

Characterization of leaf apoplastic peroxidases and metabolites in *Vigna unguiculata* in response to toxic manganese supply and silicon

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Supplementary data

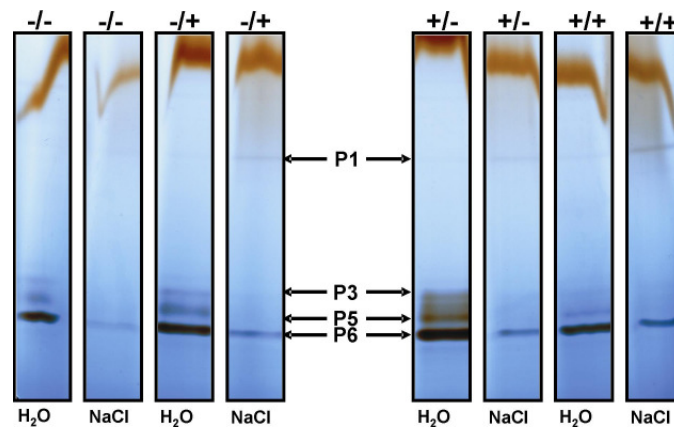


Fig. S1: Resolution of dH₂O- and NaCl-extractable AWF proteins after 0 and 4 d of Mn treatment of \pm Si treated plants (as described in “Materials and Methods”) of the Mn-sensitive cultivar TVu 91 after separation with BN-PAGE stained for guaiacol-peroxidase. The signs on the top of the lanes are as follows: -/- 0.2 μ M Mn/0 μ M Si, -/+ 0.2 μ M Mn /20 μ M Si, +/- 50 μ M Mn/0 μ M Si, +/+ 50 μ M Mn/20 μ M Si. H₂O and NaCl as indicated under the lanes describe the infiltration solution to extract AWF. 16 μ g of concentrated AWF (as determined with 2D Quant Kit) were loaded onto each lane. Guaiacol-POD staining was done in 18 mM guaiacol (in 9 mM Na₂HPO₄) and 0.03% H₂O₂ at pH 6.0.

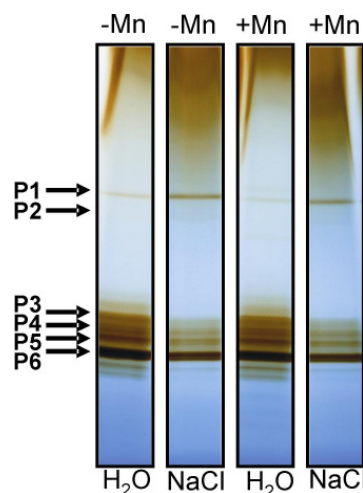


Fig. S2: Resolution of dH₂O- and NaCl-extractable AWF proteins after 0 and 4 d of Mn of the Mn-sensitive cultivar TVu 91 after separation with BN-PAGE stained for guaiacol-peroxidase. -Mn indicates 0.2 μ M Mn treatment, whereas +Mn describes 50 μ M Mn treatment for four days. H₂O and NaCl as indicated under the lanes describe the infiltration solution to extract AWF. 180 μ g of concentrated AWF (as determined with 2D Quant Kit) were loaded onto each lane. Guaiacol-POD staining was done in 18 mM guaiacol (in 9 mM Na₂HPO₄) and 0.03% H₂O₂ at pH 6.0.

Table S1: Determined extinction coefficients for the calculation of NADH-*peroxidase* activities of different POD isoenzymes supplied with different phenols in changing concentrations as shown in Fig. 4, 6 and 7.

