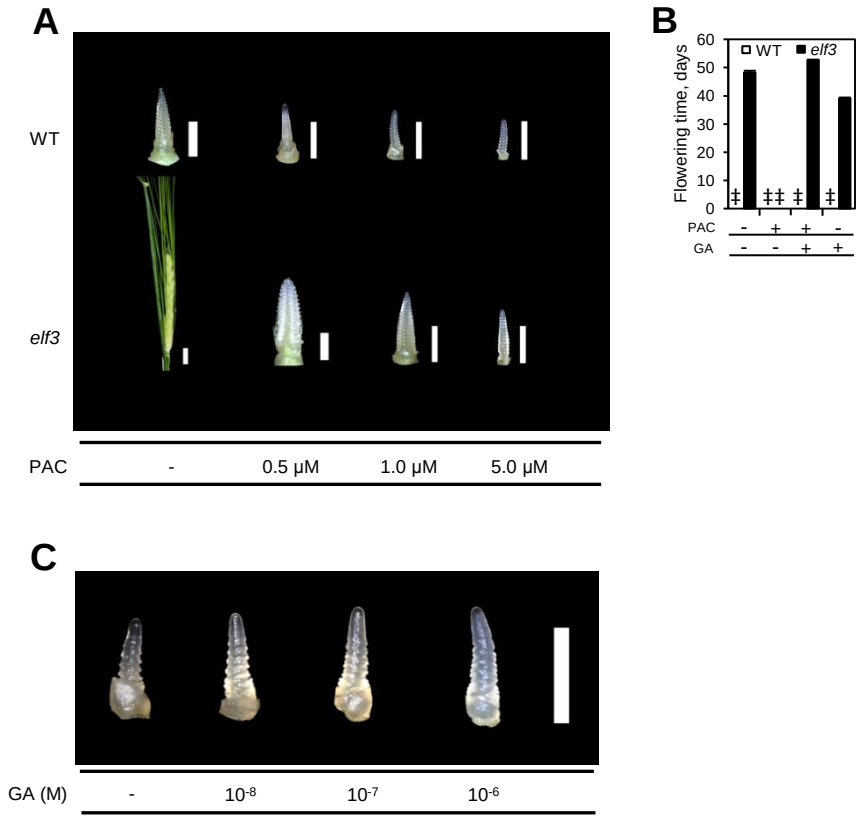
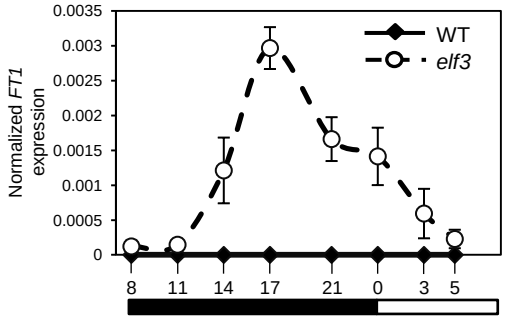
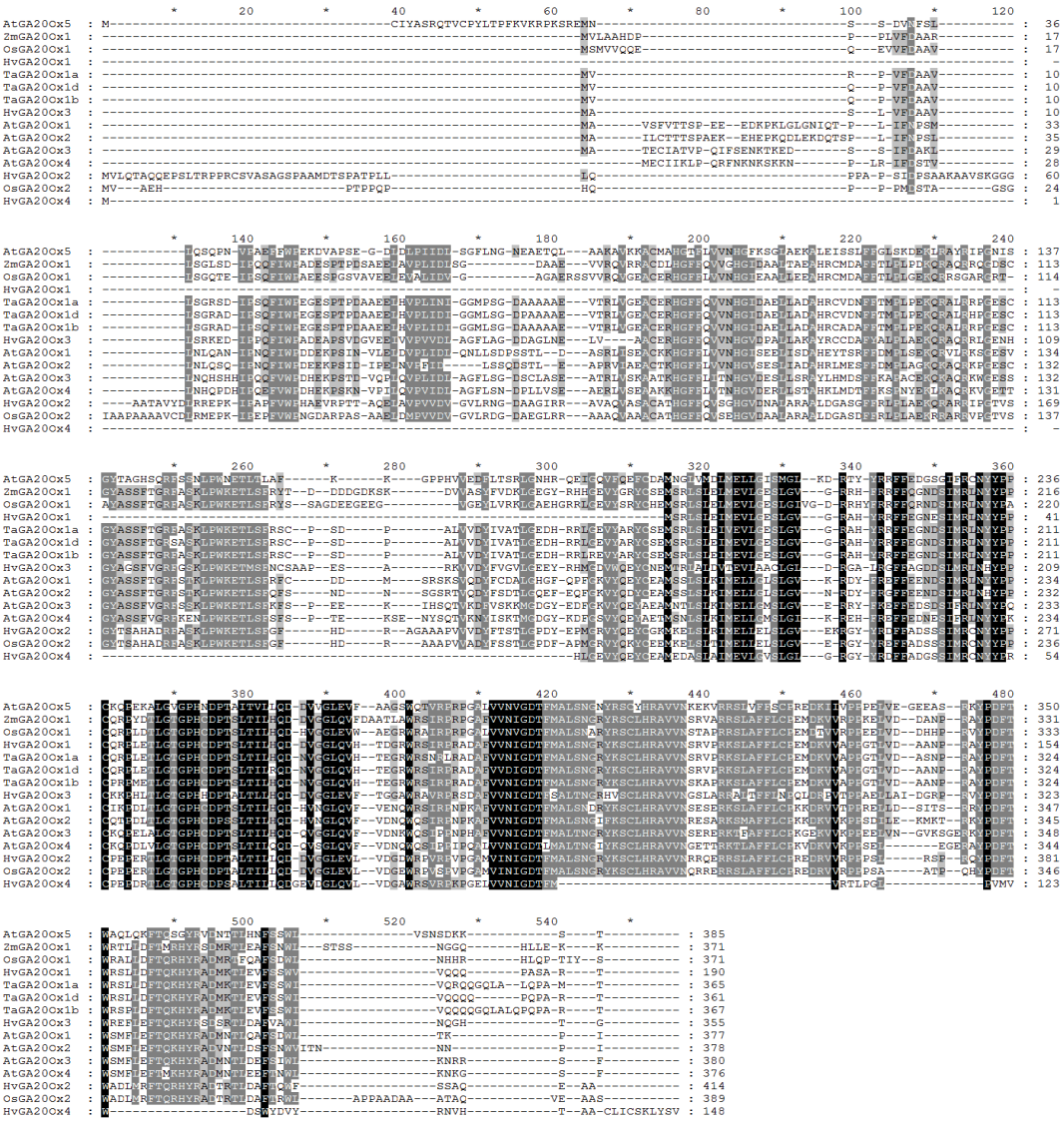
Supplemental Figures



