Supplemental Information

Wild type

MENTSPEKLDRKTQEKNRRIQMKYLSSKLFSLIPPHHHQYSAKDMVTQQDQIDQAITYIEKLKERVDVLMRRKDKII
AQGTSDDSKKFMPSTSCSNIKLPMIEVRELGSTIEVILVSCLQKKFTMQEVIIILEEEGVQVVTANFSTIGDKVYYTIHA
QVKITRLGVDASRVYLRLQNLIC*

Δnrd1-1

MENTSPEKRQENTRKEQKNSNEVSFF*ALFSYSSTPPPILS*GYGDATRPN*SSHYLH*KIERKSRCINEKEG*DYSTR
YK**FKEIHAFNIL*QY*ITYD*S*RVGFNYRGYFS*LLAKKVHHARGDNHLRGRRSSSCYR*FFNNRR*GLLYYSCSG
ENYEIRG*CIKGLFEIAKSDLL

Δnrd1-2

MENTRTGKHKKRTEEFK*SIFLLSSFLLFLHTTTNTQLRIW*RNKTKLIKPLLTLKN*KKE*MY**EGRIRL*HKVQVM
IQRNSCLQHLVAILNYL*LKLESWVQL*RLF*LVACKKSSPCKR**SS*RKKEFKLLPLIFQQSAIRFTILFMLR*KLRD*
GLMHQGSI*DCKI*FA

Supplemental Figure S1. The wild-type and mutated Nrd1 protein sequences. Mutations in the Δnrd1-1 and Δnrd1-2 lines introduce a premature stop codon at the 27th or 18th amino acid of the Nrd1 protein, respectively. Amino acids in red indicate open reading frames. The asterisks represent stop codons. In the wild-type sequence, the amino acids highlighted in yellow constitute the bHLH domain. The E (glutamic acid) and R (arginine) in blue are known to be required for binding of the E-box motif in the promoter. Based on the above, even if a truncated protein is produced in the Δnrd1-1 and Δnrd1-2 lines it would lack the E-box motif and therefore be non-functional.

A

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Nrd1           MEN---------TSPEKLDRKTQEKNRRIQMKYLSSKLFSLIPPHHHQYSAKDMVTQQDQ
Solyc03g114237 MESS---NINIVTASEKIERKTQEKIRRIQMKYLTSKLFSLIPPHHHQ-STKEVLTKQDK
Solyc03g114233 MENNSVNNIVSTSSVQKLDRKTQEKNRRIQMKYLYAKLFSLIPLNHS----KEVLTQHDH
               **:    ::   :: :*::******* ******** :******* :*     *:::*::*:

Nrd1           IDQAITYIEKLKERVDVLMRRKDKIIAQGTSDDSKKFMPSTSCSNIKLPMIEVRELGSTI
Solyc03g114237 IDAAITYIKQLKERIEVLERRKEEVVAQETCDDSKKSMPTTTCS-IKSPMVEVKELDSTL
Solyc03g114233 VDQATTFIEELKERVEVLKRRKDEVVAQIIGDDSKKSISTTTCT-IK-----VRELDSTL
               :* * *:*::*****::** ********   ***** : :*:*: **     *:**.**:

Nrd1           EVILVSCLQKKFTMQEVIIILEEEGVQVVTANFSTIGDKVYYTIHAQVKITRLGVDASRV
Solyc03g114237 EVILVSGLQKNFILQEVIKIIEQEGAQVVSANYSTIDDTIYYMIHAQVKIARLGIDASRV
Solyc03g114233 EVILTSGLQKNFTLQEVIKIIEEEGAQVVTANYSTIDGTIYYTIHAQVKITRLGIDASRI
               ****.* ***:* :**** *:*:**.***:**:***...:** *******:***:****:

Nrd1           YLRLQNLIC
Solyc03g114237 HLRLQKMVC
Solyc03g114233 HFRLQKLVS
               ::******::.
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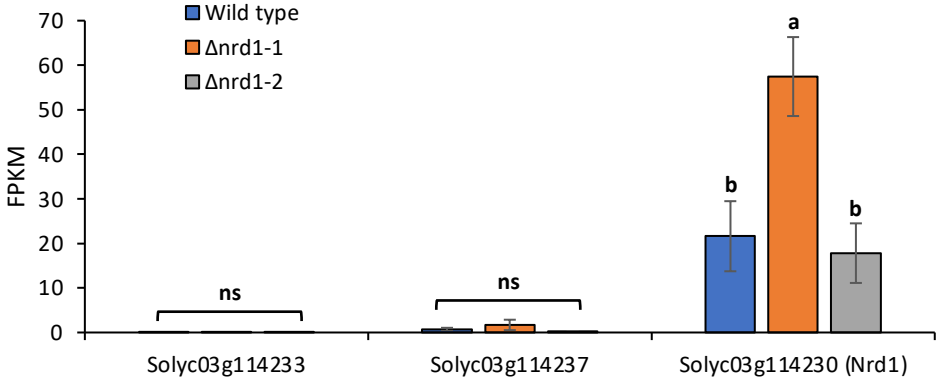
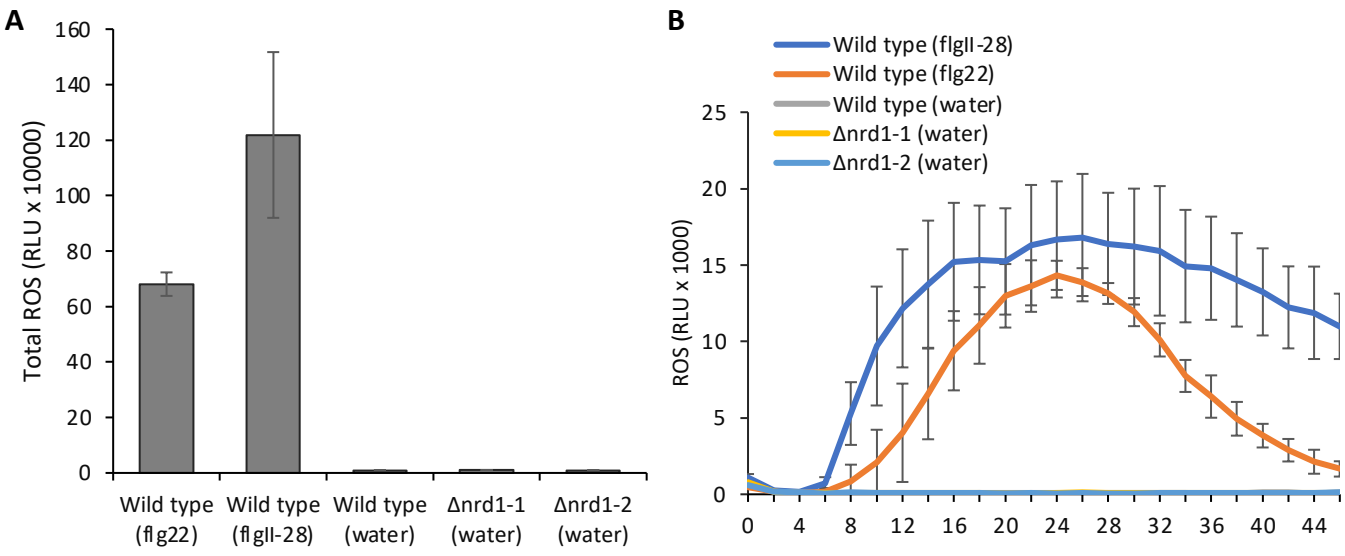
B



Supplemental Figure S2. Analysis of the two most closely related genes to Nrd1 in tomato. A, Amino acid sequence alignment of the two closest tomato Nrd1 homologs. Solyc03g114233 and Solyc03g114237 are 60.3% and 65.0% similarity to the Nrd1 protein sequence, respectively. **B**, Transcript abundance of the two closest tomato Nrd1 homologs in wildtype and Δnrd1 mutants, 6 h after treatment with 5 x 10^6 cfu/mL DC3000Δ*avrPto*Δ*avrPtoB* (DC3000ΔΔ). FPKM: fragments per kilobase of transcript per million mapped fragments. Four plants for each genotype were used. Bars show means ± standard deviation (SD). Different letters indicate significant differences based on a one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). ns, no significant difference.