Phenol ^a	Absorption	Phenol conc. [mM] ^b	NADH conc. [mM] ^c	Total conc. [mM] ^d	Extinction coefficient [ε] ^e
<i>p</i> -coumaric acid	1.979	1.66	0.22	1.88	1.13
vanillic acid	1.051	1.66	0.22	1.88	0.60
gallic acid	1.034	1.66	0.22	1.88	0.59
benzoic acid	1.026	1.66	0.22	1.88	0.58
ferulic acid	3.429	1.66	0.22	1.88	1.95
syringic acid	1.033	1.66	0.22	1.88	0.59
protocatechuic acid	1.025	1.66	0.22	1.88	0.58
caffeic acid	3.453	1.66	0.22	1.88	1.97
chlorogenic acid	3.69	1.66	0.22	1.88	2.10
<i>p</i> -hydroxybenzoic acid	0.800	1.66	0.22	1.88	0.46
<i>p</i> -coumaric acid	1.137	0.166	0.22	0.386	3.15
vanillic acid	1.046	0.166	0.22	0.386	2.90
gallic acid	1.052	0.166	0.22	0.386	2.92
benzoic acid	1.03	0.166	0.22	0.386	2.86
ferulic acid	1.484	0.166	0.22	0.386	4.12
syringic acid	1.045	0.166	0.22	0.386	2.90
protocatechuic acid	1.038	0.166	0.22	0.386	2.88
caffeic acid	1.52	0.166	0.22	0.386	4.22
chlorogenic acid	2.715	0.166	0.22	0.386	7.53
<i>p</i> -hydroxybenzoic acid	0.800	0.166	0.22	0.386	2.21
<i>p</i> -coumaric acid	1.053	0.0166	0.22	0.2366	4.77
vanillic acid	1.034	0.0166	0.22	0.2366	4.70
gallic acid	1.039	0.0166	0.22	0.2366	4.70
benzoic acid	1.04	0.0166	0.22	0.2366	4.71
ferulic acid	1.08	0.0166	0.22	0.2366	4.89
syringic acid	1.036	0.0166	0.22	0.2366	4.69
protocatechuic acid	1.032	0.0166	0.22	0.2366	4.67
caffeic acid	1.082	0.0166	0.22	0.2366	4.90
chlorogenic acid	1.195	0.0166	0.22	0.2366	5.41
<i>p</i> -hydroxybenzoic acid	0.800	0.0166	0.22	0.2366	3.62
<i>p</i> -coumaric acid	1.016	0.00166	0.22	0.22166	4.91
vanillic acid	1.017	0.00166	0.22	0.22166	4.91
gallic acid	1.021	0.00166	0.22	0.22166	4.93
benzoic acid	1.02	0.00166	0.22	0.22166	4.93
ferulic acid	1.018	0.00166	0.22	0.22166	4.92
syringic acid	1.008	0.00166	0.22	0.22166	4.87
protocatechuic acid	1.017	0.00166	0.22	0.22166	4.91
caffeic acid	0.981	0.00166	0.22	0.22166	4.74
chlorogenic acid	1.029	0.00166	0.22	0.22166	4.97
<i>p</i> -hydroxybenzoic acid	0.800	0.00166	0.22	0.22166	3.86
Phenol combination ^f	Absorption	Phenol conc. [mM] ^b	NADH conc. [mM] ^c	Total conc. [mM] ^d	Extinction coefficient [ε] ^e
<i>p</i> -coumaric acid [0.166mM] with:					
vanillic acid	1.079	0.0166	0.22	0.4026	2.87
gallic acid	1.078	0.0166	0.22	0.4026	2.87
benzoic acid	1.078	0.0166	0.22	0.4026	2.87
ferulic acid	1.118	0.0166	0.22	0.4026	2.97
syringic acid	1.072	0.0166	0.22	0.4026	2.85
protocatechuic acid	1.075	0.0166	0.22	0.4026	2.86
caffeic acid	1.120	0.0166	0.22	0.4026	2.98
chlorogenic acid	1.223	0.0166	0.22	0.4026	3.25
<i>p</i> -hydroxybenzoic acid		0.0166	0.22	0.4026	
- ^g	0.971	-	0.22	0.22	4.73

^a Phenol added to the measuring solution. Values correspond to Figs. 4, 6 and 7.

^b Phenol concentration in the measuring solution. Values correspond to Fig. 4, 6, and 7.

^c NADH concentration added to the measuring solution. Values correspond to Fig.4, 6, and 7.

^d Calculated total concentration of the absorbing substances phenol and NADH based on ^b and ^c.

^e Calculated extinction coefficient ϵ [$\text{L} (\text{mmol}\cdot\text{cm})^{-1}$ or $\text{cm}^2 (\mu\text{mol})^{-1}$] according to the Beer-Lambert law: $\text{Abs.}=\epsilon\cdot c\cdot d$

^f 0.0166 mM of each phenol was added to 0.166 mM *p*-coumaric acid, so that the end concentration of absorbing substances was 0.4026. These values correspond to Fig. 7.