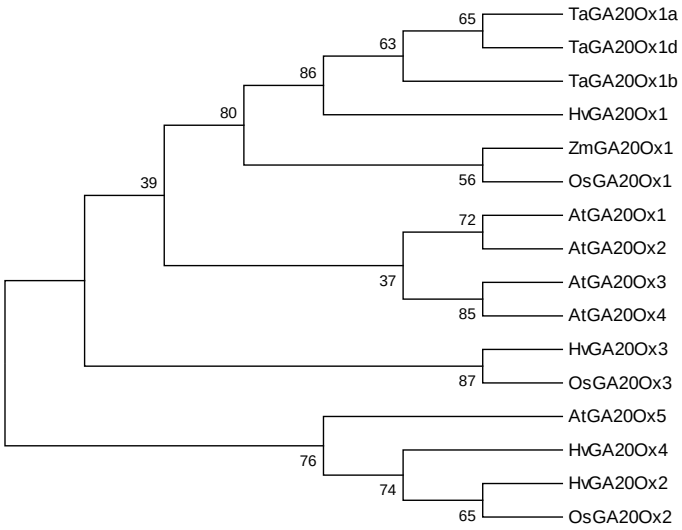
Supplemental Figure 1: Inhibition of GA biosynthesis by PAC treatment delays flowering and inflorescence development of *elf3* plants. (**A**) Inflorescence development of WT and *elf3* plants under SD conditions following treatment with varying amounts of PAC (0.5 µM, 1 µM and 5 µM). The images of inflorescences were taken on the day when the spike of the *elf3* control plant emerged from the boot. Scales: immature inflorescences, 1 mm; mature spikes, 1 cm (**B**) Flowering time of WT and *elf3* plants under SD conditions following treatment with PAC (1 µM), PAC (1 µM) and GA (10^{-8} M) or GA (10^{-8} M) (‡, plants did not flower). Data are the mean ± SEM of 8 biological replicates. (**C**) Inflorescence development of WT (Bonus) from control and GA-treated plants that were harvested at the fifth leaf stage. Scale, 1mm.



