

Profiling of Hydrophilic Proteins from *Olea europaea* Olive Pollen by MALDI TOF Mass Spectrometry

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The antigenic profile of *Olea europaea* pollen from different Mediterranean cultivars was obtained by MALDI mass spectrometry. A simple procedure of chemical fractionation of the whole antigen extract was developed, whereby less complex, or pure, fractions of antigen candidate were obtained prior to mass spectrometric analysis. Some of the features of protein structure and distribution probably depend on cultivar adaptation to the environment. The profilings of pollen proteins thus obtained allow the distinction of the analyzed cultivars into three distinct groups: (i) those characterized by a low Ole e 1 content; (ii) those over-enriched in Ole e 1 and (iii) those containing Ole e 3 and Ole e 7 only. The latter consists of at least four isoforms differing by the degree of glycosylation. These results demonstrate that the proposed experimental procedure, can supply valuable information on the antigens' micro heterogeneity.

Mass spectrometry is currently applied, with success, in protein profiling of natural matrixes.^{1,2} Our group has developed high-tech analytical methods as tools for the assessment of food quality and safety.^{3,4} In a survey of all possible candidates whose profiling could provide clues for the unambiguous identification of olive cultivars and of their subvarieties, we have undertaken a detailed analysis of olive pollen extracted from eight different typical Mediterranean cultivars.

When inhaled, olive pollen of *Olea europaea* is also important as a causative agent for type I allergy in the Mediterranean area.^{5,6} More than 30% of the population in this area is affected by type I

allergy during the pollination season, and more than 80% of the olive-tree-allergic patients are sensitive to the protein Ole e 1.⁷ Several separation methods have been employed for the isolation of the allergens, such as SDS–PAGE,^{8,9} high performance liquid chromatography (HPLC),¹⁰ immunodetection,¹¹ and gel filtration.¹² Allergenic candidates of 7, 9, 14, 15, 16, 18, and 36 kDa (Table 1), whose presence in olive pollen has been ascertained as previously mentioned, have been immunostained with sera from olive-allergic individual patients.^{13,14}

The concentration level of the major olive pollen allergens, estimated using monoclonal antibodies alone or in combination with gel scanning densitometry,^{9,15} indicates a variation between plant species.¹⁶ Cultivars and probably local variety or subvarieties of olive trees present special features that depend on their adaptation to the environment or ecotype.¹⁷ Ecosystem and crop management are factors that are able to induce changes in the allergenic profile of a given variety or cultivar.¹⁸ Therefore, pollen protein profiling could be a useful tool for cultivar discrimination. The olive (*Olea europaea*) pollens of the Mediterranean cultivars, Ottobratica (1), Carolea (2), Dolce di Rossano (3), Cassanese (4), Coratina (5), Nocellara del Belice (6), Villacidro (7), and Sino-polese (8), were selected as case studies to determine a protein

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Table 1. Olive Pollen Allergens with Clinical Relevance Developed and Maintained by Allergen Nomenclature Subcommittee of the IUIS (www.allergen.org), Including Allergens Whose IgE Reactivity Has a Prevalence of >5%

allergen name	obsolete name	MW, kDa, SDS-PAGE ^a	variants	MW (Da) ^b	p.I.	sequence ^c	accession number ^d
Ole e 1	major pollen allergen	18–21	3–3, I → V (in Ole e 1.0102, Ole e 1.0103, and Ole e 1.0105) 23–23, R → P 24–25, AG → SR (in Ole e 1.0105) 39–39, L → V (in Ole e 1.0102, Ole e 1.0103, and Ole e 1.0105) 44–44, K → R (in Ole e 1.0105) 45–45, D → E (in Ole e 1.0105) 46–46, K → I 47–47, E → K 48–48, N → K 50–50, D → S 51–51, V → I (in Ole e 1.0105) 56–56, V → I (in Ole e 1.0102, 1.0103, and 1.0105) 69–69, V → I 87–87, R → S 91–91, N → D (in Ole e 1.0105) 95–95, T → I (in Ole e 1.0105) 106–106, K → I (in Ole e 1.0102, 1.0103, and 1.0105) 108–108, N → S (in Ole e 1.0103) 111–111, N → D 121–121, G → R (in Ole e 1.0102) 123–123, F → Y (in Ole e 1.0102 and 1.0103)	16 330	6.18	C	P19963
Ole e 2	profilin-1	15–18		14 489	5.06	C	O24169
	profilin-2	15–18		14 427	5.21		O24170
	profilin-3	15–18		14 399	5.21		O24171
Ole e 3	polcalcin Ole e 3	9.2		9356	4.49		O81092
	Ole e 3 allergen (fragment)			5798			Q5DTB7
Ole e 4	major pollen allergen Ole e 4 (fragments)	32		2711	3.77	P	P80741
Ole e 5	superoxide dismutase [Cu–Zn] (fragment)	16		2973	4.65	P	P80740
Ole e 6	pollen allergen Ole e 6	10		5833	4.96	C	O24172
	pollen allergen Ole e 6 *fragment)			4986	5.43		Q84UC2
	Ole e 6 allergen (fragment)			4400	7.8	N	Q5DTB8
Ole e 7	pollen allergen Ole e 7 (fragment)	9–11	5–5, S → G (in type B) 10–10, L → K (in type B) 18–18, I → K (in type B)	9905–10 302 ^e 2199	3.56	P	P81430
Ole e 8	Ca ²⁺ -binding protein	21	43–44, GV → CA 58–58, G → A 60–60, I → M	18 907	4.51	C	Q9M7R0
Ole e 9	β-1,3-glucanase	46		48 838	5.21	C	Q94G86

^a Apparent molecular mass in SDS–PAGE. ^b Theoretical molecular weight. ^c Sequence information obtained by C, cDNA; P, peptide sequence; N, nucleotide sequence. ^d Swissprot database. ^e Mass spectrometry determination.

profile of the whole extract and to identify and characterize specific proteins without any previous chromatographic or two-dimensional gel separations. A simple procedure of chemical fractionation of

the whole antigen extract was developed, whereby less complex, or pure, fractions of antigen candidate were obtained. All fractions were directly analyzed by MALDI TOF in the linear mode.