^g Extinction coefficient ϵ calculated without phenol supply to the measuring solution

Tab.S2: Peptide sequences of apoplastic cowpea proteins. AWF proteins were separated by means of BN-PAGE and afterwards stained for guaiacol-peroxidase activity. Stained BN-bands were cut from the gel and sequenced with ESI-MS/MS. Results presented here are derived from 2 independent sequencing runs.

bande ^a	Protein name ^b	Protein accession numbers ^c	Protein molecular weight (Da) ^d	Number of unique peptides	Percentage sequence coverage ^e	Peptide sequence	Mascot Ion score	Mascot Identity score	difference score	Number of identified +1H spectra	Number of identified +2H spectra	Number of identified +3H spectra	Number of identified +4H spectra	Number of enzymatic termini	Calculated +1H Peptide Mass (AMU)
AWF _{H2O} _P1	fasciclin-like arabinogalactan protein 12 [Gossypium hirsutum]	ABV27483.1	43 894.2	1	1.88%	LADEINTR	61	47	14	0	1	0	0	2	931.4849
	Fructose-1,6-bisphosphatase, chloroplast precursor [Glycine max]	Q42796.1	43 879.4	2	7.96%	ANISNLTVGVQGANVQGEDQK	51.5	44.8	6.7	0	0	1	0	2	2 142,0792
	Fructose-1,6-bisphosphatase, chloroplast precursor [Glycine max]	Q42796.1	43 879.4	2	7.96%	YIGSLVGFDFHR	37.8	46.5	-8.7	0	0	1	0	2	1 263.6486
	fasciclin-like arabinogalactan protein FLA8 [Arabidopsis thaliana]	AAG24276.1	43 044.2	1	2.62%	VGFSAASGSK	67.5	46.1	21.4	0	1	0	0	2	967.4848
	xyloglucan endotransglucosylase/hydrolase 2 [Cucumis melo]	AB194062.1	36 316.1	1	3.88%	TDWSQAPFTASYR	90.7	45.8	44.9	0	1	0	0	2	1 529.7025
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	90	46	44	0	1	0	0	2	1 408.7479
	basic chitinase, chitinase [Arabidopsis thaliana]	NP_566426.1	36 196.3	1	5.67%	LPGYGVITNIHNGGLECGR	128	44.9	83.1	0	1	1	0	2	2 003.0385
	Os01g0357100 [Oryza sativa (japonica cultivar-group)]	NP_001043008.1	72 385.0	1	1.65%	LADEYGSSELR	72.3	49.1	23.2	0	1	0	0	2	1 209.5752
	cytosolic malate dehydrogenase [Cicer arietinum]	CAC10208.1	41 361.4	1	2.11%	ALGQISER	61.4	47.2	14.2	0	1	0	0	2	873.4795
	cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	8.14%	VFFDMTIGGQPAGR	75.8	45.7	30.1	0	2	0	0	2	1 511.7319
	alpha galactosidase [Glycine max]	AAA73963.1	46 378.6	4	10.40%	SVGNSWR	38.1	48.6	-10.5	0	1	0	0	2	805.3956
	alpha galactosidase [Glycine max]	AAA73963.1	46 378.6	4	10.40%	TFASWGIDYLK	64.8	46.5	18.3	0	2	0	0	2	1 300.6578
	alpha galactosidase [Glycine max]	AAA73963.1	46 378.6	4	10.40%	TMPGSLGHEEQDAK	36.8	46.1	-9.3	0	1	0	0	2	1 499.6801
	alpha galactosidase [Glycine max]	AAA73963.1	46 378.6	4	10.40%	YDNCENNNISPK	67.5	47.4	20.1	0	2	0	0	2	1 467.6175
	endochitinase precursor [Humulus lupulus]	AAD34596.1	33 511.4	1	4.11%	GFYTYDAFLTAAR	75.7	46	29.7	0	1	0	0	2	1 495.7221
	unnamed protein product (putative secretory peroxidase) [Vitis vinifera]	CAO48839.1	34 372.5	1	2.79%	GVEVVDTIK	53.1	46.5	6.6	0	1	0	0	2	1 023.5363
	unnamed protein product (putative Serine carboxypeptidase) [Vitis vinifera]	CAO68876.1	57 192.0	1	2.96%	NLEVGIPDLLEDGIK	58.1	45.3	12.8	0	2	0	0	2	1 624.8801
AWF _{H2O} _P3	fasciclin-like arabinogalactan protein 12 [Gossypium hirsutum]	ABV27483.1	43 894.2	1	1.88%	LADEINTR	73.1	47	26.1	0	1	0	0	2	931.4849