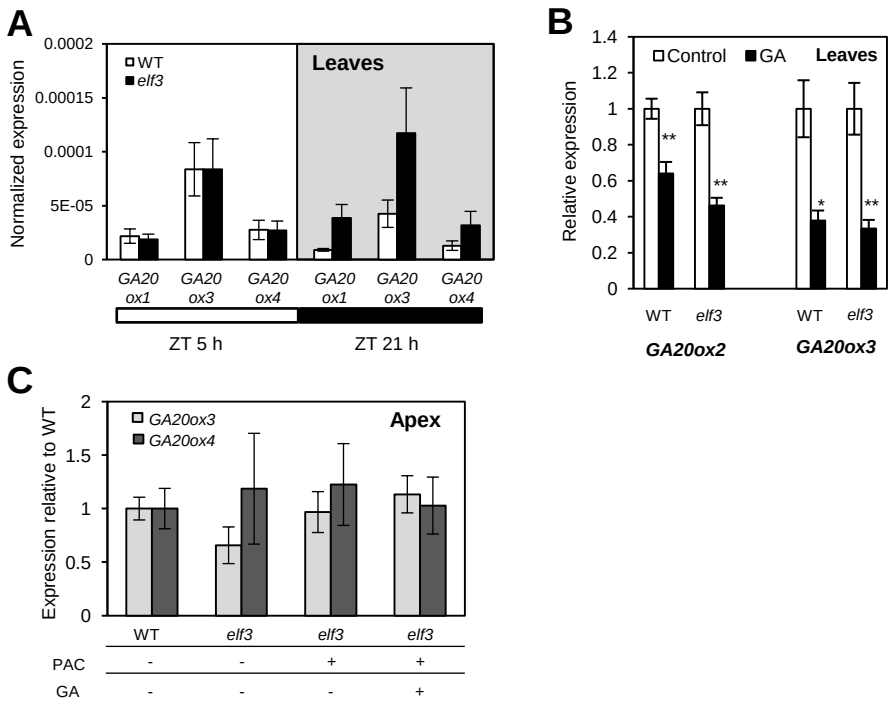
Supplemental Figure 2. *FT1* expression is elevated in the *elf3* mutant, *mat.a-8*. *FT1* expression is elevated in *elf3* leaves from plants grown under SD conditions, especially during the night-time phase of the diurnal cycle. Numbers on x-axis refer to time (hr) within 24 h cycle, with 0 h being dawn (lights on). Black and white rectangles illustrate periods of dark and light, respectively. Data are the mean ± SEM of 3 biological replicates. Transcripts are normalized to *HvGAPDH*.



