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Cell Wall Acyl-Lipids, Proteins and Polysaccharides in Mature and Germinated Olive Pollen

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Proteins, acyl-lipids and polysaccharides from cell walls of mature and germinated olive pollen were studied. In general, hemicelluloses are the most abundant polysaccharides, arabinose in mature and glucose in germinated pollen being the main components of these macromolecules. Protein content and its amino acid composition are very similar in walls from mature and germinated pollen, these compounds showing a weak acid character. Free-fatty acids are the most abundant lipid molecules in mature and germinated pollen walls and a decrease in acyl-lipids, especially in polar lipids, as well as a higher unsaturation of their fatty acid components are observed after germination.

Key words: *Olea europaea*, Pollen, Cell wall components, Germination.

Pollen represents a homogeneous cell population with numerous membrane and wall structures which confer special characteristics to germination and pollen tube development. These processes are characterized by a rapid cellular growth which involves intensive synthesis of biochemical components (10-12). The pollen cell wall is constituted by exine, resistant to acid hydrolysis, and intine, differentiated into cellulosic— and pectin-rich zones with arabinose, galactose, glucose and uronic acids as their main components (11). As pollen germinates, hemicelluloses consist mostly of glucose while pectic polysaccharides, which are made up essentially of uronic acids, are mostly located at the pollen tube tip (11). In this sense, the rapid growth of the tube is known to be related to the flexibility and plasticity of the wall structure (16).

Lipids are other important biochemical components of pollen wall. In some pollen species, such as *Brassica napus*, a high concentration of lipid compounds which could be involved in the early events of pollen adhesion, hydration and germination have been detected (3, 9).

On the other hand, wall proteins seem

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to play an important role in cell-cell recognition and form an indispensable element in the incompatibility system and in extensibility properties of the tube wall (4, 5, 20). In this respect, the presence of proteins in the walls of pollen grains and pollen tube as well as the interaction of these molecules with polysaccharides has been demonstrated (18, 20).

The aim of this work is to analyze the composition and content of some cell wall components in mature and germinated olive pollen.

Materials and Methods

Plant material. — Fresh pollen was collected from dehiscent anthers of *Olea europaea* L. cv. Marteño, dried, stored and germinated as detailed in previous works (14, 15).

Cell wall isolation. — Two grams of dried pollen were used as starting material for cell wall isolation from mature and 24 h-germinated pollen. The samples were resuspended in 60 ml of 50 mM 2-(N-morpholinol ethanesulfonic acid, pH 6.0, and ruptured by passing them three times through a French Press (AMINCO J4, Silver Spring, USA) and grinding once in a mortar. Cell walls were then isolated as described by YIQIN *et al.* (20).

Extraction and hydrolysis of cell wall polysaccharides. — Cell wall polysaccharides isolated from mature and 24 h-germinated pollen were obtained according to the method followed by WADA and RAY (19). Briefly, the isolated cell wall materials were first extracted three times for 24 h at 20 °C with dimethyl sulfoxide (DM fraction), then three times for 1 h at 96 °C with 0.5 % ammonium oxalate, and, finally three times for 24 h at 20 °C with 4 M KOH. Each extraction was followed by centrifugation at 25,000 *g* for 15 min. The ammonium oxalate-extracted fraction was dialyzed at 20 °C against distilled water for 24 h (OX fraction), and the alkali-extracted fraction, adjusted to pH 5.6 with acetic acid in an ice bath, was separated by centrifugation at 25,000 *g* for 15 min, into a precipitate (HA fraction) and a supernatant (HB fraction). Soluble fractions were precipitated by the addition of ethanol and centrifugation at 25,000 *g* for 15 min. The precipitates obtained and those corresponding to the HA fraction were washed successively with ethanol, acetone and diethyl ether, which were evaporated to dryness under vacuum. Finally, the polysaccharide fractions were hydrolyzed in 2 M trifluoroacetic acid at 123 °C for 2 h.

