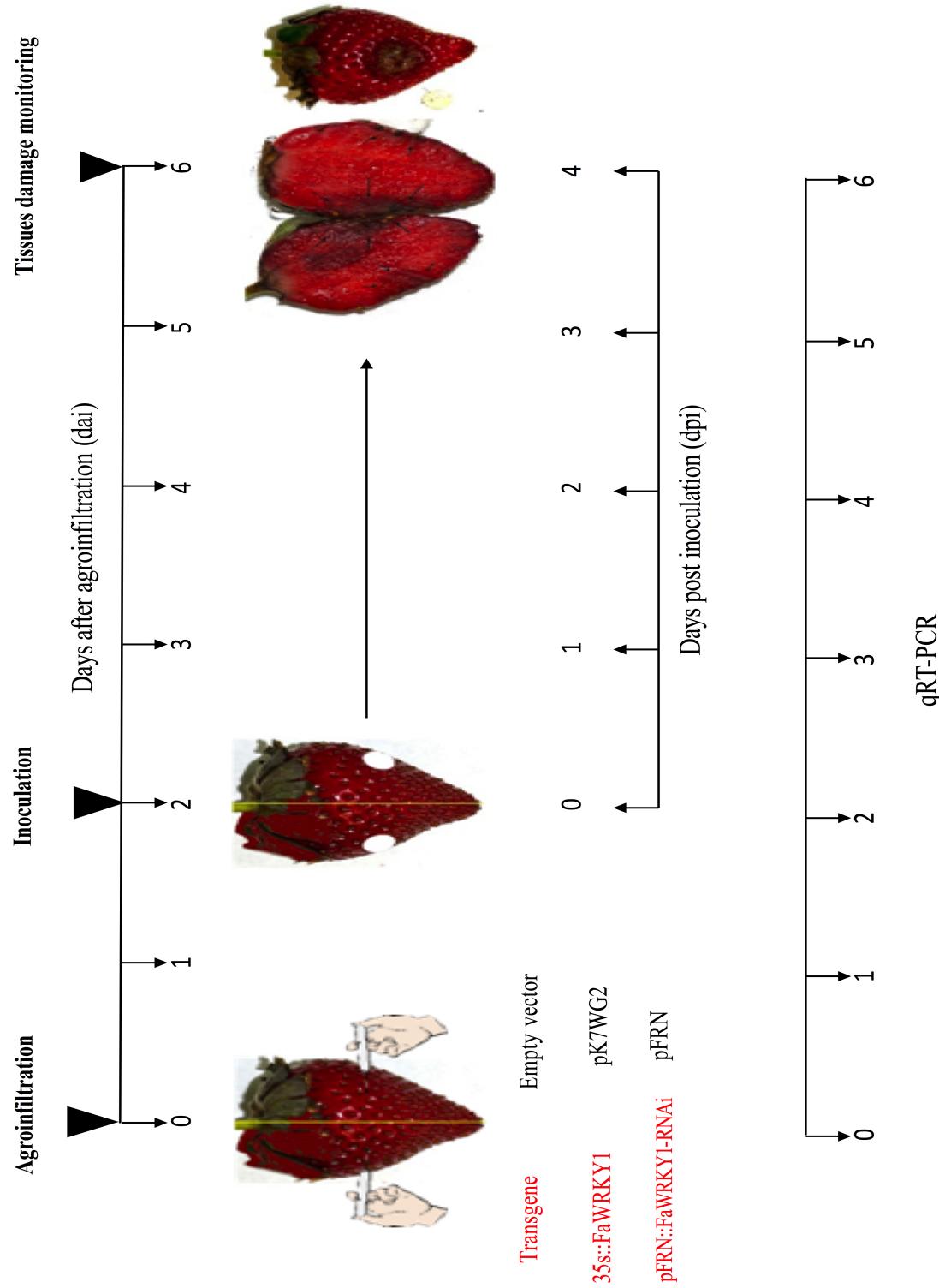


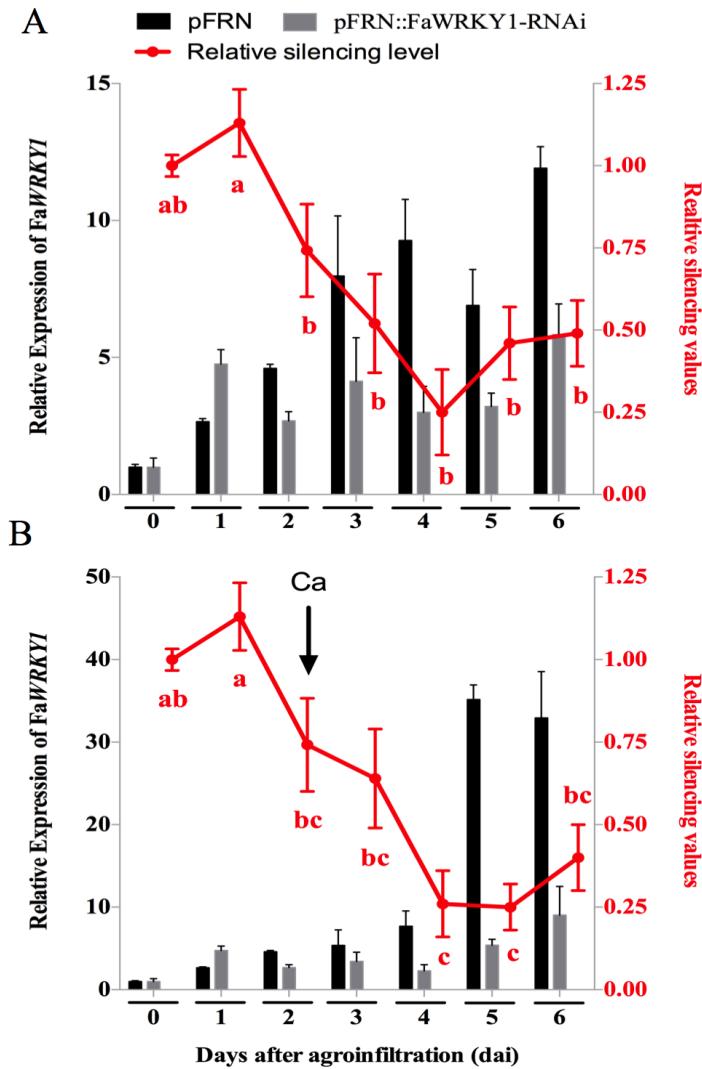
### *Supplementary Material*

## **The strawberry FaWRKY1 transcription factor negatively regulates resistance to *Colletotrichum acutatum* in fruit upon infection**

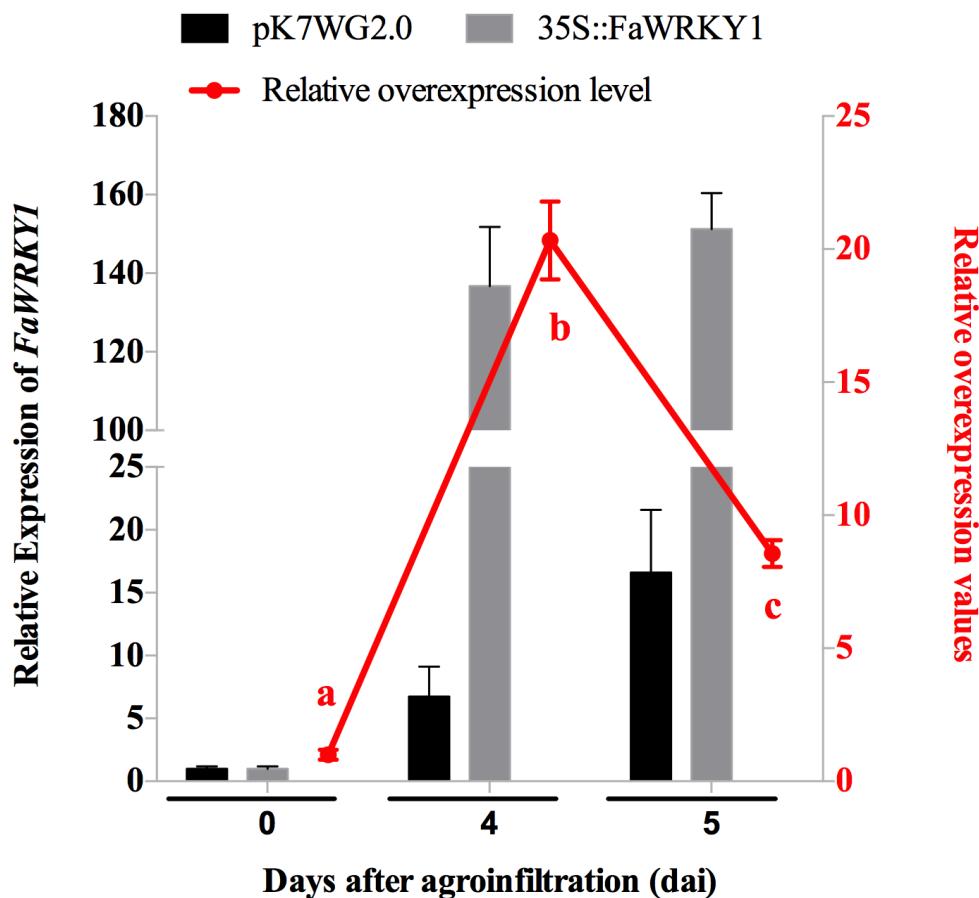
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**Supplementary Figure 1.** Experimental procedure for tissue damage assessment produced by *C. acutatum* in strawberry fruits agroinfiltrated with AGL0 bearing overexpression or silencing FaWRKY1 gene constructs