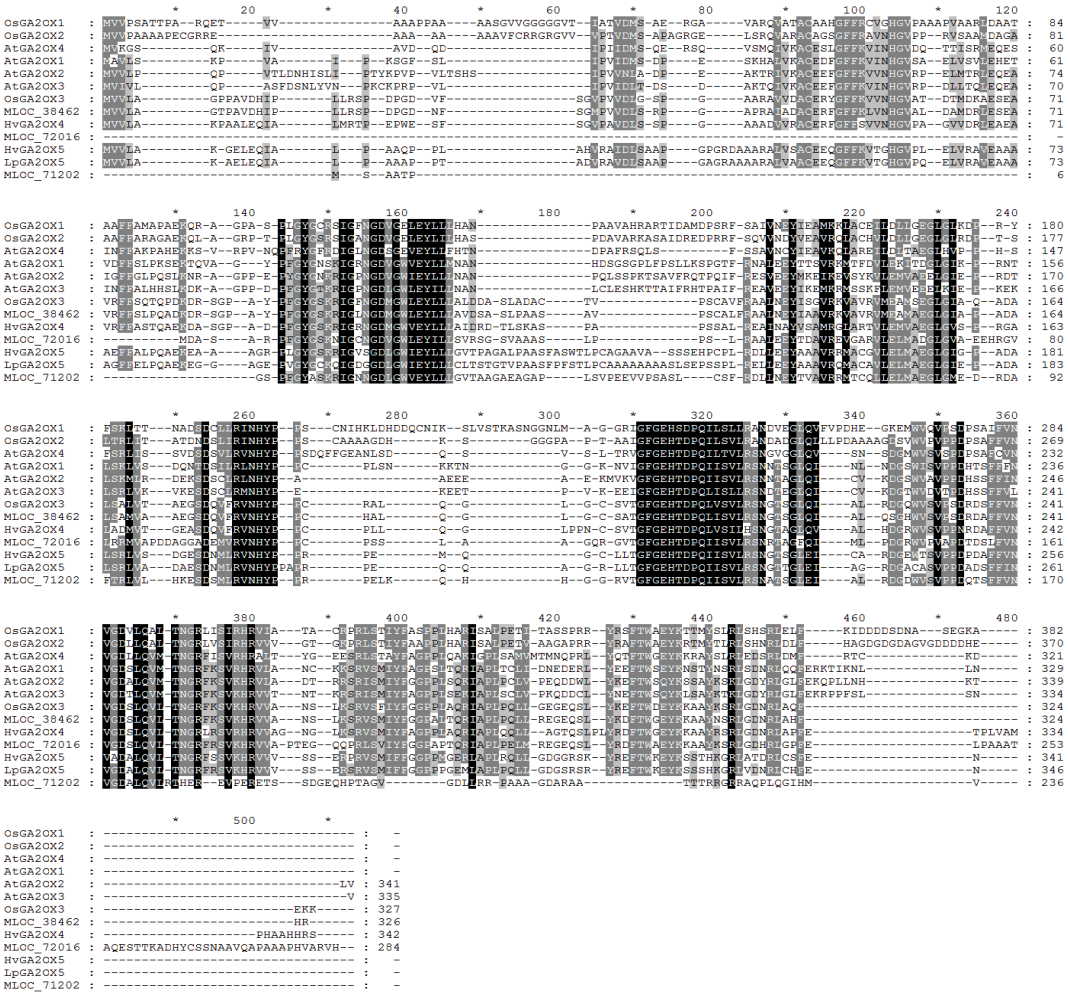
Supplemental Figure 3. Alignments of GA20oxidase amino acid sequences. Species include: *Arabidopsis thaliana* (At), *Zea mays* (Zm), *Hordeum vulgare* (Hv), *Triticum aestivum* (Ta) and *Oryza sativa* (Os). Alignments were generated with webPRANK and manually adjusted using Genedoc (Nicholas et al., 1997). Shading levels indicate degree of conservation: black - 100%, dark grey - 80%, light grey - 60%. Sequence details are given in Supplemental Table 3.



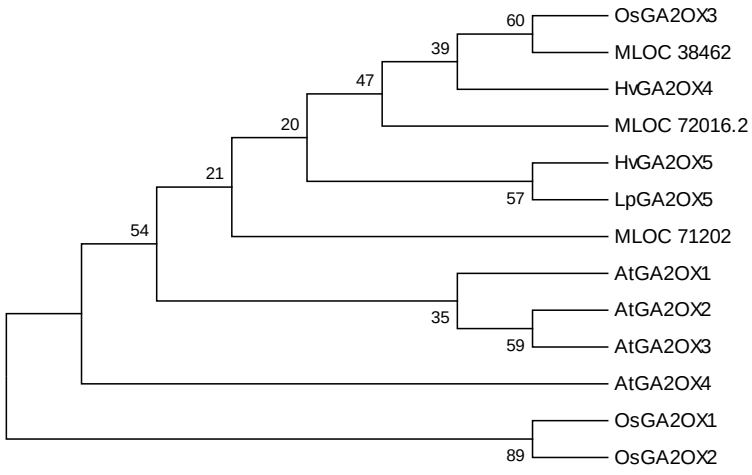
Supplemental Figure 4: Maximum Likelihood phylogenetic tree of GA20oxidases. Species include: *Arabidopsis thaliana* (At), *Zea mays* (Zm), *Hordeum vulgare* (Hv), *Triticum aestivum* (Ta), and *Oryza sativa* (Os). The unrooted tree, constructed in MEGA5 with 1000 bootstrap replicates, summarizes the evolutionary relationship among the GA20ox proteins. Parameters used for construction of tree included: JTT substitution model, Gamma distributed rates and partial deletions.



