	52
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (79) FITELSEFIPGASVR (88) EKENG DITFTEIG YTR (105) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	14432/5.85	gi 33329740	AEGLYSMLVER (79) AGFITELSEFIPGAGVR (54) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	34
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLIER (76) FITELSEFIPGASVR (88)	FMLNTVNGTTR	17
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	AEGLYSMLIER (76) FITEFSEFIPGAGVR (53)	FILNTVNGTTR	17
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362132 gi 1362137 gi 3256212 gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER (76) FITELSEFIPGAGVR (53)	FKLNTVNGTTR	18
Pi10	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) KNGDITFTEVG YTR (125) AGFINELSEFIPGASVR (71) DHKNEFCEITLISSGR (84)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER (83) KNGDITFTEVG YTR (125) AGFITELSEFIPGASVR (73)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (83) FITELSEFIPGASVR (94) EKENG DITFTEIG YTR (102) DHKNEFCEITLISSGR (84)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (83) AGFITELSEFIPGASVR (73) DKENGDTVTFTEIG YTR (115) DHKNEFCEITLISSGR (84)	FILNTVNGTTR	41

1
2
3
4

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Pi10	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER (83) DKENGDTVTFTEVG YTR (122) DHKNEFCEITLISSGR (84)	FILNTLNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	14432/5.85	gi 33329740	AEGLYSMLVER (83) AGFITELSEFIPGAGVR (65) DHKNEFCEITLISSGR (84)	FILNTVNGTTR	34
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLIER(ox) (76) FITELSEFIPGASVR (94)	FMLNTVNGTTR	17
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362132 gi 1362137	AEGLYSMLIER(ox) (76) GSITFTEVG YTR (83)	FKLNTVNGTTR	16
Pi11	Ole e 1 (<i>Olea europaea</i>)	16319/6.18	gi 14424429	LNTVNGTTR (58) AEGLYSMLVER (83) AGFITELSEFIPGASLR (74) DKENGDTVTFTEVG YTR (117) DHKNEFCEITLISSGR (90) KDCNEIPTEGWAKPSLK (52)	FKLNTVNGTTR	59
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (83) FITELSEFIPGASVR (100) EKENG DITFTEIG YTR (50) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER (83) NGDITFTEVG YTR (99) AGFINELSEFIPGASVR (70) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129 gi 33323443 gi 33325111 gi 37548749	AEGLYSMLVER (83) AGFITELSEFIPGASVR (69) DHKNEFCEITLISSGR (90) KDCNEIPTEGWAKPSLK (52)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVG YTR (76) AEGLYSMLIER (79) FITFSEFIPGAGVR (68) DHKNEFCEITLLSSSR (66)	FILNTVNGTTR	35
	Ole e 1 (<i>Olea europaea</i>)	14432/5.85	gi 33329740	AEGLYSMLVER (83) AGFITELSEFIPGAGVR (57) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	34
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134	FILNTLNGTTR (68) AEGLYSMLVER (63) AGFITELSEFIPGASLR (71) DKENGDTVTFTEVG YTR (125) DHKNEFCEITLISSGR (75)	FILNTLNGTTR	52
Be1	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER (63) AGFITELSEFIPGASVR (100) DKENGDTVTFTEIG YTR (112) DHKNEFCEITLISSGR (75)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER (63) NGDITFTEVG YTR (103) AGFITELSEFIPGASVR (100) DHKNEFCEITLISSGSK (72)	FILNTVNGTTR	42