	pepti (ISS) (putative Cyclophilin) [Ostreococcus tauri]	CAL57205.1	38 854.4	1	3.27%	IVLGLFGDDAPR	60.6	46.3	14.3	0	2	0	0	2	1 272.6955
	unnamed protein product (putative Thaumatin family) [Vitis vinifera]	CAO62993.1	26 139.4	1	4.07%	GSDGSVIGCK	77.4	46.7	30.7	0	1	0	0	2	979.452
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	71.7	46	25.7	0	1	0	0	2	1 408.7479
	putative glycine-rich RNA-binding protein [Dianthus caryophyllus]	BAF34340.1	16 872.1	1	5.68%	SITVNEAQR	65.3	46.4	18.9	0	1	0	0	2	1 104.5649
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGASILR	42.9	47.7	-4.8	0	1	0	0	2	763.4137
peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGNISPLTGSSGEIR	79.7	45.8	33.9	0	1	0	0	2	1 534.7536	
AWF _{H2O} _P4	fasciclin-like arabinogalactan protein 12 [Gossypium hirsutum]	ABV27483.1	43 894.2	1	1.88%	LADEINTR	69.5	47	22.5	0	2	0	0	2	931.4849
	pepti (ISS) (putative Cyclophilin) [Ostreococcus tauri]	CAL57205.1	38 854.4	1	3.27%	IVLGLFGDDAPR	56.1	46.3	9.8	0	2	0	0	2	1 272.6955
	oxidoreductase family protein [Arabidopsis thaliana]	NP_188715.2	38 560.4	1	3.94%	DVAVLEAMLESQAK	96.1	46.1	50	0	1	0	0	2	1 448.7308
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	76.5	46	30.5	0	1	0	0	2	1 408.7479
	hypothetical protein (putative 14-3-3 protein) [Vitis vinifera]	CAN81774.1	29 523.7	1	4.58%	TVEVELTVEER	86	46.5	39.5	0	1	0	0	2	1 432.7172
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGASILR	46.2	47.7	-1.5	0	1	0	0	2	763.4137
peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGNISPLTGSSGEIR	87.1	45.8	41.3	0	3	0	0	2	1 534.7536	
AWF _{H2O} _P5	fasciclin-like arabinogalactan protein 12 [Gossypium hirsutum]	ABV27483.1	43 894.2	1	1.88%	LADEINTR	54.4	47	7.4	0	2	0	0	2	931.4849
	unnamed protein product (putative Thaumatin family) [Vitis vinifera]	CAO62993.1	26 139.4	1	4.07%	GSDGSVIGCK	80.8	46.7	34.1	0	2	0	0	2	979.452
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	73	46	27	0	1	0	0	2	1 408.7479
	Protein P21 (putative Thaumatin family) [Glycine max]	P25096.1	25 930.1	1	4.18%	TGCNFDGSGR	81.4	46	35.4	0	2	0	0	2	1 070.4326
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGASILR	45.5	47.7	-2.2	0	1	0	0	2	763.4137
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGNISPLTGSSGEIR	83.1	45.8	37.3	0	4	0	0	2	1 534.7536
transaldolase [Lycopersicon esculentum]	AAG16981.1	55 421.0	1	2.13%	VTVSASFVSR	59	46.2	12.8	0	1	0	0	2	1 199.6423	
AWF _{H2O} _P6	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	72.9	46	26.9	0	1	0	0	2	1 408.7479
	iron-superoxide dismutase precursor [Vigna unguiculata]	AAF28773.1	27 393.8	1	6.53%	ASLGLQNVAGINLLFK	83	45.4	37.6	0	1	0	0	2	1 699.9747
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGASILR	51.8	47.7	4.1	0	2	0	0	2	763.4137
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGNISPLTGSSGEIR	105	45.8	59.2	0	4	1	0	2	1 534.7536
	ATAM11, amidase [Arabidopsis thaliana]	NP_563831.1	45 038.6	1	3.53%	LVDFSIGDTGGSVR	91.2	45.8	45.4	0	1	0	0	2	1 523.7707
	peroxidase [Populus alba x Populus tremula var. glandulosa]	AAX53172.1	33 367.4	2	7.59%	GFEVIDTIK	59.6	47.7	11.9	0	1	0	0	2	1 021.5571

