Methods S1. Supplemental Materials and Methods

Gallego-Giraldo, G., Hu, J., Urbez, C., Gomez, M.A., Sun, T-P., and Perez-Amador, M.A. Role of the gibberellin receptors GID1 during fruit-set in Arabidopsis.

Generation of DELLA-GUS gene fusion constructs

All plasmid constructs containing PCR-amplified DNA inserts were sequenced to confirm that no mutations were introduced by PCR. Oligos used are listed in Supplemental Table S2.

The making of pRGA:GUS (pRG127) was basically through introducing extra restriction digestion sites at both ends of *RGA* coding sequence in a large *RGA* genomic fragment, followed by replacing *RGA* coding region with *GUS* gene by directional cloning. First, a 4-kb *Sal*I-*Sac*I DNA fragment spanning the *RGA* locus from pRG108 (1) was inserted into pUC18 to make pRG122. A *Kpn*I site had been generated in front of *RGA* coding sequence in this DNA fragment (1). Then, PCR-based "overlap extension" mutagenesis (2) was performed to generate a *Bam*HI site at the end of *RGA* coding sequence and to remove the *RGA* stop codon in pRG102, which contains 4-kb *Sal*I-*Sac*I DNA fragment of the *RGA* locus in pUC18 (1). In detail, the first round of PCR used two sets of primers: universal M13-20 forward primer plus primer 229, and M13 reverse primer plus primer 230. The second round of PCR used the universal M13-20 forward and reverse primers. The resulting 4-kb PCR DNA was cut with *Sal*I and *Sac*I and ligated with the *Sal*I- and *Sac*I-digested vector pHXKa (pHXK with the *Bam*HI site removed) to generate pRG106. Note that the stop codon of RGA was destroyed in this construct. Next, using a native *Xho*I site in *RGA* coding sequence, an *Xho*I-*Sac*I fragment from pRG106 was inserted into pRG122 to create pRG123, which has *Kpn*I and *Bam*HI sites at 5'- and 3'- end of RGA coding sequence, respectively. A 1.8kb full-length *GUS* gene fragment was then amplified by primers GUS6 and GUS9 from pUC18GUS (3), and replaced *RGA* coding sequence in pRG123 at *Kpn*I and *Bam*HI sites, resulting in pRG124. The 4-kb SalI-SacI fragment containing *GUS* gene from pRG124 was then used to replace the original SalI-SacI fragment in pRG103 (1), which contains 15-kb *Avr*II genomic DNA fragment around the *RGA* locus in a cloning vector. The resulting pRG126 plasmid had the endogenous *RGA* coding region replaced by *GUS* gene. At last, the 15-kb *Avr*II genomic DNA from pRG126

was inserted into the *Xba*I site of binary vector pOCA28 to generate pRG127 plasmid which contains approximately 7.7 kb *RGA* promoter that is fused to *GUS* gene. Upon plant transformation, line RGA TC 1.3.1 was selected and used for GUS staining.

For making the pGAI:GUS plasmid, genomic DNA fragment for *GAI* was amplified by PCR from *Arabidopsis* BAC Clone F10B6. Primers (5'GAI5UTR*Xba*I and 3'GAI5UTR*Bam*HI) containing *Xba*I and *Bam*HI sites were used to amplify 4kb of the *GAI* promoter region for directional cloning into binary vector pBI101.1 upstream of the *GUS* reporter gene to generate pGAI:GUS. Upon plant transformation, line GAI TC 4.10.1 was selected and used for GUS staining.

To make the pRGL1:GUS plasmid, genomic DNA fragment for *RGL1* was amplified by PCR from *Arabidopsis* BAC Clone T27F4. Primers (5'RGL15UTR*Sal*I and 3'RGL15UTR*Bam*HI) containing *Sal*I and *Bam*HI sites were used to amplify 4kb of the *RGL1* promoter region for directional cloning into binary vector pBI101.1 upstream of the *GUS* reporter gene to generate pRGL1:GUS. Upon plant transformation, line RGL1 TC 1.1.2 was selected and used for GUS staining.

qRT-PCR analysis of GID1s and DELLAs

Oligos for cloning the cDNA fragments that were used to generate the standard curves for qRT-PCR data analysis are listed in the Table S3.

