

**Title:** Comprehensive transcriptome analysis and functional characterization of PR-5 for its involvement in tomato *Sw-7* resistance to tomato spotted wilt tospovirus

**Supplementary Information**

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**SUPPLEMENTARY TABLES:**

**Supplementary Table S1.** Evaluation of virus accumulation on each test plant of S-line and Sw-7 line on days post inoculation using TSWV-specific real-time quantitative RT-PCR

Treatment	Test plants and replicates	Ct (dRn) <sup>d</sup>	Mean Ct replicate	Mean Ct treatment
S-1 control <sup>a</sup>	before inoculation 1 <sup>a</sup>	No Ct	No Ct	No Ct
	before inoculation 1	No Ct		
	before inoculation 2	No Ct	No Ct	
	before inoculation 2	No Ct		
	before inoculation 3	No Ct	No Ct	
	before inoculation 3	No Ct		
Sw-7 <sup>b</sup>	before inoculation 1 <sup>b</sup>	No Ct	No Ct	No Ct
	before inoculation 1	No Ct		
	before inoculation 2	No Ct	No Ct	
	before inoculation 2	No Ct		
	before inoculation 3	No Ct	No Ct	
	before inoculation 3	No Ct		
Non-template	Non-template control 1	No Ct	No Ct	No Ct
	Non-template control 2	No Ct		
S-1 Control_4 dpi <sup>c</sup>	TSWV inoculation 1	23.12	23.50	27.02
	TSWV inoculation 1	23.88		
	TSWV inoculation 2	27.91	28.06	
	TSWV inoculation 2	28.21		
	TSWV inoculation 3	29.86	29.50	
	TSWV inoculation 3	29.14		
Sw-7_4 dpi	TSWV inoculation 1	27.33	27.22	27.43
	TSWV inoculation 1	27.10		
	TSWV inoculation 2	24.81	24.45	
	TSWV inoculation 2	24.45		
	TSWV inoculation 3	30.26	30.63	
	TSWV inoculation 3	31.00		
S-1 Control_7 dpi	TSWV inoculation 1	23.16	22.88	22.46
	TSWV inoculation 1	22.59		
	TSWV inoculation 2	18.10	18.10	
	TSWV inoculation 2	28.42		
	TSWV inoculation 3	25.89	26.40	
	TSWV inoculation 3	26.91		
Sw-7_7 dpi	TSWV inoculation 1	38.17	35.94	35.01
	TSWV inoculation 1	33.71		
	TSWV inoculation 2	34.74	34.63	
	TSWV inoculation 2	34.52		
	TSWV inoculation 3	34.31	34.47	

	TSWV inoculation 3	34.63		
S-1 Control_14 dpi	TSWV inoculation 1	16.35	16.35	22.54
	TSWV inoculation 1	16.36		
	TSWV inoculation 2	15.60	16.03	
	TSWV inoculation 2	16.45		
	TSWV inoculation 3	35.40	35.25	
	TSWV inoculation 3	35.09		
Sw-7_14 dpi	TSWV inoculation 1	32.61	29.41	33.04
	TSWV inoculation 1	26.20		
	TSWV inoculation 2	32.66	30.51	
	TSWV inoculation 2	28.35		
	TSWV inoculation 3	39.28	39.22	
	TSWV inoculation 3	39.15		
S-1 Control_21 dpi	TSWV inoculation 1	15.84	15.90	16.88
	TSWV inoculation 1	15.95		
	TSWV inoculation 2	16.74	16.43	
	TSWV inoculation 2	16.12		
	TSWV inoculation 3	15.20	18.32	
	TSWV inoculation 3	18.32		
Sw-7_21 dpi	TSWV inoculation 1	32.75	33.03	31.51
	TSWV inoculation 1	33.30		
	TSWV inoculation 2	30.76	27.49	
	TSWV inoculation 2	24.22		
	TSWV inoculation 3	No Ct	34.02	
	TSWV inoculation 3	34.02		
S-1 Control_35 dpi	TSWV inoculation 1	21.10	19.14	20.48
	TSWV inoculation 1	17.17		
	TSWV inoculation 2	21.09	18.46	
	TSWV inoculation 2	15.82		
	TSWV inoculation 3	24.89	23.85	
	TSWV inoculation 3	22.80		
Sw-7_35 dpi	TSWV inoculation 1	33.47	31.53	32.41
	TSWV inoculation 1	29.58		
	TSWV inoculation 2	34.03	34.01	
	TSWV inoculation 2	33.99		
	TSWV inoculation 3	33.75	31.71	
	TSWV inoculation 3	29.66		

<sup>a</sup>S-1 control is a TSWV susceptible breeding line, Fla. 8059 used as a recurrent parent for backcrossing.

