The tomato xylem sap protein XSP10 is required for full susceptibility to Fusarium wilt disease

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Supplementary material

Figure S1. Nucleotide sequence of the *XSP10* gene and its 5'- and 3'-flanking regions, and the deduced amino acid sequence of the XSP10. Secretion signal peptide is highlighted in bold. Intron sequence is underlined.

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ATG AAC TAC TTG TTG TGT GTT GTA GGA TTT GTT GTG CTA CTG GCC ATT GCT GGA GCT GAT GGA GCC GGT GAA
M   N   Y   L   L   C   V   V   G   F   V   V   L   L   A   I   A   G   A   D   G   A   G   E

TGC GGG AGA AAT TCT CCA GAT ATG GAA GCC ATG AAA TTG ATT CCT TGC GCA AAA GCA GCA TCG GAT GAG AAT
C   G   R   N   S   P   D   M   E   A   M   K   L   I   P   C   A   K   A   A   S   D   E   N

GCA TCC GTA TCA AGG AGT TGT TGT TTA CAA ATA CAG AAA TTG GGA CAC AAC CCG AAA TGT CTC TGT GCT GTT
A   S   V   S   R   S   C   C   L   Q   I   Q   K   L   G   H   N   P   K   C   L   C   A   V

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                                                    Y   T   L   P   *

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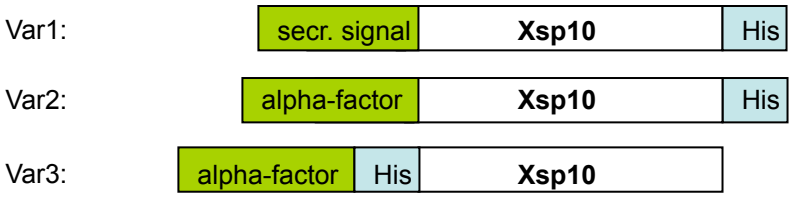
A



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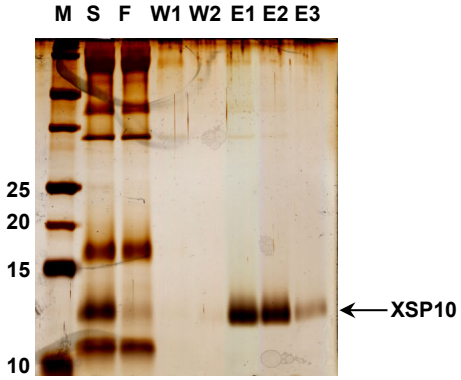

Figure S2. Recombinant XSP10 affinity purification. Recombinant variants of XSP10 used in this study. (**B**) SDS-PAGE showing affinity purification of the recombinant XSP10 (Var2). M – protein standards, S – sample (proteins precipitated from *P. pastoris* culture supernatant), F – flow through, W – wash, E – elution fractions 1-3.

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                                       *       Δ            Δ
A    Native:  1    mnyllcvvgfvvllaiagadgAGECGRNSPDMEAMKLIPCAKAASDENAS  50
                                         T1      T2            T3

                      Δ          Δ       Δ                 Δ                  ΔΔ
              51   VSRSCCLQIQKLGHNPKCLCAVMLSNTAKSSGANPEVAITIPKRCNLANR  100
                           T4       T5        T6                 T7       T8    T9

                        Δ
              101  PVGYKCGPYTLP                                        112
                           T10

     Var3:    1    mrfpsiftavlfaassalaapvntttedetaqipaeavigysdlegdfdv  50

                                                              *
              51   avlpfsnstnngllfinttiasiiaakeegvslekreaeaYVEFHHHHHH  100

                         Δ           Δ                      Δ          Δ        Δ
              101  AGECGRNSPDMEAMKLIPCAEAASDENASVSRSCCLQIQKLGHNPKCLCA  150
                                         T3               T4