Monosaccharide composition and measurement. — Monosaccharide composition of the hydrolyzed fractions was determined by gas chromatography after the formation of silylated derivatives with 0.1 ml [bis(trimethylsilyl) trifluoroacetamide] and heating at 100 °C for 45 min. The analysis of silylated compounds was carried out on a chromosorb W-HP column, 80-100 mesh, temperature programmed from 80 to 160 °C at 2 °C min^{-1}, using a flame ionization detector and a flow rate of 20 ml min^{-1} N_2. Identification and quantification of different monosaccharides was performed using standard silylated sugars determined under the same conditions as the samples. Uronic acid content was determined by the carbazole-H_2SO_4 method (1).

Amino acid analysis. — Amino acid pattern analysis of total proteins from isolated cell walls was performed by acid hydrolysis with 6 M HCl for 24 h at 110 °C under inert atmosphere of argon. The insoluble material was pelleted at 2,000 *g* for 10 min and the acid evaporated at 60 °C under vacuum. The dried residue was washed several times with distilled water and the amino acids assayed by HPLC on an ultrasphere ODS RP-18 column, using

0.05 M acetate buffer, pH 5.9, methanol and tetrahydrofurane (800/150/2.5, v/v/v) and 0.05 M acetate buffer, pH 5.9, and methanol (200/ 800, v/v) as solvents.

Lipid extraction and analysis. — Lipid extraction from walls of mature and germinated pollen was carried out as described by EVANS *et al.* (3). Briefly, cell wall material (0.3 g) was extracted twice for 10 min in 6 ml anhydrous acetone. The acetonic extracts were separated by centrifugation at 2,000 *g* for 10 min, and the solvent evaporated under a stream of argon. The non acetone-extracted wall material was dried under vacuum and resuspended in 4 ml chloroform-methanol mixture (2/l, v/v) at 0 °C for 12 h. After adding 2 ml of 1 % NaCl, the extract was homogenized with a French Press as indicated above and centrifuged at 2,000 *g* for 10 min. The lipid-containing bottom phase was recovered and the solvent removed under a stream of argon.

The different lipids were resolved by thin layer chromatography (6, 8) and the fatty acids determined by GC according to LECHEVALLIER (7) using a column of 10 % diethylene glycol succinate on Chromosorb W, 80-100 mesh, at 180 °C and 30 ml min^{-1} N_2 as the carrier gas. Methyl heptadecanoate was included in the samples as an internal standard.

Protein assay. — Protein content was determined according to either SHAFFNER and WEISSMANN (17) or BRADFORD (2).

Results

Cell walls were apparently cytoplasm- and membrane-free, since no proteins were detected in the supernatants of the last four washings carried out in the pellet obtained after centrifugation of the pollen homogenate (data not shown).

Nature and 24 h-germinated olive pollen yielded 244 mg and 266 mg of cell wall material per gram of the initial dried pollen, respectively. Polysaccharide fractionation and its monosaccharide composition obtained from above samples are shown in table I. Germination involved an increase in the content of polysaccharides, especially hemicelluloses (DM, HA and HB). Arabinose was the most abundant component of hemicelluloses (DM) and pectic polysaccharides (OX) in mature pollen, while glucose was the main sugar in the DM and hemicellulose A (HA) fractions, and at the same level as arabinose in the hemicellulose B (HB) fraction of germinated pollen. A high content of uronic acid was observed in the OX and HA fractions of germinated pollen. Germination induced general changes in sugar composition of the different polysaccharide fractions. Thus, in all the fractions, an increase in rhamnose, xylose and glucose content was detected, while arabinose in hemicelluloses (DM, HA and HB) and galactose in DM and above all, in HB fractions were particularly increased. Likewise, a decrease in the mannose content of DM, HA and OX fractions was also observed. Finally, germination also stimulated an increase in the uronic acid levels of OX and HA fractions.

In mature and germinated pollen, the wall protein content, estimated from total amino acid analysis, represented 241.1 and 296.0 μmol per g dried pollen, respectively (table II). In both types of pollen walls, the proteins showed a similar amino acid composition, except that in germinated pollen a lower percentage of cysteine and a higher percentage of hydroxyproline and methionine than in mature pollen were observed. In general, serine, threonine, alanine and, above all, aspartic acid, leucine and proline were the most abundant amino acids in wall proteins.

