SUPPLEMENTARY MATERIAL

Focusing conditions and SDS-PAGE Analysis

In the focusing, voltages of 100 V, 250 V, and 1000 V were sequentially applied for 100 Vh, 250 Vh and 1000 Vh, respectively, and finally 4000 V up to 45 000 Vh, at 20°C. Before the SDS-PAGE procedure, the focused IPG strips were equilibrated, first in a DTT equilibration buffer [2% (w/v) DTT, 6 M urea, 2% (w/v) SDS, 0.05 M Tris.Cl pH 8.8, 20% (w/v) glycerol] for 15 min, then in an iodoacetamide equilibration buffer [2.5% (w/v) iodoacetamide, 6 M urea, 2% (w/v) SDS, 0.05 M Tris.Cl pH 8.8, 20% (w/v) glycerol] for 15 min. After equilibration, the IPG strips were embedded onto 12% acrylamide 1 mm SDS-PAGE second-dimension gels. SDS-PAGE was performed using a Mini-PROTEAN 3 Dodeca cell (Bio-Rad, USA) at the constant current of 15 mA per gel. The gels were then washed in the gel-fixing solution [10% (v/v) methanol, 7% (v/v) ace

tic acid] for 30 min.

MALDI-MS and MS/MS Analyses

MALDI-MS and MS/MS analyses were performed on an Ultraflex III mass spectrometer (Bruker Daltonik, Bremen, Germany). Peptide maps were acquired in reflectron positive mode (25 kV acceleration voltage) with 800 laser shots. Twelve dominant peaks within 700 – 3600 Da mass range and minimum S/N 10 were picked out for MS/MS analysis employing LID-LIFT arrangement with 600 laser shots for each peptide. CHCA solution prepared according to Havlis *et al.* 2003 was used as the matrix in combination with AnchorChip target to enhance measurement sensitivity. Sample (1 μl) was mixed with matrix solution on the target in a 2:1 ratio. Known autoproteolytic products of trypsin were used for

internal calibration of digested peptides. In the absence of these products, an external calibration procedure was employed, using a mixture of seven peptide standards (Bruker Daltonik) covering the mass range of 1000–3100 Da. The Flex Analysis 3.0 and MS Biotools 3.1 (Bruker Daltonik) software were used for data processing.

LC-MS/MS Analysis

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis was performed using EASY-nLC system (Proxeon) on-line coupled with an HCTultra PTM Discovery System ion trap mass spectrometer equipped with nanospray (Bruker Daltonik). The reverse- phase column and precolumn used for LC separation was filled according to a previously described procedure [Planeta *et al.* 2003]

Prior to LC separation, tryptic digests were concentrated and desalted using trapping column (100 µm × 30 mm) filled with 4-µm Jupiter Proteo sorbent (Phenomenex, Torrance, CA). Sample volume was 10 µl. After washing with 0.1% formic acid, the peptides were eluted from the trapping column using an acetonitrile/water gradient (350 nL/min) onto a fused-silica capillary column (100 µm x 100 mm), on which peptides were separated. The column was filled with 3.5-µm X-Bridge BEH 130 C18 sorbent (Waters). The mobile phase A consisted of 0.1% formic acid in water and the mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient elution started at 10 % of mobile phase B and increased linearly from 10 % to 30 % during the first 10 minutes. The gradient linearly increased to 90 % of mobile phase B in the next two minutes and remained at this state for next 8 minutes. The analytical column outlet was directly connected to the nanospray ion source. Nitrogen was used as nebulizing as well as drying gas. The pressure of nebulizing gas was 8 psi. The temperature and flow rate of drying gas were set to 250 ºC and 6 L/min, respectively. The capillary voltage was 4.0 kV.

The mass spectrometer was operated in the positive ion mode in a m/z range of 300 – 1500 for MS and 100-2500 for MS/MS mode. The DataAnalysis 4.0 and MS Biotools 3.1 (Bruker Daltonik) software were used for data processing.