<sup>b</sup>Sw-7 is near-isogenic line containing Sw-7 gene for TSWV resistance through backcrossing to the Fla. 8059 for BC6 generations, theoretically 98.438% identical to the recurrent parent Fla. 8059.

<sup>c</sup>dpi: days post inoculation with TSWV.

<sup>d</sup>Viral titer measurement in TSWV-inoculated S-line and Sw-7 line was conducted using real time RT-PCR on leaf samples collected at 4, 7, 14, 21 and 35 dpi, with the corresponding Ct values presented.

**Supplementary Table S2.** Differentially expressed tomato genes to have intersected at all time points in Venn diagram

Gene accession	Gene annotation	0 dpi <sup>a</sup>	4 dpi	7 dpi	14 dpi	21 dpi	35 dpi
Solyc04g081610	Unknown Protein	11.665	11.000	11.354	10.792	10.309	10.907
Solyc04g081710	Unknown Protein	13.774	13.162	6.740	13.508	13.350	14.255
Solyc12g013530	Unknown Protein	-1.889	-2.837	-3.059	-3.837	-5.644	-4.644
Solyc12g013750	Mannan endo-1,4-beta-mannosidase 3	-6.644	-6.644	-5.767	-6.127	-6.727	-5.545
Solyc12g013960	Unknown Protein	-2.000	-2.837	-3.474	-3.644	-4.322	-3.644
Solyc12g014280	Chloroplastic group IIA intron splicing facilitator CRS1, chloroplastic	-2.059	-2.737	-3.059	-2.644	-2.943	-3.644
Solyc12g015630	Genomic DNA chromosome 5 P1 clone MDF20	-5.059	-3.837	-4.644	-5.059	-5.059	-5.059

<sup>a</sup>dpi: days post inoculation.

**Supplementary Table S3.** Selected differentially expressed photosynthetic pathway genes in *Sw-7* compared to the S-line after TSWV inoculation.

<i>S. lycopersicum</i> accession	Annotation	4 dpi <sup>a</sup>	7 dpi	14 dpi	21 dpi	35 dpi
<b>Chlorophyll binding protein</b>						
Solyc02g070950	Chlorophyll a/b binding protein	-	-	-	1.664	-
Solyc02g070970	Chlorophyll a/b binding protein	-	-	-	1.646	-
Solyc12g011450	Chlorophyll a-b binding protein 13	-	-	-	-	1.884
Solyc07g063600	Chlorophyll a-b binding protein 13	-	-	-	1.516	-
Solyc03g005780	Chlorophyll a-b binding protein 3C-like	-	-	-	1.669	-
Solyc10g006230	Chlorophyll a-b binding protein 7	-	-	-	1.411	1.888
<b>Cytochrome complex</b>						
Solyc01g007540	Cytochrome b6-f complex subunit 4	2.485	-	-	1.570	-
<b>Photosystem subunit protein</b>						
Solyc08g013670	Photosystem I reaction center subunit	-	-	-	-	1.824
Solyc12g044280	Photosystem I reaction center subunit	-	-	-	1.628	-
Solyc06g066640	Photosystem I reaction center subunit	-	-	-	1.511	-
Solyc08g006930	Photosystem I reaction center subunit X	-	-	-	1.536	-
Solyc01g008510	Photosystem II 5 kDa protein,	-	-	-	-3.474	-3.059
Solyc01g102770	Photosystem II reaction center protein Z	-	-	-	1.561	
Solyc06g084050	Photosystem II reaction center W	-	-	-	1.521	

<sup>a</sup>dpi: days post inoculation.

