

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

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S1A

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OsGID1 NLYY-----

VvGID1a NC-----

S1C

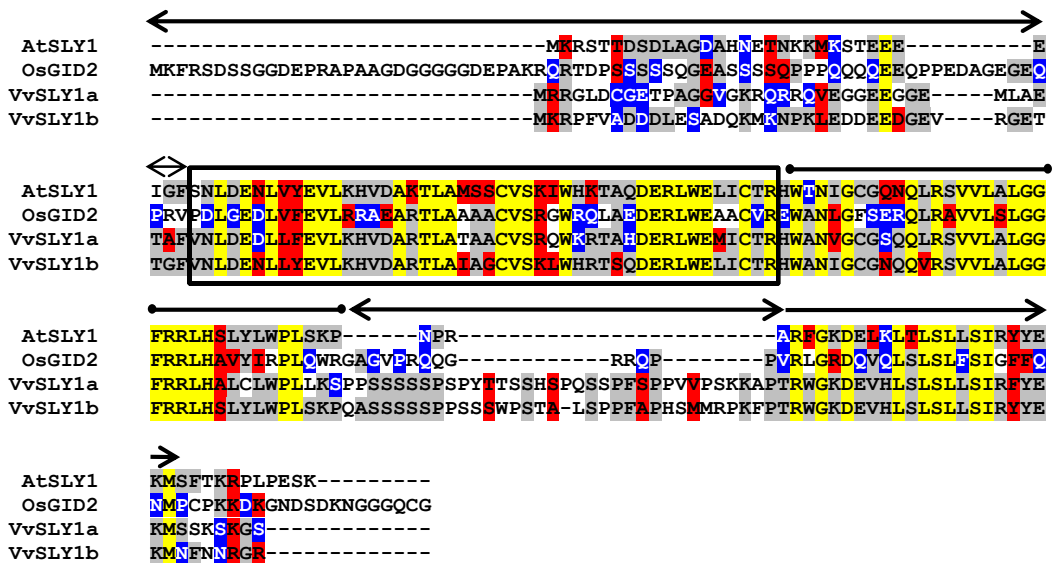


Figure S1: Amino acid sequence alignment of major GA signaling genes. Multiple alignment of amino acid sequences of Arabidopsis, rice and *V. vinifera* gene families were generated by CLUSTAL W alignment algorithm using AlignX of the Vector NTI suite. Yellow-shade: identical residues; Red-shade: similar 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