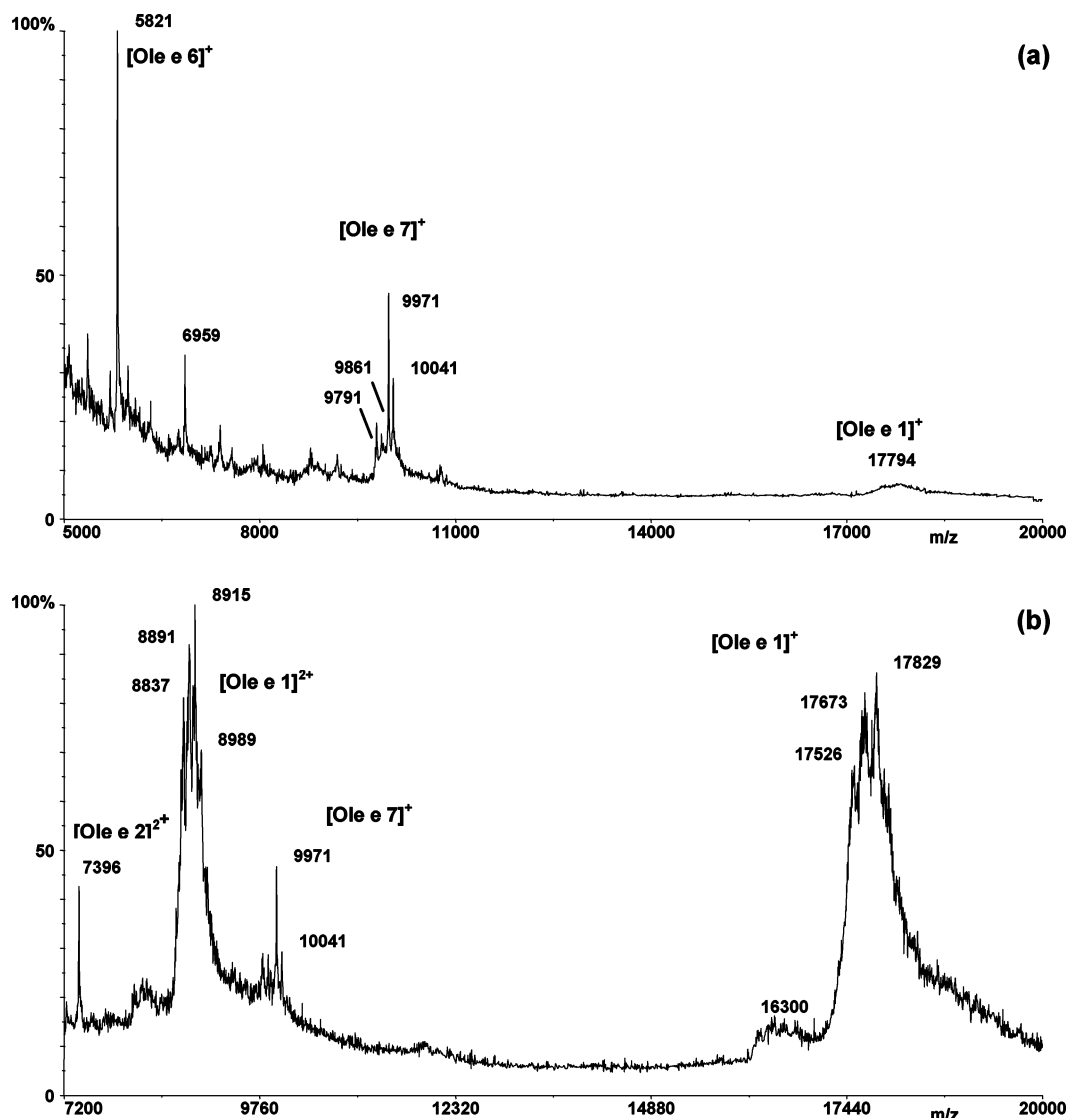
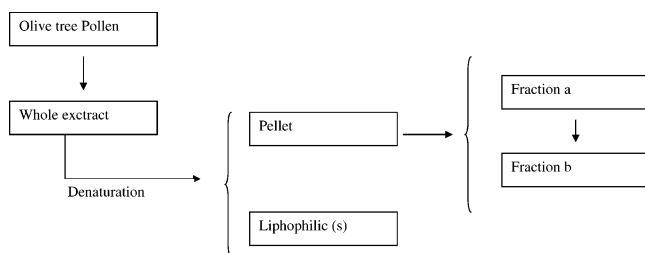


Figure 1. MALDI spectra of fractions (a) **4a** and (b) **4b**. Both fractions represent a mixture of allergens. Part (a) shows the typical pattern of Ole e 7, Ole e 6, and some polypeptides between 5 and 7 kDa; whereas part (b) shows the ion species [Ole e 2]²⁺, [Ole e 1]²⁺, [Ole e 7]⁺, and [Ole e 1]⁺.

Chart 1



EXPERIMENTAL SECTION

Materials. Olive tree pollens of cultivars 1-8 are part of the collection of the Istituto Sperimentale per l'Olivicoltura, C.da Li Rocchi, Rende (CS), Italy.

Solvents (CH₃OH, CH₃CN, CHCl₃, and H₂O, HPLC grade), ammonium bicarbonate (NH₄HCO₃, pure ≥99.5%), trifluoroacetic acid (HPLC grade, pure ≥99.0%), enzyme (trypsin and PNGase F), and α-cyano-4-hydroxy-*trans*-cinnamic acid (α-CHCA, pure ≥99.0%) were purchased from Sigma Aldrich Fluka (Milano, Italy).

Allergen Extracts. Portions (50 mg) of pollen grains (1–8) were extracted with 1 mL of aqueous 50 mM NH₄HCO₃ for 20 min at room temperature, followed by centrifugation at 14 000 rpm for 2 min. The supernatant portion was separated and stored at −20 °C. A 200-μL portion of whole extract was precipitated with 400 μL of CHCl₃/CH₃OH 1:3 (v/v), and the precipitated protein pellet was partitioned consecutively, under magnetic stirring and at room temperature, for 10 min with (a) 150 μL of 50 mM NH₄HCO₃/CH₃OH 1:1 (v/v) and (b) 150 μL of 50 mM NH₄HCO₃/CH₃CN 1:1 (v/v). The MALDI spectra of the residue of the previous extractions reveals traces of the allergens found in fraction b. Each step (a, b) was followed by centrifugation at 14 000 rpm for 1 min.

A 50-μL portion of fraction 1b (pH 8) was fully reduced by treatment with 10 μL of DTT (50 mM) under magnetic stirring for 6 h at room temperature. The sample was then incubated with 4 μL of PNGase F (0.5 unit/μL) for 3 days at 37 °C. After 78 h, the resulting mixture was digested overnight with 1 μL (10 pmol/μL) of trypsin.

