Supplementary material:

Tailoring tobacco hairy root metabolism for the production of stilbenes

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Table S1. Sequences of the primers used to amplify the genes by PCR and qPCR

PCR			
Gene	Primer Sequence	Amplicon size bp	Reference
VvSTS	FW: ATGGCTTCAGTTGAGGAATTTAGA RV: TTAATTTGTAACCGTAGGAACGCT	1179	Hidalgo et al. [1]
AtMYB12	FW: CACCATGGGAAGAGCGCCATGTTG RV: TGACAGAAGCCAAGCGACCA	1117	This work
amiRNA-CHS	FW: ACGAAGAGGAGATGACGTGTG RV: GTTTTGCCTCGGCCTTTTTCA	287	This work
rolC	FW: TAACATGGCTAGAAGACGACC RV: AAACTTGCACTCGCCATGCC	534	Slightom, et al.[2]
virD	FW: ATGTCGCAAGGCAGTAAGCCCA RV: GGAGTCTTTCAGCATGGAGCAA	438	Hirayama et al.[3]
Program: 95ºC for 5 min, 30 cycles (95ºC, 60 s; 60ºC, 40 s; 72ºC, 90 s) and an extension cycle of 10 min at 72ºC			
qPCR			
Elongation factor (qPCR)	FW: TGGTCAGGAGATTGCGAAAGAGC RV: ACGCAAAACGCTCCAATGGTG	130	Hidalgo et al.[4]
STS (qPCR)	FW: AAGGGGAAAAAGCCACCACA RV: TTCGATGGTCAAGCCAGGTC	77	Hidalgo et al.[1]
AtMYB12 (qPCR)	FW: GAGGCCACGATCGGGAATAG RV: CAGAAGCCAAGCGACCAAAG	87	This work
amiRNA-CHS (qPCR)	FW: GAGATGACGTGTGTTCCTTCG RV: GAGGAAGAAGGAGAAGAGAAGGTG	73	This work
PAL (qPCR)	FW: TCGAAGATGAATTGAAGGCTGTTTT RV: GGAATAGCAGGGTTTCCACTTTCT	82	This work
CHS (qPCR)	FW: CAGCCGCGGTCATTATAGGT RV: AGAGTTTGGGCTGCAGAGAC	82	This work
Program: 95°C for 2 min, 40 cycles (95°C, 10 s; 60°C, 20 s; 72°C, 20 s) followed by a melting curve			

Table S2. Analysis of the Pearson lineal correlation between the different parameters studied. ccP, Pearson correlation coefficient. TF, transcription factor: PAL, phenylalanine ammonia lyase; CHS, chalcone synthase, TFC, total flavonoids content; Stilb., total stilbenes content.

Related variable	**ccP**	**p value**
TF / PAL	0.970	0.001
TF / CHS	0.967	0.002
TF / TFC	0.882	0.020
TF / Stilb.	0.888	0.018
Inh / CHS	-0.665	0.072
Inh / TFC	-0.626	0.085
CHS / TFC	0.858	0.001
CHS / TPC	0.435	0.209
STS / Stilb	0.816	0.025
TF / TPC	0.423	0.403

Table S3. Metabolites identified by relevant spectra (1D and 2D NMR).

Group of compounds	Metabolite	Chemical shift (ppm)	Multiplicity/coupling constant (Hz)
Amino acids	Proline	4.08/2.31	(dd, *J* = 8.6; 6.0)/(m)
	Alanine	1.49	(d, *J* = 7.2)
	Glutamine	2.12/2.45	(m)/(m)
	Leucine	0.96/0.98	(d,*J* = 6.2)/(d, *J* = 6.4)
	Threonine	1.33	(d, *J* = 6.8)
	Valine	1.00/1.01	(d, *J* = 6.6)/(d, *J* = 7.1)
Sugar	α-Glucose	5.19	(d, *J* = 3.7)
	β-Glucose	4.58	(d, *J*= 7.9)
	Sucrose	5.41/4.17	(d, *J*=3.8)/(d,*J* = 8.6)
Organic acids	γ-Amino-butyrate (GABA)	1.9/2.32/3.01	(m)/(t,*J* = 7.5)/(t,*J* = 7.5)
	Acetic acid	1.94	(s)
	Fumaric acid	6.53	(s)
	Malic acid	2.82	(dd, *J* = 16.9; 8.1)
Phenolic acids	Cinnamic acid	7.63/7.42/7.62/6.49	(m)/(m)/(d, *J* = 16)/(d, *J* = 15.7)
	Caffeic acid	7.12/6.89/7.06	(d, *J*= 2)/(d, *J* = 8.2)/(dd, *J* = 8.4; 2)
	Sinapic acid	6.93/7.46/6.37/3.88	(s)/(d, *J* = 16)/(d, *J* = 16)/s
	p-coumaric acid	7.50/6.89/7.59?/6.49	(d, *J* = 8.49)/(d, *J* =8.2)
Others	Inositol	4.08/3.61/3.44/3.25	(t, *J* = 2.82)/(t, *J* = 9.97)/(dd, *J* = 10; 1.5?)/(t, *J* = 9.03)
	Nicotine	8.69/8.66/8.09/2.76	(d, *J* = 2.0)/(dd, *J* = 4.8; 1.4)/(dt, *J* = 4.7; 1.6)/(s)