AWF _{NaCl} _P1	peroxidase [Populus alba x Populus tremula var. glandulosa]	AAX53172.1	33 367.4	2	7.59%	GFEVIDTIK	59.6	47.7	11.9	0	1	0	0	2	1 021,5571	
	peroxidase [Populus alba x Populus tremula var. glandulosa]	AAX53172.1	33 367.4	2	7.59%	MGNISPLTGTNGEIR	57.3	45.8	11.5	0	1	0	0	2	1 559,7854	
	peroxidase [Sesamum indicum]	ABB89209.1	35 838.4	2	6.97%	IAIDMDPTTPR	73.6	47.7	25.9	0	1	0	0	2	1 245,6152	
	peroxidase [Sesamum indicum]	ABB89209.1	35 838.4	2	6.97%	VSCADILALATR	89.5	47.1	42.4	0	2	0	0	2	1 289,6889	
	pectinacetyltransferase precursor [Vigna radiata var. radiata]	CAA67728.1	43 804.9	2	8.77%	VFAAVVDDLLAK	50.7	46.6	4.1	0	1	0	0	2	1 260,7204	
	pectinacetyltransferase precursor [Vigna radiata var. radiata]	CAA67728.1	43 804.9	2	8.77%	YCDGSSFTGDVEAVDPATNLHFR	78.7	44	34.7	0	0	1	0	2	2 558,1258	
	Peroxidase 45 precursor [Arabidopsis thaliana]	Q96522.1	35 811.1	1	8.00%	GLFTSDQILFTDQR	86.6	45.3	41.3	0	2	0	0	2	1 640,8287	
	Peroxidase 45 precursor [Arabidopsis thaliana]	Q96522.1	35 811.1	1	8.00%	VSCADILALATR	89.5	47.1	42.4	0	2	0	0	2	1 289,6889	
	acid alpha galactosidase 1 [Cucumis sativus]	ABC55266.1	45 680.3	1	1.94%	VAVVLLNR	56.2	45.6	10.6	0	1	0	0	2	883,5728	
	pectin acetyltransferase [Eucalyptus globulus subsp. globulus]	ABG34280.1	38 829.9	2	6.29%	AEENPDDFNWNR	81.6	45.8	35.8	0	1	0	0	2	1 538,6663	
	pectin acetyltransferase [Eucalyptus globulus subsp. globulus]	ABG34280.1	38 829.9	2	6.29%	AIDCPYPCDK	53.5	48.9	4.6	0	1	0	0	2	1 238,5189	
	Fructose-bisphosphate aldolase, chloroplast precursor [Oryza sativa (japonica cultivar-group)]	ABA91632.2	42 913.5	1	3.26%	LASIGLENTEANR	88.3	46	42.3	0	1	0	0	2	1 387,7181	
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	15.10%	IVFELFADTTTPR	90.4	46	44.4	0	1	0	0	2	1 408,7479	
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	15.10%	TSRPVIAIDCGQLS	40.7	46.1	-5.4	0	1	0	0	2	1 474,7326	
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	9.12%	FDGLVSR	50	47.7	2.3	0	2	0	0	2	793,4209	
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	9.12%	IAINMDPTTPR	89.1	46.5	42.6	0	3	0	0	2	1 244,6311	
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	9.12%	VSCADILALATR	89.5	47.1	42.4	0	2	0	0	2	1 289,6889	
	Polygalacturonase inhibitor 2 precursor [Phaseolus vulgaris]	P58822.1	37 086.3	1	4.09%	ISGAIPDSYGSFSK	62	45.8	16.2	0	1	0	0	2	1 428,7010	
	basic chitinase, chitinase [Arabidopsis thaliana]	NP_566426.1	36 196.3	2	7.46%	IGFYQR	31.7	45.3	-13.6	0	1	0	0	2	783,4154	
	basic chitinase, chitinase [Arabidopsis thaliana]	NP_566426.1	36 196.3	2	7.46%	LPGYGVITNINNGGLECGR	103	44.9	58.1	0	1	1	0	2	2 003,0385	
	cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	8.14%	VFFDMTIGGQPAGR	79.8	45.7	34.1	0	2	0	0	2	1 511,7319	
	endochitinase precursor [Humulus lupulus]	AAD34596.1	33 511.4	1	6.01%	GFYTYDAFLTAAR	71.5	46	25.5	0	1	0	0	2	1 495,7221	
	unnamed protein product [Populus trichocarpa]	ABK94155.1	77 435.5	1	1.28%	TCAQDEVLR	53.5	46.8	6.7	0	1	0	0	2	1 091,5157	
	unnamed protein product (putative Serine carboxypeptidase) [Vitis vinifera]	CAO68876.1	57 192.0	1	2.96%	NLEVGIPDLLEDGIK	58.1	45.3	12.8	0	2	0	0	2	1 624,8801	
	AWF _{NaCl} _P2	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	6.98%	IVFELFADTTTPR	90	46	44	0	1	0	0	2	1 408,7479
		peroxidase [Sesamum indicum]	ABB89209.1	35 838.4	2	9.09%	DHPDNLSLAGDGFDTVIK	56.9	45.3	11.6	0	0	1	0	2	1 913,9248
		peroxidase [Sesamum indicum]	ABB89209.1	35 838.4	2	9.09%	VSCADILALATR	93.1	47.1	46	0	1	0	0	2	1 289,6889