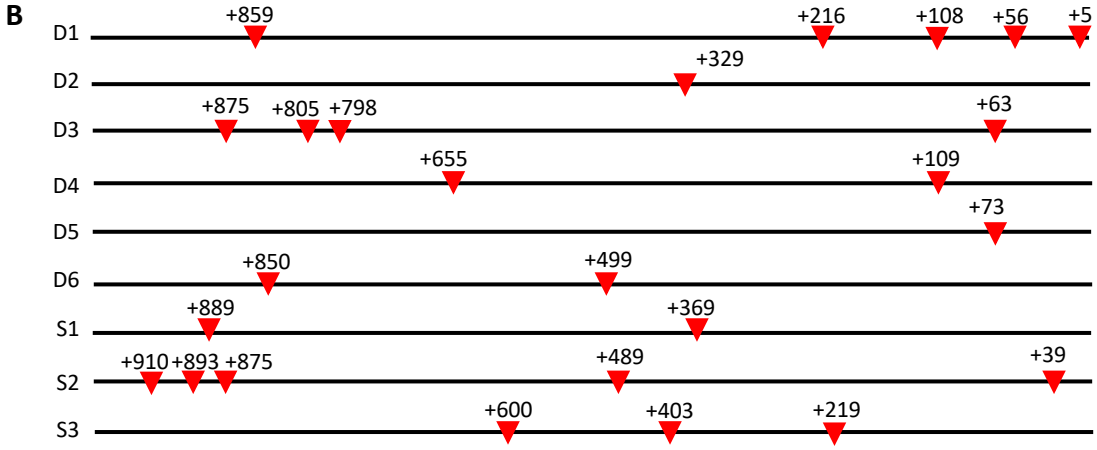


Supplemental Figure S3. The Δnrd1 mutants do not constitutively produce reactive oxygen species (ROS). RG-PtoR wild-type plants were treated with 50 nM flg22 or 50 nM flgII-28 as a positive control. Leaf discs from wild type or Δnrd1 mutants were treated with water only. Relative light units (RLU) were measured over 45 minutes. Three plants (three leaf discs per plant) were tested per treatment. Bars represent means ± standard deviation (SD) in (**A**) and (**B**). The experiment was performed twice with similar results.