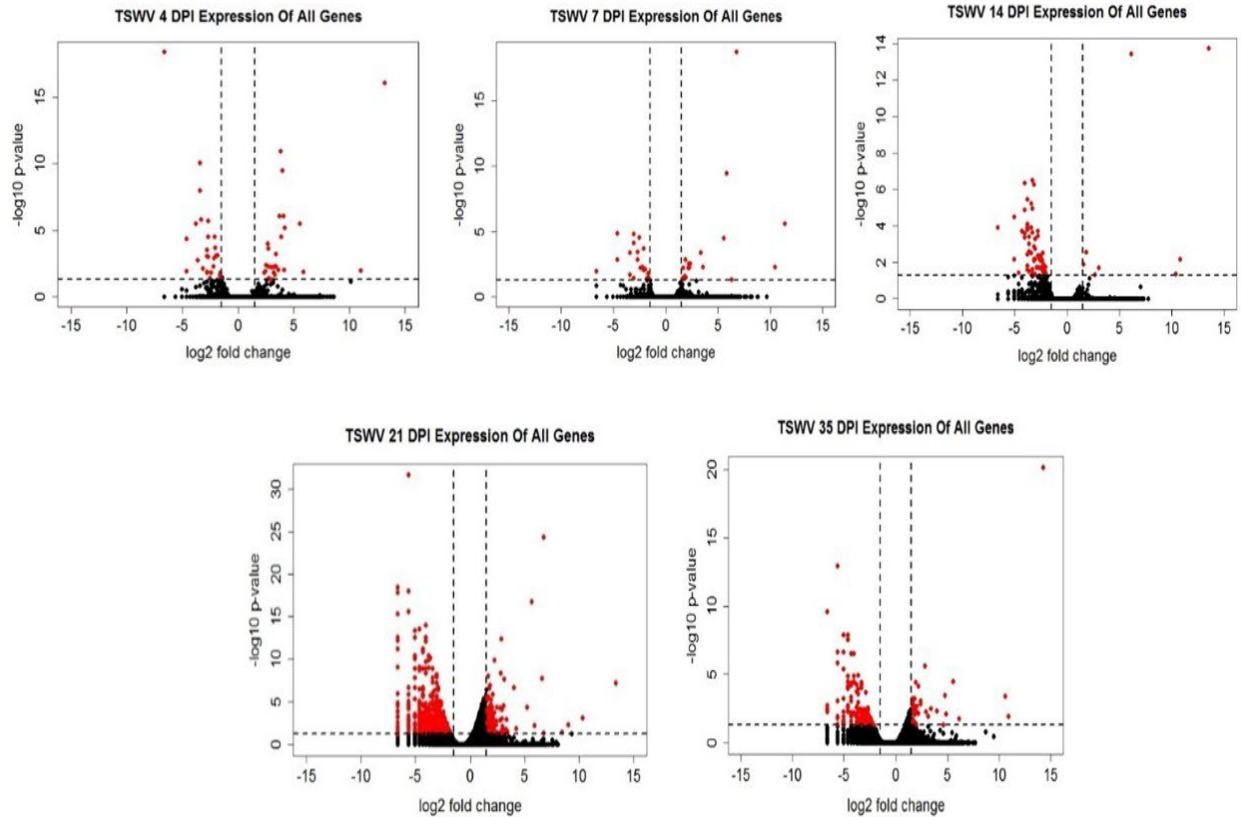
**Supplementary Table S4.** Evaluating transgenic plants expressing OLP-PR5 for their resistance to TSWV in comparison to that of the transgenic GFP and non-transgenic 'Moneymaker' plants

Treatment	Plant No.	7 dpi <sup>a</sup>	14 dpi	21 dpi	28 dpi	35 dpi	Infection status <sup>b</sup>
Transgenic GFP	1	30.66	29.25	19.10	19.33	24.38	(+)
	2	29.43	23.61	19.77	21.51	24.42	(+)
	3	No Ct	30.43	23.24	23.42	25.63	(+)
	4	34.45	No Ct	23.61	23.28	26.54	(+)
	5	34.00	20.16	20.41	20.27	22.97	(+)
Transgenic OLP-PR5	1	No Ct	34.25	No Ct	36.23	37.45	(-)
	2	36.98	36.88	No Ct	35.20	32.36	(-)
	3	No Ct	No Ct	36.11	21.32	23.63	(+)
	4	38.47	38.03	37.88	27.43	33.28	(-)
	5	37.55	22.14	20.81	22.02	24.93	(+)
Control tomato 'Moneymaker'	1	29.18	17.59	20.02	22.62	20.60	(+)
	2	19.68	18.77	20.19	20.53	20.54	(+)
	3	22.27	18.26	21.11	22.36	21.57	(+)
	4	24.83	23.20	19.50	20.43	22.91	(+)
	5	25.53	25.39	24.09	24.33	22.75	(+)
Healthy control	1	No Ct	37.08	No Ct	37.22	36.69	(-)
Positive control	1	19.10	19.81	28.81	21.92	20.90	(+)
	2	26.49	27.09	25.65	25.64	20.90	(+)
Non-template control	NTC	35.51	No Ct	No Ct	37.31	35.64	(-)

