Manuscript Title: Functional Characterization and Developmental Expression Profiling of Gibberellin Signaling Components in *Vitis vinifera*

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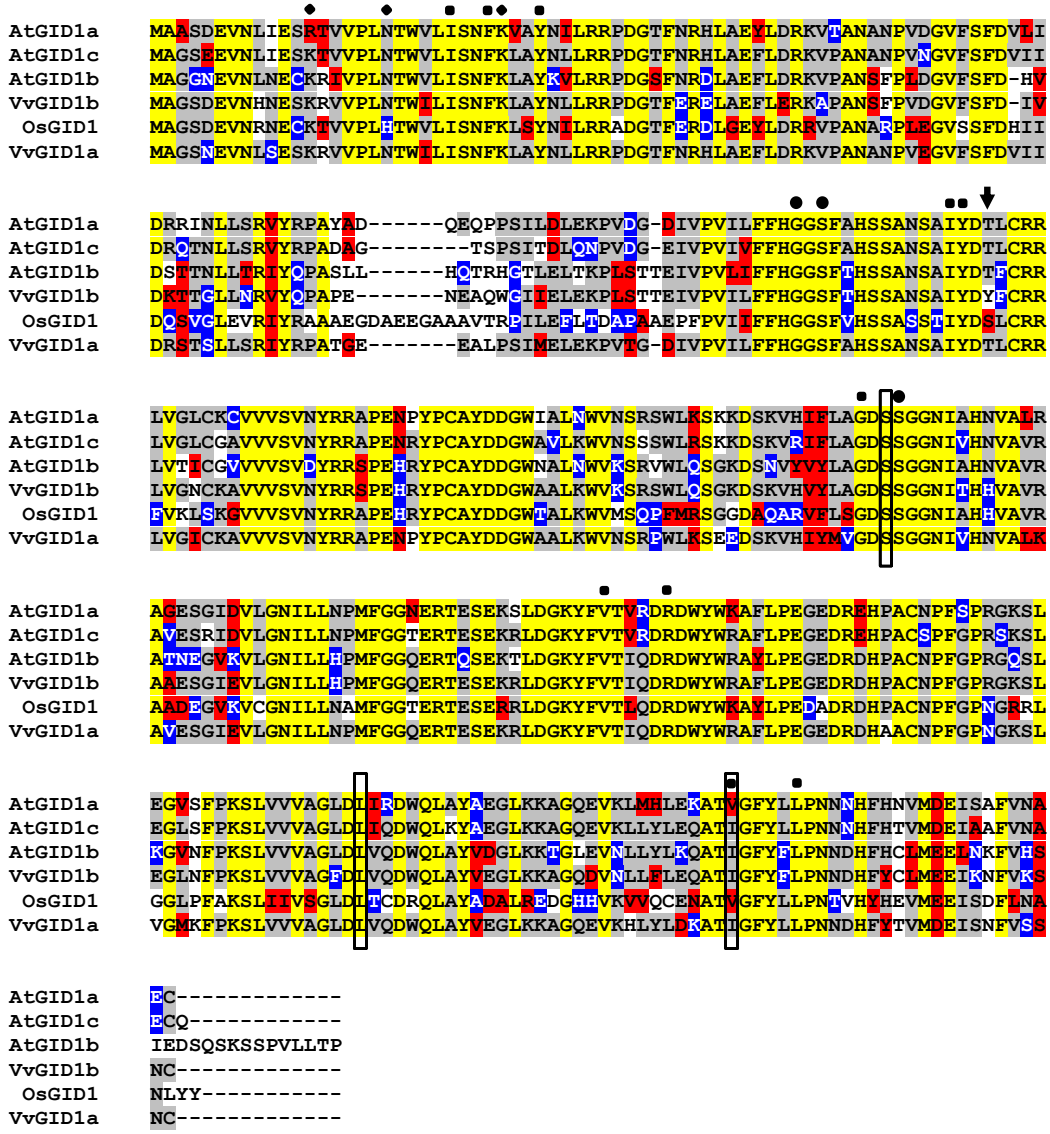
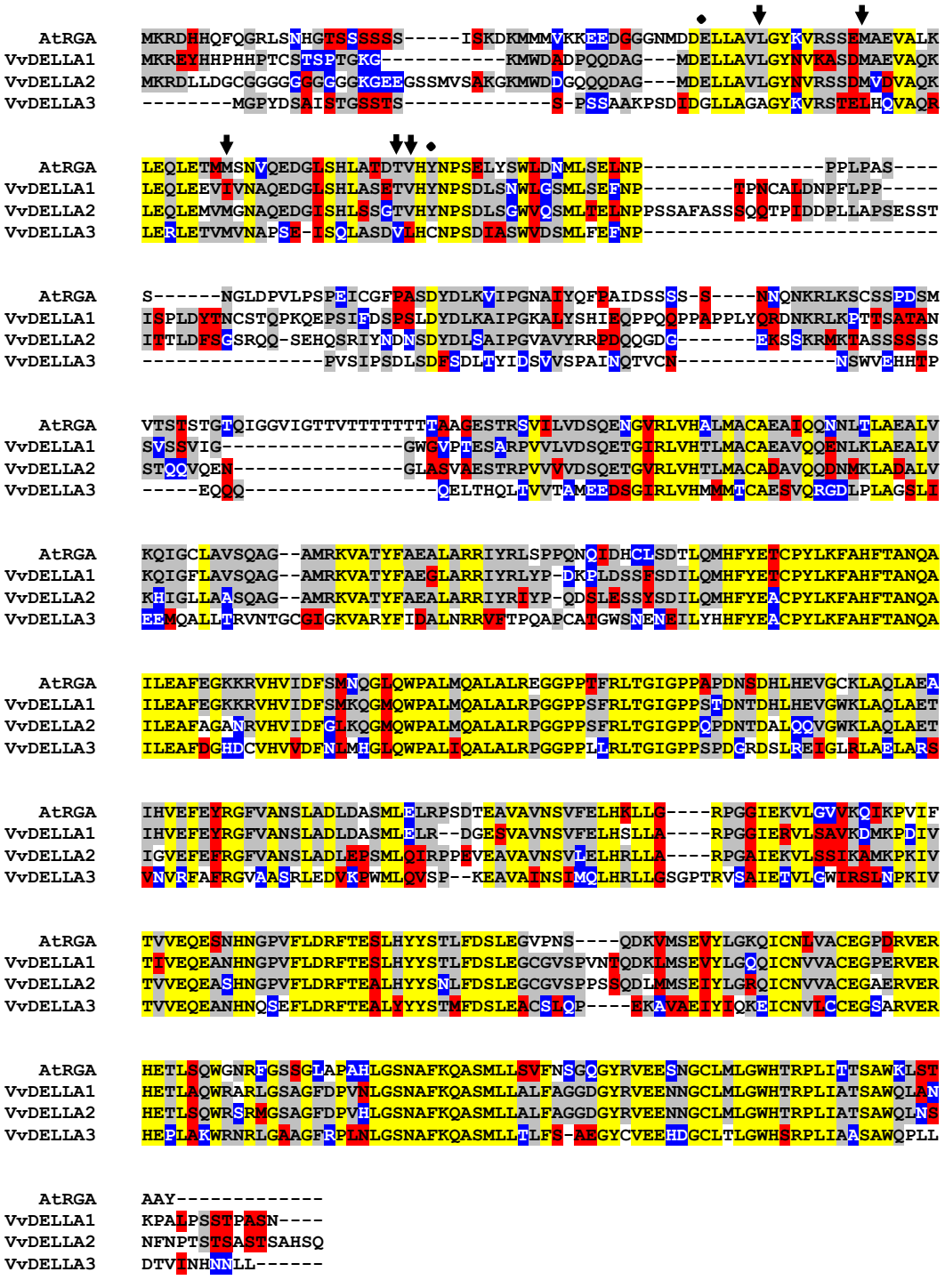
S1A