Supplemental references:

1.- Silverstone, A.L., Jung, H.-S., Dill, A., Kawaide, H., Kamiya, Y., and Sun, T.-p. (2001) Repressing a repressor: Gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. *Plant Cell* **13,** 1555–1566.

2.- Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* **1,** 51-59.

3.-Dewdney, J. (1993) Light regulation of glyceraldehydes-3-phosphate dehydrogenase genes of Arabidopsis thaliana. PhD thesis. Harvard University

Supplemental Figure Legends TPJ-00147-2014

Figure S1. *GID1* genes are differentially expressed in pistils and fruits (biological replica of Figure 1). (a) Time-course of *GID1* expression during unfertilized pistil and early fruit development. Expression is represented as the copies of cDNA per 10^3 copies of *UBQ10*. (b) *GID1* expression in ovules+funiculi (O+F) and valve (V) of hand-dissected unfertilized pistils. Expression was normalized to *UBQ10* and to the expression in the whole pistil at anthesis. Data are mean ± SD of a single representative experiment. dpa, days post-anthesis.

Figure S2. *DELLA* genes are differentially expressed in pistils and fruits (biological replica of Figure 3). (a) Time-course of *DELLA* expression during unfertilized pistil and early fruit development. Expression is represented as the copies of cDNA per 10^5 copies of *UBQ10*. (b) *DELLA* expression in ovules+funiculi (O+F) and valve (V) of hand-dissected unfertilized pistils. Expression was normalized to *UBQ10* and to the expression in the whole pistil at anthesis. Data are mean ± SD of a single representative experiment. dpa, day post-anthesis.

Figure S3. Fertility and fruit length in self-pollinated double *gid1* null mutants. Mature fruits from self-pollinated pistils were individually harvested at 10 dpa, and fruit length and seed number were measured. Ratio (seed number vs. length) was determined. Fruit length, seed number, and ratio were normalized to data from self-pollinated WT. Mean and SD were calculated from at least 50 pistils/fruits per treatment, and are shown as the percentage relative to WT. The experiment was repeated three times with similar

results. Significant differences (Student's t-test analysis) between the WT and mutants are marked with asterisk (**, p-value <0.001).

Figure S4. GID1 expression in double *gid1* null mutants. *GID1* expression was detected in double *gid1* null mutants in pistils at anthesis (a) and in 4-day old seedlings (b). Expression for each gene was normalized to *UBQ10* and to its expression in the WT. na, not assayed. Data are mean ± SEM of three biological replicas.

Figure S5. Correlation between fruit length and seed number of the double *gid1* null mutants. Flowers were emasculated one day before anthesis and hand pollinated at anthesis with different amounts of WT pollen to vary seed-set. Few flowers were also kept unpollinated. Mature fruits at 10 dpa were individually harvested, and fruit length and seed number were measured. Seed number was plotted against fruit length and correlation coefficient (R2) and adjusted regression line were calculated. More than 60 fruits and pistils were used for each genotype. The experiment was repeated twice with similar results.

Figure S6. Expression of GA20ox1-GUS and GA20ox2-GUS at 1 dpa is associated with pollen and pollen tube. Reciprocal crosses between WT and transgenic Arabidopsis carrying translational fusion constructs (a) *pGA20ox1*:*GA20ox1-GUS* (Hay *et al.,* 2002) and (b) *pGA20ox2*:*GA20ox2-GUS* (Frigerio *et al.,* 2006) were performed and siliques at 1 dpa were analyzed by X-GlcA staining. Scale bar is 500 µm.

Table S1. Primers for qRT-PCR of GID1s and DELLAs and genotyping *gid1* mutants.

Table S2. Primers for generating *DELLA-GUS* gene fusions.

Table S3. Primers for cloning *GID1s* and *DELLA* genes for qRT-PCR.

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Figure S1
Gallego-Giraldo et al.