		Probable non-specific lipid-transfer protein AKCS9 precursor [Vigna unguiculata]	Q43681.1	10 431.0	2	24.20%	KVLSNCGVTYPNC	31.6	45.7	-14.1	0	1	0	0	2	1 511,6987
		Probable non-specific lipid-transfer protein AKCS9 precursor [Vigna unguiculata]	Q43681.1	10 431.0	2	24.20%	VQEPCLNYIK	53.9	47.5	6.4	0	1	0	0	2	1 423,6716
		peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	9.12%	FDGLVSR	47.7	47.7	0	0	1	0	0	2	793,4209
		peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	9.12%	IAINMDPTTPR	81.8	46.5	35.3	0	2	0	0	2	1 228,6362
		peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	9.12%	VSCADILALATR	93.1	47.1	46	0	1	0	0	2	1 289,6889
		unnamed protein product [Vitis vinifera]	CAO17011.1	42 507.9	3	6.11%	LFSFGNLR	34.6	47.3	-12.7	0	1	0	0	2	903,5053
		unnamed protein product [Vitis vinifera]	CAO17011.1	42 507.9	3	6.11%	TTYVLALK	33.9	46.4	-12.5	0	1	0	0	2	908,5457
		unnamed protein product [Vitis vinifera]	CAO17011.1	42 507.9	3	6.11%	VINNLDER	62.1	46.7	15.4	0	1	0	0	2	972,5114
		basic chitinase, chitinase [Arabidopsis thaliana]	NP_566426.1	36 196.3	1	5.67%	LPGYGVITNINNGGLECGR	114	44.9	69.1	0	1	0	0	2	2 003,0385
		cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	8.14%	VFFDMTIGGQPAGR	71	45.7	25.3	0	1	1	0	2	1 511,7319
		iron-superoxide dismutase precursor [Vigna unguiculata]	AAF28773.1	27 393.8	2	8.98%	LVSWDVSSR	73.1	46.5	26.6	0	1	0	0	2	1 119,5798
		iron-superoxide dismutase precursor [Vigna unguiculata]	AAF28773.1	27 393.8	2	8.98%	SLEEIVTAYNK	52.9	46.2	6.7	0	1	0	0	2	1 379,7421
		pectinacetyltransferase precursor [Vigna radiata var. radiata]	CAA67728.1	43 804.9	1	3.01%	VFAAVVDDLLAK	54.2	46.6	7.6	0	1	0	0	2	1 260,7204
		endochitinase precursor [Humulus lupulus]	AAD34596.1	33 511.4	1	4.11%	GFYTYDAFLTAAR	69.8	46	23.8	0	1	0	0	2	1 495,7221
		xyloglucan endotransglucosylase/hydrolase 2 [Cucumis melo]	ABI94062.1	36 316.1	1	3.88%	TDWSQAPFTASYR	79.9	45.8	34.1	0	1	0	0	2	1 529,7025
		Phosphoglucomutase, cytoplasmic [Pisum sativum]	Q9SM60.1	63 106.2	2	4.12%	LSGTGSEGATIR	112	46.5	65.5	0	1	0	0	2	1 148,5913
		Phosphoglucomutase, cytoplasmic [Pisum sativum]	Q9SM60.1	63 106.2	2	4.12%	YDENVDAGAAK	62.2	47.5	14.7	0	1	0	0	2	1 315,5807
		putative pectin methyltransferase 3 [Linum usitatissimum]	AAG17110.1	69 430.1	1	1.90%	DITFQNTAGPSK	66.1	46.6	19.5	0	1	0	0	2	1 278,6333
pectin acetyltransferase [Eucalyptus globulus subsp. globulus]		ABG34280.1	38 829.9	2	6.29%	AEENPDDFNWNR	81.8	45.8	36	0	1	0	0	2	1 538,6663	
pectin acetyltransferase [Eucalyptus globulus subsp. globulus]		ABG34280.1	38 829.9	2	6.29%	AIDCPYPCDK	48.5	46.5	2	0	1	0	0	2	1 238,5189	
AWF _{NaCl} _P3		Probable non-specific lipid-transfer protein AKCS9 precursor [Vigna unguiculata]	Q43681.1	10 431.0	2	22.20%	KVLSNCGVTYPNC	51.6	45.7	5.9	0	1	0	0	2	1 511,6987
		Probable non-specific lipid-transfer protein AKCS9 precursor [Vigna unguiculata]	Q43681.1	10 431.0	2	22.20%	QYVNSPGAK	33.6	47.7	-14.1	0	1	0	0	2	963,49
		pepti (ISS) (putative Cyclophilin) [Ostreococcus tauri]	CAL57205.1	38 854.4	1	3.27%	IVLGLFGDDAPR	57.7	46.3	11.4	0	2	0	0	2	1 272,6955
		unnamed protein product (putative Thaumatin family) [Vitis vinifera]	CAO62993.1	26 139.4	1	4.07%	GSDGSVIGCK	61.2	46.7	14.5	0	1	0	0	2	979,452
		CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	10.50%	IVFELFADTTTPR	90	46	44	0	2	0	0	2	1 408,7479
		CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	10.50%	TAENFR	40	47.9	-7.9	0	1	0	0	2	737,3582
		ubiquitin/ribosomal protein 27a [Prunus avium]	AAG13985.1	85 258.5	1	1.18%	TLADYNIQK	57.2	46.4	10.8	0	20	0	0	2	1 065,5582

