Final Scientific Report

Cover Page

Bard Project Number: **IS-4018-07**

Title: **Characterization and manipulation of the primary components in gibberellin signaling in the grape berry**

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Abbreviations commonly used in the report, in alphabetical order:

GA: gibberellin; GA₃: gibberellic acid; PAC: paclobutrazol; SPY: SPINDLY; *st1*: *male sterility1*; VvGAI: *Vitis vinifera* GA insensitive; GID1: GA insensitive dwarf1

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Signature
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Final Scientific Report

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Short Visits & Meetings	1	1		2
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Description Cooperation: Short visit for discussions

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Summary

Seedless cultivars dominate the table grape industry. In these cultivars it is mandatory to apply gibberellin (GA) to stimulate berry development to a commercially acceptable size. These cultivars differ in their sensitivity to GA application, and it frequently results in adverse effects such as decreased bud fertility and increased fruit drop. Our long term goals are to (1) understand the molecular basis for the differential sensitivity and identify markers for selection of sensitive cultivars (2) to develop new strategies for targeted manipulation of the grape berry response to GA that will eliminate the need in GA application and the undesirable effects of GA on the vine, while maintaining its desirable effects on the berry. Both strategies are expected to reduce production cost and meet growing consumer demand for reduced use of chemicals. This approach relies on a comprehensive characterization of the central components in the GA signaling cascade in the berry.

Several key components in the GA signaling pathway were identified in Arabidopsis and rice, including the GA receptors, GID1s, and a family of DELLA proteins that are the major negative regulators of the GA response. GA activates its response pathway by binding to GID1s, which then target DELLAs for degradation via interaction with SLY, a DELLA specific F-box protein. In grape, only one *DELLA* gene was characterized prior to this study, which plays a major role in inhibiting GA-promoted stem growth and GA-repressed floral induction but it does not regulate fruit growth. Therefore, we speculated that other DELLA family member(s) may control GA responses in berry, and their identification and manipulation may result in GA-independent berry growth. In the current study we isolated two additional *VvDELLA* family members, two *VvGID1* genes and two *VvSLY* genes. Arabidopsis anti-AtRGA polyclonal antibodies recognized all three purified *VvDELLA* proteins, but its interaction with *VvDELLA3* was weaker. Overexpression of the *VvDELLAs*, the *VvGID1s*, and the *VvSLYs* in the Arabidopsis mutants *ga1-3/rga-24*, *gid1a-2/lc-2* and *sly1-10*, respectively, rescued the various mutant phenotypes. *In vitro* GA-dependent physical interaction was shown between the *VvDELLAs* and the *VvGID1s*, and GA-independent interaction was shown between the *VvDELLAs* and *VvSLYs*. Interestingly, *VvDELLA3* did not interact with *VvGID1b*. Together, the results indicate that the identified grape homologs serve as functional DELLA repressors, receptors and DELLA-interacting F-box proteins. Expression analyses revealed that (1) *VvDELLA2* was expressed in all the analyzed

tissues and was the most abundant (2) *VvDELLA1* was low expressed in berries, confirming former study (3) Except in carpels and very young berries, *VvDELLA3* levels were the lowest in most tissues. (4) Expression of both *VvGID1s* was detected in all the grape tissues, but *VvGID1b* transcript levels were significantly higher than *VvGID1a*. (5) In general, both *VvDELLAs* and *VvGID1s* transcripts levels increased as tissues aged. Unfertilized and recently fertilized carpels did not follow this trend, suggesting different regulatory mechanism of GA signaling in these stages.

Characterization of the response to GA of various organs in three seedless cultivars revealed differential response of the berries and rachis. Interestingly, *VvDELLA3* transcript levels in the GA-unresponsive berries of cv. Spring blush were significantly higher compared to their levels in the highly responsive berries of cv. Black finger. Assuming that *VvDELLA2* and *VvDELLA3* are regulating berry size, constructs carrying potential dominant mutations in each gene were created. Furthermore, constitutive silencing of these genes by mIR is underway, to reveal the effect of each gene on the berry phenotype.

ACHIEVEMENTS:

Vineyard Experimentation: Morphological response of organs to application of GA and the GA inhibitor Paclobutrazol (Not included in Proposal)

The response of organs/tissues of three cultivars ('Black finger', Thompson seedless', and 'Spring blush') to GA and PAC was monitored and phenotypic parameters were recorded at various time points during organ development (Fig. 1A-C). GA treatment resulted in a six fold increase in length of 'Black finger' rachis, but only a two fold increase in the other cultivars. The length of rachis of 'Black finger' was not significantly influenced by PAC treatment (Fig. 1A). Internodes of the three cultivars also exhibited differential response to GA application (Fig. 1B). Unlike 'Thompson seedless', internodes lengths of 'Black finger' and 'Spring blush' strongly responded to GA treatment.

A single GA application increased berry weight of 'Black finger' and 'Thompson seedless' by 3 and 1.5 folds, respectively, but had no effect on 'Spring blush'(Fig. 1C). PAC treatment significantly reduced the berry weight of 'Thompson seedless' and 'Spring Blush', as expected, but did not significantly influence the size and weight of 'Black finger' berries.

The central theme of the current study was that qualitative and quantitative variations in signaling modulators may be playing vital roles in establishing these differential sensitivities. However, quantitative differences in endo-GAs and differential penetrability of the applied GA should be considered as well. As an example, in the case of ‘Black finger’ which lack seed rudiments, the results may indicate that the berries contains very low endo-GA levels, resulting in very high sensitivity to exo-GA. and insignificant reduction in GA level in the presence of PAC.