Database Searching

MASCOT 2.2 (MatrixScience, London, UK) search engine was used for processing the MS and MS/MS data Database searches were done against the NCBI database (non redundant, all entries and restricted taxonomy to Viridiplantae; downloaded from ftp://ftp.ncbi.nih.gov/blast/db/FASTA/) and EST-Plants database (downloaded from ftp://ftp.ebi.ac.uk/pub/databases/fastafiles/emblrelease/). Databases were updated regularly. Mass tolerances of peptides and MS/MS fragments for MS/MS ion searches were 0.5 Da. Oxidation of methionine and carbamidomethylation of cysteine as optional modifications, one enzyme miscleavage and correction for one ^{13}C atom were set for all searches.

References:

J. Havlis, H. Thomas, M. Sebela, A. Shevchenko, Fast-response proteomics by accelerated in-gel digestion of proteins, Anal. Chem. 75 (2003) 1300–1306.

J. Planeta, P. Karasek, J. Vejrosta, Development of packed capillary columns using carbon dioxide slurries, J. Sep. Sci. 26 (2003) 525–530.

Effect of L80F/V84F Mutations on Cryptogein Biological Activity

Construction of L80F/V84F mutant

To introduce the mutation to the targeted codon site-directed mutagenesis was conducted as for other proteins by using a pair of specific forward-reverse oligonucleotides given in Table S2. Transformation of *Pichia pastoris* as well as expression and purification of protein was done as in other measured proteins.

Sterol-binding Activities and Affinities

The binding parameter of sterol to the protein (K_d) was calculated from the fluorescence data, and the number of binding sites was found to be approximately one per molecule. The determined value of K_d is consistent with the predicted effects of the individual mutations when no specific binding was determined.

The addition of L80F/V84F mutant stimulated a significantly reduced ability to transfer DHE between the membranes compared to wt cryptogein with rates of 0.33±0.01. On the other side this double mutant showed higher rates of PC transfer than did cryptogein with rates of 0.75±0.06 (Fig. S1). This behaviour is that found in V84F mutant.

Accumulation of Defence Gene Transcripts

By RT-qPCR assays the transcript levels for *PR2Q*, *PR3Q*, *PR5*, *GLN2*, *TuReP*, *NtPRp27* and *GeLiP* gene was quantified. The results are summarized in Table S3, showing similarity to that found in mutant V84F.

Table S1. Protein name, according to literature or NCBI database; **NCBI accession Nr**.- number of protein in NCBI database; **pI**- theoretical pI; **MW**- theoretical molecular weight; **Spot Nr** - number of spot on 2-D gel; **Score** - Mascot score; **%cov**.- percentage of coverage of the identified proteins; **In samples** - samples in which given protein was found

Protein name	NCBI accession Nr	MW [kDa]	pI	Spot Nr	MALDI-MS/MS		LC-MS/MS		In samples
					Score	% Cov	Score	% Cov	
Acidic chitinase PR-P	19771	27.5	4.9	22	660	50	164	15	WT, V84F
				24	277	12	136	5	L41F, V84F
Acidic chitinase PR-Q	19773	27.6	5.1	3	-	-	46	8	WT, V84F
				18	-	-	50	3	L41F
CBP20	632736	21.9	8.4	8	-	-	117	9	WT, L41F/V84F, V84F
				12	-	-	126	9	WT, V84F
				17	-	-	117	9	WT, L41F/V84F, V84F
Class IV chitinase	121663827	29.9	4.9	23	-	-	103	22	V84F
				5	-	-	51	7	WT, V84F
Chitinase/lysozyme	467689	42.0	9.1	12	-	-	116	3	WT, V84F
Cyclophilin-like protein	152206078	22.0	7.8	11	-	-	64	13	WT, V84F
Endochitinase A	116314	35.1	8.4	14	-	-	68	4	WT, V84F
				15	-	-	68	7	WT, V84F
Endochitinase B	116321	34.7	8.3	16	-	-	69	8	WT, V84F
Germin like protein	222051768	21.4	5.8	6	-	-	59	8	WT, L41F/V84F, V84F
Glucan endo-1,3-β-glucosidase	19859	37.8	5.2	4	176	14	259	24	WT, V84F
Glucan endo-1,3-β-glucosidase	19869	40.4	7.1	13	-	-	98	3	WT, V84F
				14	224	14	182	18	WT, V84F
				15	272	18	118	15	WT, V84F
Proteinase inhibitor I-A	547732	11.9	7.8	9	57	16	-	-	WT, V84F
Proteinase inhibitor I-B	547733	11.9	7.8	9	191	15	-	-	WT, V84F
Lignin-forming anionic peroxidase	129837	34.7	4.7	1	245	11	159	20	WT, V84F
				2	419	20	134	20	WT, V84F
				20	143	5	-	-	L41F/V84F
				21	266	11	148	10	L41F, L41F/V84F
NtPRp27	5360263	27.4	9.3	25	258	16	114	16	WT, V84F
PR-4A	19962	16.2	7.6	8	-	-	86	8	WT, L41F/V84F, V84F
PR-4B	100352	15.2	6.1	19	-	-	76	9	L41F, V84F
				21	-	-	125	9	L41F, L41F/V84F
Peptidyl-prolyl cis-trans isomerase, putative [*Ricinus communis*]	255547634	27.5	9.6	10	192	12	92	12	WT, V84F
				11	316	21	-	-	WT, V84F
Peroxidase	63002585	35.6	8.4	15	-	-	90	9	WT, V84F
Thaumatin-like protein E22	131015	24.7	5.4	22	-	-	144	33	WT, L41F, V84F
Tumor-related protein	1762933	23.4	8.5	7	178	8	-	-	WT, V84F