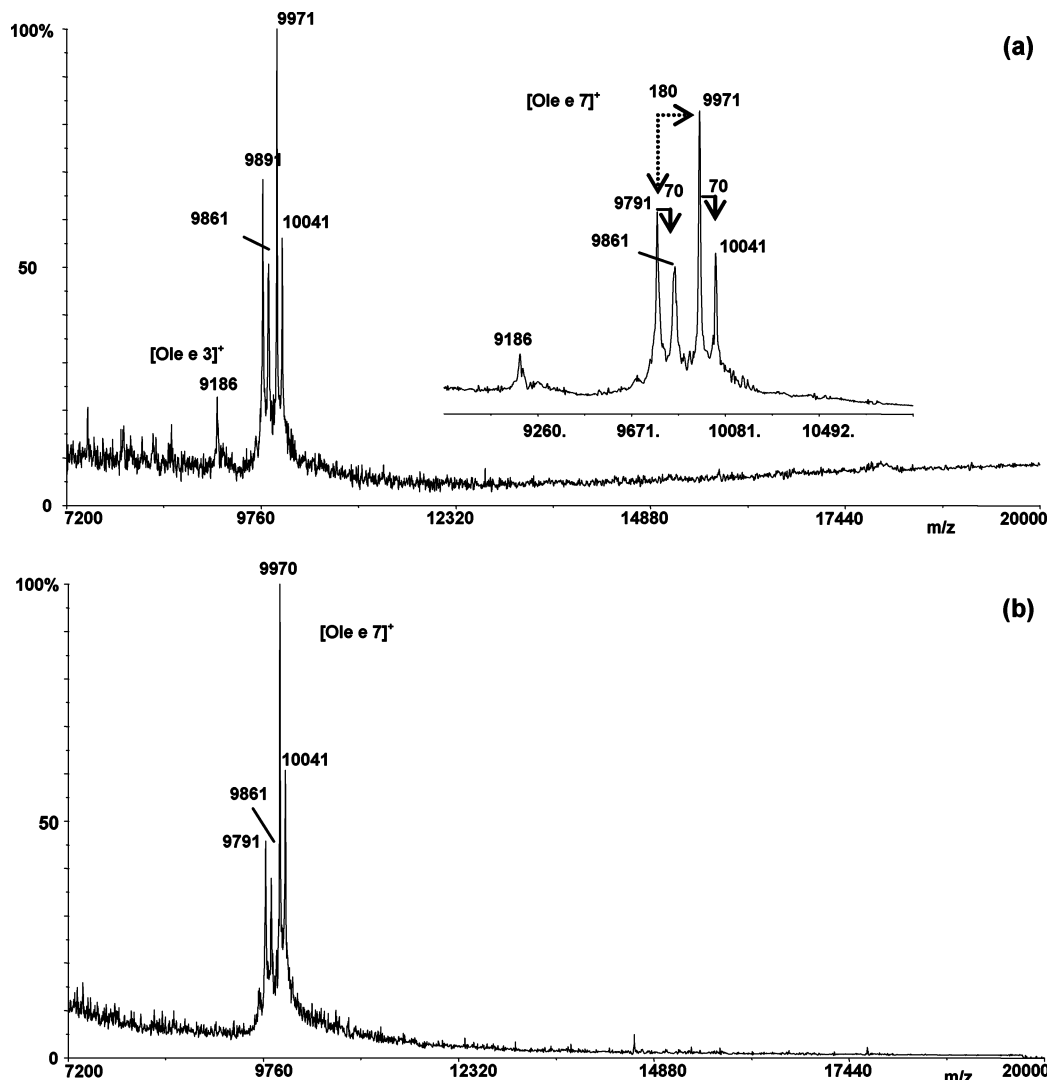


Figure 2. MALDI spectra of fractions (a) 3a and (b) 5a.

A 50- μ L portion of fraction 7b (pH 8) was fully reduced by treatment with 10 μ L of DTT (50 mM) under magnetic stirring for 6 h at room temperature. The sample was then digested overnight with 1 μ L (10 pmol/ μ L) of trypsin.

Mass Spectrometry. A 1- μ L portion of each fraction was directly analyzed by linear MALDI using α -cyano-4-hydroxy-trans-cinnamic acid (α -CHCA, 0.3% in TFA) as matrix.

MALDI-TOF analyses were performed using a 4700 Proteomics Analyzer mass spectrometer from Applied Biosystems (Foster City, CA) equipped with a 200-Hz Nd:YAG laser at 355-nm wavelength. The MS spectra were acquired in reflectron mode (20-keV accelerating voltage), with 400-ns delayed extraction, averaging 2000 laser shots with a mass accuracy of 50 ppm. A 1- μ L portion of a premixed solution of each fraction and α -CHCA (0.3% in TFA) was spotted on the matrix target, dried at room temperature, and analyzed in the mass spectrometer. Proteins were identified by searching a comprehensive protein database using Mascot programs (www.matrixscience.com). One/two missed cleavages per peptide was allowed, and an initial mass tolerance of 50 ppm was used in all searches.

Linear MALDI MS spectra were acquired averaging 2500 laser shots with a mass accuracy of 500 ppm in default calibration mode

that was performed using the following set of standards: insulin (bovine, $[M + H]^+_{\text{avg}} = 5734.59$), apomyoglobin (horse, $[M + H]^{2+}_{\text{avg}} = 8476.78$, $[M + H]^+_{\text{avg}} = 16\,952.56$), and thioredoxin (*Escherichia coli*, $[M + H]^+_{\text{avg}} = 11\,674.48$).

RESULTS AND DISCUSSION

The high solubility in aqueous medium is an important prerequisite for allergen candidates, because their biological activity better correlated to the concentration and rapid release from airborne particle than to their intrinsic properties.¹⁹ The partition coefficient of allergens and the antigenic profile of olive pollen are strongly related to the solvents used for the extraction.²⁰ Whole protein extracts can be, therefore, chemically fractionated, and the antigen contents of each fraction can be varied according to the selected experimental conditions. MALDI mass spectrometry, for its specificity and better resolution with respect to a conventional 2-D (two-dimensional gel electrophoresis)¹⁷ chromatographic approach, represents the methodology of choice to get reliable results in the profiling of olive pollen.