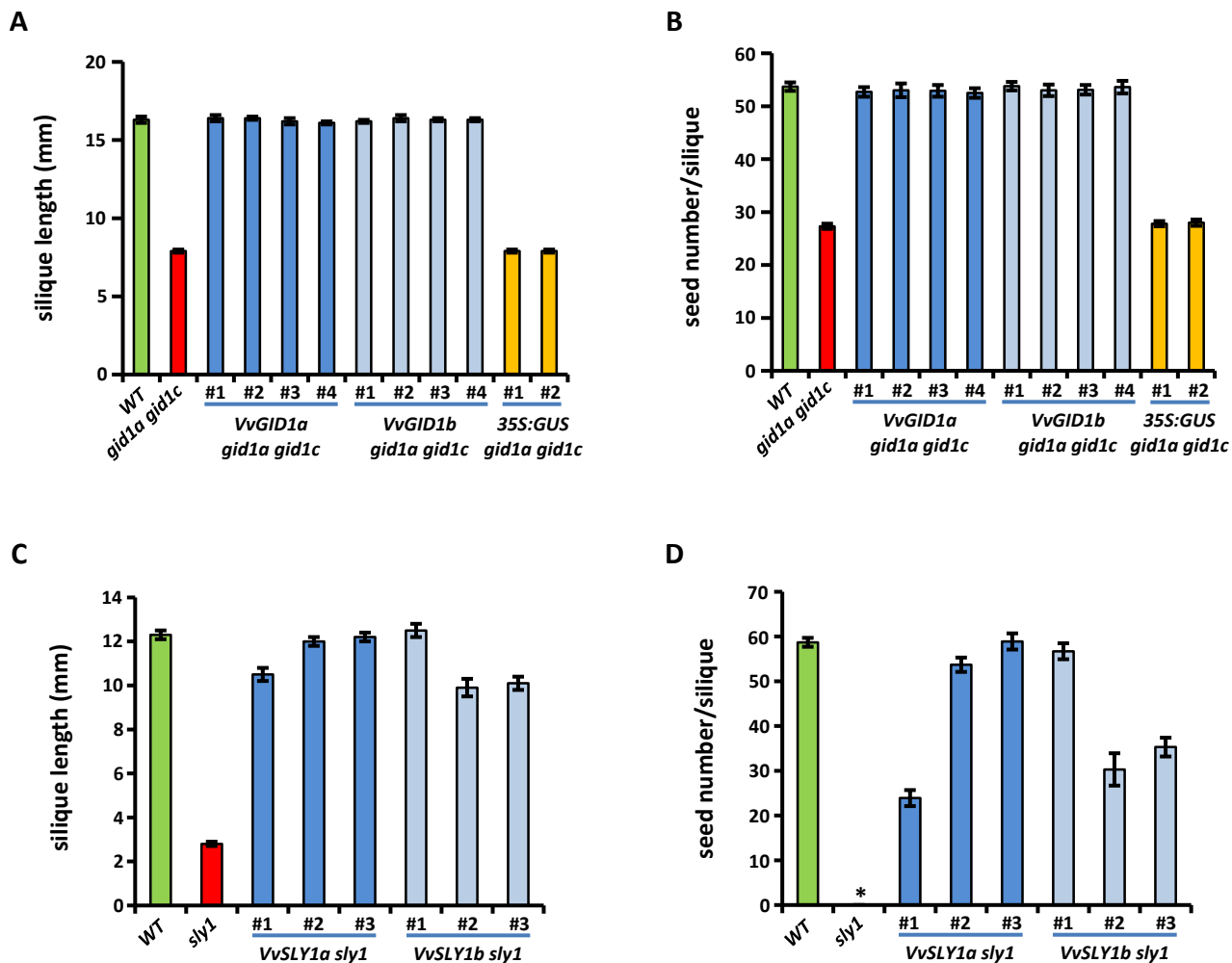


Figure S2 : Grapevine GA signaling genes rescue silique length and fertility defects of corresponding Arabidopsis mutants. (A-B) Average silique lengths (A) and seed numbers (B) of WT, *gid1a-2 gid1c-2*, and four representative *35S:VvGID1 gid1a gid1c* transgenic lines. *35S:GUS gid1a gid1c* transformants 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.