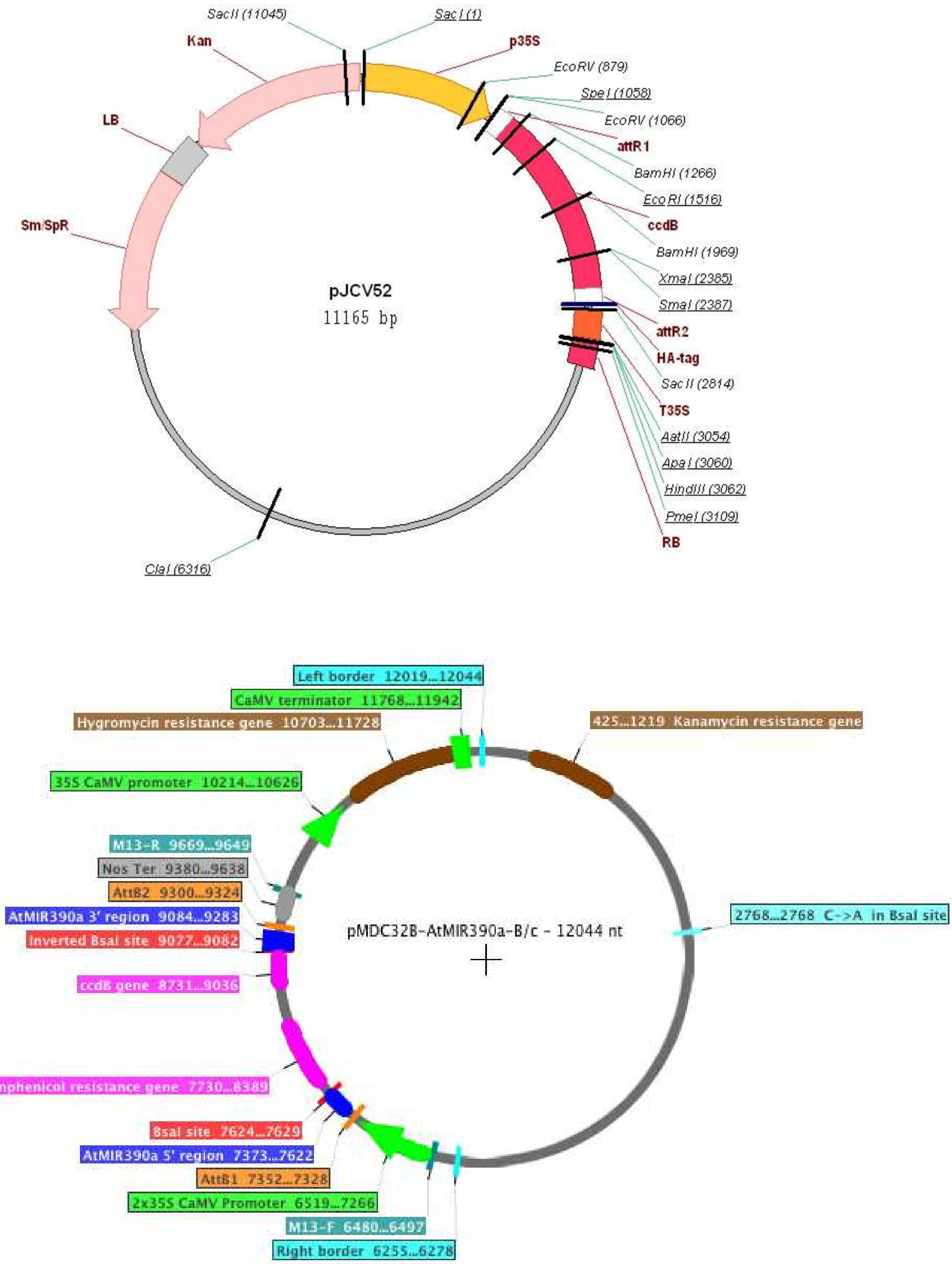


Fig. S1: Plant expression vectors pJCV52 by Karimi,et al.[5] and