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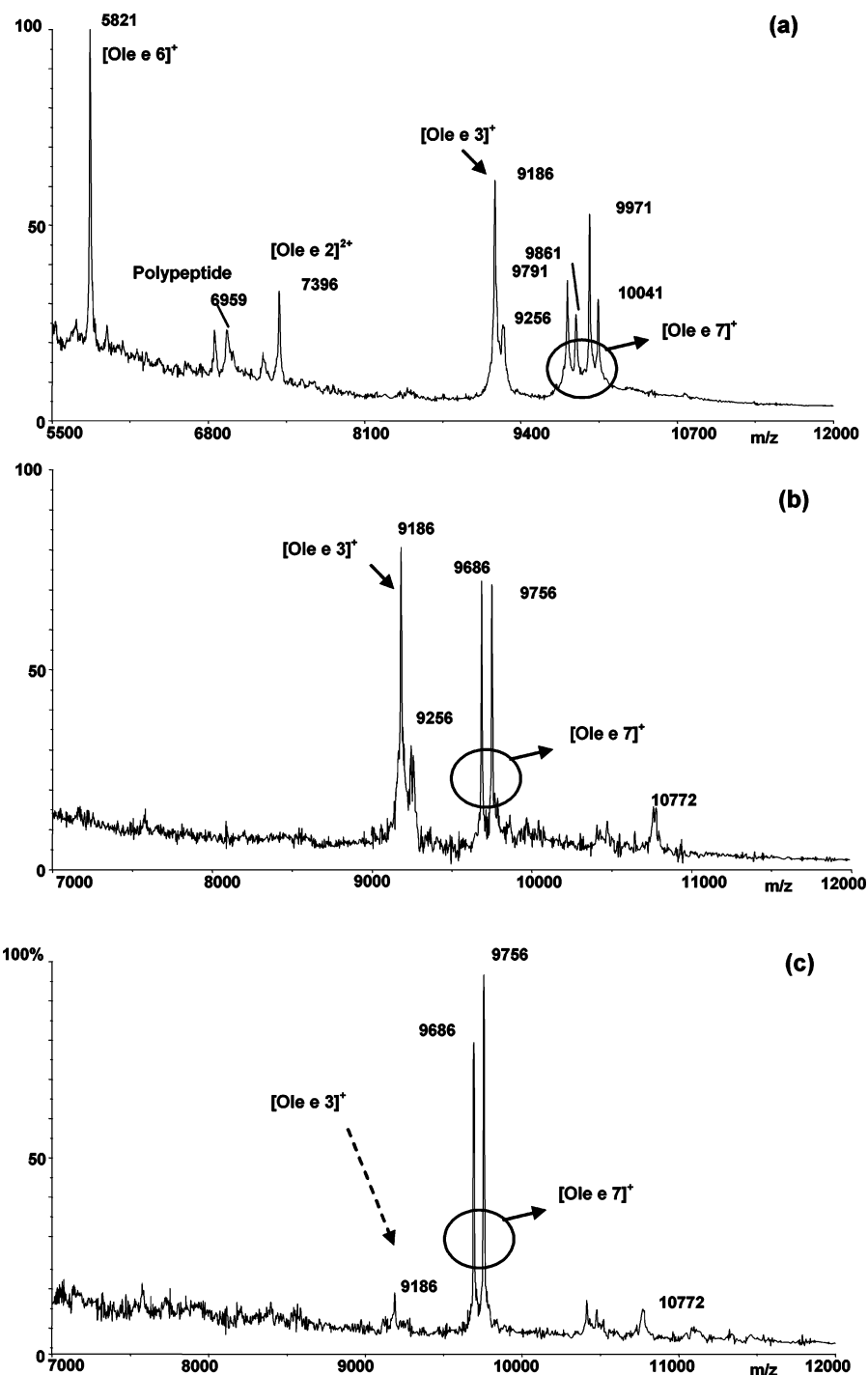


Figure 3. MALDI spectra of fractions (a) 3I-s, (b) 3II-s, and (c) 3III-s.

The use of saline solution containing sodium chloride, phosphate buffer, and borate buffer either to prepare the whole pollen extract or for its chemical fractionation are not amenable to direct MALDI-MS analysis. Accordingly, ammonium bicarbonate solutions were chosen to prepare the whole antigenic extract from pollen grain of cultivars 1–8. Ammonium bicarbonate should favor the formation of carboxylate/ammonium ion pairs, thus favoring the solubility of the proteins to be extracted in moderately polar solvents, such as acetonitrile/water mixtures. Moreover, ammonium counterions have been often used to improve the desorption of high molecular weight protein²¹ and does not interfere with the mass spectrometric analysis. A sample preparation protocol

was, therefore, devised to distribute the amount of information stored in the proteome of each olive pollen in a set of three MALDI spectra that could be independently evaluated and matched to retrieve data for their comparison. The data set displayed by the three spectra provides the entire profiling of a given entity. One lypophilic (s) and two hydrophilic fractions (a–b), respectively, can be obtained from each sample by following the procedure reported in the Experimental Section (Chart 1).²²

Accordingly the antigenic profile of Cassanese 4 displayed four allergens: Ole e 7, Ole e 6, Ole e 2, and Ole e 1 in the 5–20-kDa

(21) Rajnarayanan, R. V.; Wang, K. *J. Mass Spectrom.* **2004**, *39*, 79–85.