S1B



S1C

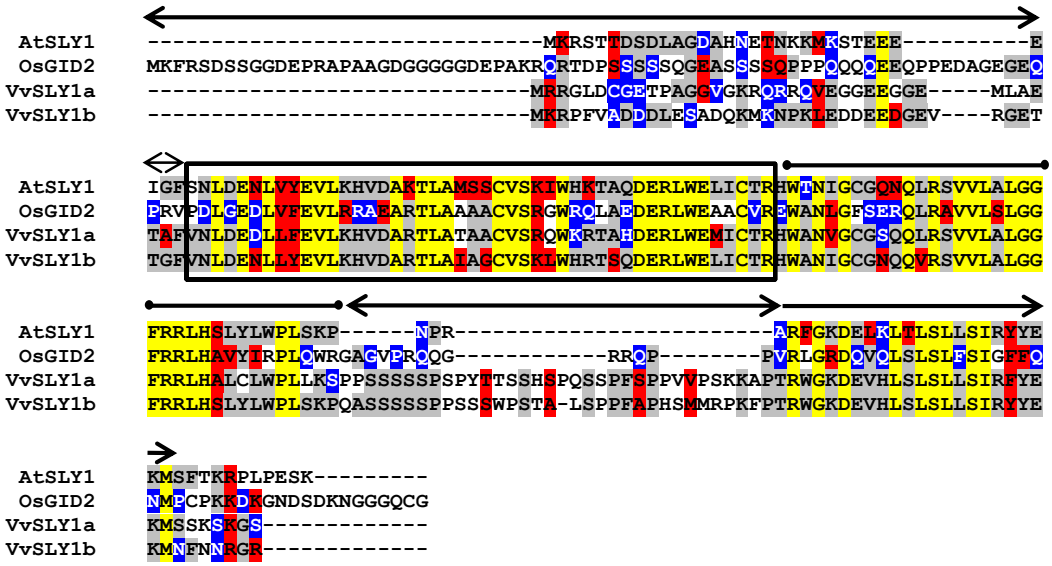


Figure S1: **Amino acid sequence alignment of major GA signaling genes.** Multiple alignment of amino acid sequences of Arabidopsis, rice and *V. vinifera* gene families were generated by CLUSTAL W alignment algorithm using AlignX of the Vector NTI suite. Yellow-shade: identical residues; Red-shade: similar residues; Blue-shade: weakly similar residues; Grey-shade: conservative residues; Unshaded: non-similar residues. For accession numbers of genes, refer to Material and Methods section. **(A)** VvGID1 paralogs (VvGID1a, VvGID1b), and orthologs from Arabidopsis (AtGID1a, AtGID1b, AtGID1c) and rice (OsGID1) share conserved domains characteristic of soluble GA receptor. Boxed: catalytic triad of Hormone Sensitive Lipases (HSL); Solid circles: Conserved residues in GID1s (the first two pair forming oxyanion with the last); Solid squares: residues essential for interacting with bioactive GA; Diamond-shaped: residues essential for GID1-DELLA interactions; Arrows: sequence variation between VvGID1a and VvGID1b in motif required for DELLA interaction. **(B)** Sequence alignment of VvDELLA paralogs [VvDELLA1 (previously characterized as VvGAI1), VvDELLA2, and VvDELLA3], and ortholog from Arabidopsis (RGA). Arrows: putative VvDELLA sequence variations in motifs required for GID1 interaction; Diamond-shaped: variations in residues required for DELLA protein stabilization. **(C)** VvSLY1 paralogs (VvSLY1a and VvSLY1b), and orthologs from Arabidopsis (AtSLY1) and rice (OsGID2). Double-headed arrows: variable regions; Boxed: F-box domain; Circle-edged lines: GGF domain; Single-headed arrows: LSL domain