pMDC32B-AtMIR390a-B/c by Carbonell et al.[6]

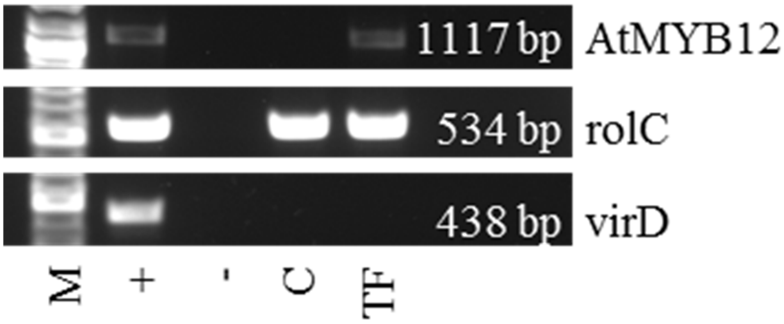
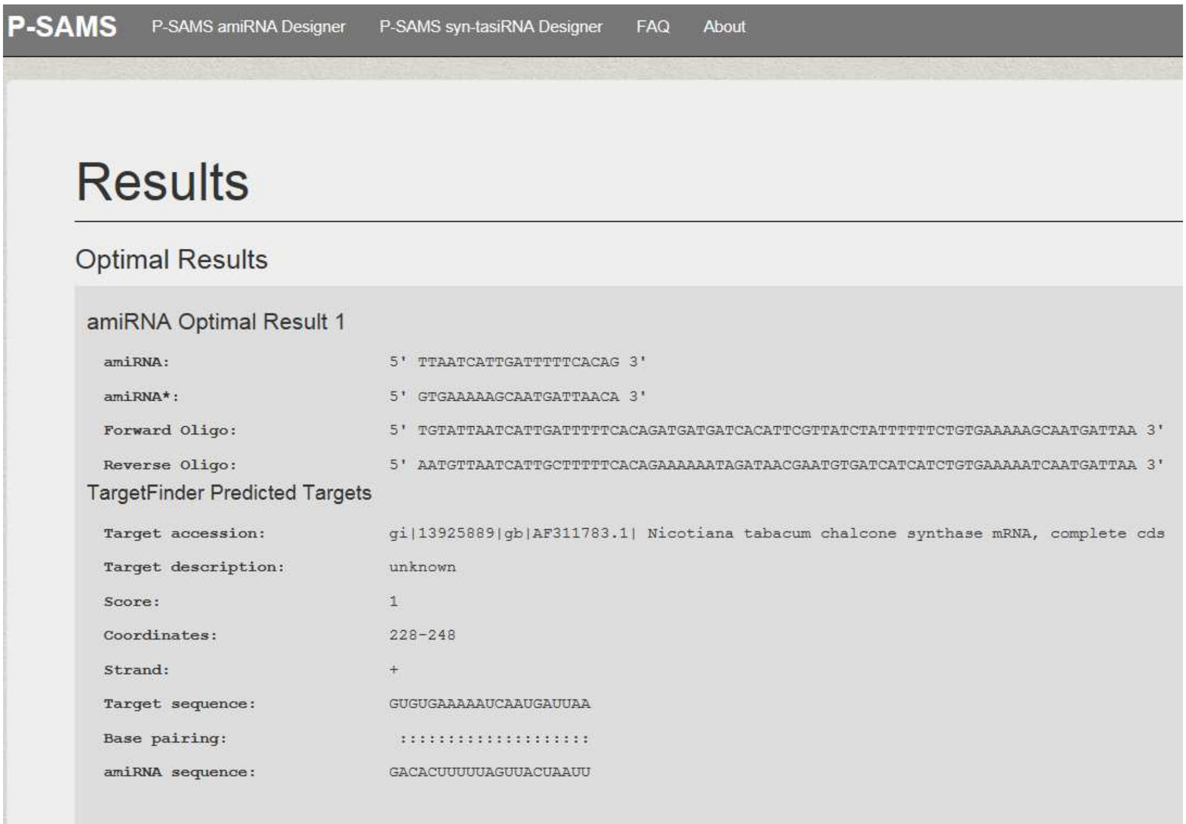