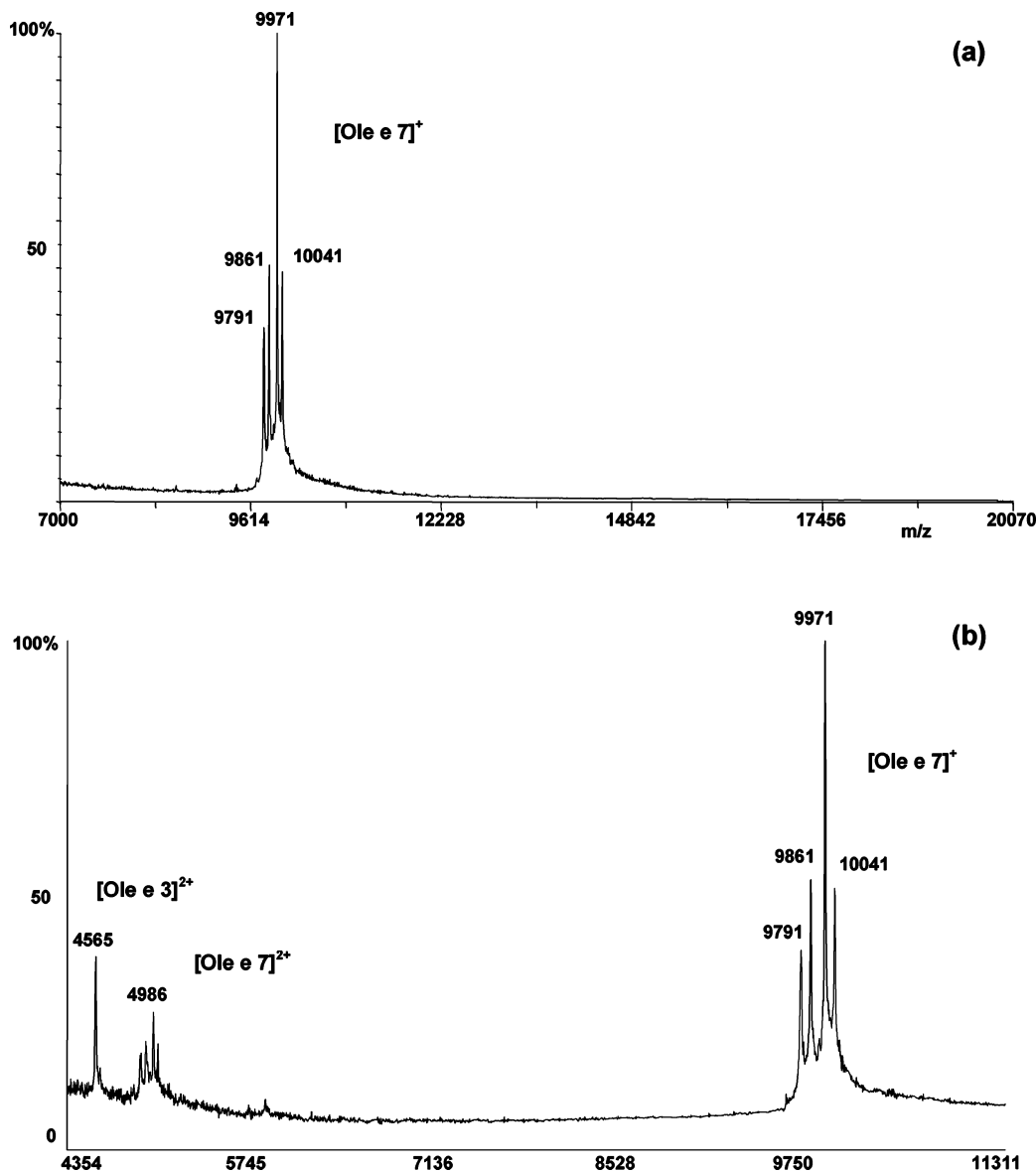


Figure 4. MALDI spectra of fractions (a) **1a** and (b) **1b**.

mass range (Figure 1). The four ion peaks at m/z 9791–10 041 mass range (Figure 1) can be ascribed to Ole e 7. In fact, the apparent SDS–PAGE molecular mass of this allergen is ~9–11 kDa (Table 1, column 3, row 8). The predicted molecular mass of the Ole e 7 fragment from the peptide sequence is 2199 Da²³ (Table 1, column 5, row 8). The only experimental values available are 9905–10 302 Da obtained by low-resolution MALDI mass spectrometry²³ (Table 1, column 5, row 8). Therefore, it can be confidently suggested that peaks in the range 9791–10 041 Da (Figure 1) should correspond to the expected Ole e 7.

The ion peak at m/z 5821 (Figure 1) was attributed to the well-known olive pollen allergen Ole e 6. This allergen has been isolated, purified and biochemically characterized,²⁴ and its specific cDNA was cloned and sequenced^{25,26} (Table 1, row 7). Considering

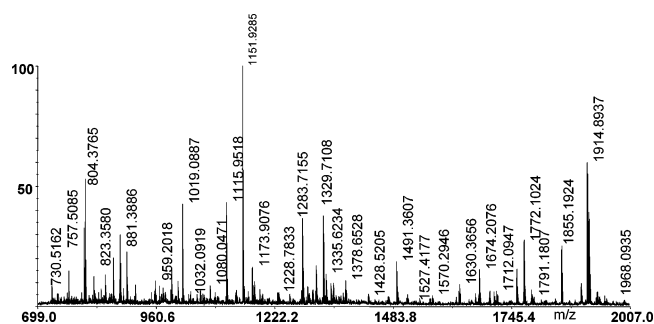


Figure 5. MALDI-MS spectra of peptide mixture of fraction **1b**.

that the value of 5833 Da (Table 1, column 5, row 7) corresponds to the predicted molecular weight from cDNA and that there are no other known allergens in this range, the observed ion peak at m/z 5821 can be ascribed to Ole e 6.

(22) Bold lower-case letters and bold numbers are used to identify the fraction and the cultivar, respectively; for example, sample **4a** represents the fraction **a** of Cassanese.

(23) Tejera, M. L.; Villalba, M.; Batanero, E.; Rodriguez, R. *J. Allergy Clin. Immunol.* **1999**, *104*, 797–802.

(24) Batanero, E.; Ledesma, A.; Villalba, M.; Rodriguez, R. *FEBS Lett.* **1997**, *410*, 293–296.