	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	5.47%	FDGLVSR	49.4	47.7	1.7	0	1	0	0	2	793.4209
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	5.47%	IAINMDPTTPR	81.3	46.5	34.8	0	2	0	0	2	1 244.6311
	aldehyde reductase [Vigna radiata]	AAD53967.1	35 565.6	1	3.69%	DPQTELLDPAVK	81.5	47.6	33.9	0	1	0	0	2	1 325.6955
	putative glycine-rich RNA-binding protein [Dianthus caryophyllus]	BAF34340.1	16 872.1	1	5.68%	SITVNEAQR	53.9	46.4	7.5	0	1	0	0	2	1 104.5649
	cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	11.60%	VFFDMTIGGQPAGR	79.2	45.7	33.5	0	2	0	0	2	1 511.7319
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	1	4.85%	MGNISPLTGSSGEIR	75.2	45.8	29.4	0	1	0	0	2	1 534.7536
	unnamed protein product [Populus trichocarpa]	ABK93386.1	83 612.9	1	0.92%	HIDETLK	63	45.7	17.3	0	2	0	0	2	855.4578
AWF _{NaCl} _P4	Os09g0537600 [Oryza sativa (japonica cultivar-group)]	NP_001063792.1	23 389.0	1	5.58%	IVIGLYGDVVPK	53.5	46.3	7.2	0	1	0	0	2	1 272.7570
	oxidoreductase family protein [Arabidopsis thaliana]	NP_188715.2	38 560.4	1	3.94%	DVAVLEAMLESQAK	107	46.1	60.9	0	1	0	0	2	1 448.7308
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	10.50%	IVFELFADTTTPR	72.9	46	26.9	0	2	0	0	2	1 408.7479
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	2	10.50%	TAENFR	37.2	47.9	-10.7	0	1	0	0	2	737.3582
	Cysteine proteinase inhibitor [Vigna unguiculata]	Q06445.1	10 740.0	1	16.50%	DVAGNQSLEIDSLAR	63.4	45.2	18.2	0	1	0	0	2	1 701.8408
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	5.47%	FDGLVSR	33.4	47.7	-14.3	0	1	0	0	2	793.4209
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	2	5.47%	IAINMDPTTPR	65.2	46.5	18.7	0	1	0	0	2	1 244.6311
	putative glycine-rich RNA-binding protein [Dianthus caryophyllus]	BAF34340.1	16 872.1	1	5.68%	SITVNEAQR	56.6	46.4	10.2	0	1	0	0	2	1 104.5649
	cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	11.60%	VFFDMTIGGQPAGR	89.8	45.7	44.1	0	2	0	0	2	1 511.7319
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGASILR	49.8	47.7	2.1	0	2	0	0	2	763.4137
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	2	7.12%	MGNISPLTGSSGEIR	56.4	45.8	10.6	0	1	0	0	2	1 534.7536
	pterocarpan reductase [Lotus japonicus]	BAF34844.1	33 974.1	1	3.23%	AGHPTFALVR	59.6	46	13.6	0	0	1	0	2	1 068.5955
	unnamed protein product [Populus trichocarpa]	ABK94155.1	77 435.5	1	1.28%	TCAQDEVLR	65.5	46.8	18.7	0	1	0	0	2	1 091.5157
