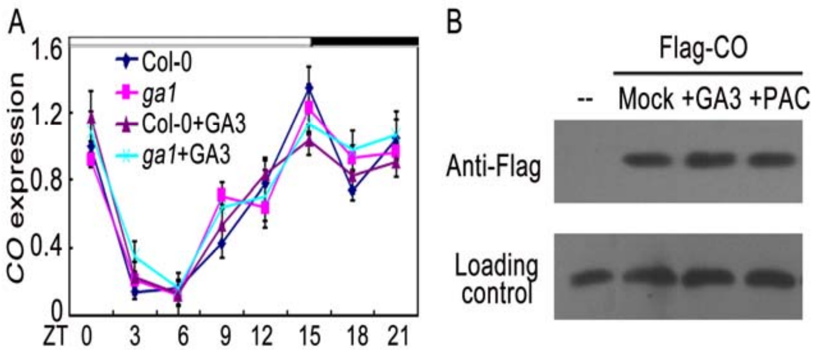
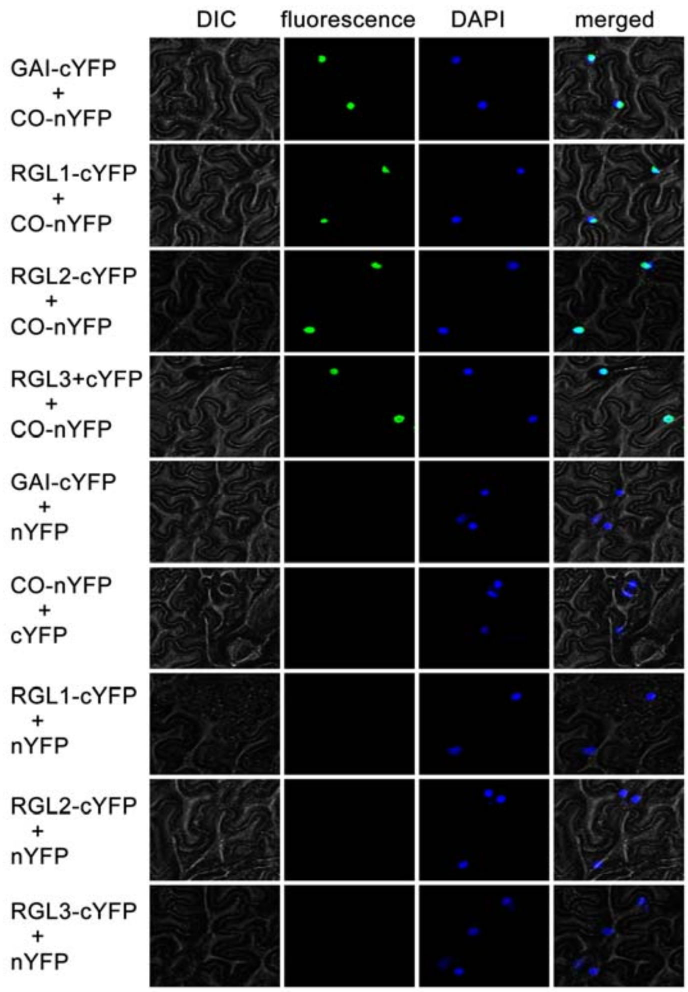
The DELLA-CO cascade integrates GA and photoperiod signaling to regulate flowering in *Arabidopsis*

