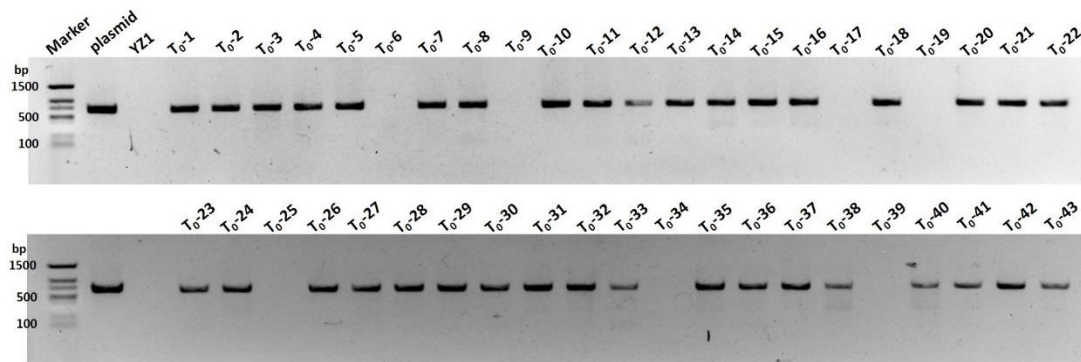


Supplementary Material

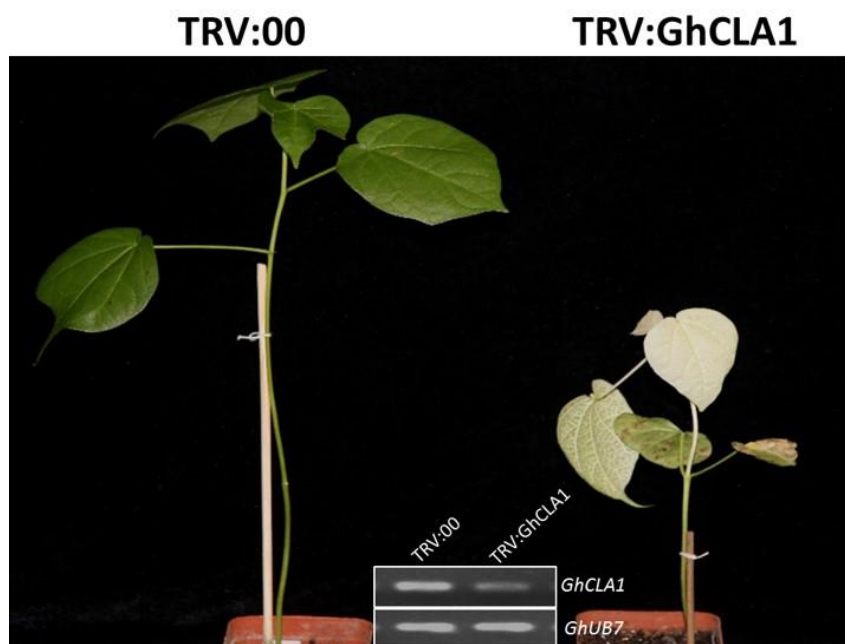
Supplementary Table S1 List of primers used in this study.

Primer ID	primer sequence (5'-3')	Description
GhCLA1-gRT1+	TATGCTCGCGGAATGATCAGGTTTTAGAGCTAGAAAT	For <i>sgRNA1-GhCLA1</i> cloning
GhCLA1-U6-29T1-	CTGATCATTCCGCGAGCATACAATCTCTTAGTCGACT	
GhCLA1-gRT2+	CATGCAAAGGGCATATGACCGTTTTAGAGCTAGAAAT	For <i>sgRNA2-GhCLA1</i> cloning
GhCLA1-U3bT2-	GGTCATATGCCCTTTGCATGTGACCAATGTTGCTCC	
GhCLA1-gRT3+	CTGCAGGCTTGGCCTGTGAGTTTTAGAGCTAGAAAT	For <i>sgRNA3-GhCLA1</i> cloning
GhCLA1-U6-29T3-	TCACAGGCCAAGCCTGCAGCAATCTCTTAGTCGACT	
GhEF1-gRT1+	GGCTTGTCTGAAGGCCTCTGTTTTAGAGCTAGAAAT	For <i>sgRNA1-GhEF1</i> cloning
GhEF1-U3bT1-	AGAGGCCTTCAGACAAGCCTGACCAATGTTGCTCC	
GhPDS-gRT1+	AAGCGAGAGATGTTCTAGGGTTTTAGAGCTAGAAAT	For <i>sgRNA1-GhPDS</i> cloning
GhPDS-U6-29T1-	CCTAGAACATCTCTCGCTTCAATCTCTTAGTCGACT	
GhPDS-gRT2+	TGGATGGAAACCCTCCCGAGGTTTTAGAGCTAGAAAT	For <i>sgRNA2-GhPDS</i> cloning
GhPDS-U6-29T2-	CTCGGGAGGGTTTCCATCCACAATCTCTTAGTCGACT	
GhPDS-gRT3+	ATCACTGGGGGGTGAGGTCGTTTTAGAGCTAGAAAT	For <i>sgRNA3-GhPDS</i> cloning
GhPDS-U3b-T3-	GACCTCACCCCCCAGTGATTGACCAATGTTGCTCC	
GhPDS-F1	TGCATGATCCATCACTCAAGTTT	For mutant detection of <i>GhPDS</i>
GhPDS-R1	GAACGAAAGGCCCTTCTTTC	