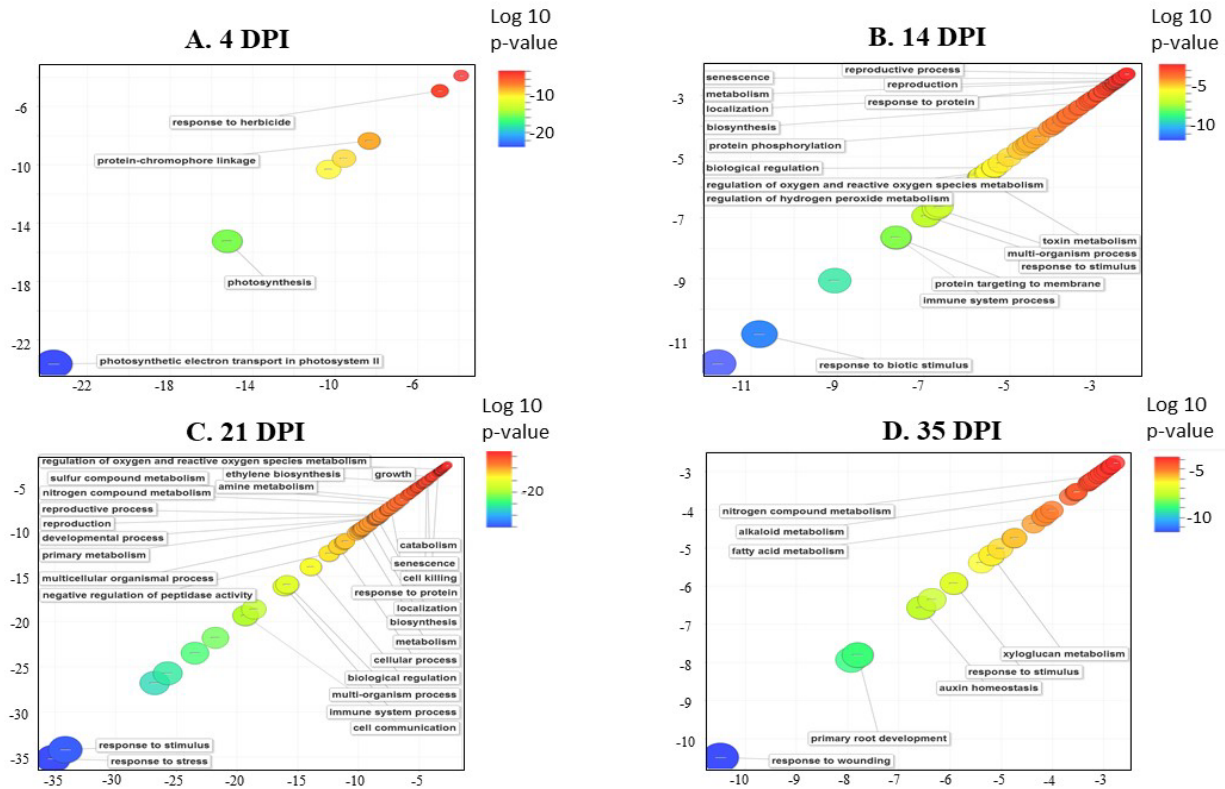
<sup>a</sup>dpi: days post inoculation.

<sup>b</sup>Ct values >35.00 to No Ct were considered negative (-); Ct values <30 were considered positive (+); Ct values 30-35 were considered border lines.