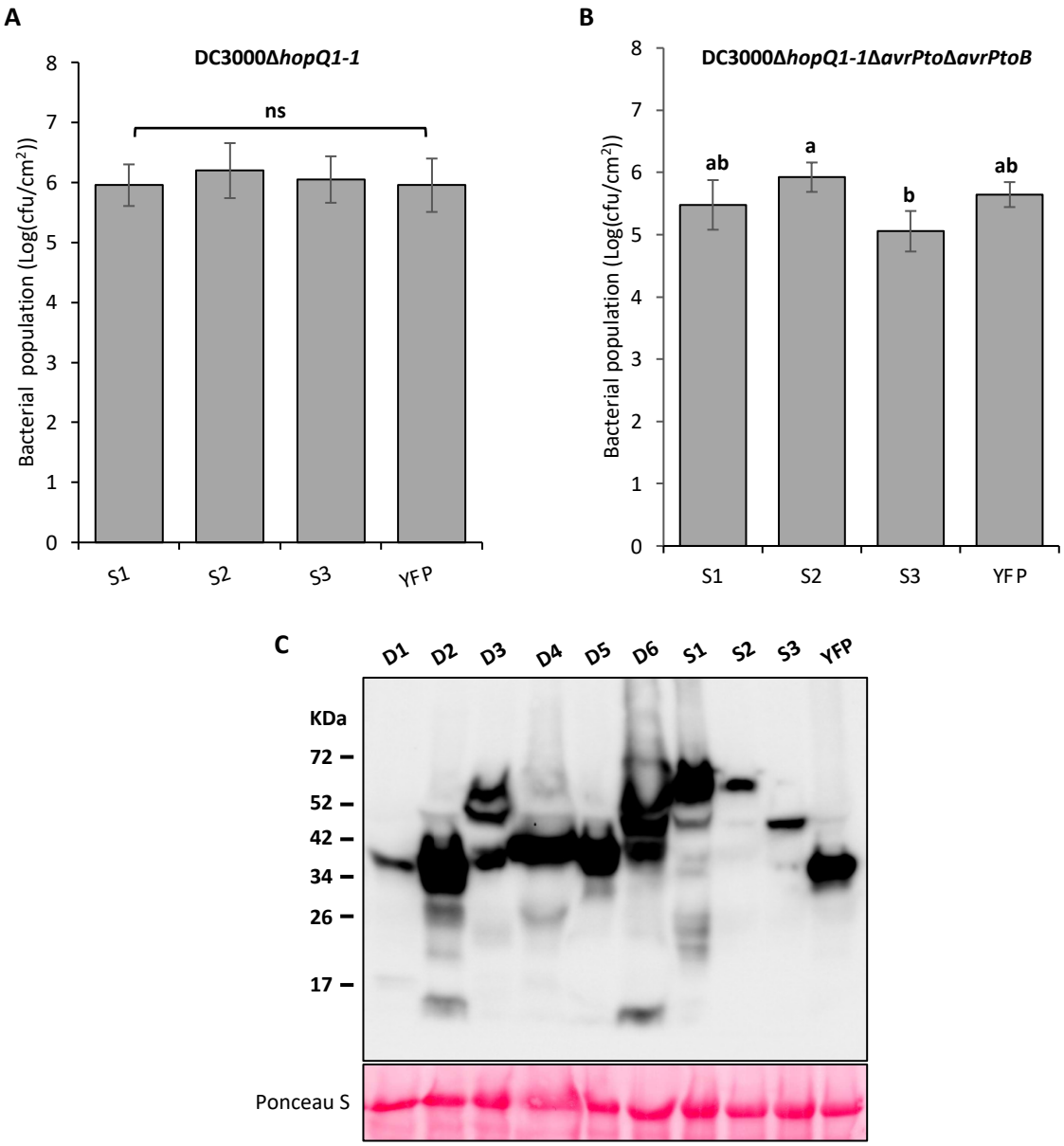
A

Gene name	Position of the predicted element	Predicted E-box sequence
D1	+859	CATTTG
	+216	CATATG
	+108	CAATTG
	+56	CACGTG
	+5	CAACTG
D2	+329	CACTTG
D3	+875	CAGCTG
	+805	CACATG
	+798	CAATTG
	+63	CATATG
D4	+655	CAAGTG
	+109	CACGTG
D5	+73	CACCTG
D6	+850	CATATG
	+499	CAATTG
S1	+889	CATTTG
	+369	CATATG
S2	+910	CAAATG
	+893	CACTTG
	+875	CAAATG
	+489	CAGTTG
	+39	CATATG
S3	+600	CAAGTG
	+403	CAAATG
	+219	CATGTG

B



Supplemental Figure S4. Predicted E-box elements (CANNTG) in Nrd1-regulated putative defense and susceptibility genes. A-**B**, 1 kb DNA sequence upstream of the 5' untranslated region (5'UTR) or coding region (CDS) of each gene was analyzed by PlantPan2.0 (Chow et al., 2016) to identify potential bHLH-binding elements, denoted with inverted red triangles. The first nucleotide of the promoter sequence upstream of 5'UTR or CDS is designated as "+1".