Data of acyl-lipid content in pollen walls are shown in table III. Free fatty acids were the most abundant lipid molecules, with germination leading to a decrease in acyl- lipid content in terms of

Table I. *Sugar composition of different polysaccharide fractions of cell walls from mature and germinated olive pollen.*
Values are means of three independent experiments not differing by more than 10 % from the mean.

Sugar	Monosaccharide content (μg g^{-1} dried pollen)							
	DM fraction		OX fraction		HA fraction		HB fraction	
	Mature	Germinated	Mature	Germinated	Mature	Germinated	Mature	Germinated
Arabinose	401,0	1168,7	167,8	76,3	28,4	751,4	n.d.	5559,5
Rhamnose	5,0	232,2	33,8	95,0	8,7	718,0	n.d.	2986,9
Xylose	20,6	750,8	7,5	65,5	6,3	718,1	n.d.	3248,5
Mannose	33,7	n.d.	26,3	7,2	14,1	n.d.	n.d.	n.d.
Galactose	35,6	n.d.	22,1	47,5	9,1	n.d.	n.d.	4251,4
Glucose	114,3	5588,3	19,9	89,1	39,6	1886,9	n.d.	5755,8
Uronic acids	14,4	n.d.	98,0	220,6	n.d.	100,2	n.d.	n.d.
Total	624,6	7740,0	375,4	601,2	106,2	4174,6		21802,1

n.d., not detected; DM, hemicellulose fraction; HA, hemicellulose A fraction; HB, hemicellulose B fraction; OX, pectic polysaccharide fraction.

Table II. *Amino acid composition of cell walls isolated from mature and germinated olive pollen.*
Values are means of three independent experiments with standard error.

Amino acid	Amino acid percentages		Amino acid content (μmol g^{-1} dried pollen)	
	Mature	Germinated	Mature	Germinated
Aspartic acid	8.5	10.0	20.7 ± 1.7	29.6 ± 3.7*
Glutamic acid	5.6	5.4	13.5 ± 2.4	16.0 ± 2.4
Hydroxyproline	0.5	1.0***	1.2 ± 0.2	3.0 ± 0.3***
Serine	7.0	7.8*	16.9 ± 0.5	23.1 ± 1.2*
Asparragine	3.7	4.5	9.2 ± 1.6	13.3 ± 2.0*
Glycine	4.5	4.1	10.8 ± 1.9	12.1 ± 1.8
Histidine	3.0	4.0*	7.2 ± 0.5	11.8 ± 1.3**
Treonine	6.5	7.0	15.7 ± 0.9	20.7 ± 2.1*
Alanine	7.6	6.9	18.3 ± 1.9	20.4 ± 3.0
Arginine	5.3	5.5	12.8 ± 0.9	16.3 ± 1.2*
Proline	10.5	10.7	24.9 ± 1.8	31.8 ± 3.2*
Tyrosine	2.2	2.5	5.3 ± 0.6	7.4 ± 1.0*
Valine	5.6	6.2	13.7 ± 1.2	18.3 ± 1.2*
Methionine	0.7	1.5**	1.7 ± 0.2	4.4 ± 0.5***
Cysteine	5.7	0.9***	14.0 ± 1.4	2.7 ± 0.3***
Isoleucine	5.5	5.9	13.3 ± 0.7	17.5 ± 1.1**
Leucine	9.0	9.3	21.2 ± 0.9	27.5 ± 2.3*
Phenylalanine	3.7	3.2	8.9 ± 1.2	9.5 ± 1.0
Lysine	4.9	3.6*	11.8 ± 1.1	10.6 ± 1.2
Total			241.1	296.0

t Student test, 4 degrees of freedom. Differences between means:
* significative (p = 0.05)
** very significative (p = 0.01)
*** highly significative (p = 0.001)

Table III. *Acyl-lipid composition of cell walls isolated from mature and germinated olive pollen.* Values are means of three independent experiments with standard error. Statistic significance as in table I.