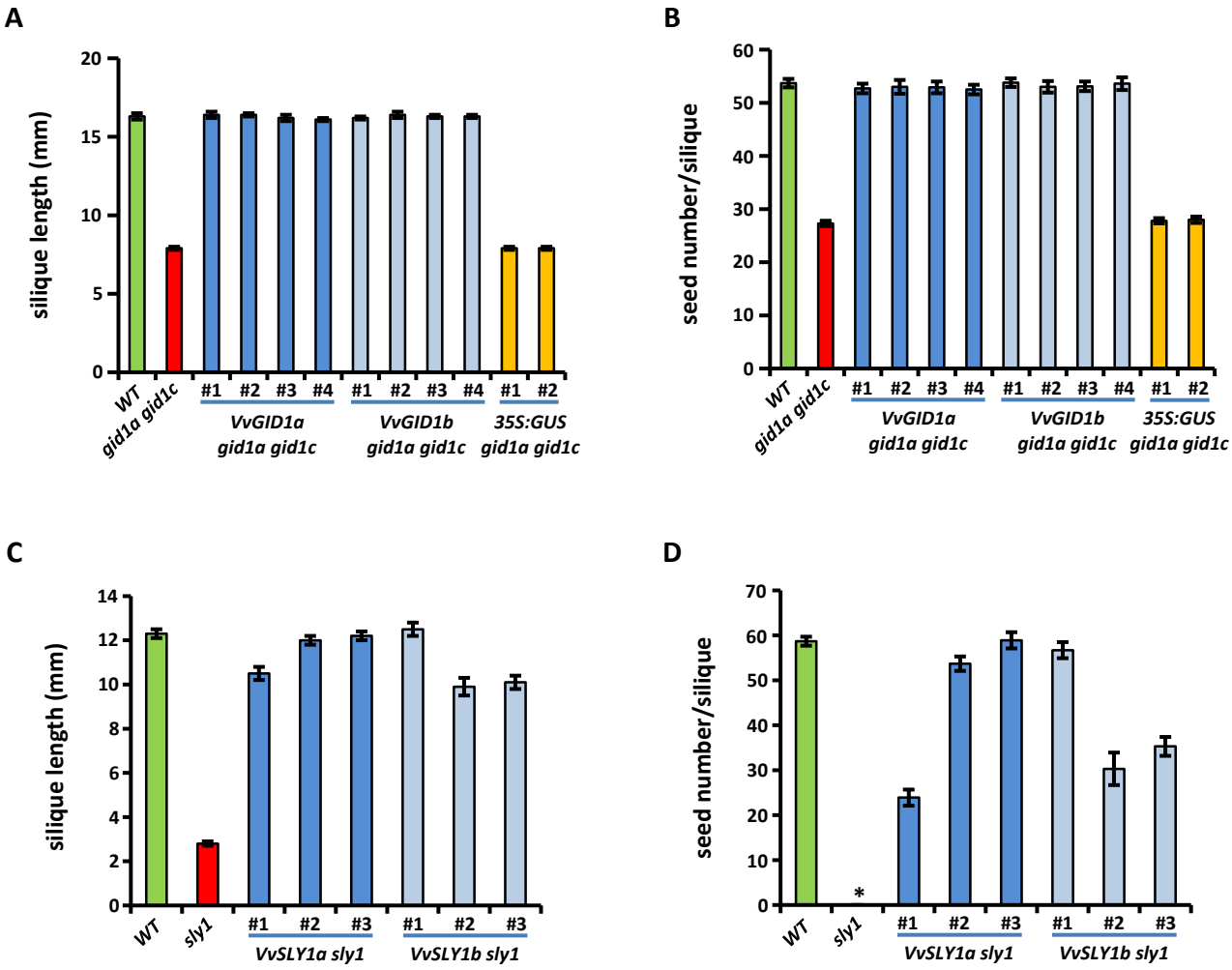


Figure S2 : **Grapevine GA signaling genes rescue silique length and fertility defects of corresponding Arabidopsis mutants. (A-B)** Average silique lengths (A) and seed numbers (B) of WT, *gid1a-2 gid1c-2*, and four representative *35S:VvGID1 gid1a gid1c* transgenic lines. *35S:GUS gid1a gid1c* transformants were included as negative control. **(C-D)** Average silique lengths (C) and seed numbers (D) of WT, *sly1-10*, and three representative *35S:VvSLY1 sly1-10* transgenic lines. In A-D, the length and seed number of 10th silique on the main stem were measured, and both were significantly different from the corresponding mutant ($n \geq 9$; $p < 0.01$). In contrast, the *35S:GUS gid1a gid1c* lines have no phenotypic difference from *gid1a gid1c* ($n \geq 10$). Parameters for VvGID1, VvSLY1 transformants were measured at 51 and 87 days, respectively. Asterisk: no viable seed.