Supplemental Figure S5. Transient overexpression of putative susceptibility genes proteins in *Nicotiana benthamiana* leaves followed by a bacterial pathogenicity assay and confirming expression of the D and S proteins. A-B, Five-week-old *Nicotiana benthamiana* plants were syringe-infiltrated with *Agrobacterium* strains (1D1249) containing a binary expression vector expressing each gene (OD = 0.5). Two days later, the same agro-infiltrated spots were syringe-infiltrated with 5 x 10^4 cfu/mL DC3000*ΔhopQ1-1* (**A**) or 5 x 10^4 cfu/mL DC3000*ΔhopQ1-1ΔavrPtoΔavrPtoB* (**B**). Bacterial populations were measured two days after infiltration. cfu, colony-forming unit. **A-B**, Three plants were tested with each gene in each experiment. Bars represent means ± SD. Different letters indicate significant differences based on a one-way ANOVA followed by Student's *t* test ($p < 0.05$). ns, no significant difference. **C**, Protein expression by western blotting. Proteins were extracted from *N. benthamiana* leaves two days after agroinfiltration. S proteins with an HA epitope tag were detected by immunoblotting with α-HA antibody.

Agp1 protein sequence:

MALSHPMTIFSLFLTFLALTAAQSPMMAPTMPPSTMSMPPTTSTTTPPPMSSMSPPPSAMSPTPSTMSP
PPMSPMTPSMSPMGPMTPTMSPMDSPPAPAGPGMAPGMSTPGPAPGPMGGESMASPPPSSGFVHG
ISISMAMVAIIGSVALFF

Position	Score	Comment
4	0.006	
8	0.018	
11	0.037	
15	0.017	
20	0.041	
24	0.130	
30	0.713	Positive
34	0.767	Positive
35	0.795	Positive
37	0.887	Positive
41	0.920	Positive
42	0.768	Positive
43	0.930	Positive
44	0.885	Positive
45	0.895	Positive
46	0.882	Positive
51	0.827	Positive
52	0.890	Positive
54	0.826	Positive
58	0.872	Positive
61	0.749	Positive
63	0.652	Positive
65	0.910	Positive
66	0.776	Positive
68	0.882	Positive
73	0.628	Positive
76	0.522	Positive
78	0.874	Positive
80	0.721	Positive
86	0.465	
88	0.680	Positive
90	0.637	Positive
94	0.738	Positive
108	0.876	Positive
109	0.710	Positive
121	0.428	
124	0.312	
128	0.073	
129	0.014	
136	0.011	
138	0.007	
147	4.1E-06	

Supplemental Figure S6. Prediction of glycosylation sites in the Agp1 protein. Glycosylation sites were predicted using NetOGlyc-4.0 (Steentoft et al., 2013) with a cutoff score higher than 0.5 (marked as “Positive”). The first amino acid of Agp1 protein is designated as position “1”. Positive sites of glycosylated amino acids are highlighted in red and underlined.

Supplemental Table S1. Summary of disease assays with the Δnrd1 mutant plants.

Bacterial strains	**Inoculum (cfu/mL)[1]**	**Disease symptom (compared to wild type)**	**Bacterial growth (compared to wild type)**
DC3000	$5x10^6$	ns[2]	ns
DC3000ΔavrPtoΔavrPtoB	$5x10^4$	More resistant	Less bacterial growth
NYS-T1	$1x10^4$	ns	ns
T1	$2x10^4$	ns	ns
Pst14ΔavrPto	$2x10^4$	ns	ns
Pst25ΔavrPto	$2x10^4$	ns	ns
Xanthomonas campestris pv. *vesicatoria*	$5x10^4$	ns	ns

[1]cfu, colony-forming unit
[2]ns, no significant difference

Supplemental Table S2. The 51 *Nrd1*-regulated putative defense and susceptibility genes identified by RNA-Seq.

