

**Table 1.** *Proteins identified in the apoplastic fluids of rosettes*

Proteins were extracted, separated and identified by MALDI-TOF-MS or LC/MS/MS. Functions or putative functions were defined after a bioinformatic analysis, following the TIGR ([www.tigr.org](http://www.tigr.org)) database annotation and the CAZy ([afmb.cnrs-mrs.fr/CAZY/](http://afmb.cnrs-mrs.fr/CAZY/)) nomenclature for carbohydrate-linked enzymes.

Proteins were distributed in six functional classes.

Protein identification	Spot numbers <sup>c</sup>	Gene Acc. No. <sup>d</sup>	Signal peptide	Theoretical <sup>e</sup>		Experimental		MOWSE Score <sup>f</sup>	% coverage
				MM (kDa)	pI	MM (kDa)	pI		
<b>Cell wall modifying proteins</b>									
Homolog to glycoside hydrolase family 38 ( $\alpha$ -mannosidase)	1	At5g13980	1-21	116.6	5.9	183.2	5.8	1991	6.0
Homolog to glycoside hydrolase family 17 ( $\beta$ -1,3-D-glucosidase) (BG2) (PR2)	9, 10, 15 <sup>a</sup> , 16 <sup>a</sup> , 20 <sup>a</sup> , 21 <sup>a</sup> / 2-D	At3g57260	1-30	37.3	4.6	66.3-11.3	4.2-5.6	1.631 e+006	32.0
Homolog to glycoside hydrolase family 17 ( $\beta$ -1,3-D-glucosidase) (BG3) (PR3)	14 <sup>a</sup> , D9-10	At3g57240 <sup>g</sup>	1-32	34.1	9.3	31.5-32.9	nd / 5.3	1.818 e+006	46.0
Homolog to glycoside hydrolase family 17 ( $\beta$ -1,3-D-glucosidase)	A25 <sup>a</sup>	At3g55430	1-28	48.4	10.2	30.9	nd	1491	13.0
Homolog to glycoside hydrolase family 17 ( $\beta$ -1,3-D-glucosidase)	C19, D11	At4g16260 [29]	1-21	33.6	5.0	29.8-31.8	nd	1.818 e+006	46.0
Homolog to glycoside hydrolase family 1 ( $\beta$ -glucosidase)	A10, A11, B7	At3g18080	1-23	58.9	9.7	55.1-57.1	nd	7.719 e+004	18.0
Homolog to glycoside hydrolase family 3 ( $\beta$ -xylosidase) (XYL4)	8 <sup>a</sup> , D4 <sup>a</sup>	At5g64570	1-38	84.3	8.8	55.6-63.2	6.2	1.937 e+009	21.0
Homolog to glycoside hydrolase family 3 ( $\beta$ -xylosidase) (AtBXL7)	A8	At1g78060	1-19	83.9	9.1	63.5	nd	4.754 e+004	19.0