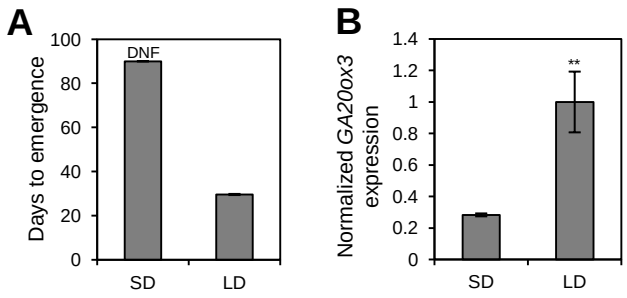
Supplemental Figure 5. *GA20oxidase* expression analysis. (**A**) Quantification of transcript levels for *GA20ox1*, *GA20ox3* and *GA20ox4* shows elevated transcript levels during the night-time (ZT 21 h) but not during the day (ZT 5 h). Black and white rectangles illustrate periods of dark and light, respectively. Data are the mean ± SEM of 3 biological replicates. (**B**) Quantification of *GA20ox2* and *GA20ox3* expression in leaves of control and GA_3 treated (10^{-4} M) WT and *elf3* plants. Data are the mean ± SEM of 3 biological replicates. (*, $P < 0.05$; **, $P < 0.01$). (**C**) Quantification of *GA20ox3* and *GA20ox4* in the developing apex of WT and *elf3* plants, and also in *elf3* plants that have been treated with PAC (1 µM) or PAC (1 µM) + GA_3 (10^{-7} M). *GA20ox1* transcripts were not detected. Data are the mean ± SEM of 3 biological replicates, each containing 6 developing inflorescences. All data are from plants grown under SDs.



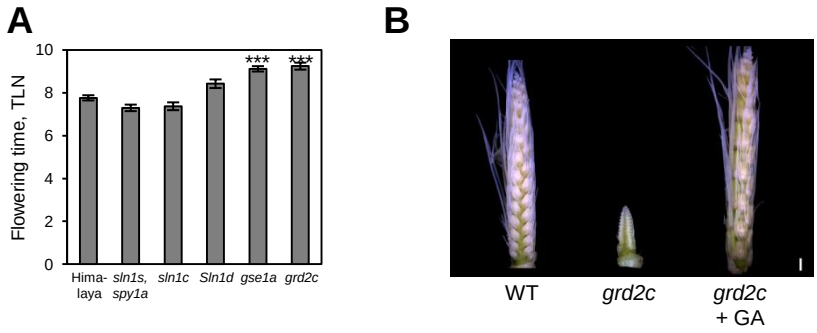
Supplemental Figure 6. Alignments of GA2oxidase amino acid sequences. Species include: *Arabidopsis thaliana* (At), *Hordeum vulgare* (Hv and MLOC), *Lolium perenne* (Lp) and *Oryza sativa* (Os). Alignments were generated with webPRANK and manually adjusted using Genedoc (Nicholas et al., 1997). Shading levels indicate degree of conservation: black - 100%, dark grey - 80%, light grey - 60%. Sequence details are given in Supplemental Table 3.



Supplemental Figure 7: Maximum Likelihood phylogenetic tree of GA2oxidases. Species include: *Arabidopsis thaliana* (At), *Hordeum vulgare* (Hv, MLOC acession numbers), *Oryza sativa* (Os) and *Lolium perenne* (Lp). The unrooted tree, constructed in MEGA5 with 1000 bootstrap replicates, summarizes the evolutionary relationship among the GA2ox proteins. HvGA2ox3 (MLOC 38462; this study), *HvGA2ox4* and *HvGA2ox5* (Spielmeyer et al., 2004) were named based on their similarity to rice genes. Parameters used for construction of tree included: JTT substitution model, Gamma distributed rates and partial deletions.