Gene ID	RNA-Seq data from this study						RNA-Seq data from Rosli et al.[1]
	FPKM in Δnrd1-1	Δnrd1-1 /wld type	Adjusted P	FPKM in Δnrd1-2	Δnrd1-2 /wild type	Adjusted P	
Solyc05g024190 (D1)[2]	**3.7**	**2.8**	**0.0013**	**5.1**	**3.9**	**0.0079**	**flgII-28 suppressed**
Solyc07g061790 (D2)	**216.42**	**4.4**	**2.45E-05**	**324.36**	**6.5**	**2.66E-08**	**flgII-28 and csp22 suppressed/ ETI suppressed**
Solyc02g077330 (D3)	**2.05**	**2.7**	**0.01**	**2.64**	**3.5**	**0.011**	**flgII-28/flg22/csp22 suppressed/ ETI suppressed**
Solyc12g009650 (D4)	**1544.34**	**2.2**	**6.79E-13**	**1630.19**	**2.3**	**2.97E-05**	**flgII-28 and csp22 suppressed/ ETI-suppressed**
Solyc11g019910 (D5)	**56.23**	**2.2**	**0.005**	**68**	**2.6**	**5.81E-05**	**flgII-28 and csp22 suppressed/ ETI-induced**
Solyc08g078020 (D6)	**696.82**	**3.2**	**3.81E-11**	**800.1**	**3.6**	**3.40E-09**	**flgII-28 and csp22 suppressed/ ETI suppressed**
Solyc11g021060	917.98	4.4	1.01E-06	985.23	4.7	2.45E-09	csp22 suppressed/ETI suppressed
Solyc12g011010	2.29	3.0	0.033	3.1	4.0	0.0066	ETI suppressed
Solyc01g104720	502.78	2.2	0.0015	762.9	3.4	8.57E-12	csp22 suppressed/ETI suppressed
Solyc03g019890	26.54	2.2	1.61E-14	32	2.6	1.20E-18	csp22 suppressed/ETI suppressed
Solyc06g160050	732.67	39.7	8.94E-10	1134.4	61.5	3.29E-08	No record
Solyc05g021245	16.12	2.6	0.004	97	15.8	1.57E-21	No record
Solyc02g161340	10.46	5.0	4.87E-15	10.62	5.1	6.12E-14	No record
Solyc04g009850	17.36	4.0	1.23E-07	12.97	3.0	4.40E-05	flgII-28 and csp22 induced/ ETI-induced
Solyc12g017800	4.08	408.0	1.81E-32	4.12	412.0	1.07E-30	Not expressed
Solyc05g024230	7.65	NA*	1.80E-11	9.74	NA	1.68E-13	Induced in hpBti9 and one hpPti line
Solyc01g066790	103.32	430.5	6.94E-40	42.82	178.4	1.20E-18	Induced in one hpPti line
Solyc05g055460	1.38	46.0	0.0003	2.05	68.3	0.00015	Not induced
Solyc07g045127	0.64	64.0	0.0005	0.55	55.0	0.00073	No record
Solyc09g082340	0.51	51.0	0.0002	0.57	57.0	0.00099	Not induced
Solyc08g150133	0.13	13.0	0.016	0.24	24.0	0.00292	No record
Solyc07g026710	4.67	9.7	0.005	10.61	22.1	1.05E-13	Not induced
Solyc03g096430	23.19	105.4	3.31E-12	4.99	22.7	5.85E-08	Not induced
Solyc12g036740	1.44	12.0	0.022	2.62	21.8	0.00418	Not induced
Solyc07g055490	0.56	18.7	8.11E-06	0.45	15.0	0.00468	Not induced
Solyc12g087830	25.9	4.6	0.024	70.28	12.5	7.02E-23	Not clear
Solyc07g056704	0.54	6.8	0.0002	1.01	12.6	2.50E-08	No record
Solyc03g121900	11.03	6.9	5.33E-12	18.23	11.5	6.67E-16	Induced in one hpPti line

Continued

[1] Rosli et al., (2013). Genome Biology 14:R139

[2] Shown in bold are the genes selected for further functional characterization based on criteria discussed in the Results.