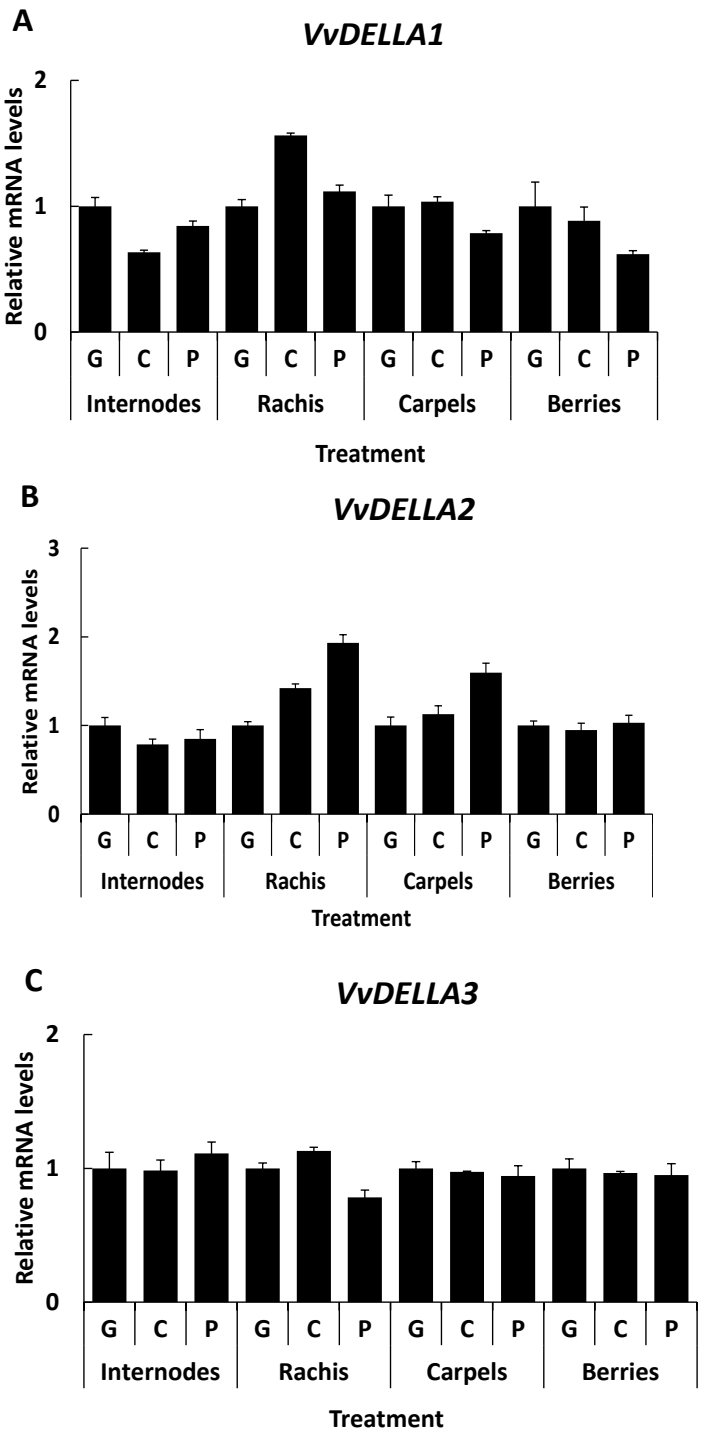


Figure S3: **GA regulation of expression of *VvDELLA1* (A), *VvDELLA2* (B), and *VvDELLA3* (C).** Organs were dipped or sprayed until run-off with a single GA_3 (G), paclobutrazol (P), or Triton X-100 (C) treatment. Tissues/organs were sampled 6 h after GA treatment (121 µM for internodes and rachises, and 90 µM for carpels and berries), and 102 h after PAC treatment. Total RNA was extracted from pooled samples of each treatment. The absolute mRNA levels of each gene were determined by real-time quantitative RT-PCR (qRT-PCR) and normalized against *VvGAPDH*. Absolute expressions of gene, in any organs/tissues are shown relative to values of GA-treated. The values represent the mean ± SE of three biological repeats with two technical repeats each. Results were reproducible in successive growing seasons.