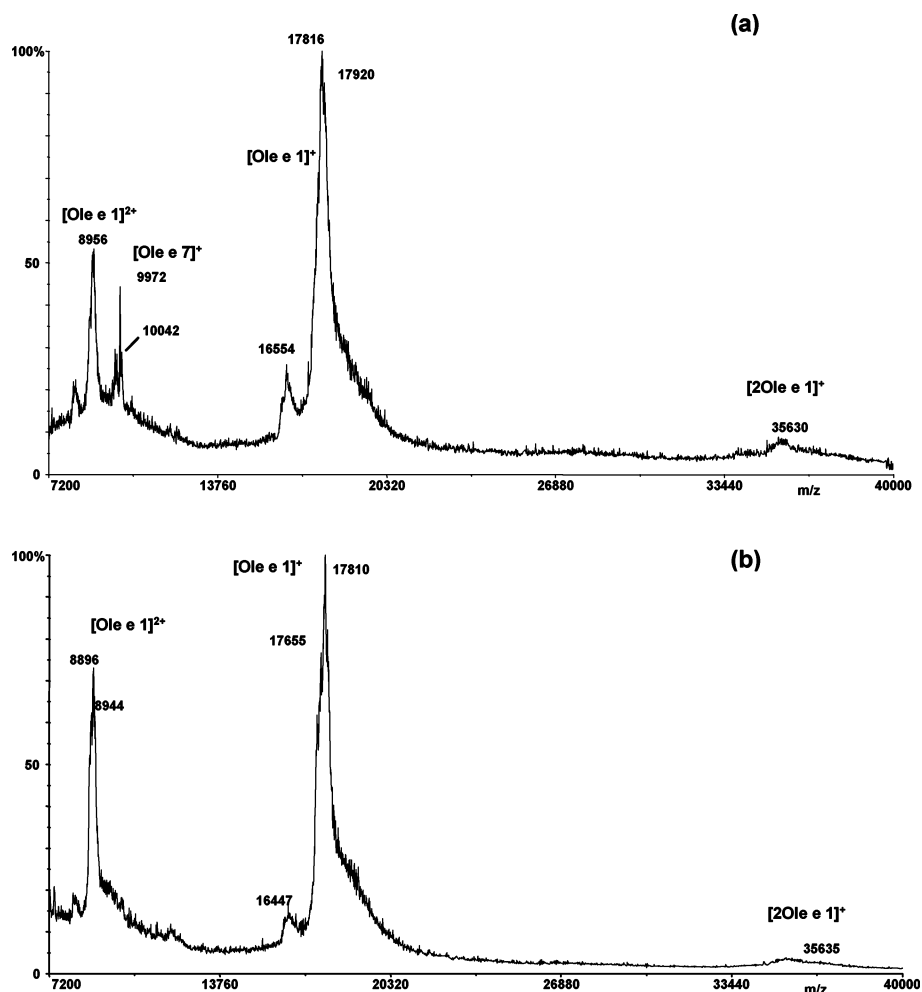


Figure 6. MALDI spectra of fractions (a) **5b** and (b) **7b**.

Table 2

	1	MADDPQEVAE	HERIFKRFDA	NGDGKISSSE	LGETLKTLS	VTPEEIQRMM
	51	AEIDTDGDGF	ISFEFTVFA	RANRGLVKDV	AKIF	
start–end	obs	M_r (exptl)	M_r (calcd)	ΔM_r	miss	sequence
1–16	1914.89	1913.88	1913.89	0.01	1	MADDPQEVAEHERIFK
18–25	823.37	822.35	822.35	0.01	0	FDANGDGK
18–36	1968.09	1967.08	1966.95	0.13	1	FDANGDGKISSSELGETLK
37–48	1329.71	1328.69	1328.69	–0.01	0	TLGSVTPEEIQR
72–78	757.50	756.49	756.46	0.03	1	ANRGLVK

The doubly charged ion at m/z 7396 probably corresponds to Ole e 2, since it is known that this allergen consists of 134 amino acids,^{27,28} and the predicted average molecular mass is 14.4 kDa (Table 1, row 3).

The mono e doubly charged ions at m/z 16 300–17 829 and at m/z 8837–8989, respectively, were ascribed to Ole e 1 (Table 1, row 2).

(25) Barral, P.; Tejera M. L.; Trevino M. A.; Batanero E.; Villalba M.; Bruix M.; Rodriguez R. J. *Protein Expression Purif.* **2004**, *37*, 336–343.

(26) Trevino, M. A.; Garcia-Mayoral, M. F.; Barral, P.; Villalba, M.; Santoro, J.; Rico, M.; Rodriguez, R.; Bruix, M. J. *Biol. Chem.* **2004**, *279*, 39035–39041.

(27) Martinez, A.; Asturias, J. A.; Monteseirin, J.; Moreno, V.; Garcia-Cubillana, A.; Hernandez, M.; de la Calle, A.; Sanchez-Hernandez, C.; Perez-Formoso, J. L.; Conde, J. *Allergy* **2002**, *57*, 17–23.

The two a and b fractions (Figure 1) show a significantly different antigenic pattern; a complete pool of Ole e 1 isoforms is, in fact, predominant in hydrophilic fraction **4b**.

The MALDI spectrum of fraction **4s** is characterized by the presence of ion peaks corresponding to low molecular weight proteins as a consequence of the fractionation procedure which lowers the solubility of lower molecular weight proteins in aqueous ammonium bicarbonate.

The MALDI spectra of the first hydro-soluble fractions of 1, 3, and 5–8 showed similar protein expression. In particular, the MS spectrum of fraction **5a** (Figure 2b) shows four peaks in the mass range 9.7–10 kDa, whereas that of 3a (Figure 2a) displays one

(28) Asturias, J. A.; Arilla, M. C.; Gomez-Bayon, N.; Martinez, J.; Martinez, A.; Palacios, R. J. *Allergy Clin. Immunol.* **1997**, *100*, 365–372.

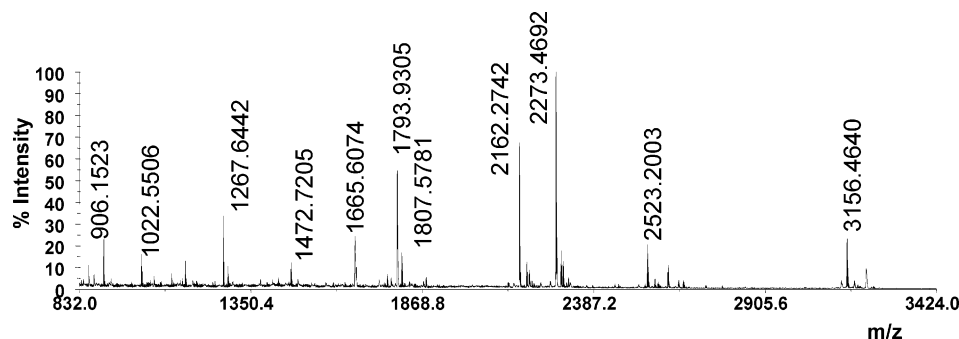


Figure 7. MALDI-MS spectra of peptide mixture of fraction **7b**.