Supplemental Table S2 (continued). The 51 *Nrd1*-regulated putative defense and susceptibility genes identified by RNA-Seq.

Gene ID	RNA-seq in this study						Rosli's RNA-seq data
	FPKM in Δnrd1-1	Δnrd1-1 /wild type	Adjusted P	FPKM in Δnrd1-2	Δnrd1-2 /wild type	Adjusted P	
Solyc12g062200	18.92	7.2	0.0095	27.17	10.4	0.0004	Not clear
Solyc03g096420	4.19	17.5	5.19E-05	2.14	8.9	0.0015	Not induced
Solyc07g056708	0.31	7.8	0.037	0.36	9.0	0.0161	No record
Solyc00g011660	0.48	4.4	0.004	0.85	7.7	7.83E-05	Not induced
Solyc05g051310	1.23	12.3	3.12E-05	0.72	7.2	0.0421	Not induced
Solyc08g067530	1.82	4.8	0.0023	2.31	6.1	0.0005	Not clear
Solyc09g089780	22.22	10.7	3.47E-06	12.96	6.2	0.0015	csp22 and flg22 induced
Solyc01g079920	0.53	8.8	1.57E-05	0.34	5.7	0.0043	csp22 and flg22/flgII-28 induced
Solyc01g107370	4.36	5.5	4.09E-06	3.9	4.9	1.75E-05	Not induced
Solyc07g055690	337.65	12.2	7.22E-09	133.88	4.8	0.0059	csp22 and flg22/flgII-28 induced/ ETI induced?
Solyc10g084600	9.14	3.9	0.009	7.95	3.4	0.0147	ETI suppressed?
Solyc07g009380	397.73	3.9	2.02E-11	319.41	3.1	4.35E-07	Not clear
Solyc09g091060	3.39	4.5	3.92E-05	2.01	2.7	0.0008	Not induced
Solyc12g011200	6.75	10.7	2.01E-08	1.68	2.7	0.034	Not induced
Solyc02g065470	346.18	3.4	5.52E-09	249.33	2.4	0.0091	ETI induced
Solyc03g112030 (S1)	**1.74**	**0.31**	**0.0238**	**2.31**	**0.41**	**0.0078**	**flgII-28 induced/ETI induced**
Solyc02g088210 (S2)	**5.29**	**0.46**	**0.0006**	**4.11**	**0.36**	**5.14E-09**	**csp22 induced/ETI induced**
Solyc05g007440 (S3)	**7.36**	**0.29**	**3.05E-30**	**8.87**	**0.35**	**4.42E-19**	**ETI suppressed**
Solyc08g074710	0.07	0.03	0.0021	0.46	0.20	0.0164	Not expressed
Solyc02g030520	0.11	0.03	0.0067	0.24	0.06	0.0432	Not expressed
Solyc07g064370	0.02	0.02	0.0001	0.03	0.02	0.0322	Not induced
Solyc07g064380	0.03	0.02	0.0009	0.01	0.01	0.0014	Not induced
Solyc06g043150	0.23	0.21	0.0037	0.48	0.44	0.0354	Not induced

Supplemental Table S3. Primers used in this study.