Homolog to glycoside hydrolase family 3 ( $\beta$ -xylosidase) (AtBXL5)	A1	At3g19620	1-23	85.5	9.5	84.6	nd	2600	13.0
Homolog to glycoside hydrolase family 3 ( $\beta$ -glucosidase)	B4	At5g20950 [29]	1-19	67.9	9.8	70.8	nd	6.136 e+004	16.0
Homolog to glycoside hydrolase family 10 ( $\beta$ -xylanase)	14 <sup>a</sup>	At4g33810		61.3	6.0	31.6	5.3	6.290 e+006	14.0
Homolog to glycoside hydrolase family 31 ( $\alpha$ -xylosidase) (XYL1)	C2, C3, A1, A2 <sup>a</sup> , B2, B3	At1g68560 [29]	1-27	102.4	6.2	102.2-87.5	nd	3.729 e+008	20.0
Homolog to glycoside hydrolase family 35 $\beta$ -galactosidase (BGAL1)	A3, A4 <sup>a</sup>	At3g13750 [29]	1-32	93.6	9.5	86.3-74.3	nd	9.564 e+004	11.0
Homolog to glycoside hydrolase family 35 ( $\beta$ -galactosidase) (BGAL6)	C4, A5, A6 <sup>a</sup>	At5g63800	1-28	79.7	9.5	74.2-69.9	nd	8.855 e+006	23.0
Homolog to glycoside hydrolase family 35 ( $\beta$ -galactosidase) (BGAL8)	D7 <sup>a</sup> , D8 <sup>a</sup>	At2g28470	1-29	93.2	9.0	38.2-39.8	nd	3.495 e+004	11.0
Homolog to glycoside hydrolase family 27 ( $\alpha$ -galactosidase/melibiose)	2-D <sup>a</sup>	At5g08380	1-27	45.7	6.3	34.7-19.2	8.5-5.9	7.000 e+06	30.0
Homolog to glycoside hydrolase family 27 ( $\alpha$ -galactosidase/melibiose)	A19 <sup>a</sup>	At3g26380	1-32	75.5	9.6	38.8	nd	3.194 e+004	11.0

Homolog to glycoside hydrolase family 32 ( $\beta$ -fructofuranosidase)	A7	At3g13790	1-25	66.3	9.6	68.5	nd	3.106 e+004	26.0
Homolog to glycoside hydrolase family 20 (N-acetyl- $\beta$ -glucosaminidase)	A8	At1g65590	1-25	60.0	9.1	63.5	nd	3.772 e+008	30.0
Homolog to glycoside hydrolase family 79 (endo- $\beta$ -glucuronidase/heparanase)	C12, A16 <sup>a</sup>	At5g07830	1-24	60.2	9.7	44.1	nd	3.017 e+004	17.0
Homolog to glycoside hydrolase family 28 (polygalacturonase)	C5, C6	At4g23500	1-21	54.7	5.4	62.3-66.6	nd	3092	18.0
Homolog to $\alpha$ -expansin (AtEXP11)	A30	At1g20190	1-20	26.7	9.8	22.6	nd	3718 PSD	18.0
Expansin-related protein 3	A37	At2g18660	1-26	14.5	10.3	13.5	nd	225 PSD	23.0
Homolog to pectin methylesterase	D5, B5, B6 <sup>a</sup>	At1g76160 [29]	1-23	60.0	8.5	59.9-64.2	nd	7.139 e+008	41.0
Homolog to pectin methylesterase	A9, B6	At1g41830 [29]	1-24	60.5	9.7	59.9-60.1	nd	5.920 e+008	35.0
Homolog to carbohydrate esterase family 8 (pectin methylesterase)	A38 <sup>a, b</sup>	At4g14310	1-40	60.1	9.6	12.7	nd	LC/ MS/MS	
Homolog to carbohydrate esterase family 13 (pectin acetylerase)	A19, A20 <sup>a</sup>	At5g45280	1-23	42.0	9.9	37.4-38.8	nd	7746	17.0

<b>Glycoside hydrolase family 16 (xyloglucan endotransferase) (ext-EXGT-A1) (At-XTH4)</b>	<b>A27<sup>a</sup>, A32<sup>a</sup></b>	At2g06850	1-24	34.3	9.7	20.5-27.9	nd	LC/MS/MS	
<b>Defense proteins</b>									
Homolog to protease inhibitor (Kunitz) family (tomato miraculin)	C27, B26	At1g17860	1-21	22.0	9.8	19.0-19.4	nd	9094	33.0
Homolog to cysteine protease inhibitor (cystatin) family	C33, B29	At4g16500	1-22	12.5	9.7	11.7-12.4	nd	2580	41.0
Homolog to cysteine protease inhibitor (cystatin)	D13	At5g47550	1-27	13.3	9.5	15.0	nd	8142	30.0
<b>Homolog to <i>Pisum sativum</i> disease resistance protein family</b>	<b>B23, A32</b>	At1g65870	1-19	20.4	9.5	20.6	nd	LC/MS/MS	
Homolog to pectin methylesterase inhibitor (PMEI) family	C29	At1g47960	1-26	18.0	9.7	15.8	nd	1072	33.0
Homolog to berberine-bridge enzyme (S)-reticuliln:oxygen oxidoreductase (FAD-linked oxidoreductase family)	11-13 <sup>a</sup>	At2g34790 [29]	1-27	60.0	5.9	50.0-65.2	5.1-5.2	1.577 e+004	15.0