Fig. S2: PCR analysis of the genomic DNA of HR lines. (+) positive control (corresponding *A. rhizogenes* used for the infections), (-) negative control (DNA of *Nicotiana tabacum* wild type plant). C, wild type hairy root (control), TF, hairy root line carrying the AtMYB12 transcript

Supplementary information (1) for the design of amiRNA-CHS.

The amiRNA for *Nicotiana tabaccum* CHS, was designed from the gb|AF311783.1 sequence by the web tool: (http://p-sams.carringtonlab.org/amirna/designer) as described by Carbonell et al.[6]. The optimal amiRNA CHS sequence result was:



Results

Optimal Results

amiRNA Optimal Result 1

```
amiRNA:                  5' TTAATCATTGATTTTTCACAG 3'
amiRNA*:                 5' GTGAAAAAGCAATGATTAACA 3'
Forward Oligo:           5' TGTATTAATCATTGATTTTTCACAGATGATGATCACATTCGTTATCTATTTTTTCTGTGAAAAAGCAATGATTAA 3'
Reverse Oligo:           5' AATGTTAATCATTGCTTTTTCACAGAAAAAATAGATAACGAATGTGATCATCATCTGTGAAAAATCAATGATTAA 3'
```

TargetFinder Predicted Targets

```
Target accession:        gi|13925889|gb|AF311783.1| Nicotiana tabacum chalcone synthase mRNA, complete cds
Target description:      unknown
Score:                   1
Coordinates:             228-248
Strand:                  +
Target sequence:         GUGUGAAAAAUCAAUGAUUAA
Base pairing:             ::::::::::::::::::::
amiRNA sequence:         GACACUUUUUAGUUACUAAUU
```

amiRNA optimal results:
amiRNA: 5' TTAATCATTGATTTTTCACAG 3'
amiRNA*: 5' GTGAAAAAGCAATGATTAACA 3'

Forward Oligo: 5'
TGTATTAATCATTGATTTTTCACAGATGATGATCACATTCGTTATCTATTTTTTC
TGTGAAAAAGCAATGATTAA 3'
Reverse Oligo: 5'
ATGTTAATCATTGCTTTTTCACAGAAAAAATAGATAACGAATGTGATCATCATC
TGTGAAAAATCAATGATTAA 3'

TargetFinder Predicted Targets
Target accession: gi|13925889|gb|AF311783.1| *Nicotiana tabacum* chalcone synthase mRNA, complete cds
Score: 1
Coordinates: 228-248

Strand: +
Target sequence: GUGUGAAAAAUCAAUGAUUAA
Base pairing: : : :: : : :: : : :: : : : :: : :: :
amiRNA sequence: GACACUUUUUAGUUACUAAUU

Supplementary information (2). Sequence of *Nicotiana tabacum* chalcone synthase mRNA, complete cds

>gi|13925889|gb|AF311783.1| Nicotiana tabacum chalcone synthase mRNA, complete cds
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TTAAGGAAAAATTTAAGCGCATGTGTGAAAAATCAATGATTAAGAAAAGGT
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ACATGGCACCTTCCCTTGATGCTAGACAAGACATAGTGGTGGTTGAAGTGC
CAAAACTTGGCAAAGAGGCAGCCCAAAAAGCCATCAAAGAATGGGGCCAG
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TGCCCGGGTGTGACTACCAACTCACTAAGCTACTCGGGCTCCGTCCATCGGT
CAAGCGGTTCATGATGTACCAACAAGGTTGCTTTGCCGGTGGCACGGTACTC
CGGATGGCTAAGGACTTGGCCGAAAACAACAAGGGCGCTCGAGTCCTTGTT
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Supplementary information (3). Sequence of *Vitis vinifera* stilbene synthase clone

