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Supplementary Dataset S1. DNA sequences of target genes in CBW used to construct the cotton transformation vector. Italicized headers indicate the complete transcript sequence obtained from the CBW's transcriptome. Headers in bold letters indicate sense strand sequences of DNA encoding the dsRNA molecules.

Gene	Primers (5' – 3')	Organism	Used for	Amplicon length (bp)	Efficiency (Miner)
Target1	F- gactacacccggctgatgagt R- atccgccgatccgtacttac	*G. hirsutum*	PCR	407	--
Target2	F- gttctgccatggggtagagct R- atccgccgatccgtacttac	*G. hirsutum*	qPCR	106	1.85
UBQ14	F- caacgctccatcttgtcctt R- tgatcgtctttcccgtaagc	*G. hirsutum*	qPCR	75	1.82
PP2A1	F- gatccttgtggaggagtgga R- gcgaaacagttcgacgagat	*G. hirsutum*	qPCR	100	1.86
CHS2	F- aaggcattaacggtgacgac R-tccaagtcgttgatgactgc	*A. grandis*	qPCR	121	1.91
ETHR	F- ggtttattgctgcggtgtttac R-gatagtgttggccgaggtatg	*A. grandis*	qPCR	110	1.91
Vg	F- tcatcaaatctatatggctggttatgac R-gctacaggactaattgccataacatcac	*A. grandis*	qPCR	201	1.88
RPS11	F- acccacggttttccttaacc R-gacgactccggtgaggatac	*A. grandis*	qPCR	123	1.89
RPS26	F- tcccaaaagacaaggcaatc R-ccttcttgctcctgttacgc	*A. grandis*	qPCR	150	1.96
AHAS	F-gtcactgggttaatatctctcgaatcttgca R-cctacttccaatgtctgattagtgcttctgg	*G. hirsutum*	qPCR	257	1.99
UBC1	F-tggcattatattgtcattgttactatcc R-accatgttatcttattctaagacaagctc	*G. hirsutum*	qPCR	121	1.99

Supplementary Table S1. List of primers used in PCR and qPCR experiments. Target 1: fragment for PCR confirmation in the transformation vector. Target 2: loop region of the dsRNA-EF fragment. *UBQ14*: ubiquitin-14. *PP2A1*: serine/threonine-protein phosphatase PP2A-1. *CHS2*: chitin synthase 2. *ETHr*: ecdysis-triggering hormone receptor. *Vg*: vitellogenin. *RPS11*: ribosomal protein 11. *RPS26*: ribosomal protein 26. F: forward primer. R: reverse primer.

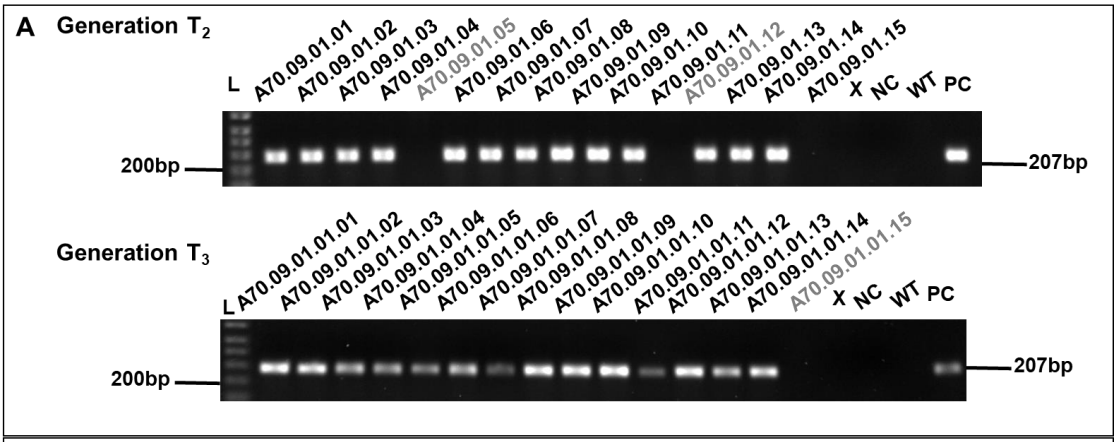
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B

Generation		# flower buds per plant	# seeds/cotton boll	Weight of fiber (g)
T0	GM	22.6 (± 1.42)	26 (± 1.63)	2.18 (± 0.16)
	WT	22.1 (± 1.88)	26.6 (± 0.96)	2.38 (± 0.17)
T1	GM	23.3 (± 1.80)	25.9 (± 1.10)	2.28 (± 0.20)
	WT	22.2 (± 1.81)	26.6 (± 0.95)	2.34 (± 0.19)
T2	GM	23.3 (± 1.85)	25.4 (± 0.69)	2.18 (± 0.13)
	WT	22.2 (± 1.81)	24.7 (± 1.05)	2.24 (± 0.120)