Homolog to berberine-bridge enzyme (S)-reticulic: oxygen oxidoreductase (FAD-linked oxidoreductase family)	A14	At5g44400	1-29	60.4	10.1	48.9	nd	3.332 e+08	28.0
Pathogenesis-related protein 5 (PR-5), thaumatin family	17-19 <sup>a</sup> , C24 / 2-D	At1g75040	1-20	25.3	4.5	16.5-23.0 / 22-23.4	nd / 4.1-4.6	2489	25.0
Homolog to lipase	A19, A20 <sup>a</sup> , A21 <sup>a</sup>	At1g28600	1-22	43.6	9.6	36.3-38.8	nd	8922	20.0
Homolog to GDSL-motif lipase/hydrolase protein family	A23-A25 <sup>a</sup> , B18	At1g29670	1-24	39.8	9.6	30.9-32.6	nd	2.493 e+004	23.0
Germin-like protein (subfamily 2 member 2)	A33	At1g02335	1-22	21.0	9.4	21.1	nd	LC/MS/MS	
Homolog to germin (subfamily 2 member 4) (GLP10)	A28, B21, B23	At3g62020	1-21	23.5	9.7	24.8-26.0	nd	959	27.0
Germin-like protein (AtGER3)	C25, C26, C28, A33, A34, B23, B24, B25 / 2-D	At5g20630	1-20	21.8	6.2	18.4-21.1 / 16.7-19.6	nd / 5.0-8.9	2737	22.0
Homolog to germin (subfamily 1 member 10)	B22	At5g38930	1-24	23.6	7.9	22.8	nd	7720	31.0
Homolog to germin (subfamily 1 member 11)	C24, B27	At5g38940	1-24	23.6	9.7	17.1-23.0	nd	8137	21.0

Homolog to copper amine oxidase	A4	At4g14940	1-17	73.8	9.5	77.4	nd	1.333 e+004	11.0
Pathogenesis-related protein 1 (PR-1)	B28	At2g14610	1-26	17.6	9.8	14.6	nd	835	18.0
Homolog to glycoside hydrolase family 18 (chitinase/lysozyme)	C16	At4g19810	1-24	41.1	9.4	35.9	nd	3.740 e+004	31.0
<b>Proteins containing domains for interaction with other proteins or polysaccharides</b>									
Homolog to polygalacturonase Inhibiting Protein PGIP1 (LRR protein FLR1)	C20, A24, A25 <sup>a</sup> , B19	T23B7.10	1-23	35.8	9.7	29.7-31.7	nd	5.636 e+004	31.0
PGIP2	C17, C18	At5g06870 [28, 29]	1-21	37.0	9.8	33.9-34.7	nd	5.258 e+005	45.0
Homolog to curculin-like (mannose-binding) lectin family	C17 <sup>a</sup> , B16 <sup>a</sup> / 2- D <sup>a</sup>	At1g78830 [28, 29]	1-22	50.3	9.5	34.5-34.7 / 32.3	nd / 4.9	1.509 e+004	17.0
Homolog to curculin-like (mannose-binding) lectin family	D6	At1g78850 [28, 29]	1-22	49.0	9.0	49.5	nd	109	8.0
Homolog to curculin-like (mannose-binding) lectin family	D6	At1g78860 [29]	1-22	49.2	5.7	49.5	nd	109	8.0