                           Δ                  ΔΔ                Δ
              151  VMLSNTAKSSGANPEVAITIPKRCNLANRPVGYKCGPYTLP           191
                      T6              T7               T9
```

B

	Predicted* Mw	Observed holomass A (+n)**	Observed holomass B (+n)
Native	9567.0	n.m.	n.m.
Var1	10389.9	10553.0 (+1)	10877.0 (+3)
Var2	10928.5	10919.9	11244.3 (+2)
Var3	10928.5	10920.3	11082.5 (+1)

* Mw should be considered 8 Da smaller for each variant due to the reduction of four Cys-bonds

** Number in brackets represents number of attached hexose residues (Mw=162.2)

C

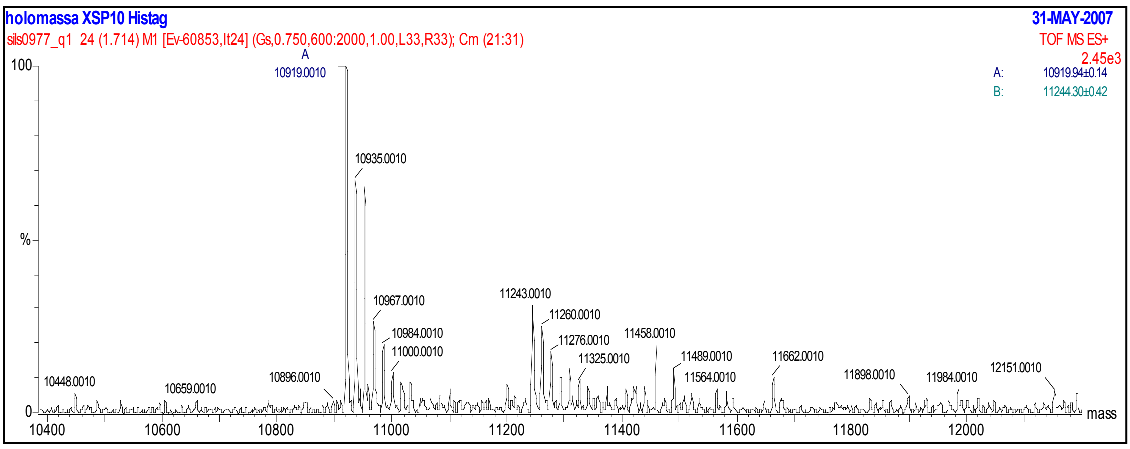

Figure S3. MS analysis of recombinant XSP10. (**A**) Amino acid sequence of native and recombinant XSP10 fused with yeast alpha-factor (Var3). Predicted tryptic peptides are numbered (T1-T10), Δ indicates their carboxyl-terminal amino acids; * corresponds to the presumed cleavage site of the signal peptide (lower case). Peptides in Var3 XSP10 that