Acyl-lipid	Lipids percentage		Lipid content (μg g^{-1} dried pollen)	
	Mature	Germinated	Mature	Germinated
PL+GL	13.9	4.3***	645.8 ± 85.4	115.7 ± 17.7***
MG	6.9	5.7	321.1 ± 50.6	150.9 ± 11.9**
DG	21.8	20.0	1009.9 ± 133.9	523.0 ± 78.2**
FFA	45.8	57.3**	2116.1 ± 197.9	1520.0 ± 179.8*
TG	11.6	12.7	538.1 ± 76.0	338.4 ± 46.9*
Total			4631.0	2648.0

PL+GL, Phospholipids + glycolipids; MG, monoacylglycerols; DG, diacylglycerols; FFA, free fatty acids; TG, triacylglycerols.

Table IV. *Fatty acid composition of lipids in the cell walls isolated from mature and germinated olive pollen.* Values are means of three independent experiments with standard error. Statistic significance as in table I.

Fatty acid	Percentage of fatty acids		Fatty acid content (μg g^{-1} dried pollen)	
	Mature	Germinated	Mature	Germinated
12:0	10.2	6.0*	394.0 ± 70.9	171.3 ± 25.8**
14:0	14.8	11.2	568.4 ± 73.8	316.1 ± 40.6**
16:0	31.9	38.9	1225.8 ± 139.9	1100.2 ± 85.5
18:0	4.3	5.3	166.1 ± 24.1	149.8 ± 12.7
18:1	11.1	6.8*	425.9 ± 60.9	193.6 ± 15.8**
18:2	16.9	23.7*	646.7 ± 72.5	666.4 ± 65.7
20:0	2.0	—	77.2 ± 9.0	n.d.
18:3	6.8	8.1	262.3 ± 24.7	228.2 ± 16.2
20:1	2.0	—	76.6 ± 8.4	n.d.
Total			3843.0	2825.6*

n.d., not detected; 12:0, lauric acid; 14:0 myristic acid; 16:0, palmitic acid; 18:0 stearic acid; 18:1 oleic acid; 18:2 linoleic acid; 20:0, eicosanoic acid; 18:3, linolenic acid, 20:1, cis-9 eicosanoic acid.

microgram per gram of dried pollen, while the percentage of these lipids remained practically unchanged, except for the slight increase in free fatty acids and the strong decrease in glycolipids and phospholipids. Lipids from mature and germinated pollen were highly saturated (ca. 60 %), palmitic acid being the main fatty acid (table IV); however, germination gave rise to a general diminution in the amount of saturated fatty acids.

Discussion

In a previous work, a high germination percentage for olive pollen (ca. 65 %) was obtained (15). In this study, subsequent changes in lipid content and sugar composition of pollen walls due to germination were remarkable. Thus, as in other plant species, the sugar composition of olive pollen walls differs markedly from mature and germinated grains. The rise in

wall hemicelluloses and the high content in glucose of these polysaccharides after germination may be related to active synthesis of callose, a hemicellulosic glucan which constitutes the most abundant component in *Lilium* and *Camellia* tube walls (11, 13).

Amino acid composition of olive pollen wall proteins was similar to that observed in *Lilium longiflorum* by YI-QIN *et al.* (20). In several species of pollen, this composition indicates a weak acid character of these proteins. In agreement with the data obtained in *Lilium* by YI-QIN *et al.* (20), who suggested that proteins with a high hydroxyproline content are presumably involved in tube growth, the amount of the hydroxyproline of the wall proteins in olive pollen increased after germination.

One of the most outstanding events in the olive pollen wall after 24 hours of germination is the decrease in polar lipid content (phospholipids and glycolipids), which could correspond to the decrease in total phospholipid content detected in the whole pollen cell previously described (14). Likewise, the high content in free fatty acids of cell wall relative to the content of this fraction in the lipids of mature and germinated olive pollen (14) also indicates that most of these free fatty acids are located in the cell wall. It may be suggested that the high percentage of free fatty acids in walls of mature and germinated pollen could influence the properties of this structure.