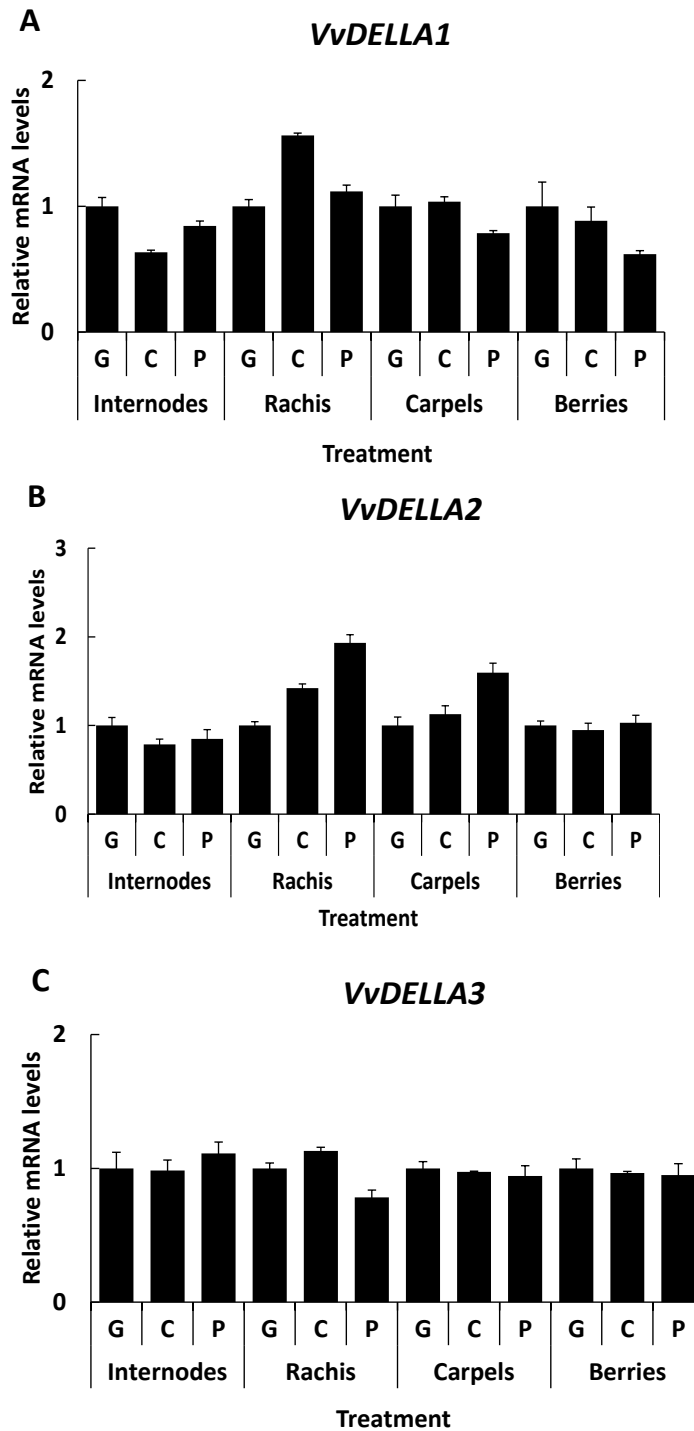


Figure S3: **GA regulation of expression of *VvDELLA1* (A), *VvDELLA2* (B), and *VvDELLA3* (C).** Organs were dipped or sprayed until run-off with a single GA₃ (G), paclobutrazol (P), or Triton X-100 (C) treatment. Tissues/organs were sampled 6 h after GA treatment (121 μM for internodes and rachises, 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.

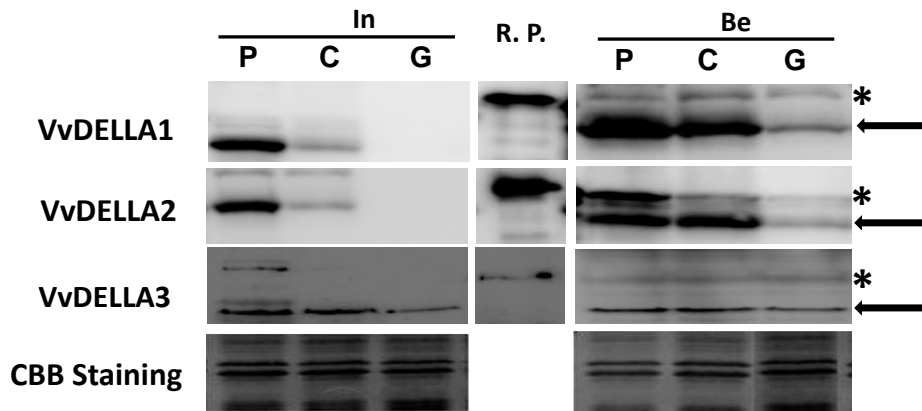


Figure S4: **GA₃-induced degradation of VvDELLA proteins in cv. Thompson seedless.** Western blot analyses of VvDELLA proteins in organs using protein-specific, affinity-purified, anti-VvDELLA polyclonal antibodies. Total proteins were extracted from internodes (In) and berries (Be) treated with PAC (P, 0.8 mM) and GA₃ (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.

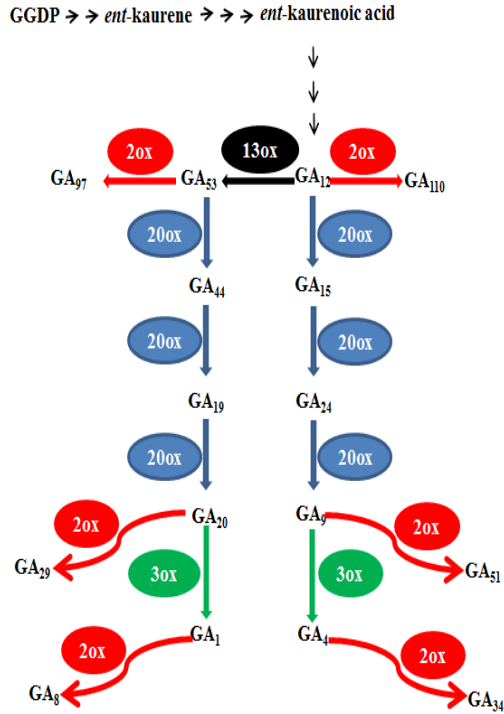


Figure S5: Schematic representation of major GA metabolism pathways in plants.