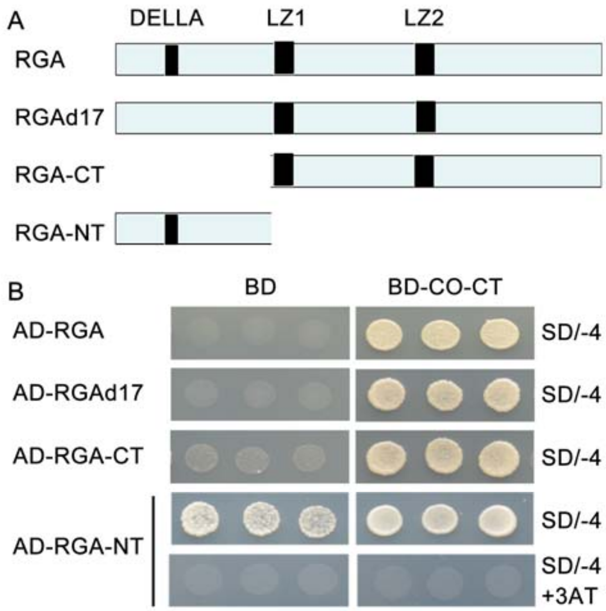
Houping Wang, Jinjin Pan, Yang Li, Dengji Lou, Yanru Hu, and Diqiu Yu



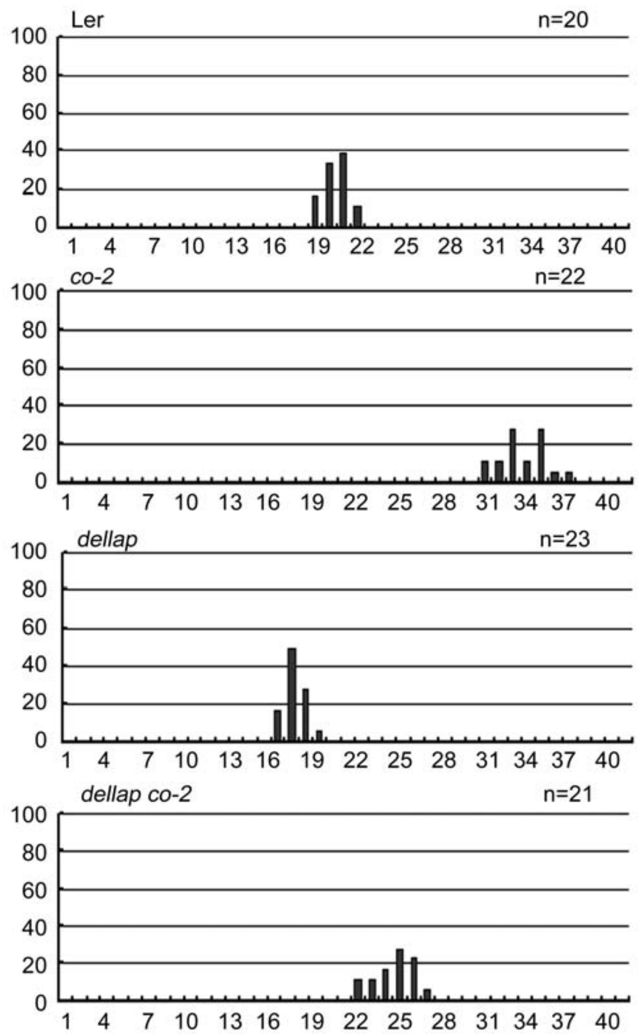
Supplemental figure 1. CO transcription and protein abundance are not regulated by GA pathway. (A) qRT-PCR analysis of *CO* expression in response to GA3 in *ga1* mutant and wild type (Col-0) under LDs. (B) Western blot analysis of CO protein accumulation in tobacco leaves. Flag-fused CO was transiently expressed in tobacco leaves. Infected leaves were treated with diluted ethanol (MocK), 10 μM GA, and 10 μM paclobutrazol 40h after infiltration. After 8 hours, those leaves were homogenized in extraction buffer, and Flag-CO was detected using an anti-Flag antibody.