GhPDS-F2	ATTCATTTTCGTGTTTCATTCATTTGTAT	For mutant detection of <i>GhPDS</i>
GhPDS-R2	GAAAGTTCAACATCCATCAGCTAT	For mutant detection of <i>GhCLA1</i>
GhCLA1-F1	GGATCTGAAAGGTGAAAGGAATC	
GhCLA1-R1	TACCGTGATACTTGTGAGCAGCT	For mutant detection of <i>GhCLA1</i>
GhCLA1-F2	TTTACTGGTGCCTCGATATCTGA	
GhCLA1-R2	CATCGTGTACGACCTGTTGCAG	For mutant detection of <i>GhCLA1</i>
GhCLA1-F3	CACGGTAACATACAGAATAAGCC	
GhCLA1-R3	ACGTCATGTACGACCTGTTGC	For mutant detection of <i>GhEF1</i>
GhEF1-F	TGGTATCACCATTGATATTGCCT	
GhEF1-R	ATGACCTGAGAAGTGAAGTTTGC	For mutant detection of <i>NPT II</i>
NPT II .-F	GGCACAACAGACAATCGGC	
NPT II -R	CGTAAAGCACGAGGAAGCG	For RT-PCR analysis
Cas9-RT-F	TCAACGTACATATCCCTACCG	
Cas9-RT-R	AGGCTCAAGACTTACGCTCAT	



Supplementary Figure S1 PCR analysis of genomic DNA (43 transgenic lines) to detect the plant-selectable marker gene *NPT II*. Lanes: Marker, molecular weight markers; Plasmid, positive control vector pYLCRISPR/Cas9-N; YZ1, genomic DNA of wild type plant; T₀-T₄₃, independent transformed kanamycin-resistant calluses lines.



Supplementary Figure S2 Silencing of *GhCLA1* in cotton by tobacco rattle virus (TRV)-mediated virus-induced gene silencing (VIGS). Ten-day-old seedlings were used for infiltration with *A. tumefaciens* carrying *TRV:CLA1* vector. The photobleaching phenotype was appeared after two weeks inoculation. RT-PCR analysis indicated the expression of *CLA1* was reduced after inoculation.

Supplementary Note S1 Sequencing information of *GhCLA1* transgenic lines using CRISPR/Cas9 induced stable transformation.

Line-1

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (8/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1
02 (3/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
03 (4/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2

Line-2

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (3/22) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
02 (10/22) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC-T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
03 (9/22) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1

Line-3

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (3/9) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
02 (4/9) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0
03 (2/9) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-5

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (7/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0
02 (6/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
03 (4/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1

Line-7

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (10/13) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
02 (3/13) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1

Line-8

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (4/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
02 (13/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0

Line-10

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (1/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
02 (16/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0

Line-13

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (7/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0
02 (4/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
03 (4/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-14

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (1/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TG-AGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -3
02 (8/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
03 (7/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-16

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (5/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT--AAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
02 (11/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1

Line-20

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (14/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
02 (2/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2

Line-22

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (16/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-26

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (4/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0
02 (4/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
03 (1/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1
04 (1/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
05 (1/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTG-----GCCTTCAAACCTTTTGTGCAATCTACTCATCATTCAT -4

Line-27

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (18/20) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0
02 (2/20) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1

Line-28

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (8/14) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
02 (4/14) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
03 (2/14) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0

Line-30

WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
01 (2/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1
02 (9/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
02 (4/15) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-31
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (5/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
 02 (11/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0

Line-35
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTG-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (8/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
 02 (1/11) GAACAACATGCTGTACACCTTTGCTGCAGGCT-----ACTCATCATTCAT -36
 03 (2/11) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGATGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1

Line-38
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGT-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (9/13) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1
 02 (3/13) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2
 02 (1/13) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-T-GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-40
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTG-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (6/10) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT +1
 02 (3/10) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
 02 (1/10) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2

Line-41
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (17/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1

Line-42
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (12/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
 02 (4/16) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCC--TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2

Line-43
 WT GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT
 01 (2/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT-TGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -1
 02 (4/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCTGTGAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT 0
 03 (11/17) GAACAACATGCTGTACACCTTTGCTGCAGGCTTGGCCT--GAAGGCTTGAAACCTTTTGTGCAATCTACTCATCATTCAT -2