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, GA_{13ox} produces GA_{53} (thick black arrow), the main precursor of bioactive GA_1 in the 13-hydroxylated pathway. Through a series of reaction catalyzed by two types of 2-ODDs, GA_{20ox} (solid blue arrow) and GA_{30ox} (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, GA_{20ox} (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
<i>VvGID1a</i>	CACCATGGCCGGGAGT	AAGTCTATTAACAGTTAGAACTCAC	√	
<i>VvGID1b</i>	CACCATGGCCGGGAGT	TTAACAGTTAGATTTTCACGAAATTC	√	
<i>VvDELLA1</i>	CACCATGAAGAGGGAGTATCA	ACTCAGTTGGAGGCAGGTGT	√	
<i>VvDELLA2</i>	CACCATGAAGAGAGACCTCCT	TCACTGAGAATGAGCAGAGGT	√	
<i>VvDELLA3</i>	CACCATGGGGCCTTACGAC	GAGGAGATTATTATGAT	√	
<i>VvSLY1 a</i>	CACCATGAGGCGAGGACTGGA	TTAACTGCCTTTGCTTTTGGA	√	
<i>VvSLY1 b</i>	CACCATGAAGCGACCTTTTG	TCAACAATAGAGGGAGATGA	√	
<i>VvGID1a</i>	AGGCTCTTGTGGCAGCATG	CTCCTTATGGCCGGAAGTCA		√
<i>VvGID1b</i>	AACTGCCTCTTTCGGTCAAG	AGTTGGTTCAGCCAAGTCTCA		√
<i>VvDELLA1</i>	CGACTCCTCGTTCTCCGATATT	GATAGCTTGATTGGCGGTGAAG		√
<i>VvDELLA2</i>	TGGTGGACGTGGCTCAGA	ACAGTCCCAGAGGAGAGATGAGA		√
<i>VvDELLA3</i>	CTTGGAGCAGCAGGGTTCAG	ACCCCTCAGCCGAGAACAG		√
<i>VvSLY1 a</i>	CGTCGTCTCACTCTCCTCAGT	AAGATGAACCTCGTCCTTTCC		√
<i>VvSLY1 b</i>	GATCTGGAAAGCGCAGATCAG	TACAGCAGATTCTCGTCCAGATTC		√
<i>VvGAPDH</i>	TTCTCGTTGAGGGCTATTCCA	CCACAGACTTCATCGGTGACA		√

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

Gene	Sequence	
	Forward (VvGene-1)	Reverse (VvGene-2)
<i>VvGID1a</i>	CGAGGAATTCATGGCCGGGAGTAATGAAGTCAAC	GCAGGGATCCTTAACAGTTAGAACTCACAAAGTTAC
<i>VvGID1b</i>	CGAGGAATTCATGGCCGGGAGTGATGAAGTCAAC	GCAGGGATCCTTAACAGTTAGATTTACGAAATTCCTTATC
<i>VvSLY1a</i>	CTTCGAATTCATGAGGCGAGGACTGGACTGCG	CCTTGGATCCTTAAGTGCCTTTGCTTTTGGAAGTCT
<i>VvSLY1b</i>	CTTCGAATTCATGAAGCGACCTTTTGTGCCGAC	CCC GGATCC TCATCTCCCTCTATTGTTGAAAT
<i>VvDELLA1</i>	GGCAGCATATGAAGAGGGAGTATCATCATC	GCAGGGATCCTCAGTTGGAGGCAGGTGTGG
<i>VvDELLA2</i>	GCAGCATATGATGAAGAGAGACCTCCTAGACGGTTG	GCAGGGATCCTCACTGAGAATGAGCAGAGGTGGAG
<i>VvDELLA3</i>	GCAGCATATGATGGGGCCTTACGACTCTGCCATC	GCAGGAATTCCTTAGAGGAGATTATTATGATTATAAC

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)
<i>VvGID1a</i>	AM468374	14	1,934	1,032	38.4
<i>VvGID1b</i>	AM479851	7	1,817	1,032	38.9
<i>VvDELLA1</i>	AM459432.1	1	1,770	1,770	64.8
<i>VvDELLA2</i>	AM470304.2	14	1,830	1,830	66.1
<i>VvDELLA3</i>	AM484828.1	11	1,596	1,596	58.6
<i>VvSLY1a</i>	AM445694.2	7	552	552	20.2
<i>VvSLY1b</i>	AM450967	18	552	552	20.9