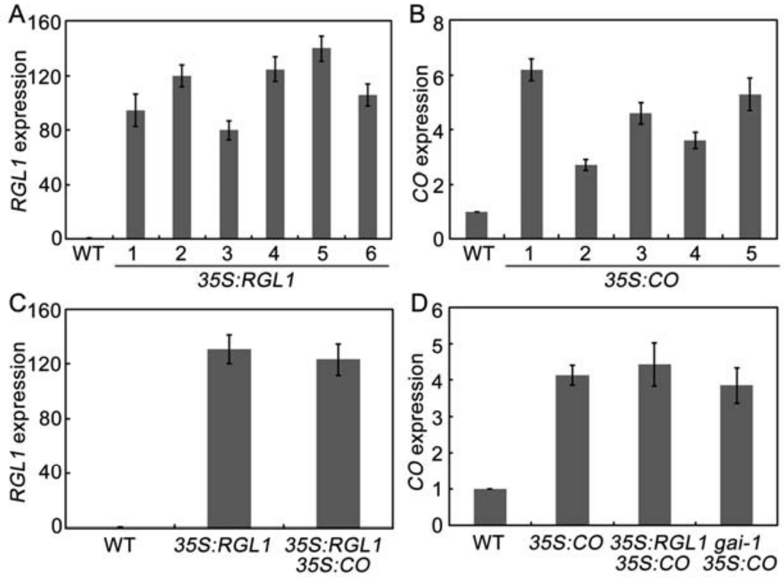
Supplemental figure 2. BiFC assay showing the fluorescence complementations of the cYFP fused with DELLAs and the nYFP fused with CO. 4',6-diamidino-2-phenylindole (DAPI) staining marks the nucleus.



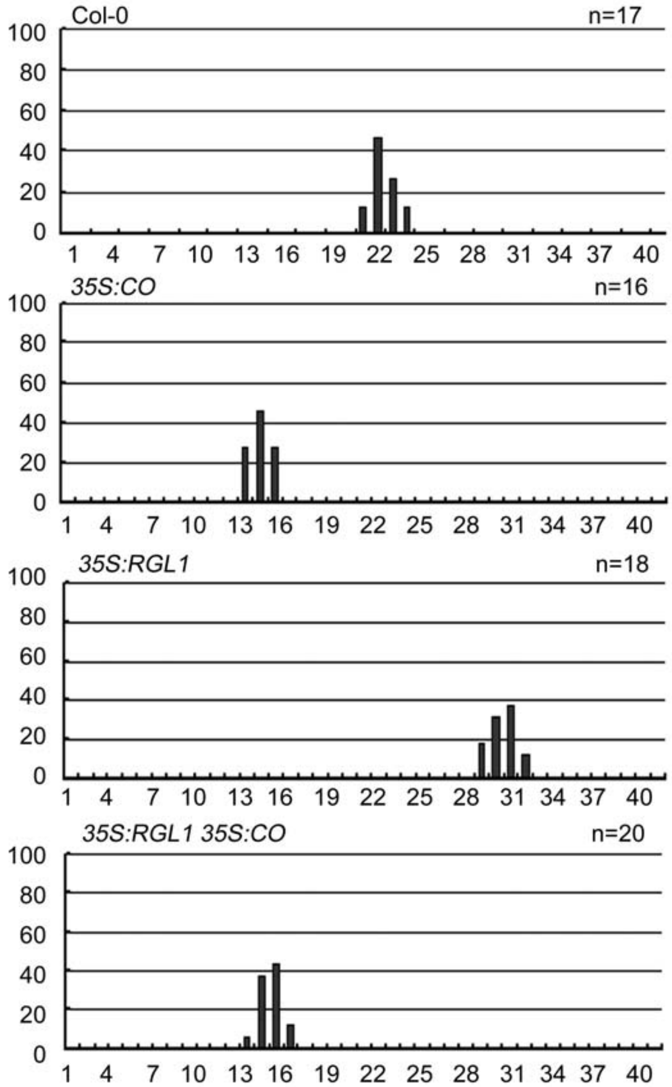
Supplemental figure 3. C-terminal parts of RGA contribute to the interaction between RGA and CO. (A) Schematic diagram of the constructs used in yeast two-hybrid assays in (B). (B) Interactions were indicated by the ability of cells to grow on selective media lacking Leu/Trp/His/Ade (SD/-4) or grow on selective media lacking containing 10 mM 3-aminotriazole (3AT). The empty pGBKT7 vector (BD) was used as a negative control.