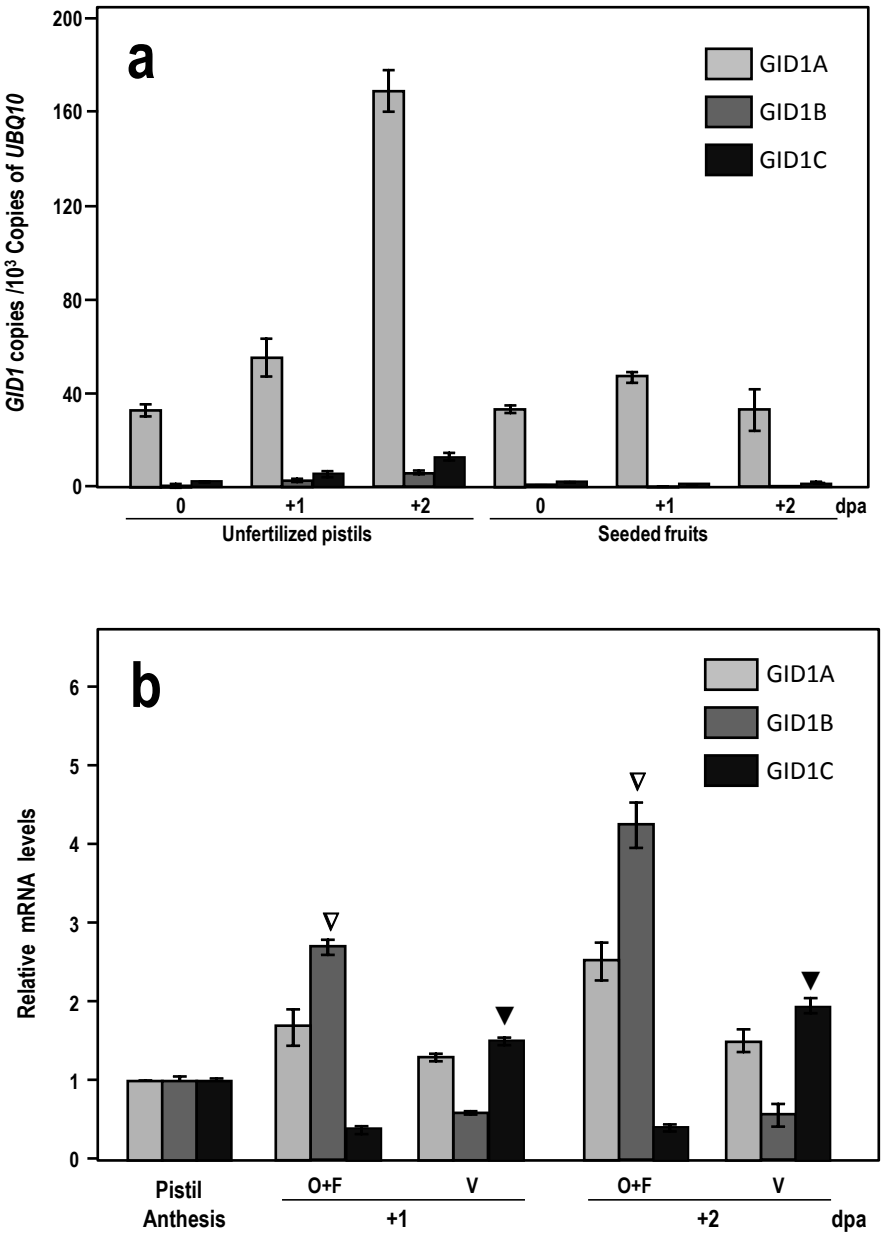

Figure S2
Gallego-Giraldo et al.