Table S2. Sequences of the oligonucleotides used for mutagenesis of cryptogein and qPCR primer sequences.

Gene	Primer sequence (5´ → 3´)
L41F	F*: ACGGCCAAGGCCTTCCCCACCACGGCG R*: CGCCGTGGTGGGGAAGGCCTTGGCCGT
V84F	F*: CGGCCTGGTACTCAACTTCTACTCGTACGCGAACG R*: CGTTCGCGTACGAGTAGAAGTTGAGTACCAGGCCG
L80F	F*: GTCCCCACGAGCGGCTTCGTACTCAACGTGTAC R*: GTACACGTTGAGTACGAAGCCGCTCGTGGGGAC
GeLiP	F*: GTCCAAGATTTCTGCGTCGC R*: TTTCCAGCTGCACTAAGCCC
GLN2	F*: TCTGTGTATGCTGCCCTCGAG R*: CCAGGCTTTCTTGGGCTACC
NtPRp27	F*: ATTGTACCACGAGAGCACCCA R*: GGTTTCACCCAGTGGCTAGGT
PR2Q	F*: TCCAGCAGATGTTGTGTCGCT R*: GGCTTGGCTAGCAGCAACATT
PR3Q	F*: TCTGGATCACCAATGGCATT R*: AGAAGCCATTGGCAGGACAT
PR5	F*: CCGAGGTAATTGTGAGACTGGAG R*: CCTGATTGGGTTGATTAAGTGCA
TuReP	F*: TCACCTGCGAACCCTAACGA R*: CACGCCCTGGATTTCCTTCT
EF-1α	F*: TGTGATGTTTTTGTTCGGTCTTTAA R*: TCAAAAGAAAATGCAGACAGACTCA

* F is the forward primer and R is the reverse primer, respectively.

Table S3. *Accumulation of defence-related genes.* Effect of L80F/V84F mutant on accumulation of transcripts for PR and other defence related proteins. Gene expression relative to a control was calculated by the ΔΔC(t) method. The values given in the table are the logarithm of the relative increase (logR) and its standard deviation (SD). More than a two-fold increase in gene expression was taken as significant. A.N. = accession number of gene in NCBI database.

Gene	**A.N.**	logR	SD
PR2Q β-1,3-glucanase	X54456	1.41	0.05
GLN2 β-1,3-glucanase	X53600	0.98	0.05
PR3Q Chitinase	X51425	1.31	0.07
PR5 Thaumatin-like protein	X12739	1.23	0.05
TuReP Tumor-related protein	FG644925	0.80	0.05
NtPRp27	FG633857	1.04	0.06
GeLiP Germin-like protein	AB449366	0.80	0.06