Supplemental Figure 8. Flowering time and induction of *GA20ox3* in photoperiod responsive spring barley grown under SD and LD photoperiods. (**A**) Quantification of flowering time for photoperiod responsive spring barley (CSIRO B07) grown under short day (SD) and long day (LD) photoperiods. SD grown plants did not flower during the course of the experiment (90 days; DNF). Data are the mean ± SEM of 14 biological replicates. (**B**) *GA20ox3* expression in photoperiod responsive spring barley increases under LD conditions. This data is from RNA extracted from leaf samples at the fourth leaf stage, harvested at ZT 16 h. Data are the mean ± SEM of 3 biological replicates (**, $P < 0.01$).



Supplemental Figure 9. Flowering of GA biosynthesis and signaling mutants. (**A**) Flowering time phenotypes of WT (Himalaya) and mutant plants with constitutive GA responses (*Sln1s*, *spy1a* and *sln1c*) or compromised GA responses (*Sln1d*, *gse1a* and *grd2c*), grown under LDs (TLN, total leaf number). Data are the mean ± SEM of 10 biological replicates (***, $P < 0.001$). (**B**) Inflorescence development in the *grd2c* mutant is restored by application of GA_3 (10^{-4} M). Inflorescences were viewed at the sixth leaf stage. Scale: 1 mm.

Supplemental Tables

Supplemental Table 1. Measurements of GA_{19}, GA_{20}, GA_1 and GA_8 levels from WT and *elf3* plants.

	WT	*elf3*	*t-test*
GA_{19}	69.9 (5.96)	91.4 (4.84)	$P < 0.05$
GA_{20}	7.77 (1.19)	25.9 (0.63)	$P < 0.001$
GA_1	2.6 (0.59)	13.8 (1.05)	$P < 0.001$
GA_8	26.95 (2.91)	52.3 (2.63)	$P < 0.001$

The units for these measurements are ng g^{-1} of dry weight. Values given are the mean of four biological replicates and the standard errors are written in parentheses. Leaf tissue was harvested from plants at the fourth leaf stage at ZT 0 h.