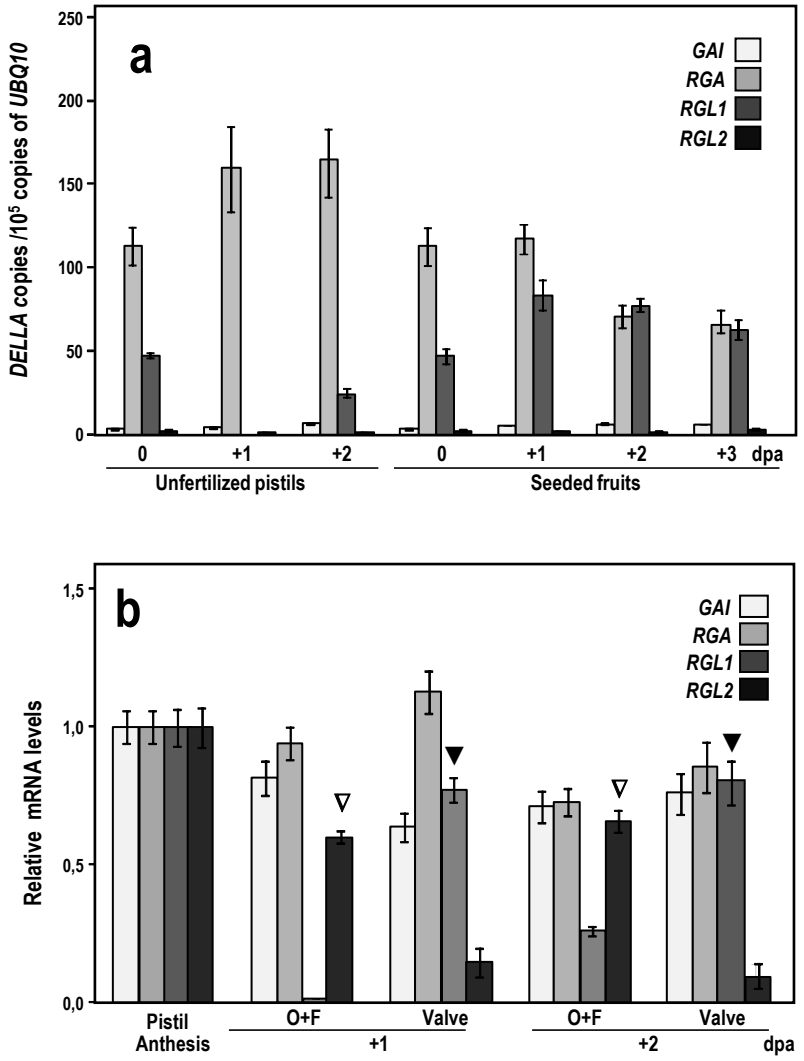

Figure S3
Gallego-Giraldo et al.

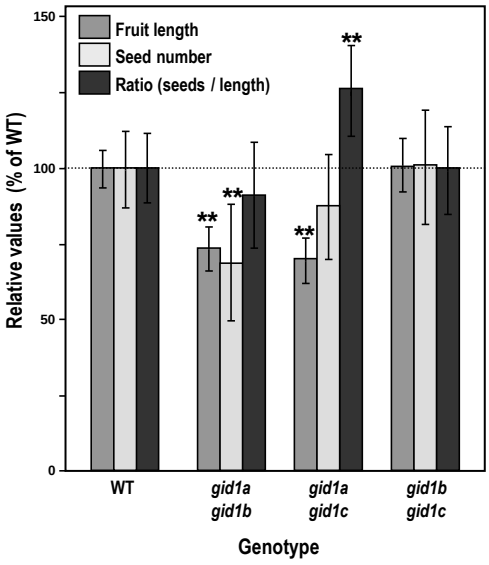

Figure S4
Gallego-Giraldo et al.

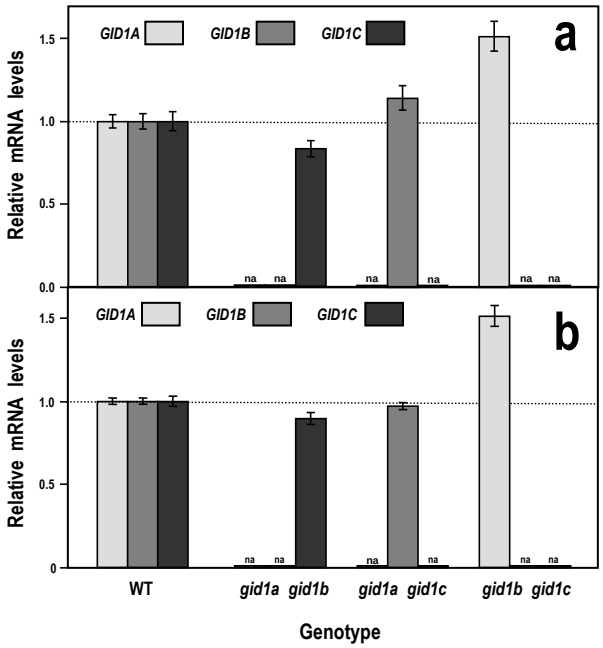

Figure S5
Gallego-Giraldo et al.