Although this work contributes to the knowledge of the biochemical composition of olive pollen wall, further efforts are needed to elucidate the role of the changes in some components during germination.

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Resumen

Se estudian comparativamente las proteínas, lípidos acílicos y polisacáridos de paredes de polen de olivo maduro y germinado. Las hemicelulosas son los polisacáridos más abundantes, siendo la arabinosa su principal componente en paredes de polen maduro y la glucosa en polen germinado. Tanto en polen maduro como en germinado, el contenido en proteínas de la pared y su composición aminoacídica es muy similar, mostrando un débil carácter ácido. Los ácidos grasos libres son los constituyentes lipídicos más abundantes de paredes de polen maduro y en germinado, observándose en paredes de grano de polen germinado una disminución en el contenido de lípidos acílicos, especialmente fosfolípidos y glucolípidos, así como un incremento de la insaturación de sus ácidos grasos.

Palabras clave: *Olea europaea*, Polen, Componentes pared celular, Germinación.

References

1. Bitter, T. and Muir, H. M.: *Anal. Biochem.*, 41, 330-334, 1962.
2. Bradford, M. M.: *Anal. Biochem.*, 72, 248-254, 1976.
3. Evans, D. E., Rothnie, N. E., Palmer, M. V., Burke, D. G., Sang, J. P., Knox, R. B., Williams, E. G., Hilliard, E. P. and Salisbury, P. A.: *Phytochemistry*, 26, 1895-1897, 1987.
4. Honson, T, and Wada, S.: *Plant Cell Physiol.*, 21, 511-524, 1980.
5. Lamport, D. T. A. and Catt, J. W.: In "Encyclopedia of Plant Physiology" (Pierson, A. and Zimmerman, M. H., eds). Springer-Verlag, Berlin, 1981. vol. 13. pp. 133-165.
6. Lepage, M.: *Lipids*, 2, 244-250, 1967.
7. Lechevallier, D.: *C. R. Acad. Sci. Paris*, 263, 1849-1852, 1966.
8. Mangold, H. K.: *J. Am. Oil Chem. Soc.*, 41, 762-773, 1961.
9. Mattsson, O.: In "Pollen Biology and Implications for Plant Breeding" (Mulcahy, D. L. and Ottaviano, E., eds). Elsevier Science Publishing Co., Amsterdam, 1983. pp. 257-264.
10. Mollenhauer, M. M. and Morre, D. J.: In "The

Biochemistry of Plant" (Stumpf, P. K. and Conn, E. E. eds.). Academic Press Inc., New York, 1980. vol. 1. pp. 437-488.
11. Nakamura, N. and Suzuki, H.: *Phytochemistry*, 20, 981-984, 1981.
12. Picton, J. M. and Steer, M. W.: *J. Theor. Biol.*, 98, 15-20, 1982.
13. Rae, A. L., Harris, P. J., Bacic, A. and Clarke, A. E.: *Planta*, 166, 128-133, 1985.
14. Rodríguez-Rosales, M. P. and Donaire, J. P.: *New Phytol.*, 108, 509-514, 1988.
15. Rodríguez-Rosales, M. P., Roldán, M., Belver, A. and Donaire, J. P., *Plant Physiol. Biochem.*, 27, 723-728, 1989.
16. Roggen, H. P. and Stanley, R. G.: *Physiol. Plant.*, 24, 80-84, 1971.
17. Schaffner, W. and Weissmann, C.: *Anal. Biochem.*, 56, 502-514, 1973.
18. Steer, M. W. and Steer, J. M.: *New Phytol.*, 111, 323-258, 1989.
19. Wada, S. and Ray, P. M.: *Phytochemistry*, 17, 923-931, 1978.
20. Yi-qin, L., Croes, A. F. and Linskens, H. F.: *Planta*, 158, 422-427, 1983.