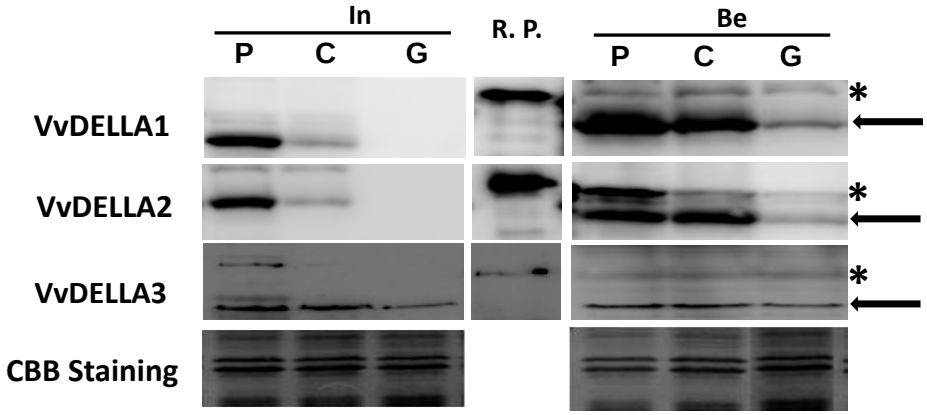


Figure S4: **GA_3-induced degradation of VvDELLA proteins in cv. Thompson seedless.** Western blot analyses of VvDELLA proteins in organs using protein-specific, affinity-purified, anti-VvDELLA polyclonal antibodies. Total proteins were extracted from internodes (In) and berries (Be) treated with PAC (P, 0.8 mM) and GA_3 (G, 121 µM for internodes, and 90 µM for berries). Control (C) samples were treated with Triton X-100 (0.025%). Recombinant full-length proteins (R.P.) (3.75 ng each of VvDELLA1 and VvDELLA2, and 37.5 ng of VvDELLA3) were used as size controls. Coomassie Brilliant Blue-stained (CBB) proteins were used as loading control. In all lanes except R.P., solid black arrows show band of interest, and Asterisked-bands indicate non-specific proteins detected by the anti-VvDELLA antibodies. Differences in sizes of R.P. and endogenous VvDELLA proteins result from tags on the R.P.

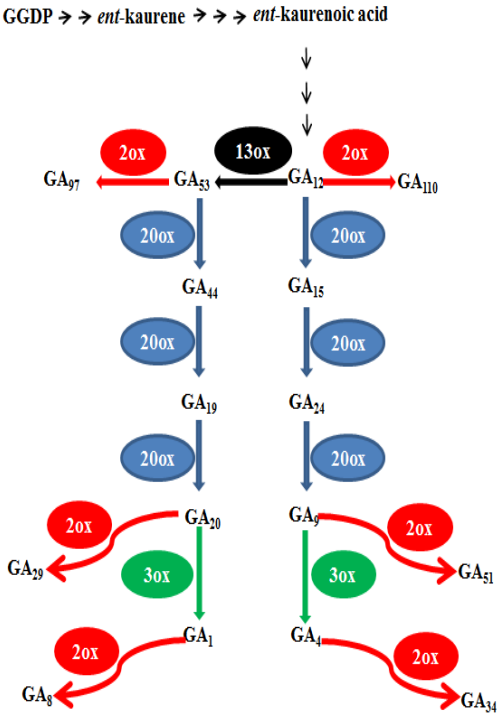


Figure S5: **Schematic representation of major GA metabolism pathways in plants.**
Number of short black arrows indicates number of intermediate molecules in the multiple reactions. Geranylgeranyl diphosphate (GGDP) is converted to *ent*-kaurene and then to *ent*-kaurenoic acid. *ent*-Kaurenoic acid is converted to GA_{12} in stepwise reactions by *ent*-kaurenoic acid oxidases. Using GA_{12} as substrate, GA13ox produces GA_{53} (thick black arrow), the main precursor of bioactive GA_1 in the 13-hydroylated pathway. Through a series of reaction catalyzed by two types of 2-ODDs, GA20ox (solid blue arrow) and GA3ox (solid green arrow), GA_{12} and GA_{53} are converted to bioactive GA_4 and GA_1, respectively. Deactivation of GA_1 and GA_4 and their precursors proceed through the action of another 2-ODD, GA2ox (solid red arrows).

Table S1: Primers used for gene isolation and gene expression analyses by qRT-PCR