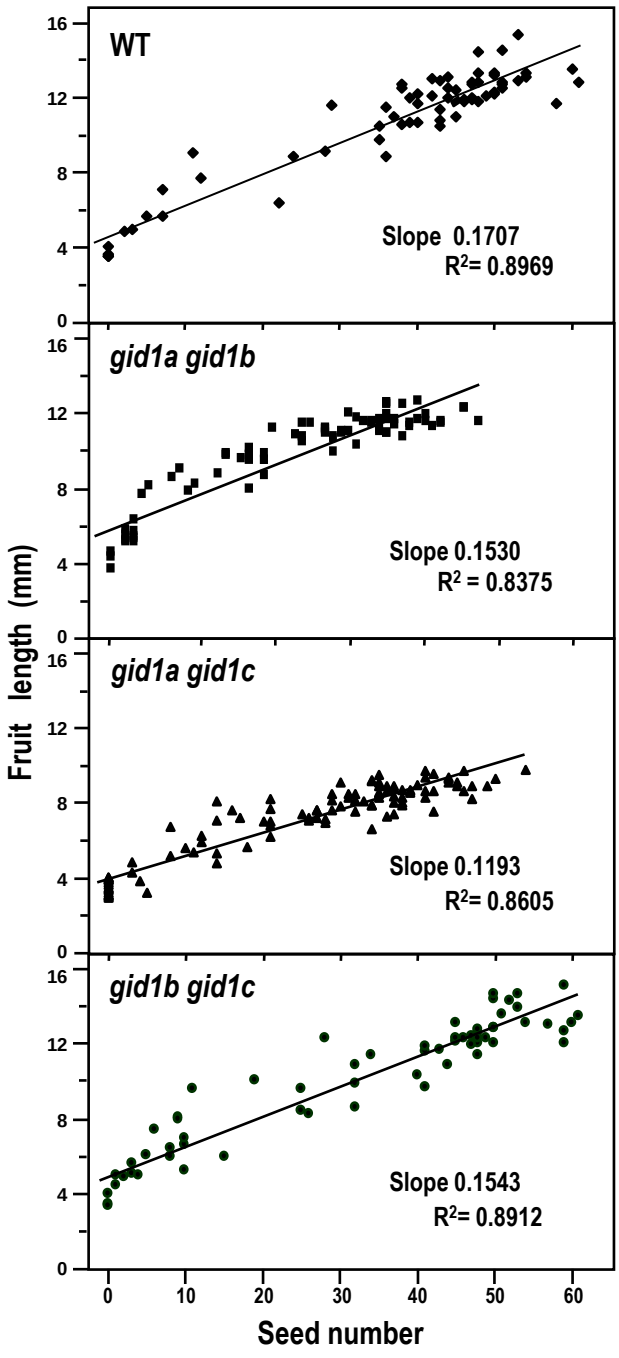

Figure S6
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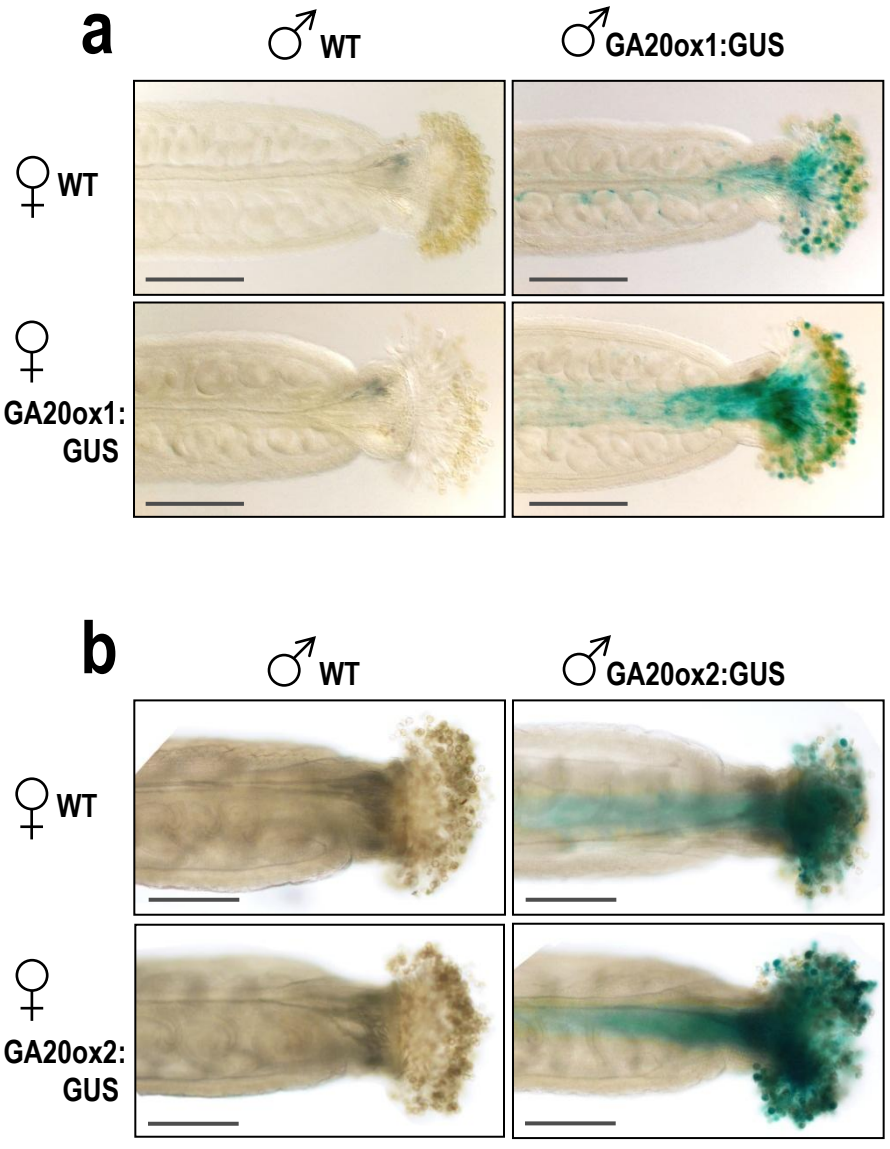

Table S1. Primers for qRT-PCR of GID1s and DELLAs and genotyping *gid1* mutants.

Gene	Code	Oligo	Oligo sequence	Reference
qPCR analysis				
GID1A	*At3g05120*	GID1A-qF	AAACGCTAAAGCTTGTGGAAGAA	This paper
		GID1A-qR	CCTTTCCAAGTCTCTAAACGCCT	This paper
GID1B	*At3g63010*	GID1B-qF	TTCTGTGAGGTCTTGGTTGCGC	This paper
		GID1B-qR	TGCCGATAACCGAAGTGGTCTCTC	This paper
GID1C	*At5g27320*	GID1C-qF	ACAAACGGGTGATATCCACCA	This paper
		GID1C-qR	AAAACGAGAATCTCGGGCTTTC	This paper
GAI	*At1g14920*	GAI-qF	CCTCCGTCGTCTAACGCC	This paper
		GAI-qR	GTTGACTCAGCCGTCGCTGTAG	This paper
RGA	*At2g01570*	RGA-qF	AGAAGCAATCCAGCAGA	This paper
		RGA-qR	GTGTACTCTCTTCTTACCTTC	This paper
RGL1	*At1g66350*	RGL1-qF	GAGTTCATCAATGACGACGGT	This paper