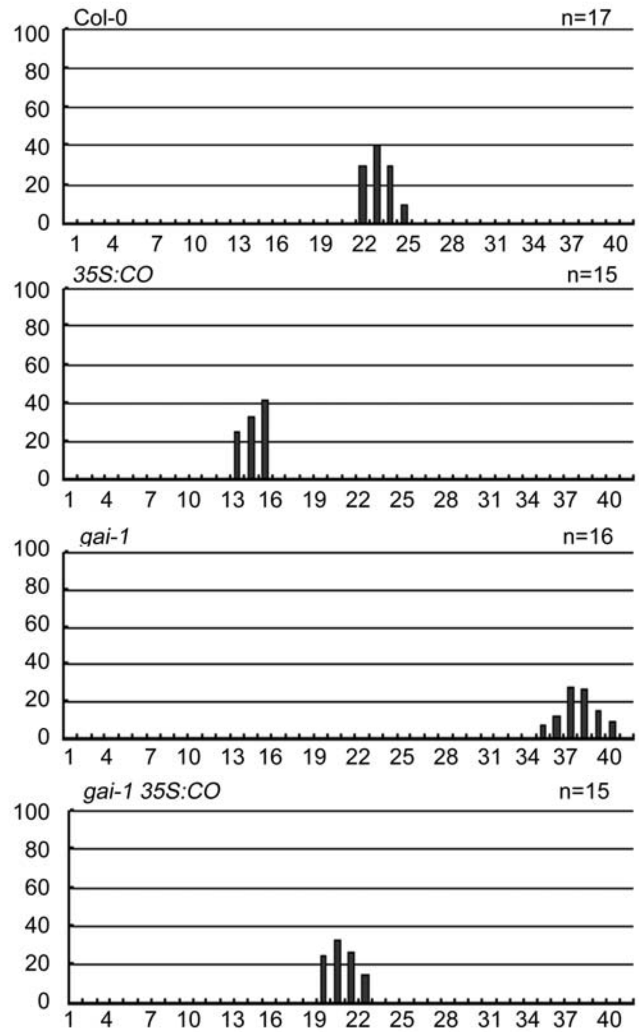
Supplemental figure 4. Flowering time of Ler, *co-2, dellap,* and *dellapco-2* under LDs. The x axis indicates the number of days. The y axis indicates the percentage of plants that flower with a given of days.



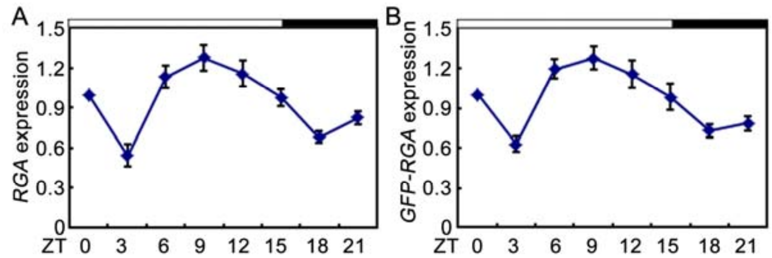
Supplemental figure 5. Overexpression lines for *RGL1* and *CO*. (A) Analysis of *RGL1* expression levels in wild type (WT) and *35S:RGL1* transgenic plants. (B) Analysis of *CO* expression levels in WT and *35S:CO* transgenic plants. (C) Analysis of *RGL1* expression levels in WT, *35S:RGL1*, and *35S:RGL1 35S:CO* transgenic plants. (D) Analysis of *CO* expression levels in WT, *35S:CO*, *35S:RGL1 35S:CO*, and *gai-1 35S:CO* transgenic plants. The *ACTIN2* gene was used as an internal control. Total RNA was extracted from 10-d-old plants at ZT 16 grown under LD. Error bars indicate SD from three independent RNA extracts.