Table 3.

accession	description	mass	p.I.	sequence coverage, %	no. mass values matched
gi 13195753	main olive allergen (<i>Olea europaea</i>)	14 575	7.74	83	10
gi 1362133	major allergen OLE26, common olive (fragment)	15 288	7.74	62	6
gi 14424429	major pollen allergen (allergen Ole e 1) (Ole e I)	16 319	6.18	50	6

Table 4.

start–end	obs	M_r (exptl)	M_r (calcd)	ΔM_r	miss	sequence
1–29	3234.45	3233.45	3233.57	–0.12	2	QVYCDTCRAGFITELSEFIPGASVRLQCK
9–25	1793.94	1792.93	1792.94	–0.01	0	AGFITELSEFIPGASVR
9–31	2523.20	2522.20	2522.32	–0.12	2	AGFITELSEFIPGASVRLQCKEK
32–45	1600.80	1599.80	1599.79	0.01	1	KNGDITFTEVGYTR
33–45	1472.72	1471.72	1471.69	0.02	0	NGDITFTEVGYTR
46–56	1267.64	1266.64	1266.63	0.01	0	AEGLYSMLVER
46–73	3156.46	3155.46	3155.53	0.07	2	AEGLYSMLVERDHKNEFCEITLISSGSK
74–89	1787.91	1786.90	1786.86	0.05	0	DCNEIPTTEGWAKPSLK
90–100	1235.67	1234.66	1234.67	–0.00	0	FILNTVNGTTR
101–109	1022.55	1021.54	1021.56	–0.02	0	TVNPLGFFKK

additional peak at m/z 9186. A closer inspection of the main four peaks present in that mass range shows a difference of 70 and 180 mass units between two adjacent peaks and couples, respectively.

The allergens in the 9–10-kDa range (Table 1, column 3) that have already been found in olive tree pollen are Ole e 3²⁹ and Ole e 7.

Ole e 7 consists of one polymorphic polypeptide chain of ~10 kDa, which accounts for 88 amino acid residues, as deduced from the elution profile on HPLC and determined by mass spectrometry²³ (Table 1, column 5, row 8). Data obtained from Edman degradation of NH₂-terminal amino acids²³ indicate that the number of isoforms of Ole e 7 is four and differs by the exchange of three amino acids at the positions 5 (S–G), 10 (L–K), and 18 (I–K), leading to a difference of –30, +15, and +15 mass units, respectively (Table 1, column 4, row 8). Therefore, the observed difference of 70 mass units (Figure 2a) could be associated with the combined effect of a different peptide sequence, to the presence of calcium adducts, or both, whereas the difference of 180 should be associated with a hexose unit. The four ion peaks in the m/z 9.7–10-kDa range can be attributed to Ole e 7 because of its known polymorphism and its very high solubility in saline solution.

Additional information was obtained for cultivar 3, whose pollen was consecutively extracted at room temperature with ammonium

bicarbonate for 20, 60, and 120 min. The whole extracts **3I**, **3II**, and **3III** thus obtained were subsequently partitioned into three fractions (see Chart 1) generically identified as **I-s**, **I-a**, and **I-b**, respectively.

The MALDI-MS spectrum of lipophilic fraction 3I-s, (Figure 3a) shows ion peaks at m/z 5821 and 7396, likely corresponding to the species [Ole e 6]⁺ and [Ole e 2]²⁺, respectively,^{24–28} those at m/z 9186 and 9256 ($\Delta m = 70$ Da), which can be attributed to [Ole e 3]⁺; and that at m/z 9.7–10 kDa, which reasonably corresponds to [Ole e 7]⁺. Two other different polypeptides at m/z 9686 and 9756, observed in fractions **3II-s** and **3III-s**, respectively (Figure 3b and c), belongs to the isoform pool of Ole e 7.

The antigen Ole e 3 (Table 1, row 4), known also as Poc3_Oleeu, could exist in the two cDNA-derived isoforms²⁹ whose 30-u difference is due to the replacement, at the positions 43 and 80, of the amino acid P with L and of V with I, respectively. The MALDI spectra of the two fractions **3I** and **3II-s** displayed two peaks, at m/z 9186 and 9256, differing by 70 u (Figure 3a and b). Taking into account the calcium-binding properties²⁹ of Ole e 3, the observed difference likely proves the identification in the natural matrix of both the expected isoforms, as calcium ion adducts.

Ole e 3 and Ole e 7 are the only proteins identified in the whole extract of cultivar Ottobratica (1). MALDI spectra of fractions **1a** (Figure 4a) show in the mass range 9.7–10 kDa the typical pattern of Ole e 7, previously discussed, whereas that of fraction **1b**

(29) Ledesma, A.; Villalba, M.; Batanero, E.; Rodriguez, R. *Eur. J. Biochem.* **1998**, *258*, 454–459.