		RGL1-qR	GTACTCTGAGTCAGGCTT	This paper
RGL2	*At3g03450*	RGL2-qF	CACCAAAACCACTACCAGC	This paper
		RGL2-qR	CTATCCACACAACTTCGGG	This paper
UBQ10	*At4g05320*	UBQ10-qF	GGCCTTGTATAATCCCTGATGAATAAG	Czechowski *et al.,* 2005
		UBQ10-qR	AAAGAGATAACAGGAACGGAAACATAGT	Czechowski *et al.,* 2005
Genomic DNA genotyping*				
GID1A	*At3g05120*	GID1A-F	GGGTTAACTCGAGATCTTGGC	This paper
		GID1A-R	CGCCGAAATCTCATCCATAACA	This paper
		LBA1	TGGTTCACGTAGTGGGCCATCG	Griffiths *et al.,* 2006
GID1B	*At3g63010*	GID1B-F	TCTCCTGTCCACCAAACATTG	Griffiths *et al.,* 2006
		GID1B-R	CTGGGTTTTGGAGACTATGGC	Griffiths *et al.,* 2006
		SALT-3	CTTATTTCAGTAAGAGTGTGGGGTTTTGG	Griffiths *et al.,* 2006
GID1C	*At5g27320*	GID1C-F	ACCACCACCTCTTATTAGCTC	This paper
		GID1C-R	CAGGGGAACTATACACAAACACAAC	This paper
		LBA1	TGGTTCACGTAGTGGGCCATCG	Griffiths *et al.,* 2006