1
2
3
4

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER (63) FITESEFIPGASVR (94) EKENGDTTFTEIGYTR (103) DHKNEFCEITLISSGR (75)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	16404/5.43	gi 1362136	AEGLYSMLIER (62) DEFCEITLISSSR (75) FITESEFIPGASVR (94)	FMLNTVNGTTR	26
	Ole e 1 (<i>Olea europaea</i>)	14866/5.89	gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER (62) FITESEFIPGAGVR (51) DHKNEFCEITLISSSR (66)	FMLNTVNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	AEGLYSMLIER (60) FITESEFIPGAGVR (50) DHKNEFCEITLISSSR (66)	FILNTVNGTTR	28
	Thioredoxin- dependent peroxidase (<i>Plantago lanceolata</i>)	17381/5.34	gi 52851172	FALLVEDLK RFALLVEDLK VILFAVPGAFTPTCSMK	None	16
Be2	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127	FILNTVNGTTR (86) AEGLYSMLVER(ox) (74) AGFITESEFIPGASVR (88) DKENGDTVTFTEIGYTR (127) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	48
	Ole e 1 (<i>Olea europaea</i>)	16318/5.73	gi 1362135 gi 37724593 gi 37724597	FILNTVNGTTR (86) AEGLYSMLVER(ox) (74) AGFITESEFIPGASLR (79) DKENGDTVTFTEVGYTR (99) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	48
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	FILNTVNGTTR (86) AEGLYSMLVER(ox) (74) FITESEFIPGASVR (76) EKENGDTTFTEIGYTR (103) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	47
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	FILNTVNGTTR (86) AEGLYSMLVER(ox) (74) NGDTTFTEVGYTR (104) DHKNEFCEITLISSGR (94)	FILNTVNGTTR	37
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVGYTR (53) FILNTVNGTTR (86) AEGLYSMLIER(ox) (63) DGENGKITFTEVGYTR (78) DHKNEFCEITLISSSR (54)	FILNTVNGTTR	37
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	FILNTVNGTTR (86) AEGLYSMLVER(ox) (74) NGDTTFTEVGYTR (104) AGFITESEFIPGASVR (88) DHKNEFCEITLISSGSK (56)	FILNTVNGTTR	50
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362132 gi 1362137	AEGLYSMLIER(ox) (63) GSITFTEVGYTR (63)	FKLNTVNGTTR	16
	Thioredoxin- dependent peroxidase (<i>Plantago lanceolata</i>)	17381/5.34	gi 52851172	FALLVEDLK (52) RFALLVEDLK (50) VILFAVPGAFTPTCSMK (53) GVDEILCISVNDPVMK(ox) (80)	None	27

1
2
3
4
5

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Be3	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (69) AGFITELSEFIPGASVR (85) DKENGDTVTFTEIGYTR (124) DHKNEFCEITLISSGR (91)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (69) FITELSEFIPGASVR (66) EKENGDTTFTEIGYTR (103) DHKNEFCEITLISSGR (91)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593	AEGLYSMLVER(ox) (69) AGFITELSEFIPGASLR (50) DKENGDTVTFTEVG YTR (117) DHKNEFCEITLISSGR (91)	FILNTLNGTTR	44
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (69) NGDITFTEVG YTR (100) DHKNEFCEITLISSGR (69)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (69) NGDITFTEVG YTR (100) AGFITELSEFIPGASVR (85) DHKNEFCEITLISSGSK (71)	FILNTVNGTTR	42
	Thioredoxin- dependent peroxidase (<i>Plantago major</i>)	17381/5.34	gi 52851172	FALLVEDLK (52) VILFAVPGAFTPTCSMK (60) GVDEILCISVNDPFVMK (88)	None	26
Be4	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (68) AGFITELSEFIPGASVR (75) DKENGDTVTFTEIGYTR (125) DHKNEFCEITLISSGR (99)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (68) FITELSEFIPGASVR (88) EKENGDTTFTEIGYTR (106) DHKNEFCEITLISSGR (99)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (68) DKENGDTVTFTEVG YTR (99) DHKNEFCEITLISSGR (99)	FILNTLNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (68) NGDITFTEVG YTR (90) DHKNEFCEITLISSGR (99)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (68) NGDITFTEVG YTR (90) AGFITELSEFIPGASVR (75) DHKNEFCEITLISSGSK (82)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	DGENGKITFTEVG YTR (61) DHKNEFCEITLLSSSR (78)	FILNTVNGTTR	22
	Thioredoxin- dependent peroxidase (<i>Plantago major</i>)	17381/5.34	gi 52851172	FALLVEDLK (55) RFALLVEDLK (54) GVDEILCISVNDPFVMK (86)	None	16
Be5	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (74) FITELSEFIPGASVR (88) EKENGDTTFTEIGYTR (108) DHKNEFCEITLISSGR (75)	FILNTVNGTTR	39