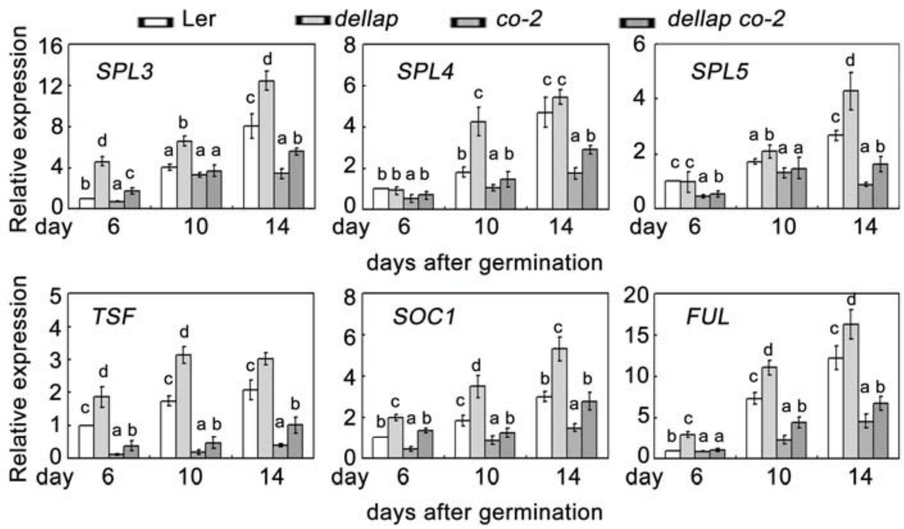
Supplemental figure 6. Flowering time of Col-0, *35S:CO, 35S:RGL1,* and *35S:CO 35S:RGL1* plants under LDs. The x axis indicates the number of days. The y axis indicates the percentage of plants that flower with a given of days.



Supplemental figure 7. Flowering time of Col-0, *35S:CO*, *gai-1*, and *gai-1 35S:CO* plants under LDs. The x axis indicates the number of days. The y axis indicates the percentage of plants that flower with a given of days.



Supplemental figure 8. qRT-PCR of *RGA* and *GFP-RGA* expression. (A) qRT-PCR of *RGA* relative to *IPP2* under LDs. Total RNA was extracted from 10-d-old Ler seedings. (B) qRT-PCR of *GFP-RGA* relative to *IPP2* under LDs. Total RNA was extracted from homozygous *proRGA:GFP-RGA* transgenic plants. A *GFP-RGA-* specific primer was used to detect *GFP-RGA* transcripts. Error bars indicate SD from three independent RNA extracts.



Supplemental figure 9. Expression of multiple flowering-related genes was co-regulated by DELLA and CO. The aerial part of Ler, *co-2, dellap,* and *dellap co-2* plants was collected at 6, 10, and 14 day after germination. The *ACTIN2* gene was used as an internal control. Error bars indicate SD from three independent RNA extracts. Different letters above columns indicate significant differences based on Tukey's test ($P < 0.05$).

Supplemental Table 1. Primers used in this study.