Supplemental Table 2. Oligonucleotide sequences used in qRT-PCR assays

Gene	Orientation	Sequence	Source
HvFT1	Sense	ATGAGGACCTTCTACACGCT	Hemming et al., 2012
EU331775.1	Anti-sense	GGCTCTCGTACCACATCACC	
HvVRN1	Sense	GGAAACTGAAGGCGAAGGTTGA	Greenup et al., 2010
AY785826.1	Anti-Sense	TGGTTCTTCCTGGCTCTGATATGTT	
HvGA20 ox.1	Sense	GGTACAAGAGCTGCCTCCAC	This paper
MLOC_16059	Anti-sense	CACCACCTTGTCCATCTCG	
HvGA20 ox.2	Sense	CTACGAGCCAATGGGGAG	This paper
MLOC_56462	Anti-Sense	CCAGCAGCTCCATGATCCT	
HvGA20 ox.3	Sense	GGGGATGTGTGGCAGGAAT	This paper
MLOC_66389	Anti-sense	CAGCCGCATCAGGGAGTC	
HvGA20 ox.4	Sense	GGACGGCGAGGTGGAC	This paper
MLOC_34543	Anti-Sense	AAGGTGTCGCCGATGTTTAC	
HvLFY1	Sense	CCGCCTCTTCCTGCTCC	This paper
MLOC-14305	Anti-sense	GAACACCTGGTTCGTCACCT	
HvSOC1	Sense	CTCGTCGTCTTCTCCCCTC	This paper
JN673265	Anti-Sense	TTGCTGCAAATCTGGCTGTA	
HvPAP2	Sense	TGAAGAGAAAGGAGCAAATGTTAC	This paper
MLOC_64157	Anti-sense	GATCAGGTGTCGCCTCCAC	
HvFPF1-Like3	Sense	CGCTACTACGAGAACCGAGACATC	Hemming et al., 2012
BM377273	Anti-Sense	AAGTGGTGCCTGTTCTTGACG	
HvMADS8	Sense	AGCAGCAGCAACAACAACAAC	This paper
MLOC_61901	Anti-sense	CTTGGGGCTGGTTTTGAGTA	
HvMADS14	Sense	GGAAACTGAAGGCGAAGGT	This paper
MLOC_1852	Anti-Sense	CTGCTGCTCCAGTTGCTG	
HvSPL11	Sense	CGTCGTCATTGATGTTTGATG	This paper
MLOC_62426	Anti-Sense	CTTGAAGTCAGATGGGCTGTC	
HvSPL12	Sense	GGATGTCTACGATGTTTTATGATG	This paper
MLOC_11199	Anti-sense	TCCCATGAAGAACTTCCACAG	
HvSPL14	Sense	CAGCATGGTCACTTCTCAGG	This paper
MLOC_61297	Anti-Sense	TACAGGGACCAGTTGGACGA	
HvACTIN	Sense	GCCGTGCTTTCCCTCTATG	Hemming et al., 2012
AY145451.1	Anti-Sense	GCTTCTCCTTGATGTCCCTTA	
HvGAPDH	Sense	AGGCTATCAAGGCTGCTTCC	This paper
AB120301.1	Anti-Sense	ACCCCACTCGTTGTCATACC	
HvGA3ox1	Sense	GCACTACCGCCACTTCTCTG	This paper
AY551430.1	Anti-Sense	ACGAGGAACAGCTCCATCAG	
HvGA3ox2	Sense	GAAGCAGGTTTAACGCAAGA	This paper
AY551431.1	Anti-Sense	TCTCTCTTCGGGGTCTCTTC	
HvGA2ox3	Sense	GCAGGTGCTGACCAACG	This paper
MLOC_38462	Anti-Sense	GGTGCAATCCTCTGTGTCAA	
HvGA2ox4	Sense	GACTCCCTCCAGGTTCTGAC	This paper
AY551432.1	Anti-Sense	CGGCGAAGTAGATCATCG	
HvGA2ox5	Sense	CGAGGGTGTCCATGATCTTC	This paper
AY551433.1	Anti-Sense	TTGTGGGTGCTGCTCTTGTA	
MLOC_71202	Sense	GCCTAGAGATCGCCCTGC	This paper
	Anti-Sense	CTTTTTGACCATCATCCTGC	
MLOC_72016.2	Sense	CTCCCTCCAGGTGCTGAC	This paper
	Anti-Sense	GCCGAAGTAGATCACCGACA	

Genes from barley (*Hordeum vulgare*) with their oligonucleotide sequences as designed using public available sequences (GenBank or Ensembl Plant), which are provided below gene names.

Supplemental Table 3. Gene identifiers of the *GA20ox* and *GA2ox* genes in barley (*Hordeum vulgare*), Arabidopsis (*Arabidopsis thaliana*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), Maize (*Zea mays*) and *Lolium perenne* that were used for multiple sequence alignments and constryuction of phylogenetic trees.

Gene	Gene Locus ID
HvGA20ox1	MLOC_16059
HvGA20ox2	MLOC_56462
HvGA20ox3	MLOC_66389
AtGA20ox1	At4g25420
AtGA20ox2	At5g51810
AtGA20ox3	At5g07200
AtGA20ox4	At1g60980
AtGA20ox5	At1g44090
TaGA20ox1a	JF930282
TaGA20ox1b	FR716526
TaGA20ox1d	FR716527
ZmGA20ox1	NM_001254854
OsGA20ox1	U50333
OsGA20ox2	AY114310
HvGA2ox3	MLOC_38462
HvGA2ox4	AY551432.1
HvGA2ox5	AY551433.1
AtGA2ox1	At1g78440
AtGA2ox2	At1g30040
AtGA2ox3	At2g34555
AtGA2ox4	At1g47990
OsGA2ox1	AB059416
OsGA2ox2	AB092484
OsGA2ox3	AB092485
LpGA2ox5	EF687858

Supplemental Methods

GA feedback experiment

WT and *elf3* plants were grown on New Growool Propagating Blocks (Growool Horticultural Systems, New South Wales, Australia) under SD conditions. At the fourth-leaf stage, plants were treated with GA_3 (10^{-4} M) at ZT 0 h, or with a control mock treatment. After 3 h exposure to the treatments (ZT 3 h), the youngest emerging leaf was harvested for RNA extraction. Four biological replicates were harvested for each genotype under each treatment condition. Quantitative real-time PCR was performed as described in materials and methods, using oligonucleotides shown in Supplemental Table 2.