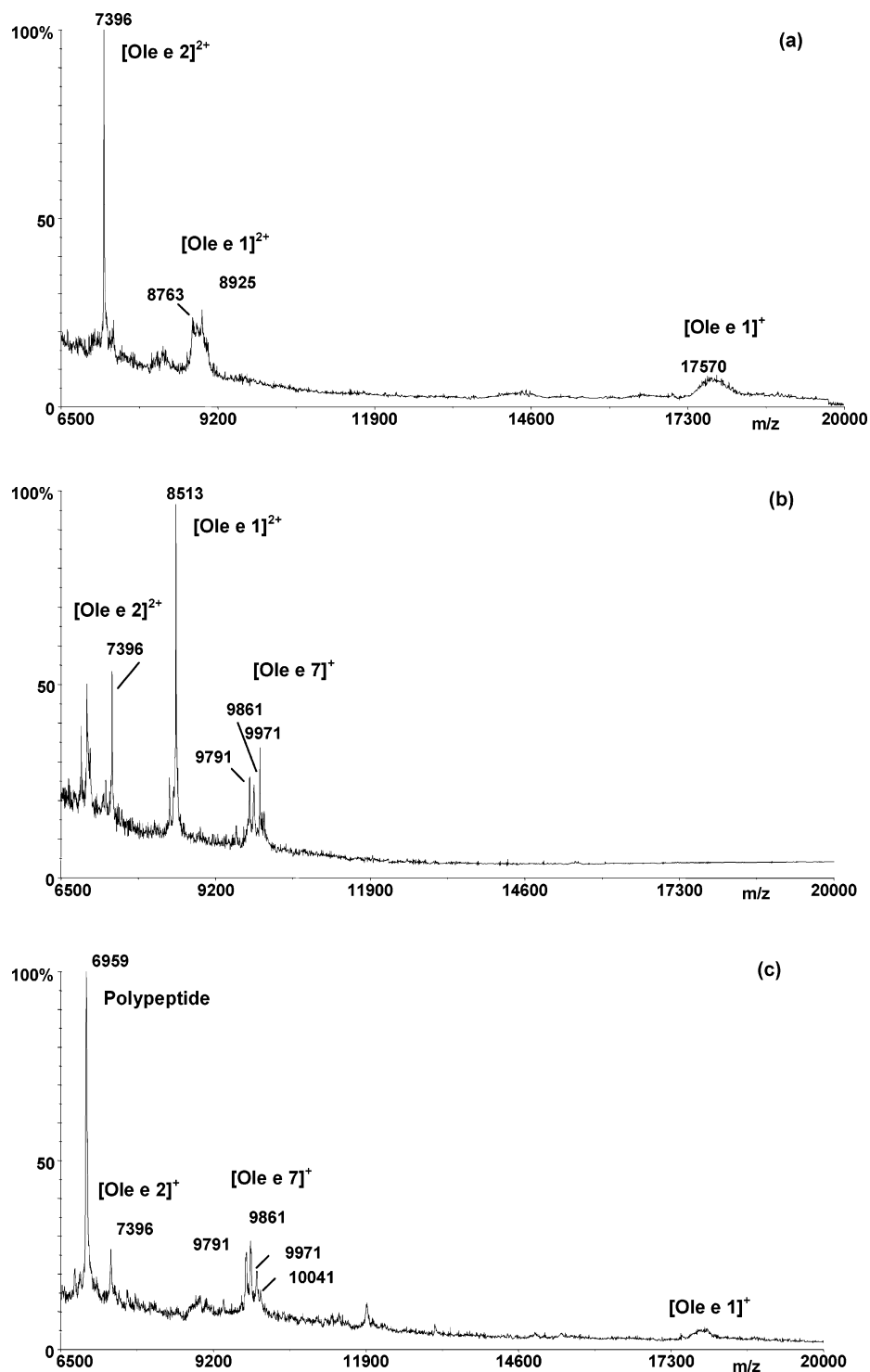


Figure 8. MALDI spectra of fractions (a) **2b**, (b) **3b**, and (c) **8b**.

(Figure 4b) contains an additional peak at m/z 4565, probably corresponding to $[\text{Ole e } 3]^{2+}$.

The presence of Ole e 3 in fraction 1b has been proved by a peptide mass fingerprinting (PMF) approach by tryptic digestion following deglycosylation by PNGase F. Database searching of the MALDI reflectron spectrum of the tryptic digest (Figure 5) confirms that one component of the fraction **1b** is Polcalcine Ole e 3 (calcium-binding pollen allergen Ole e 3) (spt|O81092) with sequence coverage of 50%. The Ole e 3 identified sequences, detected by MALDI TOF, are shown in Table 2.

A particular case is represented by the cultivar Carolea (2). Its fraction **2a** is characterized, in fact, by only one abundant doubly charged ion peak at m/z 7230, probably corresponding to Ole e 2.

The antigenic profile of Coratina and Villacidro hydrosoluble **b** fractions, **5b** and **7b**, respectively, (Figure 6a,b) shows a similar overenrichment of Ole e 1 allergens. The fraction **7b** (Figure 6b) contains the ionic species at m/z 8896, 17810, and 35635 only, corresponding to the doubly and monocharged and dimeric forms of Ole e 1, respectively. The peaks at m/z 8277 and 16447

represent probably the doubly and the monocharged deglycosylated forms of the same allergen.

Ole e 1 is the major allergen of *Olea europaea* (Table 1, row 2) and one of the best characterized allergens of the Oleaceae family; it has been isolated, purified, and biochemically characterized,^{7,30} and its specific cDNA was cloned and sequenced^{31,32} (GenBank/ EMBL Data Bank accession numbers S75766 and X76395, respectively). Ole e 1 shows 85–95% identity with Lol p 11, Lig v 1, Syr v 1, Fra e 1 and Pla l 1 allergens and 36–38% identity with the deduced amino acids sequences from *LAT52*, *Zmc13*, and *OSPSG* genes from tomato anthers and maize and rice pollens, respectively.³³

Fraction **7b** was used for the identification of Ole e 1 by peptide mass fingerprinting after tryptic digest. The MALDI reflectron spectrum (Figure 7) of tryptic peptides of intact Ole e 1 displays ion peaks corresponding to protonated tryptic peptides of the Ole e 1 family, with mass errors of 50 ppm. Database searching indicates that the major components of the mixture are (i) main olive allergen (*O. europaea*), (ii) major allergen OLE26, and (iii) major pollen allergen (allergen Ole e 1) with sequence coverages of 83, 62, and 50%, respectively (Table 3). The tryptic fragments of main olive allergen detected by MALDI-TOF are shown in Table 4.

The fractions **2b**, **3b**, and **8b** did not show any enrichment in Ole e 1, as previously observed for **5** and **7**, whereas they contain the antigenic mixture of Ole e 1, Ole e 2, Ole e 7 and one unidentified polypeptide at *m/z* 6959 (Figure 8a–c).

The significant differences observed in the MALDI-MS spectra of **1b8b** fractions suggest that the antigenic profile of the analyzed pollens can be divided into three distinct groups: (i) those with

low Ole e 1 content, represented by Carolea (2), Dolce di Rossano (3), and Sinopolese (8); (ii) those characterized by the overenrichment of Ole e 1, corresponding to Cassanese (4), Coratina (5), Nocellara del Belice (6), and Villacidro (7); and (iii) that containing Ole e 3 and Ole e 7 only, which is represented by the cultivar Ottobratica (1).

CONCLUSION

The mass spectral profiling of the examined samples (1–8) has to take into account that different allergenic and antigenic pattern can be found in the pollen of olive trees of the same species¹⁶ and, also, of different cultivars¹⁸ of the 260 existing varietal species.³⁴ Moreover, environmental adaptation and factor management could significantly affect qualitatively and quantitatively the protein profile of a given pollen. The experimental approach used so far is based on the SDS–PAGE or 2-D chromatographic separation followed by an immunoassay test.²³ The lack of sensitivity of those methods prevents the identification of cDNA-derived variants. The approach here proposed aims at characterizing olive cultivars from the profiling of the allergens of olive pollen by matching MALDI-TOF spectra of specific fractions containing chemically homogeneous proteins. The separation of proteins of different hydrophilicity from the lipophilic ones prevents the suppression effect typical of desorption methodologies²¹ and makes more effective the matching of MALDI spectra of different pollen fractions. The olive tree pollens (1–8) are distinguishable by the overenrichment of Ole e 1. An exception is represented by Ottobratica (1) which contains only Ole e 3 and Ole e 7. The data here presented indicate clearly that two isoforms of Ole e 3 are present in the natural matrix and that Ole e 7 consists of four isoforms characterized by a different glycosilation degree. These results demonstrate that the proposed experimental procedure can supply valuable information on the antigens' micro heterogeneity.

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