1
2
3
4

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Be5	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (74) AGFITESEFIPGASLR (66) DKENGDTVTFTEVG YTR (117) DHKNEFCEITLISSGR (75)	FILNTLNGTTR	55
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (74) AGFITESEFIPGASVR (67) DKENGDTVTFTEIG YTR (113) DHKNEFCEITLISSGR (75)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (74) NGDITFTEVG YTR (99) AGFINELSEFIPGASVR (65) DHKNEFCEITLISSGR (75)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVG YTR (69) AEGLYSMLIER(ox) (72) FITEFSEFIPGAGVR (56) DHKNEFCEITLLSSSR (78)	FILNTVNGTTR	35
	Ole e 1 (<i>Olea europaea</i>)	14866/5.89	gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER(ox) (72) FITESEFIPGAGVR (58) DHKNEFCEITLLSSSR (78) KDCDEIPVEGWVKPSLK (56)	FMLNTVNGTTR	44
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (74) NGDITFTEVG YTR (99) AGFITESEFIPGASVR (67) DHKNEFCEITLISSGSK (66)	FILNTVNGTTR	42
Be6	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (74) FITESEFIPGASVR (75) EKENG DITFTEIG YTR (105) DHKNEFCEITLISSGR (98) KDCDEIPIEGWAKPSLK (60)	FILNTVNGTTR	51
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (74) AGFITESEFIPGASLR (89) DKENGDTVTFTEVG YTR (113) DHKNEFCEITLISSGR (98)	FILNTLNGTTR	55
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131	ITFTEVG YTR (71) AEGLYSMLIER(ox) (76) FITEFSEFIPGAGVR (81) DHKNEFCEITLLSSSR (60)	FILNTVNGTTR	35
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (74) AGFITESEFIPGASVR (69) DKENGDTVTFTEIG YTR (120) DHKNEFCEITLISSGR (98)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (74) NGDITFTEVG YTR (91) AGFINELSEFIPGASVR (79) DHKNEFCEITLISSGR (98)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (74) NGDITFTEVG YTR (91) AGFITESEFIPGASVR (69) DHKNEFCEITLISSGSK (79)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	15538/7.55	gi 1362128 gi 1362132 gi 1362137	AEGLYSMLIER(ox) (76) GSITFTEVG YTR (75) FITESEFIPGAGVR (58)	FKLNTVNGTTR	27
Be7	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (72) AGFITESEFIPGASLR (84) DKENGDTVTFTEVG YTR (94) DHKNEFCEITLISSGR (86)	FILNTLNGTTR	44