Homolog to legume lectin protein family	C21, C22, A24-26, A29 <sup>a</sup> , A33 <sup>a</sup> , A34 <sup>a</sup> , B19, D11	At3g15356 [29]	1-19	29.7	9.5	20.1-31.6	nd	1.106 e+004	33.0
Homolog to legume lectin protein family	C22, A27, A36 <sup>a</sup> , B20, D12	At3g16530	1-19	30.5	7.9	15.1-28.8	nd	5.333 e+008	56.0
Homolog to legume lectin protein family	A27, A32 <sup>a</sup> , A35 <sup>a</sup> , B20	At5g03350	1-19	30.1	9.8	17.6-28.3	nd	2.981 e+005	42.0
Homolog to legume lectin protein family	A24	At1g53070 [28]	1-23	30.3	9.3	31.7	nd	1.293 e+007	30.0
Homolog to disease resistance protein (Leucine Rich Repeat or LRR domains)	C8, C9, A12, A13 <sup>a</sup> , B8	At1g33590 [29]	1-24	51.7	9.9	50.3-52.5	nd	1.343 e+010	45.0
Homolog to disease resistance protein (LRR domains)	A15 <sup>a</sup> , B9	At1g33600	1-25	52.5	10.4	47.6-48.8	nd	1.039 e+004	19.0
Homolog to disease resistance protein (LRR domains)	C15, A19, A20 <sup>a</sup>	At3g20820	1-19	39.8	9.9	37.4-38.8	nd	4.582 e+006	35.0
Homolog to disease resistance protein (LRR domains)	C1, A1, A2 <sup>a</sup> , B2	At2g34930	1-28	100.2	9.6	95.7-102.2	nd	4.397 e+008	25.0
Homolog to disease resistance protein (LRR domains)	C7	At5g23400 [29]	1-26	64.1	10.5	58.2	nd	1.446 e+004	14.0

Homolog to extracellular dermal glycoprotein (EDGP)	A15	At1g03230	1-23	46.1	10.3	47.6	nd	1.335 e+005	22.0
Homolog to extracellular dermal glycoprotein (EDGP)	C10, B10, B11	At1g03220 [29]	1-23	35.8	9.7	46.0-49.0	nd	2.192 e+005	24.0
<b>Fasciclin-like arabinogalactan protein (FLA1)</b>	<b>B23<sup>a</sup></b>	At5g55730	1-24	44.9	6.2	20.6	nd	LC/ MS/MS	
<b>Proteinases</b>									
Subtilisin-like serine protease (ARA12)	2-5, 6 <sup>a</sup> , 7 <sup>a</sup> , D1-4	At5g67360	1-25	79.5	5.8	63.2-89.2	6.2-7.0	9.573 e+008	17.0
Homolog to subtilisin serine-protease	A5, A6 <sup>a</sup> , A8 <sup>a</sup> , B4	At2g05920 [29]	1-26	80.0	9.3	55.6-89.2	nd	1.448 e+006	17.0
Homolog to subtilisin serine protease	A3	At1g01900	1-22	81.1	9.2	84.6	nd	6.358 e+04	11.0
Homolog to subtilisin serine protease XSP1	A4	At4g00230	1-20	80.3	9.9	77.4	nd	3.215 e+05	17.0
Homolog to Asp protease	C10, C11, B11	At1g09750	1-23	47.4	9.1	46.0-49.1	nd	983	11.0
Homolog to Asp protease	A17, B12, B13	At5g07030	1-19	46.9	10.8	36.5-42.9	nd	3.582 e+005	30.0
Homolog to cysteine proteinase XCP2	A36 <sup>a</sup>	At1g20850	1-26	39.7	4.9	15.1	nd	411 PSD	13.0
Homolog to cysteine proteinase RD21A	2-D	At4g11310	1-23	39.9	5.5	41.1	5.2	1188	10.0