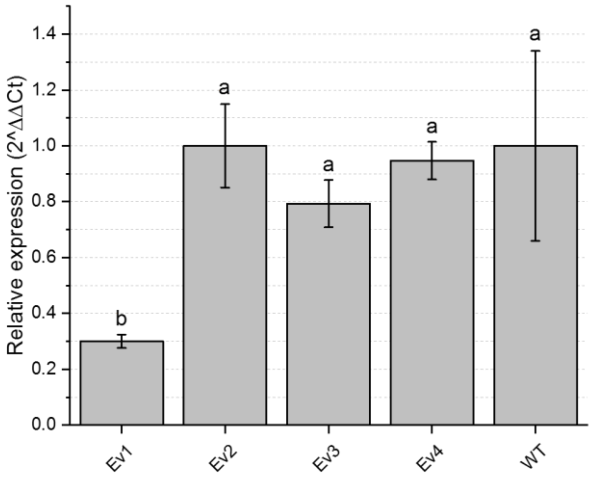
Supplementary Figure S1. Comparative phenotypic analyses between GM and WT plants. **A.** Phenotypic comparison between 60-day-old GM- and wild-type (WT) plants. **B.** Table of bud production 40 days after flowering in T0, T1, and T2. Number of seeds per cotton boll. Fiber weight per boll of different generations (Five bolls from 10 plants in each generation were used for data on weight and number of seeds). WT: wild-type plants (untransformed); GM: cotton genetically modified. Bars indicate standard error (SE, 95% confidence).



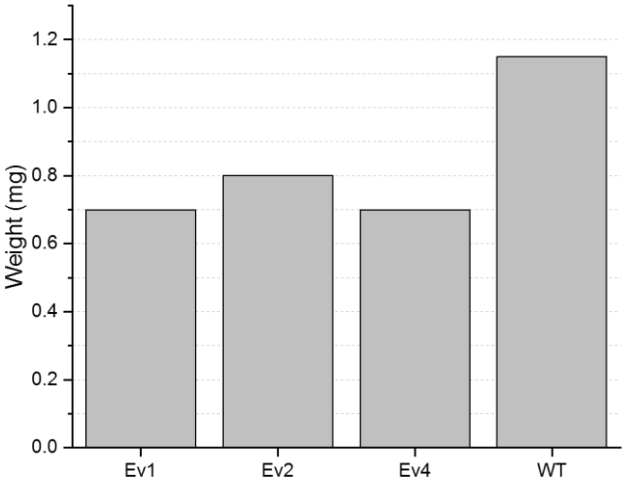
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#Plant ID	Generation	Number of Plants	Number Plants PCR Positive	% Segregation
A70.09.01	T_2	15	13	86.6
A71.41.01	T_2	15	12	80.0
A71.48.01	T_2	15	11	73.3
A73.22.01	T_2	15	12	80.0
A70.09.01.01	T_3	15	14	93.3
A71.41.01.01	T_3	15	13	86.3
A71.48.01.01	T_3	15	14	93.3
A73.22.01.01	T_3	15	13	86.3

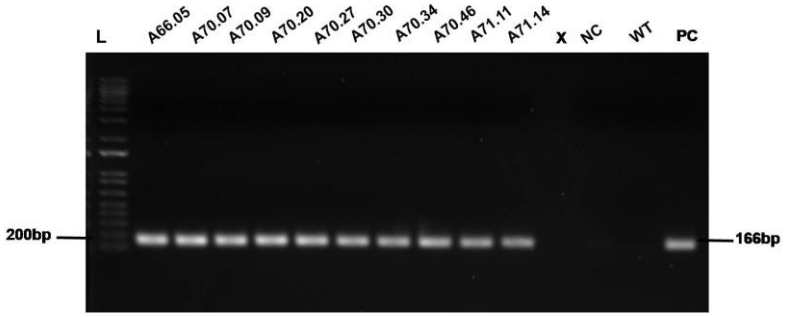
Supplementary Figure S2. Confirmation of cotton plant transformation in different generations through molecular analysis. A. Detection of the dsRNA fragment in GM cotton plants via PCR. Genomic DNA was extracted from leaves of T2 and T3 selected plants for amplification of the gene of interest (207 bp). **B.** Segregation transgene analyses on T2 and T3 generation cotton plants. Legend – Black letters: positive plants; Gray letters: negative plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; L: 1.0 kb ladder; NC: negative control (ultrapure water). The X represents an empty well.