Use	Gene name	Primers (5'->3')
qRT-PCR	FT	qFT-F: CTGGAACAACCTTTGGCAAT qFT-R: TACACTGTTTGCCTGCCAAG
	CO	qCO-F: CTACAACGACAATGGTTCCATTAAC qCO-R: CAGGGTCAGGTTGTTGC
	RGA	qRGA-F: AACGCGTTTAAGCAAGCGAG qRGA-R: GTTTCCAAGCGGAGGTGGTA
	GAI	qGAI-F: TGGCTTGTGATGGACCTGAC qGAI-R: AATATGTGCAGCCGCAAACC
	RGL1	qRGL1-F: GGAAACTTGGTCAGCTTGCG qRGL1-R: GAAGACCGAGTTAACCGCCA
	RGL2	qRGL2-F: TTTCGTCTCACCGGAATCGG qRGL2-R: TCCGATAAACTCTCAGCGGC
	RGL3	qRGL3-F: TTCGACTCGCTCGAAGATGG qRGL3-R: TCTCGTGTCGCTCGATCCTA
	SOC1	qSOC1-F: ACGAGAAGCTCTCTGAAAAG qSOC1-R: GAACAAGGTAACCCA ATGAAC
	TSF	qTSF-F: GAGTCCAAGCAACCCTCACCA A qTSF-R: CACAATACGATGAATTCCCGAG
	SPL3	qSPL3-F: CTCATGTTCGGATCTCTGGTC qSPL3-R: TTTCCGCCTTCTCTCGTTGTG
	SPL4	qSPL4-F: CTCTCAGGACTTAACCAACGC qSPL4-R: CAGAGCTCTTCCTTCTTCGC
	SPL5	qSPL5-F: AAGGCATCTGCTGCGACTGTTG qSPL5-R: TCCTCCTCCTCTCATTGTGTCC
	FUL	qFUL-F: TTGCAAGATCACAACAATTCGCTTCT qFUL-R: GAGAGTTTGGTTCCGTCAACGACGAT
	IPP2	qIPP2-F: GTATGAGTTGCTTCTCCAGCAAAG qIPP2-R: GAGGATGGCTGCAACAAGTGT
	GFP-RGA	qGFP-RGA-F: ATGGTCCTGCTGGAGTTCGT qGFP-RGA-R: ATGATGATGAAGAAGAAGTCCCG
Yeast Two Hybrid	CO	BD-CO-CT-F: ATAGAATTCTATCTAAACCTTGTGGATTACAACTC BD-CO-CT -R: ATAGGATCCTCAGAATGAAGGAACAATCCCA
	RGA	AD-RGA-F: ATACATATGATGAAGAGAGATCATCACCAATTCC AD-RGA-R: ATAGGATCCGTACGCCGCCGTCGAGAGTT AD-RGAd17: ATACATATGATGAAGAGAGATCATCACCAATTCC TTCAAAGCAACCTCGTCCATGTTACCTCCACCGTCTT GAGGTTGCTTTGAAACTCGAACAAT ATAGGATCCGTACGCCGCCGTCGAGAGTT AD-RGA-NT-F: ATACATATGATGAAGAGAGATCATCACCAATTCC AD-RGA-NT-R: ATAGGATCCCGTTGTCGTGGTGGTTGTCGTC AD-RGA-CT-R: ATACATATGACGGCGGCGGGTGAGTC AD-RGA-NT-R: ATAGGATCCGTACGCCGCCGTCGAGAGTT
	GAI	AD-GAI-F:ATACATATGATGAAGAGAGATCATCATCATCATC AD-GAI-R: ATAGGATCCCTAATTGGTGGAGAGTTTCCAAG