Homolog to serine carboxypeptidase, peptidase S10	A31	At5g42240	1-28	49.8	6.2	22.1	nd	LC/MS/MS	
Homolog to signal peptidase subunit family	A31	At3g05230	1-26	19.0	9.7	22.1	nd	PSD	
		and/or At5g27430	1-26	19.2	9.7	22.1	nd	PSD	
<b>Miscellaneous</b>									
Homolog to apospory-associated protein	A23, A24 <sup>a</sup> , B17	At4g25900 [28, 29]	1-20	36.2	10.4	31.7-33.1	nd	3.859 e+007	45.0
Homolog to CLAVATA1 <sup>b</sup> (putative receptor kinase)	A5 <sup>a</sup> , A6 <sup>a</sup>	At5g05160	1-27	70.5	6.4	69.9-72.4	nd	8.451 e+004	24.0
Homolog to fimbrin protein (AtFim <sup>b</sup> )	2-D <sup>a</sup>	At2g04750		73.4	5.9	67.5	6.3	3099	10.0
Cellulose synthase, catalytic subunit (IRX1) <sup>b</sup>	A31 <sup>a</sup>	At4g18780		111.5	6.7	22.1	nd	PSD	
Homolog to FAD-linked oxidoreductase family <sup>b</sup>	A5 <sup>a</sup>	At2g46740		64.9	9.2	72.4	nd	1.802 e+004	19.0
Non specific lipid-transfer protein 5 (LPT5)	C34	At3g51600	1-25	9.9	12.7	12.0	nd	LC/MS/MS	
Homolog to bacterial spore coat protein	A7	At1g71040	1-25	66.1	9.6	68.5	nd	5.552 e+006	28.0

<b>Homolog to MD-2-related lipid recognition (ML) domain protein</b>	<b>B23</b>	At3g44100	1-21	16.1	9.7	20.6	nd	LC/ MS/MS	
<b>Unknown function</b>									
Expressed protein (Domain of Unknown Fonction or DUF642)	A20-A22 <sup>a</sup> , A30 <sup>a</sup> , A32 <sup>a</sup> , A36 <sup>a</sup> , B15	At3g08030 [28, 29]	1-21	39.0	8.0	15.1-37.4	nd	1.534 e+005	28.0
Expressed protein	C13, C14, A18, B13	At5g11420	1-22	39.6	9.0	40.2-41.5	nd	9.073 e+004	25.0
Expressed protein (DUF288) <sup>b</sup>	A5 <sup>a</sup>	At3g57420		87.6	5.9	72.4	nd	2.418 e+004	16.0
Expressed protein (DUF26)	C23	At3g22060	1-27	27.8	10.1	24.1	nd	6578	23.0
Expressed protein	A4	At1g21670	1-21	77.3	9.8	74.3	nd	2112	9.0
<b>Expressed protein</b>	<b>A32</b>	At1g76020	1-15	25.3	9.2	20.5	nd	LC/ MS/MS	
<b>Intracellular proteins</b>									
Precursor of RUBISCO 3b small subunit	22 <sup>a</sup>	At5g38410		20.3	9.4	11.9	5.6	1.914 e+005	50.0
Homolog to proteinase inhibitor	2-D	At2g38870		7.6	7.3	9.2	5.2	1.019 e+04	70.0

Homolog to NADH dehydrogenase	2-D	At5g08740		56.0	9.0	65.2	8.0	7.435 e+04	17.0
Homolog to translation initiation factor 3 (IF-3)	A31 <sup>a</sup>	At4g30690		31.8	10.0	22.1	nd	1578	13.0
Thioredoxin m (trx-m4)	C30 <sup>a</sup>	At3g15360		21.1	9.9	15.3	nd	2921	21.0
Thioredoxin m (trx-m1)	C31 <sup>a</sup>	At1g03675		19.6	9.9	14.1	nd	1.264 e+004	22.0
Thioredoxin h	C32	At5g42980		13.1	4.7	13.5	nd	583	38.0
Ubiquitin extension protein (UBQ) (seven possible genes)	C35 <sup>a</sup> , D14 <sup>a</sup>					10.6-11.6	nd	nd	