*, For genotyping the wt locus, the forward oligo was combined with corresponding reverse oligo. For genotyping the mutant allele, LBA1 (*gid1a-1* and *gid1c-1*) or SALT3 (*gid1b-1*) oligos were combined with the corresponding reverse oligo.

Table S2. Primers for generating *DELLA-GUS* gene fusions.

Gene	Oligo name	Oligo sequence	Purpose
GAI	5'GAI5UTRXbaI	5′-AAGATTCTAGAAGAAGTAATAAAATGTAAATTC	GAI-GUS constructs
	3'GAI5UTRBamHI	5′-TCTCTGGATCCGGTTGGTTTTTTTTCAG	
RGA	229	5′-GGATCCAGTACGCCGCCGTCGA	RGA-GUS constructs
	230	5′-GCGGCGTACTGGATCCGACTCGAA	
RGL1	5'RGL15UTRSalI	5′-TAGGAGTCGACATTAACGATATTATTATCG	RGL1-GUS constructs
	3'RGL15UTRBamHI	5′-TCTCTGGATCCTAAAATTGATTTGCAAGA	
GUS	GUS6	5′-ATGCGGTACCTGATCCATGGTACGTCCTGTAGA	GUS gene amplification
	GUS8	5′-TGCGGATCCAGGGAGGTTCCATGGTACGTCCTGTA	
	GUS9	5′- ATGCGGATCCTCATTGTTTGCCTCCCTGCTG	

Table S3. Primers for cloning *GID1s* and *DELLA* genes for qRT-PCR.

Gene	Oligo name	Oligo sequence	Purpose
GID1A	GID1A-Fw	5′- GTTAACACTGGGTTAGAGAAAG	GID1A cDNA cloning
	GID1A-Rv	5′- GTAAAATCCATTAGAGTACAATG	
GID1B	GID1B-Fw	5′- GAATAAGTTTGTGCACTCCATAG	GID1B cDNA cloning
	GID1B-Rv	5′- GGAAGTGAGAAACTACAACCAC	
GID1C	GID1C-Fw	5′- CCAATGAGAACACTCTTATC	GID1C cDNA cloning
	GID1C-Rv	5′- GAAAATAAGATCACTTTTAGG	
GAI	GAI-Fw	5′- TAAGCAGTCCTAACCGATCCC	GAI cDNA cloning
	GAI-Rv	5′- TAAATCCGCCGCGCGAGAG	
RGA	RGA-Fw	5′- CTTCATCATCCCCAAACACAC	RGA cDNA cloning
	RGA-Rv	5′- TCGATTCAGTTCGGTTTAGGTC	
RGL1	RGL1-Fw	5′- CAATTATTATGACACTCCCGTG	RGL1 cDNA cloning
	RGL1-Rv	5′- CCTCAAGTATCGCTTGATTC	
RGL2	RGL2-Fw	5′- CCTTACCAACCCATGAAGTAAAG	RGL2 cDNA cloning
	RGL2-Rv	5′- CTCAAAGATACGCACAAGGTCC	
UBQ10	UBQ10-Fw	5′- ACCATCACCCTTGAAGTGGAAAG	UBQ10 cDNA cloning
	UBQ10-Rv	5′- AAAAGATTAACATGTTCAAGCCATCCT	