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Be7	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (72) FITEELSEFIPGASVR (71) EKENGDTTFTEIGYTR (77) DHKNEFCEITLISSGR (86)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (72) NGDITFTEVG YTR (80) AGFINELSEFIPGASVR (64) DHKNEFCEITLISSGR (86)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129 gi 33323443 gi 33325111 gi 33329730 gi 33329734 gi 33329742 gi 33329744 gi 33329750 gi 37548749	AEGLYSMLVER(ox) (72) AGFITEELSEFIPGASVR (69) DHKNEFCEITLISSGR (86)	FILNTVNGTTR	30
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131 gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER(ox) (74) NEFCEITLLSSSR (80)	FILNTVNGTTR	16
	Cyclophilin (<i>Phaseolus vulgaris</i>)	18148/8.36	gi 829119	FADENFVK (70) VFFDMTIGGQ PAGR (85) HVVFGQVVEGLDVVK (135) VIPNFM CQGGDFTAGNGTGGESIYGAK (105)	None	37
	Cyclophilin (<i>Kandelia candel</i>)	18234/8.68	gi 37722431	FADENFVK (70) IVMELFADV VPR(ox) (92) HTGPGILSMANAGPGTNGSQFFICTAK (94)	None	27
Ar1	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (72) AGFITEELSEFIPGASVR (91) DKENG DVTTFTEIGYTR (113) DHKNEFCEITLISSGR (85)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (72) DKENG DVTTFTEVG YTR (132) DHKNEFCEITLISSGR (85)	FILNTLNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (72) EKENG DITFTEIGYTR (112) DHKNEFCEITLISSGR (85)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (72) NGDITFTEVG YTR (97) DHKNEFCEITLISSGR (85)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131 gi 33329754 gi 33329756 gi 33329758	NEFCEITLLSSSR (90)	FILNTVNGTTR	8
Ar2	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (74) AGFITEELSEFIPGASVR (82) DKENG DVTTFTEIGYTR (110) DHKNEFCEITLISSGR (96)	FILNTVNGTTR	41

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Ar2	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (74) FITEELSEFIPGASVR (66) EKENGDTFTEIGYTR (98) DHKNEFCEITLISSGR (96)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (74) DKENGDTVTFTEVG YTR (112) DHKNEFCEITLISSGR (96)	FILNTLNGTTR	31
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (74) NGDITFTEVG YTR (103) DHKNEFCEITLISSGR (96)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (74) NGDITFTEVG YTR (103) AGFITEELSEFIPGASVR (82) DHKNEFCEITLISSGSK (75)	FILNTVNGTTR	42
Ar3	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (75) AGFITEELSEFIPGASLR (73) DKENGDTVTFTEVG YTR (119) DHKNEFCEITLISSGR (90)	FILNTLNGTTR	44
	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (75) AGFITEELSEFIPGASVR (71) DKENGDTVTFTEIGYTR (112) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	15491/6.34	gi 1362129	AEGLYSMLVER(ox) (75) FITEELSEFIPGASVR (82) EKENGDTFTEIGYTR (96) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (75) NGDITFTEVG YTR (109) DHKNEFCEITLISSGR (90)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (75) NGDITFTEVG YTR (109) AGFITEELSEFIPGASVR (71) DHKNEFCEITLISSGSK (90)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16474/5.91	gi 1362131 gi 3256212 gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER(ox) (74) DHKNEFCEITLLSSSR (54)	FILNTVNGTTR	18
Ar4	Ole e 1 (<i>Olea europaea</i>)	16536/6.13	gi 2465127 gi 2465129	AEGLYSMLVER(ox) (76) AGFITEELSEFIPGASVR (84) DKENGDTVTFTEIGYTR (115) DHKNEFCEITLISSGR (109)	FILNTVNGTTR	41
	Ole e 1 (<i>Olea europaea</i>)	16605/5.73	gi 2465131 gi 1362129	AEGLYSMLVER(ox) (76) FITEELSEFIPGASVR (81) EKENGDTFTEIGYTR (116) DHKNEFCEITLISSGR (109)	FILNTVNGTTR	39
	Ole e 1 (<i>Olea europaea</i>)	15384/6.90	gi 1362134 gi 1362135 gi 14424429 gi 37724593 gi 37724597	AEGLYSMLVER(ox) (76) DKENGDTVTFTEVG YTR (110) DHKNEFCEITLISSGR (109)	FILNTLNGTTR	31

1

Table S2. (Continued)