Gene	Sequence		Purpose	
	Forward	Reverse	Gene isolation	qRT-PCR
VvGID1a	CACCATGGCCGGGAGT	AAGTCTATTAACAGTTAGAACTCAC	√	
VvGID1b	CACCATGGCCGGGAGT	TTAACAGTTAGATTTCACGAAATTC	√	
VvDELLA1	CACCATGAAGAGGGAGTATCA	ACTCAGTTGGAGGCAGGTGT	√	
VvDELLA2	CACCATGAAGAGAGACCTCCT	TCACTGAGAATGAGCAGAGGT	√	
VvDELLA3	CACCATGGGGCCTTACGAC	GAGGAGATTATTATGAT	√	
VvSLY1 a	CACCATGAGGCGAGGACTGGA	TTAACTGCCTTTGCTTTTGGA	√	
VvSLY1 b	CACCATGAAGCGACCTTTTG	TCAACAATAGAGGGAGATGA	√	
VvGID1a	AGGCTCTTGTGGCAGCATG	CTCCTTATGGCCGGAAGTCA		√
VvGID1b	AACTGCCTCTTTCGGTCAAG	AGTTGGTTCAGCCAAGTCTCA		√
VvDELLA1	CGACTCCTCGTTCTCCGATATT	GATAGCTTGATTGGCGGTGAAG		√
VvDELLA2	TGGTGGACGTGGCTCAGA	ACAGTCCCAGAGGAGAGATGAGA		√
VvDELLA3	CTTGGAGCAGCAGGGTTCAG	ACCCCTCAGCCGAGAACAG		√
VvSLY1 a	CGTCGTCTCACTCTCCTCAGT	AAGATGAACCTCGTCCTTTCC		√
VvSLY1 b	GATCTGGAAAGCGCAGATCAG	TACAGCAGATTCTCGTCCAGATTC		√
VvGAPDH	TTCTCGTTGAGGGCTATTCCA	CCACAGACTTCATCGGTGACA		√

Table S2: Primers used for cloning genes to yeast two-hybrid vectors

Gene	Sequence	
	Forward (VvGene-1)	**Reverse (VvGene-2)**
VvGID1a	CGAGGAATTCATGGCCGGGAGTAATGAAGTCAAC	GCAGGGATCCTTAACAGTTAGAACTCACAAAGTTAC
VvGID1b	CGAGGAATTCATGGCCGGGAGTGATGAAGTCAAC	GCAGGGATCCTTAACAGTTAGATTTCACGAAATTCTTTATC
VvSLY1a	CTTCGAATTCATGAGGCGAGGACTGGACTGCG	CCTTGGATCCTTAACTGCCTTTGCTTTTGGAACTC
VvSLY1b	CTTCGAATTCATGAAGCGACCTTTTGTTGCCGAC	CCC GGATCC TCATCTCCCTCTATTGTTGAAAT
VvDELLA1	GGCAGCATATGAAGAGGGAGTATCATCATC	GCAGGGATCCTCAGTTGGAGGCAGGTGTGG
VvDELLA2	GCAGCATATGATGAAGAGAGACCTCCTAGACGGTTG	GCAGGGATCCTCACTGAGAATGAGCAGAGGTGGAG
VvDELLA3	GCAGCATATGATGGGGCCTTACGACTCTGCCATC	GCAGGAATTCTTAGAGGAGATTATTATGATTTATAAC

Table S3: Amount of deuterated GA species used as the Internal Standard (IS) mix during extraction of endogenous gibberellins in different organs of grapes. Cat. 1= Berries (30 d); Cat. 2= Young leaves, Mature leaves, Young tendrils, mature tendrils, carpels, berries (0 d), berries (10 d); Cat. 3= Young rachis, mature rachis; Cat. 4=Young internodes

GA species	**Amount (ng)/ extraction**			
	Cat. 1	**Cat. 2**	**Cat. 3**	**Cat. 4**
GA_1	0.025	5	0.5	5
GA_4	0.125	0.25	0.025	0.25
GA_{53}	0.025	5	0.5	5
GA_{44}	0.025	5	0.5	5
GA_{19}	0.025	5	0.5	5
GA_{20}	0.025	5	0.5	5
GA_{29}	0.025	5	0.5	5
GA_8	0.025	5	0.5	5
GA_{12}	0.125	0.25	0.025	0.25
GA_{15}	0.125	0.25	0.025	0.25
GA_{24}	0.125	0.25	0.025	0.25
GA_9	0.125	0.25	0.025	0.25
GA_{51}	0.125	0.25	0.025	0.25
GA_{34}	0.125	0.25	0.025	0.25

Table S4: GA signaling genes of *Vitis vinifera* cv. Thompson seedless

Gene name	NCBI Locus ID	Chromosome	Size of gene (bp)	mRNA size (bp)	Protein size (kDa)
VvGID1a	AM468374	14	1,934	1,032	38.4
VvGID1b	AM479851	7	1,817	1,032	38.9
VvDELLA1	AM459432.1	1	1,770	1,770	64.8
VvDELLA2	AM470304.2	14	1,830	1,830	66.1
VvDELLA3	AM484828.1	11	1,596	1,596	58.6
VvSLY1a	AM445694.2	7	552	552	20.2
VvSLY1b	AM450967	18	552	552	20.9