were sequenced with tandem MS are indicated (T3, T4, T6, T7, T9). (**B**) Holomass analysis of the variants of XSP10 used in this study. (**C**) Example of holomass spectra for the Var2 XSP10 after MaxEnt1 processing.

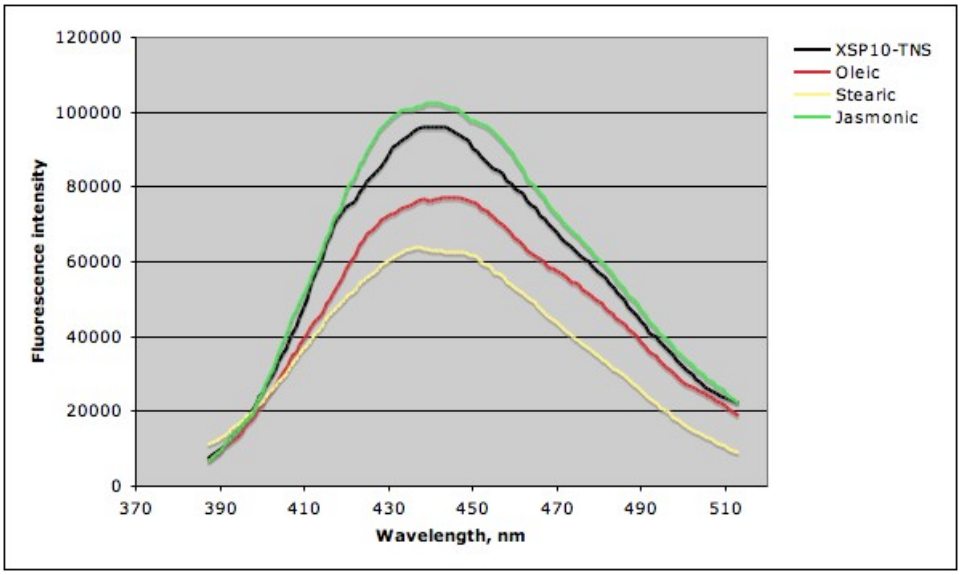

Figure S4. Displacement of the TNS from XSP10 by stearic, oleic, and jasmonic acids. Fluorescence emission spectras are shown for the XSP10 (100 nM) with TNS (5 μM) and after addition of FAs or JA (10 μM).

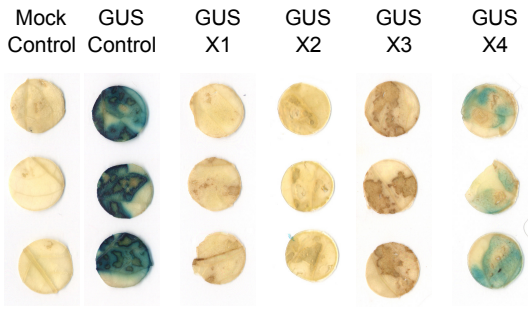

Figure S5. Transient *GUS* expression is silenced in T1 progeny of hpXSP10 lines. Young tomato leaves were infiltrated with *A. tumefaciens* strain C58C1 harboring plasmid pTFS40, resulting in transient *GUS* expression from the CaMV 35S promoter. Two days after infiltration leaf disks were punched and stained for GUS activity. *GUS* expression appeared absent or strongly reduced in hpXSP10 lines as compared to the GCR161 control. A partially silenced hpXSP10 line (X4) is shown for comparison.