Spot ^{a)}	Matched protein (Species)	Mass (Da)/pI ^{b)}	Accession No. ^{c)}	Identified peptides (Mascot ion scores)	Glycopeptide	% ^{d)}
Ar4	Ole e 1 (<i>Olea europaea</i>)	15438/8.24	gi 1362130	AEGLYSMLVER(ox) (76) NGDITFTEVGYTR (103) DHKNEFCEITLISSGR (109)	FILNTVNGTTR	29
	Ole e 1 (<i>Olea europaea</i>)	15288/7.74	gi 1362133 gi 13195753	AEGLYSMLVER(ox) (76) NGDITFTEVGYTR (103) AGFITESEFIPGASVR (84) DHKNEFCEITLISSGSK (74)	FILNTVNGTTR	42
	Ole e 1 (<i>Olea europaea</i>)	16454/5.91	gi 3256212 gi 33329754 gi 33329756 gi 33329758	AEGLYSMLIER(ox) (68) FITESEFIPGAGVR (50) DHKNEFCEITLLSSSR (57)	FMLNTVNGTTR	28

a) Spots are named accordingly to Fig. 1.

b) Theoretical molecular mass (Da)/Theoretical isoelectric point.

c) Accession number in NCBI nr database after BLAST homology search. All accession numbers corresponding to Ole e 1 proteins identified with the same set of peptides are grouped (for each group the first accession number is in bold).

d) Percentage of amino acids in reference proteins covered by matching peptides from nano-LC-MS/MS analysis.

2

3

Table S3. List of N-glycans identified by nano-LC-MS/MS in three olive cultivars.

Spot ^{a)}	Peptide ^{b)}	Identified N-linked glycans ^{c)}
Pi1	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
	FMLNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
	FMLNTVNGTTR (Met oxidized)	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Pi2	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
	FMLNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
Pi3	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
	FILNTVNGTTR	Man ₅ GlcNAc ₂ Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc

Table S3. (Continued)

Spot ^{a)}	Peptide ^{b)}	Identified N-linked glycans ^{c)}
Pi4	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Pi5	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
	FILNTVNGTTR	Man ₅ GlcNAc ₂ Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Pi6	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Pi7	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ Man ₉ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc

Table S3. (Continued)

Spot ^{a)}	Peptide ^{b)}	Identified N-linked glycans ^{c)}
Pi8	LNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂
	FILNTVNGTTR	Man ₄ GlcNAc ₂ Man ₅ GlcNAc ₂ Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Pi9	LNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂
	FILNTVNGTTR	Man ₄ GlcNAc ₂ Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Pi10	FILNTVNGTTR	Man ₇ GlcNAc ₂
	FMLNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FMLNTVNGTTR (Met oxidized)	Man ₅ GlcNAc ₂ Man ₇ GlcNAc ₂
Pi11		Non glycosylated
Be1	FILNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
Be2	FILNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
Be3	LNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
	FILNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
Be4	FILNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
Be5	LNTVNGTTR	Man ₇ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc Man ₃ XylGlcNAcFucGlcNAc

1
2
3

Table S3. (Continued)

Spot^{a)}	Peptide^{b)}	Identified N-linked glycans^{c)}
Be6	LNTVNGTTR	Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ Hex ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc GlcNAc ₂ Man ₃ XylGlcNAcFucGlcNAc
Be7	LNTVNGTTR	GlcNAcMan ₃ XylGlcNAc ₂
	FILNTVNGTTR	Man ₆ GlcNAc ₂ Man ₇ GlcNAc ₂ HexGlcNAcMan ₃ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
Ar1	FILNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂
Ar2	FILNTVNGTTR	Man ₇ GlcNAc ₂
Ar3	LNTVNGTTR	Man ₅ GlcNAc ₂ Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FKLNTVNGTTR or FILNTLNGTTR	Man ₇ GlcNAc ₂
	FILNTVNGTTR	Man ₇ GlcNAc ₂ Man ₈ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAcMan ₃ XylGlcNAcFucGlcNAc
Ar4	LNTVNGTTR	Man ₇ GlcNAc ₂ GlcNAcMan ₃ XylGlcNAc ₂ GlcNAc ₂ Man ₃ XylGlcNAc ₂
	FILNTVNGTTR	Man ₇ GlcNAc ₂

a) Spots are named accordingly to Fig. 1.

b) Putative N-glycosylated peptide.

c) Hex indicates that a mannose or galactose residue is present in the N-glycan motif.