**Supplementary Figure 2. Effect of transient silencing of *FaWRKY1* gene in Strawberry Fruit by agroinfiltration and *C. acutatum* infection.** Gene expression kinetic analysis of *FaWRKY1* by qRT-PCR in each of the two strawberry fruit halves agroinfiltrated with pFRN (black line) and pFRN::FaWRKY1-RNAi (grey line), respectively, and non-inoculated (A) or inoculated with *C. acutatum* (B). In the graphics, standard value 1 at T0 was added to better illustrate changes. Data were normalized with respect to the transcript level of the housekeeping *elongation factor 1α* and *actine 1* genes, and scored during six days. Values are the means of three biological replicates. In red, the level of *FaWRKY1* silencing was calculated as a ratio value between the gene expression values found within the pFRN::FaWRKY1 fruit half with respect to the corresponding pFRN half. Means followed by the same letters in each trait are not significantly different at 5% level, according to tukey's test.



**Supplementary Figure 3. Effect of transient ectopic expression of *FaWRKY1* gene in strawberry fruit by agroinfiltration and *C. acutatum* infection.** Gene expression kinetic analysis of *FaWRKY1* by qRT-PCR in each of the two strawberry fruit halves agroinfiltrated with pK7::WRKY1 (35s::FaWRKY1) (grey line) or pK7WG2.0 (black line), respectively, and inoculated with *C. acutatum*. In the graphics, standard value 1 at T0 was added to better illustrate changes. Data were normalized with respect to the transcript level of the housekeeping elongation factor 1 $\alpha$  and actine 1 genes. Values are the means of three biological replicates. In red, the level of overexpression of *FaWRKY1* was calculated over time as a ratio value between the gene expression values found within the pK7::WRKY1 fruit half with respect to the corresponding pK7WG2.0 half. Means followed by the same letters in each trait are not significantly different at 5% level, according to tukey's test.