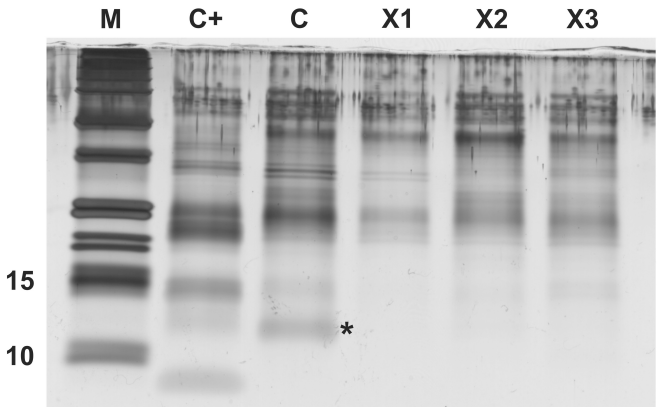

Figure S6. XSP10 abundance is reduced in hpXSP plants.
Xylem sap of 8 week-old mock-inoculated control (**C**), *hpXSP10* plants (X1, X2 and X3) and Fol007-infected control plants (C+) was concentrated 20-fold by acetone precipitation and loaded on 15% Tris-Tricine SDS-PAGE. Loading was normalised on the amount of protein.
M: protein standards, * marks XSP10 protein.

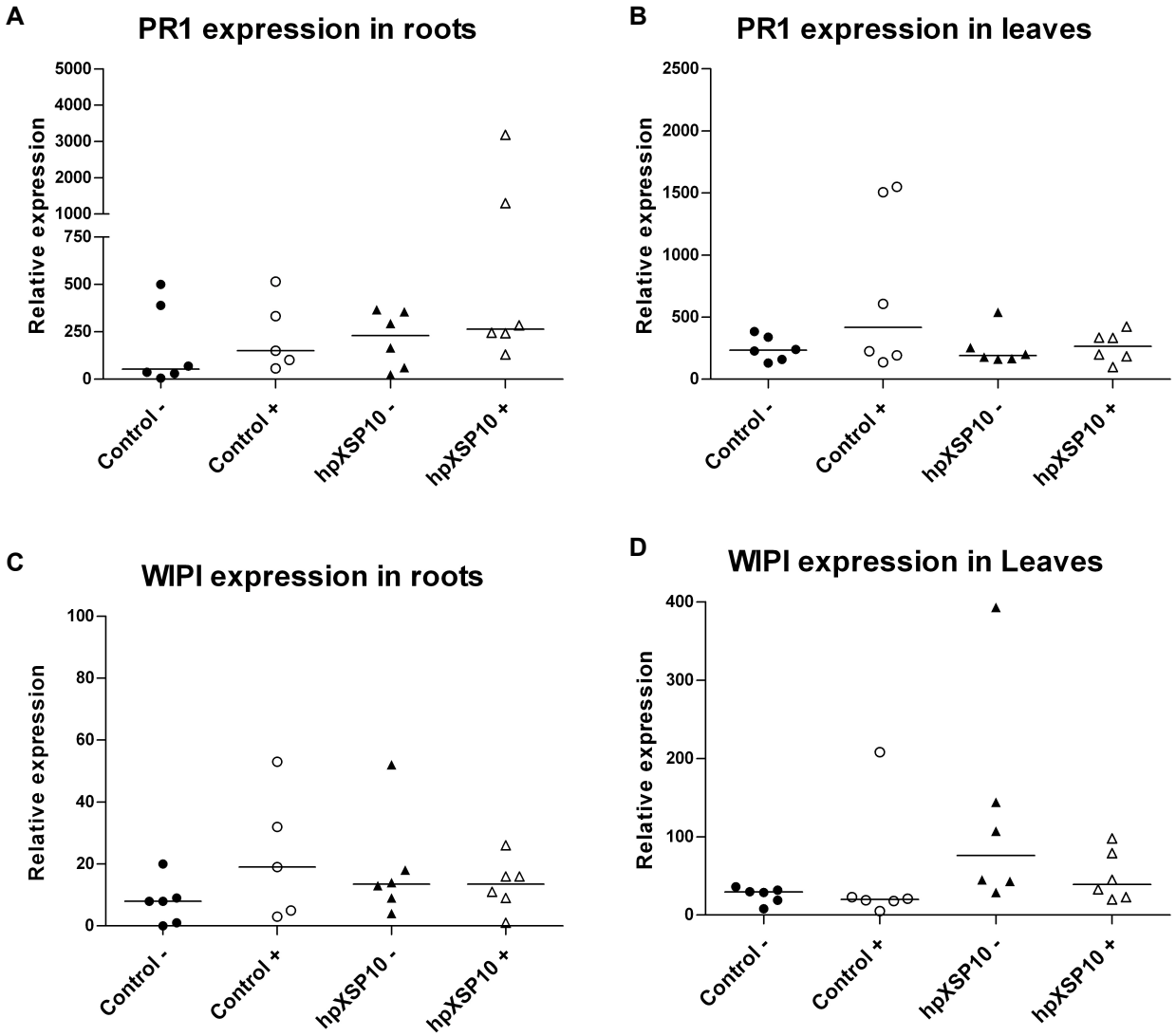

Figure S7. *PR1 and WIPI* transcription levels are not significantly altered in roots and leaves of hpXSP plants upon Fol007-infection.

Roots and leaves were collected three weeks after mock or Fol007-inoculation of ten-days-old seedlings**.** Transcript levels were determined by real-time qPCR relative to *alpha-tubulin* in roots and *RCE* in leaves of mock-inoculated (-) or Fol007 infected (+) control and hpXSP plants. Median and individual expression of 6 plants per condition are shown.