ATGGCTTCAGTTGAGGAATTTAGAAACGCTCAACGTGCCAAGGGTCCGGCCA
CCATCCTAGCCATTGGCACAGCTACCCCCGACCACTGTGTCTACCAGTCTGAT
TATGCTGATTACTATTTCAGGGTCACTAAGAGCGAGCACATGACTGAGTTGAA
GAAGAAGTTCAATCGCATATGTGACAAATCAATGATCAAGAAGCGTTACATTC
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Supplementary information (4). Blast result for aligned sequences of VvSTS and NtCHS

Score	Expect	Identities	Gaps	Strand
493 bits(546)	5e-143	820/1170(70%)	26/1170(2%)	Plus/Plus

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1012
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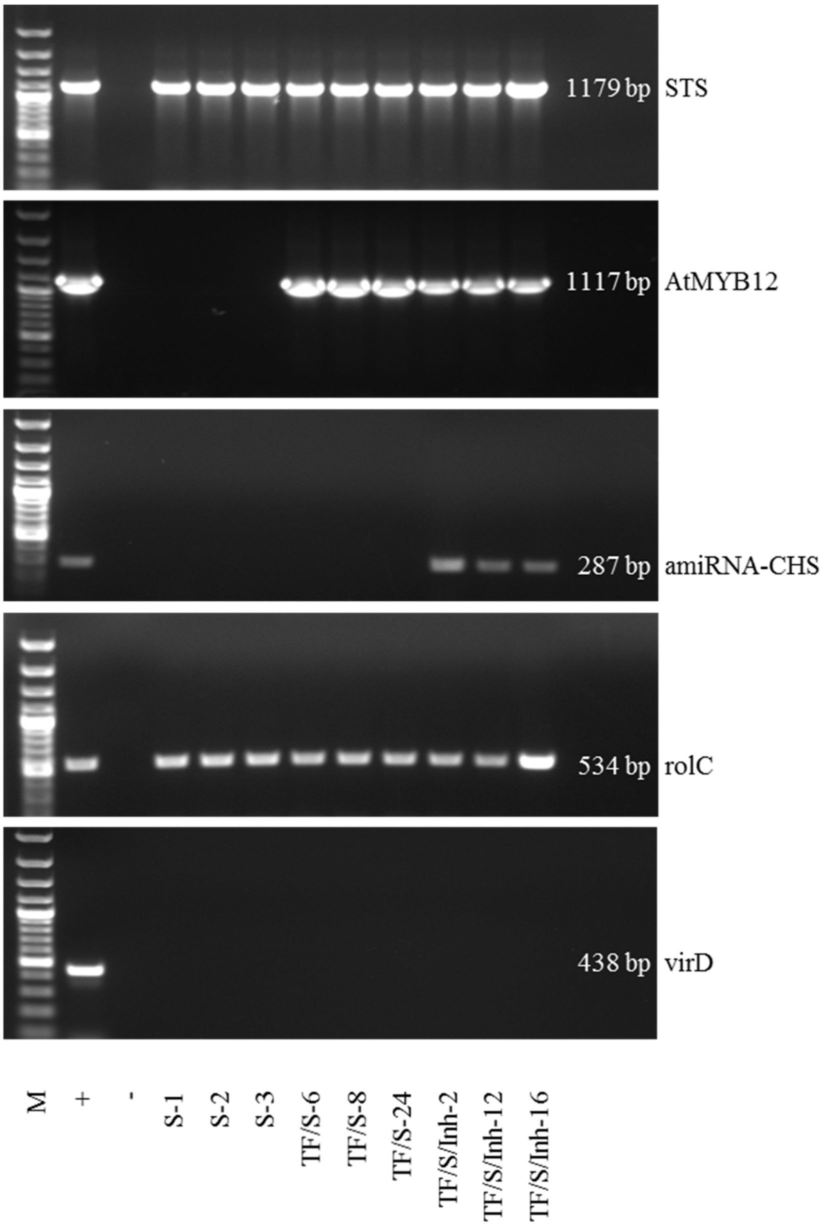
Unprocessed original scans for all of the blots in figure 2



References

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