## SUPPLEMENTARY FIGURES:

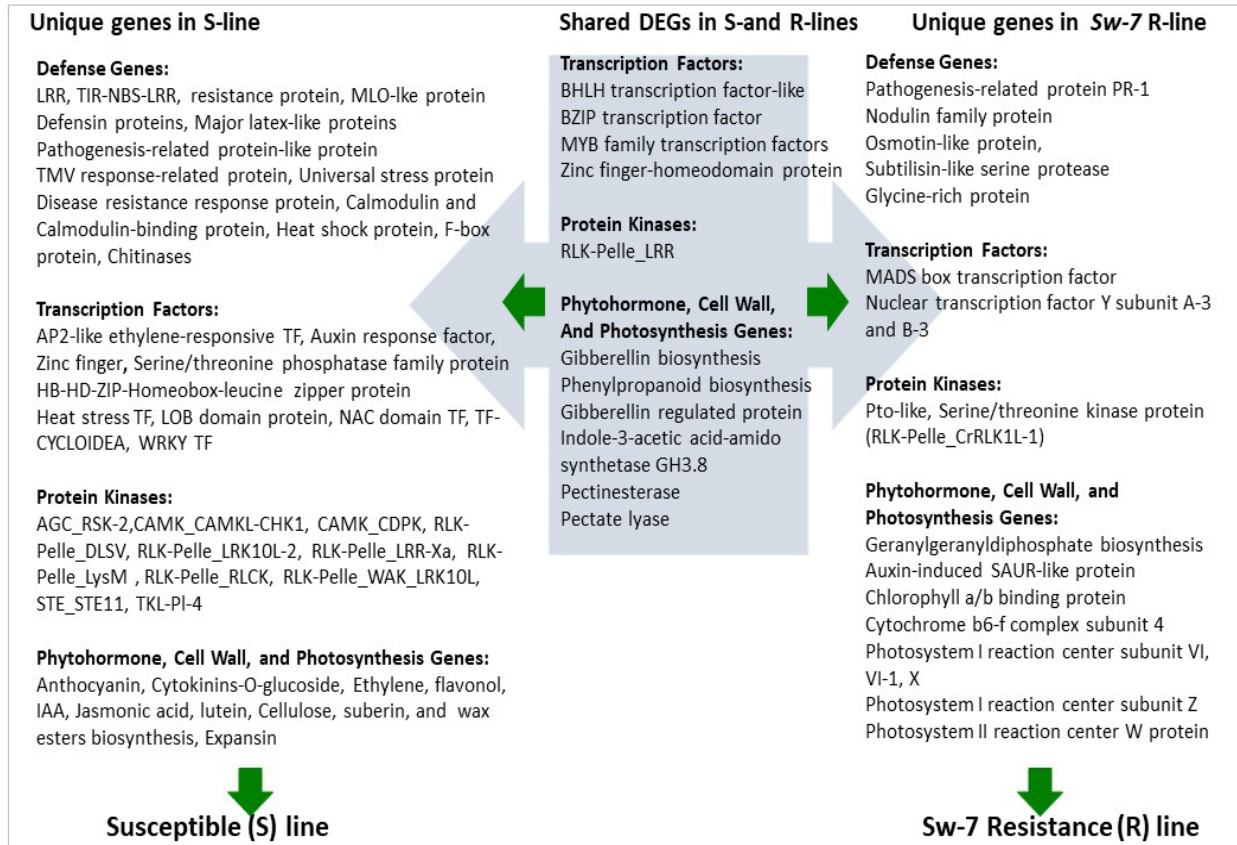


**Supplementary Figure S1.** The Volcano plots illustrate the distribution of all expressed genes and highlighted DEGs with the number of up- and down-regulated genes for each time point on 4, 7, 14, 21 and 35 dpi. Red dots on the right indicate *Sw-7* induced genes and red dots on the left indicate suppressed in *Sw-7*. X-axis represents  $-\log_{10}(\text{p-value})$  and y-axis represents  $\log_2(\text{fold change})$ . Black horizontal dotted lines show the p-value cut off at 0.05. Black vertical dotted lines were drawn using  $\log_2(\text{fold change})$  cut off at -1.5 and 1.5 demonstrating suppressed and induced genes respectively in the *Sw-7* line.



**Supplementary Figure S2.** Scatterplot showing cluster representatives of differentially expressed genes. Figures were generated with the REVIGO program using the list of enriched GO terms at each time point.





**Supplementary Figure S3.** Summary of the differentially expressed genes (DEGs). We have classified DEGs in four major groups: including a) Defense genes, b) Transcription factors, c) Protein kinases, and d) Phytohormones, cell wall and photosynthesis genes. In each category, certain group of genes are induced in the TSWV-resistant (*Sw-7*) plants (right panel), or in the susceptible (*S*) line (left panel). We have placed a small group of gene members, expressed in both the resistance (*Sw-7*) and the susceptible line in the middle panel.