GA extraction and quantification

For each replicate, tissue was ground using liquid nitrogen and freeze-dried. The dry material was weighed and 25 mL of cold (-20 ^{o}C) 80% methanol was added, followed by deuterated internal standards ([$^{2}H_2$]GA_1, [$^{2}H_2$]GA_8, [$^{2}H_2$]GA_{19} and [$^{2}H_2$]GA_{20}). After extraction overnight (4 ^{o}C), samples were centrifuged at 4300 rpm for 15 min. Aliquots of the supernatant were then dried and purified using Sep-Pak C18 cartridges, loading the extract in 0.4% acetic acid in water, and eluting GAs with 60% methanol in 0.4% acetic acid. The eluate was dried and purified further as described previously (McAdam and Brodribb, 2013). The eluate was dried again and derivatised by adding 20 µL di-isopropyl ethylamine and 100 µL 500 mM 2-bromoethyl triethylammonium bromide in 85% acetonitrile, and heating at 80 ^{o}C for 2 h. After drying, re-suspension in 1% acetic acid and centrifugation (3 min, 13000 rpm), the supernatant was analysed by ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) using the instrument described previously (Tivendale et al., 2012).

The UPLC program was 1% acetic acid in water for 30 sec followed by a linear gradient to 15% acetonitrile at 2 min, then to 100% acetonitrile at 11 min and this was followed by immediate reequilibration to starting conditions for 3 min. The flow rate was 0.35 mL/min, the column was held at 35 °C, and the sample compartment was at 6 °C. Approximate retention times for derivatised GA_8, GA_1, GA_{19} and GA_{20} were 3.5, 4.2, 5.4 and 5.7 min, respectively.

The mass spectrometer was operated in positive ion electrospray mode with a needle voltage of 2.8 kV, and MRM was used to detect all analytes. For all GAs except GA_{19} a "pseudo MS^3" strategy was used, in which the protonated molecules underwent "in-source" fragmentation through the use of a relatively high cone voltage resulting in elimination of trimethylamine (59 Da) to create an intense ion 27 Da above the molecular weight of the GA. This ion was then selected in Q1 for MS/MS fragmentation. The cone voltage was 75 V in these cases. The cone voltage for GA_{19} was 45 V.

The ion source temperature was 130 °C, the desolvation gas was N_2 at 950 L h^{-1}, the cone gas flow was 100 L h^{-1}, and the desolvation temperature was 450 °C. Data were processed using MassLynx software. MRM transitions were monitored in two separate time windows, switching at 4.8 min to the GA_{19} and GA_{20} channels, with a dwell time of 36 msec per channel. The channels used for quantification were as follows, with the transition for endogenous GA given first followed by that for the corresponding deuterated GA: GA_8, 391.2 to 153.05 and 393.2 to 153.05 (collision energy (CE) 40 V); GA_1, 375.2 to 153.05 and 377.2 to 153.05 (CE 35 V); GA_{19}, 448.3 to 299.1 and 450.3 to 301.1 (CE 30 V). For endogenous GA_{20}, peak areas were summed for the transitions 359.2 to 179.1 (CE 35 V), 359.2 to 241.15 (CE 30 V), 359.2 to 269.15 (CE 25 V) and 359.2 to 313.2 (CE 25 V); and for deuterated GA_{20},

summed for the transitions 361.2 to 179.1, 361.2 to 243.15, 361.2 to 271.15 and 361.2 to 315.2.

GA application to the *grd2c* mutant

GA treatments were performed to *grd2c* mutant plants grown under LD conditions (in Conviron CMP6050 growth cabinets) using GA_3 at a concentration of 10^{-4} M in 95 % ethanol, which was applied to emerging leaf at the site of the ligule from the youngest fully emerged leaf. GA_3 was applied at three-day intervals from the third leaf stage until the sixth leaf stage. Control mock treatments were also performed in parallel. Images were taken of the inflorescence at the sixth leaf stage.

Supplemental References

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