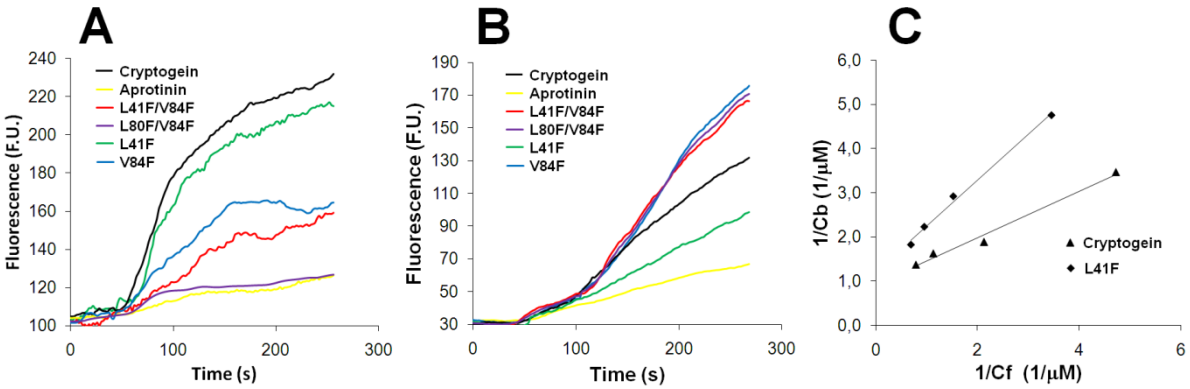

Fig. S1. *Transfer of sterols and fatty acids catalyzed by proteins and binding plots*

A. Transfer of sterols between DHE and stigmasterol micelles catalyzed by cryptogein, L41F, L41F/V84F, V84F, L80F/V84F mutants and aprotinin, measured by changes in DHE fluorescence. Donor micelles contained DHE (0.63 µM), acceptor micelles contained stigmasterol (3 µM) in MES buffer. The transfer was induced by adding elicitins or aprotinin (0.5 µM). B. Transfer of fatty acids between NBD-PC vesicles and PC vesicles catalyzed by cryptogein, L41F, L41F/V84F, V84F, L80F/V84F mutants and aprotinin monitored by measuring changes in NBD-PC fluorescence. Donor vesicles contained NBD-PC (0.63 µM), while acceptor vesicles contained PC (3 µM), in MES buffer. The transfer was induced by adding elicitins or aprotinin (0.5 µM). ([a] addition of donor vesicles, [b] addition of elicitins). C. Binding plots of the titration of cryptogein and L41F mutant by DHE. C_b and C_f are the concentrations of bound and free DHE or NBD-PC, respectively. Experiments were performed with 1.0 µM proteins.

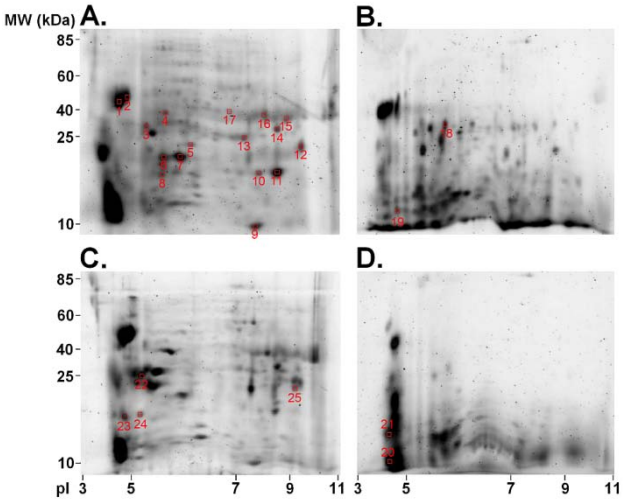

Fig. S2. *Two-dimensional gel electrophoresis proteome maps of intercellular fluid determined 48 hours after application of cryptogein and its mutants.* Identified proteins with qualitative changes between individual samples are indicated. For the first dimension of separation, 80 μg of protein was applied to each IPG strip (7 cm, pH 3–10 NL). For separation in the second dimension, 12% SDS–PAGE was carried out. Proteins were visualized by SYPRO Ruby staining. Isoelectric points (pI) and molecular weights (MW, kDa) are marked. (A) wt cryptogein (B) L41F (C) V84F (D) L41F/V84F