Primer name	Primer sequence	Purpose	Solyc ID
S1_CDS	F:TTCGAATTCCAAGCTTGCCCATGATTTCCTTATTTGCGGTATTTCC	To clone the coding region of S1 into pJLSmart	Solyc03g112030
	R:ATAGGAATTCGGATCCGCCCGAATGCGTGGAGTTGCAACGAC		
S2_CDS	F:TTCGAATTCCAAGCTTGCCCATGAAATTCGGTAAAGAGTTCACAAC	To clone the coding region of S2 into pJLSmart	Solyc02g088210
	R:ATAGGAATTCGGATCCGCCCGTTCCGGTGGATGGAAATCCTC		
S3_CDS	F:TTCGAATTCCAAGCTTGCCCATGGCGACGGAGCTAGAAGAATTG	To clone the coding region of S3 into pJLSmart	Solyc05g007440
	R:ATAGGAATTCGGATCCGCCCGCGAAGATGTCTCTGCTAACGAAG		
D1_CDS	F:TTCGAATTCCAAGCTTGCCCATGTCAATATGGGGAAAAATGTTCTTTC	To clone the coding region of D1 into pJLSmart	Solyc05g024190
	R:ATAGGAATTCGGATCCGCCCGACTCCTATCTTCACCAGTTACCG		
D2_CDS	F:TTCGAATTCCAAGCTTGCCCATGCTTAGTGTAAGTATGAATCTGTTG	To clone the coding region of D2 into pJLSmart	Solyc07g061790
	R:ATAGGAATTCGGATCCGCCCGAATGGCAAGTGCTTTTTCCAAATCA		
D3_CDS	F:TTCGAATTCCAAGCTTGCCCATGCTGATGAGGCTTCTGATTATC	To clone the coding region of D3 into pJLSmart	Solyc02g077330
	R:ATAGGAATTCGGATCCGCCCGGTCTAGTAAATCAACATTAATAGCTTTAAG		
D4_CDS	F:TTCGAATTCCAAGCTTGCCCATGGAGTTCTCTAAGATAACTTCACTTC	To clone the coding region of D4 into pJLSmart	Solyc12g009650
	R:ATAGGAATTCGGATCCGCCCGAATTTCAGATTGGAAACAAGTGTAGCC		
D5_CDS	F:TTCGAATTCCAAGCTTGCCCATGGAAAATTATAGTCCTTATAATTCCAA	To clone the coding region of D5 into pJLSmart	Solyc11g019910
	R:ATAGGAATTCGGATCCGCCCGATAAAGGTGATTTATGAAAGCTAAAGCA		
D6_CDS	F:TTCGAATTCCAAGCTTGCCCATGGCTCTCTCACATCCTATGAC	To clone the coding region of D6 into pJLSmart	Solyc08g078020
	R:ATAGGAATTCGGATCCGCCCGGAAAAAAAGTGCTACACTTCCAATAATTG		
Agp1-SP-L12H	F:ATTTTCTCTCATTTTCTTACATTTTTAGC	For mutagenesis in the signal peptide sequence of Agp1	Solyc08g078020
	R:TGTCATAGGATGTGAGAG		
Agp1-SP-T20KA22H	F:TCACCAATCCCCCATGATGGC	For mutagenesis in the signal peptide sequence of Agp1	Solyc08g078020
	R:GCCTTGAGGGCTAAAAATGTAAGAAAAAG		
Agp1-GPI-S128KS129K	F:TCCACCTCCAAAGAAGGGATTTGTTCATGGAATTAG	For mutagenesis in the GPI anchor sequence of Agp1	Solyc08g078020
	R:GAAGCCATTGACTCACCAC		
Agp1-GPI-F151KF152K	F:TGTAGCACTTAAGAAGCGGGCGGATC	For mutagenesis in the GPI anchor sequence of Agp1	Solyc08g078020
	R:CTTCCAATAATTGCTACC		
Agp1-ΔSP	F:TTCGAATTCCAAGCTTGCCCATGCAATCCCCCATGATGGCCCCA	To delete the signal peptide sequence of Agp1	Solyc08g078020
	ATAGGAATTCGGATCCGCCCGGAAAAAAAGTGCTACACTTCCAATAATTG		
Agp1-ΔGPI	R:TTCGAATTCCAAGCTTGCCCATGGCTCTCTCACATCCTATGAC	To delete the GPI anchor sequence of Agp1	Solyc08g078020
	ATAGGAATTCGGATCCGCCCGTGGAGGTGGAGAAGCCATTGAC		