	RGL2	AD-RGL2-F: ATACATATGATGAAGAGAGGATACGGAGAAACA AD-RGL2-R: ATAAGATCTGAGTTTCCACGCCGAGGTT
BiFC	CO	YN-CO-F: ATAGGATCCATGTTGAAACAAGAGAGTAACGACA YN-CO-R: ATATCTAGAGAATGAAGGAACAATCCCATATC
	RGA	YC-RGA-F: ATATCTAGAATGAAGAGAGATCATCACCAATTCCA YC-RGA-R: ATAGGATCCGTACGCCGCCGTCGAGAGTT
	GAI	YC-GAI-F: ATATCTAGAATGAAGAGAGATCATCATCATCATC YC-GAI-R: ATAGGATCCCTAATTGGTGGAGAGTTTCCAAG
	RGL1	YC-RGL1-F: ATATCTAGAATGAAGAGAGAGCACAACCACC YC-RGL1-R: ATAGGATCCTTATTCCACACGATTGATTCGC
	RGL2	YC-RGL2-F: ATACCTAGGATGAAGAGAGGATACGGAGAAACA YC-RGL2-R: ATAAGATCTGAGTTTCCACGCCGAGGTT
	RGL3	YC-RGL3-F: ATACCTAGG ATGAAACGAAGCCATCAAGAAACG YC-RGL3-R: ATAAGATCTCCGCAACTCCGCCGCTAGT
Co-IP	CO	Flag-CO-F: ATAGGATCCATGTTGAAACAAGAGAGTAACGACA Flag -CO-R: ATATCTAGAGAATGAAGGAACAATCCCATATC
	RGA	myc-RGA-F: ATATCTAGAATGAAGAGAGATCATCACCAATTCCA myc-RGA-R: ATAGGATCCGTACGCCGCCGTCGAGAGTT
Transient expression assay	FT	pFT-F: ATAAAGCTTAGCTTTATGAGCTTAAACATATGCA pFT-R: ATACCCGGGCTTTGATCTTGAACAAACAGGTGG
	CO	35S:CO-F: ATATCTAGAATGTTGAAACAAGAGAGTAACGACA 35S:CO-R: ATAGGATCCTCAGAATGAAGGAACAATCCCA
	RGA	35S:RGA-F: ATATCTAGAATGAAGAGAGATCATCACCAATTCCA 35S:RGA-R: ATAGGATCCTCAGTACGCCGCCGTCGAG 35S:RGAd17: ATATCTAGAATGAAGAGAGATCATCACCAATTCCA TTCAAAGCAACCTCGTCCATGTTACCTCCACCGTCTT GAGGTTGCTTTGAAACTCGAACAAT ATAGGATCCGTACGCCGCCGTCGAGAGTT
	RGL1	35S:RGL1-F: ATATCTAGAATGAAGAGAGAGCACAACCACC 35S:RGL1-R: ATAGGATCCTTATTCCACACGATTGATTCGC 35S:RGL1d17: ATATCTAGAATGAAGAGAGAGCACAACCACC AACTCCGGCAGCTTCTTCTTTA GCTGCCGGAGTTGACGTGGCACACAAGCTTGA ATAGGATCCTTATTCCACACGATTGATTCGC
Transgenic Plants	CO	35S:CO-F: ATAGAGCTCATGTTGAAACAAGAGAGTAACGACA 35S:CO-R: ATAGGATCCGAATGAAGGAACAATCCCATATC
	RGL1	35S:RGL1-F: ATA GAGCTCATGAAGAGAGAGCACAACCACC 35S:RGL1-R: ATAGGATCCTTCCACACGATTGATTCGCC
Pull-Down Assay	CO	GST-CO-F: ATAGGATCCATGTTGAAACAAGAGAGTAACGACA GST-CO-R: ATAGAATTCGAATGAAGGAACAATCCCATATC
	RGA	His-RGA-F: ATAGGATCCATGAAGAGAGATCATCACCAATTCCA His-RGA-R: ATAGTCGACTCAGTACGCCGCCGTCGAG
Identification of mutants	*rga-t2*	906F：GCCGGAGCTATGAGAAAAGTGG DS3-2: CCGGTATATCCCGTTTTCG 2076R: AAGAATTTTAAACAAGTGAACG DS5-3: CGGTCGGTACGGGATTTTCC
	gai-t6	N6: TAGAAGTGGTAGTGG B34:ACGGTCGGTACGGGATTT DL6: TTGCAGCAGCAATAACAGAGTCTAGC DL71: CGTTACCGACCGTTTTCATCCCTA
	rgl1-1	1670F: AAGCTAGCTCGAAACCCCAAAT Ds5-P1: CATGGGCTGGGCCTCAGTG DS3-2: CCGGTATATCCCGTTTTCG 2295R: CCACAGAGCGCGTAGAGGATAAC

	rgl2-1	856F: GCTGGTGAAACGCGTGGGAACA DS3-2: CCGGTATATCCCGTTTTCG 1883R: ACGCCGAGGTTGTGATGAGTG DS5-3: CGGTCGGTACGGGATTTTCC