**Figure S1.** Mass spectra of Lys-C peptides originated from **(a)** non-treated and **(b)** *Nt*Phyt-treated CRT3 bands (arrows in Figure 1A); **(c)** non-treated and **(d)** *Nt*Phyt-treated CRT3 D420E bands (lanes 3 and 4 in Figure 2A). Positions of the characteristic C-terminal semi-LysC peptides and of the N-terminal peptides are marked in red.

**Figure S2.** MS/MS fragmentation spectra of the characteristic C-terminal semi-LysC peptides of CRT3 (**(a)** non-treated, **(b)** *Nt*Phyt-treated) and CRT3 D420E mutant (**(c)** non-treated, **(d)** *Nt*Phyt-treated) marked in Figure S1.

**Figure S3.** Sequence coverage of the apoplastic 26 kDa CRT3 fragment originated from EGFP\_CRT3\_C6 protein with mass spectrometry-identified peptides.

Sequences of tryptic (blue upper bracket) and Glu-C (red bottom bracket) peptides for which identity was confirmed by MS/MS fragmentation are shown. Boundaries of the N-, P-, and C-terminal domains of *Nt*CRT3 are indicated below the amino acid sequence.



**Table S1.** List of the primers used in this study.

|  |  |  |
| --- | --- | --- |
| # | Primer name | Sequence |
| 1 | CRT3\_Pst700\_dir  | AAGCCTGCAGACTGGGAAGAC |
| 2 | CRT3\_D420E\_Sac\_rev  | CGGAGCTCTTAAAGTTCATCATGGTAGTCTTCCATATAATC |
| 3 | CRT\_Kpn\_Nco\_dir | CAAGGTACCATGGCTCTCTCTGAGCATAAAC |
| 4 | CRT\_SP\_Sal\_rev | GTGGTCGACGAAAATCTCAGATGCTGAAG |
| 5 | CRT\_LF\_Apa\_Nde\_dir | GAGGGCCCGCATCTCATATGTCTGAGATTTTCTTTGAAGAAAG |
| 6 | CRT\_1-420\_Sac\_rev | CGGAGCTCTTAATCCATATAATCGTGGTGATAGC |
| 7 | EGFP\_Sal\_dir | GAGGTCGACAAATCTGAGATGGTGAGCAAGGGCGAG |
| 8 | EGFP\_Apa\_rev | GTGGGCCCACCTGGAGACTTGTACAGCTCGTCCATGC |
| 9 | pLH\_seq\_dir | GACCTCGAGAATTCTCAAC |
| 10 | NtPhyt-His\_rev | GGGAGCTCTTAATGGTGATGATGATGGTGCAGAGGATCCACTCCTATG |