**Table S1.** Primers and sequences used in this study.

Name	Description / Orthologs	Sequence (5' - 3')		Observations
<i>FaWRKY1*</i>	WRKY DNA-binding protein 75 Fv gene07210 / AT5G13080	sense chain anti-sense chain	ACAGCAGTAAGATTAGGGATGAAGAAGGGAG GCTTCTTCACATTGCAACCCCTGATCGTG	Amil-Ruiz et al., 2013 ** Amil-Ruiz et al., 2016 * RT-qPCR
<i>FaCHI4-2*</i>	Chitinase class IV Fv gene02717 / AT3G54420	sense chain anti-sense chain	TGCCGGCAAGACCTCTACACTAGACAG TGTGAAGGATGTGTCGAGTAGGTGG	
<i>FaACTIN**</i>	Actin Fv gene26612 / AT3G12110	sense chain anti-sense chain	GGGCCAGAAAGATGCTTATGTCGG GGGCAACACGAAGCTCATGTAGAACAG	
<i>FaEF1a**</i>	Elongation factor 1-alpha Fv gene28639 / AT5G60390	sense chain anti-sense chain	TGGATTGAGGGTGACAACATGA GTATACATCTGAAGTGGTAGACGGAGG	
<i>FaICS1</i>	ICS1 Fv gene25950 / AT1G74710	sense chain anti-sense chain	TTGGTCAGTGTGCTGGTGT GTAGCCAAGGCCTCCCAT	
<i>FaCAT</i>	Catalase 2 Fv gene10917 / AT4G35090	sense chain anti-sense chain	CCTGCCCTTATTGTCCTGG GTGAGCAGACTTGGGAGCAT	
<i>FaWHY1</i>	Whirly1 Fv gene04012 / AT1G14410	sense chain anti-sense chain	TTTCGGAAGAGACGACTCGC ACATAAAACCTAGCCGGCCC	
<i>FaWHY2</i>	Whirly2 Fv gene31174 / AT1G71260	sense chain anti-sense chain	ACACAAAGCACCGGCTTTTC AGAGAGTGCAGCTTGCCTT	
<i>FaJAZ1</i>	<i>FvTIFY10A</i> Fv gene12541 / AT1G19180	sense chain anti-sense chain	TGGGAGATCTGAACCTCGTC TTCCCTCGGTTCTCCATCAC	This work RT-qPCR
<i>FaJAZ4</i>	<i>FvTIFY6B</i> Fv gene06180 / AT3G17860	sense chain anti-sense chain	AGAAGTGCTGGTGCACATG TGGGCATAAAATCTGGAGGAC	
<i>FaJAZ5</i>	<i>FvTIFY11A-Like</i> Fv gene05383 / AT1G17380	sense chain anti-sense chain	CACCATGAACTTGCTCAACG GAAAGGTGCGCTGAAGACGGAG	
<i>FaJAZ7</i>	<i>FvTIFY5B</i> Fv gene24321 / AT2G34600	sense chain anti-sense chain	GGATGAGCAGACCAGACAGG AAACATAAACCCGGCCATCG	
<i>FaJAZ8.1</i>	<i>FvTIFY5A</i> Fv gene30624 / AT1G30135	sense chain anti-sense chain	GAGGAGGAACGTCAATTGG AAGAGGGAAGCCGGAATTAG	
<i>FaJAZ9</i>	<i>FvTIFY6B</i> Fv gene09356 / AT5G13220	sense chain anti-sense chain	GGATGAGCAGACCAGACAGG AAACATAAACCCGGCCATCG	
<i>FaJAZ10</i>	<i>FvTIFY9</i> Fv gene07265	sense chain anti-sense chain	TTCCAGAACGTTCTCGAACG GATTTCTGGCTGCAATCAC	
<i>FaJAZ12</i>	<i>FvTIFY3B</i> Fv gene12975 / AT5G20900	sense chain anti-sense chain	GAAGCGTAGGGACAGATTGG AACCGGAAGAAGCATCATTG	
<b>pFRN::FaWRKY1 (RNAi)</b>	FaWRKY1-RNAi	sense chain anti-sense chain	ATGGATACCTACCCAGCATCTA TCCCTTCTCATCCCTAAATC	Cloning of a 272 bp fragment from <i>FaWRKY1</i> in pFRN
<b>35S::FaWRKY1 (Overexpression)</b>	WRKY DNA-binding protein 75	sense chain anti-sense chain	GGGGACAAGTTGTACAAAAAAGCAGGCTCAACAA TGGATACCTACCCAGCATCTA GGGGACCACTTGTACAAGAAAGCTGGGCTTACAA AGCACGCACAGCAGGCA	Encinas-Villarejo et al., 2009
<b>pCaMV35s::GUS</b>	Cauliflower mosaic virus (CaMV) promoter	sense chain anti-sense chain	GGGGACAAGTTGTACAAAAAAGCAGGCTACTAGAG CCAAGCTGATCTCCTT GGGGACCACTTGTACAAGAAAGCTGGGTCGACTA GAATAGTAAATIGTAATG	Cloning of CaMV promoter (1035 bp) in pKGWFS7.0
<b>FaWRKY1-RNAi</b>	WRKY DNA-binding protein 75	ATGGATACCTACCCAGCATCTAATCTCTCATCAACACCACCTCTGCT GCTGCTTCTCGCTGTCATTGAAACATGGTGAACACTCATCCTCATCATGCT TACGGTAACGATCATCAGTACCAAGCTAGCAATAACAAGGAAAATGGGTT CTTGGGGCTGATGTCAGAGATGGAGGTTCGAACAGCATGAGTAGTATTAC CCAGCAGAGTATGAAAGCTTGGGGAGGGTGAAGTAATACAGCAGTAA GATTAGGGATGAAGAAGGGA		<i>FaWRKY1</i> fragment of 272 bp for RNAi gene silencing

**Table S2:** Distribution of fruit number according to season and constructs used in the present study

Construct	Purposes	1° year		2° year		Total	
		+I	-I	+I	-I	+I	-I
pFRN::FaWRKY1-RNAi	Expression	24	24	-	-	120	24
	Statistic	48	-	48	-		
pFRN (RNAi control)	Expression	24	24	-	-	120	24
	Statistic	48	-	48	-		
Total per year		192		96		288	
35S::FaWRKY1	Expression	24	24	-	-	120	24
	Statistic	48	-	48	-		
pK7WG2 (OE control)	Expression	24	24	-	-	120	24
	Statistic	48	-	48	-		
Total per year		192		96		288	

Fruits were inoculated with *C. acutatum* (+I) or non-inoculated (-I).