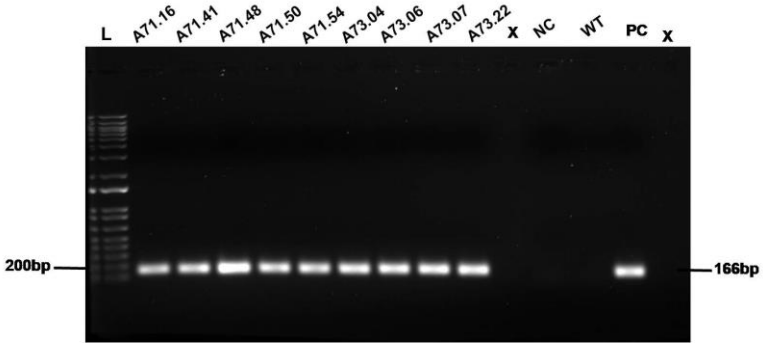
Supplementary Figure S3. Relative expression of CHS2 in CBW females fed GM cotton plants expressing dsRNA molecules after emerging from buds, 30 days after oviposition. Individuals fed on transgenic plants from different events (Ev) are compared with insects fed on wild-type cotton (WT). Lowercase letters indicate significant differences between treatments (t test, bonferroni corrected; p value < 0.05).



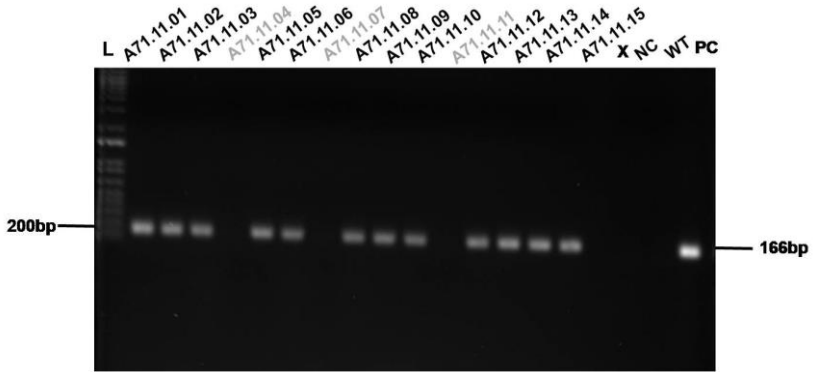
Supplementary Figure S4. Effects of dsRNA-expressing cotton plants in *A. grandis* **larvae.** The mean weight of larvae was determined by measuring a pool of 15 first instar larvae and dividing the total value by the number of individuals.



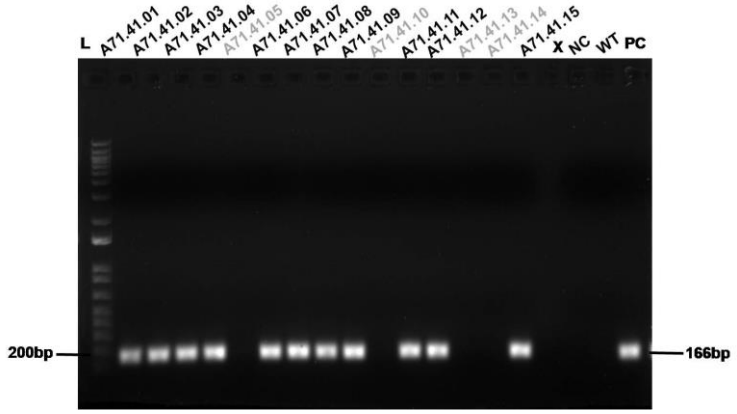
Supplementary Figure S5. Original image of the agarose gel 1% shown in figure 3A (first line). Black letters: positive plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; NC: negative control (ultrapure water). The X represents an empty well.



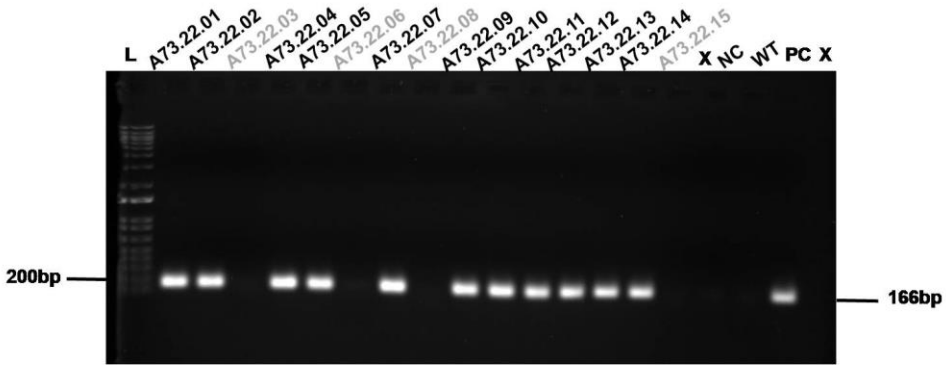
Supplementary Figure S6. Original image of the agarose gel 1% shown in figure 3A (second line). Black letters: positive plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; NC: negative control (ultrapure water). The X represents an empty well.