	Os01g0840100 [Oryza sativa (japonica cultivar-group)]	NP_001044757.1	71 715.0	1	1.99%	TTPSYVAFDTSER	67.2	45.9	21.3	0	2	0	0	2	1 473.6861
pterocarpan reductase [Lotus japonicus]	BAF34842.1	36 176.4	1	3.09%	VIIIGDGNPK	58.9	45.9	13	0	1	0	0	2	1 025.5998	
AWF _{NaCl} _P5	unnamed protein product (putative Thaumatococcus) [Vitis vinifera]	CAO62993.1	26 139.4	1	4.07%	GSDGSVIGCK	76.2	46.7	29.5	0	2	0	0	2	979.452
	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	76.6	46	30.6	0	2	0	0	2	1 408.7479
	peroxidase2 [Medicago sativa]	CAC38106.1	35 992.3	1	3.34%	IAINMDPTTPR	58.7	46.5	12.2	0	1	0	0	2	1 244.6311
	putative glycine-rich RNA-binding protein [Dianthus caryophyllus]	BAF34340.1	16 872.1	1	5.68%	SITVNEAQR	60.8	46.4	14.4	0	1	0	0	2	1 104.5649
	Protein P21 (putative Thaumatococcus) [Glycine max]	P25096.1	25 930.1	1	4.18%	TGCNFDGSGR	86	46	40	0	1	0	0	2	1 070.4326
	cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	8.14%	VFFDMTIGGQPAGR	90.1	45.7	44.4	0	2	0	0	2	1 511.7319
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	1	4.85%	MGNISPLTGSSGEIR	87.1	45.8	41.3	0	1	0	0	2	1 534.7536
	Os01g0840100 [Oryza sativa (japonica cultivar-group)]	NP_001044757.1	71 715.0	1	1.99%	TTPSYVAFDTSER	67.5	45.9	21.6	0	1	0	0	2	1 473.6861
AWF _{NaCl} _P6	CYP1 (putative cyclophilin_ABH_like) [Vigna radiata]	BAB82452.1	18 188.7	1	6.98%	IVFELFADTTTPR	76.5	46	30.5	0	1	0	0	2	1 408.7479
	putative glycine-rich RNA-binding protein [Dianthus caryophyllus]	BAF34340.1	16 872.1	1	5.68%	SITVNEAQR	73.2	46.4	26.8	0	1	0	0	2	1 104.5649
	cyclophilin [Phaseolus vulgaris]	CAA52414.1	18 141.5	1	8.14%	VFFDMTIGGQPAGR	56.4	45.7	10.7	0	1	0	0	2	1 511.7319
	peroxidase [Spinacia oleracea]	CAA71493.1	33 435.5	1	4.85%	MGNISPLTGSSGEIR	88.6	45.8	42.8	0	2	0	0	2	1 534.7536

^a AWF_{H2O} and AWF_{NaCl} describe the apoplast extraction solution from which the BN-PAGE followed by protein sequencing was performed, respectively.

Numbers P1 to P6 correspond to the numbers given in Fig. 3.

^b Identities are based on sequence comparisons using the NCBI green plants protein database

^c Accession numbers are based on sequence comparisons using the NCBI green plants protein database

^d Molecular weights (MW) are based on sequence comparisons using the NCBI green plants protein database

^e Protein sequence coverage obtained with the peptides identified by mass spectrometry