<sup>a</sup> protein identified as a proteolytic digest

<sup>b</sup> protein with at least one predicted trans-membrane domain

<sup>c</sup> spot numbers refer to Figures 1 and 3

<sup>d</sup> numbers between brackets refer to previous identification of CWPs by a proteomic approach

<sup>e</sup> theoretical molecular masses (MM) and pIs have been calculated for mature proteins

<sup>f</sup> MOWSE Score is given by the MS-FIT software. LC/MS/MS and PSD results are given in Tables 2 and 3, respectively

<sup>g</sup> mass spectrometry data indicate that the encoded protein is longer than predicted in databanks (NCBI, TIGR) at its N-terminus  
2-D (spot column) indicates a protein identified after separation on a 2-D gel not shown in this paper.

Proteins written in bold characters were identified by LC/MS/MS

**Table 2.** *List of data for proteins identified by LC/MS/MS*

Spot number	Gene Acc. No.	Peptide sequence	Mascot Score <sup>a</sup>	Number of proteins sharing the sequenced peptide <sup>a</sup>
A31	At5g42240	LLTFATVR LPGQPTVGFK ELAQDLNFK	13 18 28	1 1 1
A27 A32	At2g06850	TGQPAILQTNVFTGGK IYLWFDPSK TGQPAILQTNVFTGGK	95 71 82	1 1 1
A32	At1g76020	IVELGTVSLGNSYQSVLK	64	1
A33	At1g02335	IGEVFVFPR	30	1
A38	At3g14310	IGATSDLQSVK	77	1
A32 B23	At1g65870	LTVGPEITSEEVGR LTVGPEITSEEVGR	81 95	1 1
B23	At5g55730	VFAETLLTNP GASK	64	1
B23	At3g44100	ISDPVVS GAAATFK	91	1
C34	At3g51600	QQACRCIQGAAR	39	1

<sup>a</sup> These results were given by the Mascot software

**Table 3.** *List of data for proteins identified by PSD*

Spot number	Gene Acc. No.	Peptide sequence	Matched ions <sup>a</sup>	MH+ error (ppm) <sup>a</sup>
A30	At1g20190	GGIVPVVFQR	10/11	0.01
A31	At3g05230 At5g27430	GKDFNLTLHWHVMPK	11/25	17.0
A31	At4g18780	GLDGIQGPVYVGTGTVFRR	17/21	17.0
A36	At1g20850	ALAHQPLSVAIDASGR	13/13	-0.8
A37	At2g18660	AVYYDPPYTR	8/8	-4.1

<sup>a</sup> These results were given by the MS-TAG software.

**Table 4.** Comparison of CWP functional class distribution between 4 week-old rosettes and 7 day-old cell suspension cultures

Functional classes were subdivided according to the predicted biological activity of the corresponding proteins. A few proteins showing very diverse putative activities were not included. CWPs extracted from 4 week-old rosettes (this study) and 7 day-old cell suspension cultures [29] were counted in each functional class (Total). The number of proteins exclusively extracted from rosettes (Rosettes) or from cell suspension cultures (Cells) as well as the number of proteins found to be common to both plant materials (Common) are reported.

Functional class	Biological activity	Total	Rosettes	Cells	Common
Cell wall modifying proteins	Glycoside hydrolases	26	19	4	3
	Expansins	4	2	2	0
	Esterases	6	2	2	2
	Oxidases	12	0	12	0
Defense-related proteins	Inhibitors (proteases/esterases)	3	3	0	0
	Oxidases	6	2	3	1
	Chitinases	3	1	2	0
	Lipid hydrolases	2	2	0	0
Proteins with interacting domains	Germin-like	6	5	1	0
	Lectin domains	8	3	0	5
Proteinases	LRR domains	8	4	1	3
		15	10	4	1
Miscellaneous		14	7	6	1
Unknown		9	5	3	1