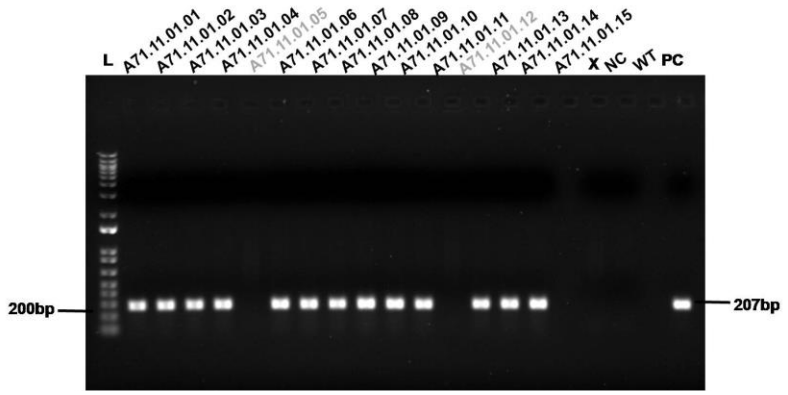
Supplementary Figure S7. Original image of the agarose gel 1% shown in figure 3B (first line). Black letters: positive plants; Gray letters: negative plants; CP: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; CN: negative control (ultrapure water). The X represents an empty well.



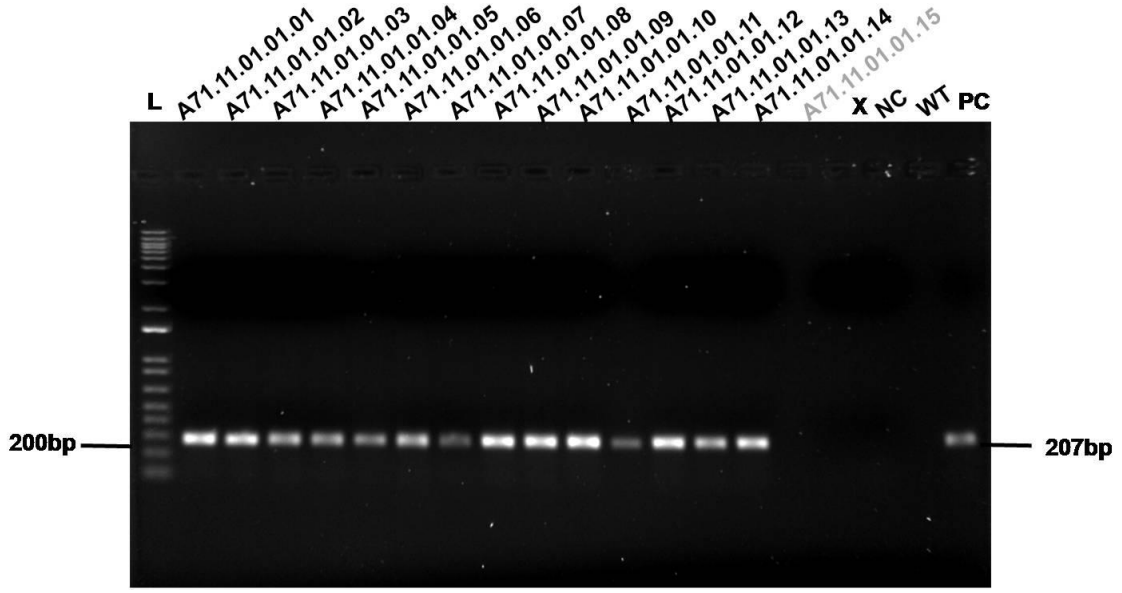
Supplementary Figure S8. Original image of the agarose gel 1% shown in figure 3B (second line). Black letters: positive plants; Gray letters: negative plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; NC: negative control (ultrapure water). The X represents an empty well.



Supplementary Figure S9. Original image of the agarose gel 1% shown in figure 3B (third line). Black letters: positive plants; Gray letters: negative plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; NC: negative control (ultrapure water). The X represents an empty well.



Supplementary Figure S10. Original image of the agarose gel 1% shown in figure S1A (first line). Black letters: positive plants; Gray letters: negative plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; NC: negative control (ultrapure water). The X represents an empty well.



Supplementary Figure S11. Original image of the agarose gel 1% shown in figure S1A (second line). Black letters: positive plants; Gray letters: positive plants; PC: positive control (vector with transformation cassette); WT: wild-type plants; M: 1.0 kb ladder; NC: negative control. The X represents an empty well.