Specific Aim A1: Identification and cloning of all grapevine DELLA genes

Prior to commencing this research, the only *DELLA* gene characterized in grapevine was responsible for inhibiting GA-promoted stem growth and GA-repressed floral induction, but did not regulate fruit size. Therefore, we speculated that other DELLA family member(s) exists, which control GA responses in the berry and participate in the control of GA responses of other organs as well. Bioinformatic analyses duly identified two new genes encoding for DELLA protein, VvDELLA2 and VvDELLA3 (Table 1). Full and partial-length open reading frames for the three *DELLA* genes were cloned into appropriate GATEWAY-based vectors for over-expression (OE) and protein induction assays, respectively.

Table 1: Amino acid sequence homology of VvDELLA, AtGAI and AtRGA

	VvDELLA1	VvDELLA2	VvDELLA3
VvDELLA2	68%		
VvDELLA3	48%	48%	
AtGAI	46%	43%	38%
AtRGA	44%	60%	46%

Specific Aim A2 & A3: Analysis of the spatial and temporal expression pattern of DELLA genes/proteins

Pairs of gene-specific primers with comparable efficiencies were designed and used to determine, by quantitative real-time PCR (qRT-PCR), the absolute mRNA levels of each *VvDELLA*, in selected organs/tissues, at different phenological stages (Fig. 2). In most tissues and developmental stages, *VvDELLA2* was highest expressed. Except for carpels and berries,

mRNA levels of *VvDELLA3* were lowest in all organs analyzed. *VvDELLA* transcript levels increased as tissues aged, except unfertilized and recently fertilized carpels (berries at 0 d) that did not follow this trend. Corroborating findings of others, *VvDELLA1* was expressed highest in rachis, but lowest in berries. *VvDELLA3* was expressed highest in unfertilized and recently fertilized carpels compared to other organs. Unlike *VvDELLA1*, transcript levels of *VvDELLA2* remained constant during ‘Black finger’ and ‘Spring blush’ berry development suggesting that this gene is required in the first phase of berry development. While transcript levels of *VvDELLA3* decreased during ‘Black finger’ berry development, they remained constant in ‘Spring Blush’, suggesting that level of its product may explain in part the differential sensitivity of these cultivars to GA. We raise here the assumption that higher endo-GA levels in ‘Spring blush’ berries may lead to higher DELLA degradation, and subsequently feedback up-regulation of *VvDELLA3* mRNA. However, direct connection between higher level of *VvDELLA3* and lower sensitivity of ‘Spring blush’ berries should not be ruled out at this stage, despite the common understanding that DELLA is regulated mainly in the protein level.

Western blot analyses showed that the anti-AtRGA polyclonal antibodies could recognize over-expressed *VvDELLA* proteins (Fig. 3). This antiserum detects *VvDELLA1* and *VvDELLA2* at the same strength as AtRGA, while its recognition of *VvDELL3* is about 30-fold weaker. To generate gene-specific antibodies that will not cross-react with non-targets, sequences encoding differential domains of the three genes were cloned into pET-DEST42 protein expression vector. All three partial proteins have been induced and purified (Fig. 4) and production of rabbit polyclonal antibody is currently underway. Using the resultant antibodies, we will have the final quantitative data of spatial and temporal expressions of each DELLA protein during the first quarter of 2012.

Specific Aim A4: Functional analysis of berry-expressed *DELLA* genes by complementation of the Arabidopsis *rga gai gal* mutant

35S:*VvDELLA* constructs were transformed into the Arabidopsis mutant *gal-3/rga-24*. Only 1-2 stable homozygous lines were obtained for each DELLA construct. These transformants either showed phenotypes of partially rescued *gal-3/rga-24* (in the case of *VvDELLA1* and *VvDELLA3*, Fig. 5) or more severe form of *gal-3* (*VvDELLA2*, no picture yet), indicating that the three putative *VvDELLAs* are functional DELLA repressors. More homozygous lines were initially isolated in this experiment, but became unstable as indicated by loss of kanamycin

resistance. These plants had phenotype similar to *gal-3/rga-24*. This is probably caused by transgene silencing due to the stress caused by VvDELLA over-expression. This is similar to our previous data showing that *35S:RGA* was frequently silenced in transgenic Arabidopsis lines. We will have the final quantitative data of phenotype analysis of the stable homozygous VvDELLA lines by December, 2011.

Specific Aim A5: Investigation of the function of berry-expressed DELLA genes in grape

This mission was delayed until we gained better understanding of the behavior of the signaling components in *V. Vinifera*. Based on the data described above, and in light of the lack of tissue specific promoters and the long term challenge in production of transgenic vines, we decided on the preferred strategy and started promoting it.

Though all three VvDELLAs are redundantly expressed in the young berries, VvDELLA1 is non-functional in berries. A two-tier approach was selected to identify and manipulate the VvDELLA that regulate berry size. First, PCR-based overlap-extension mutagenesis was performed to generate the 51-bp deletion of the DELLA and TVYNP motifs (identical to the mutation in *gai-1*) of VvDELLA2 and VvDELLA3 genes to create *vvdella2* and *vvdella3*, respectively. The resulting 1791 bp PCR product of VvDELLA2 and 1548 bp product of VvDELLA3 (containing *vvdella2* and *vvdella3* mutations, respectively) were hooked to 2.5kb and 3kb DNA fragments upstream of the respective genes, assuming that it contains the relevant promoter elements. The *pVvDELLA2::vvdella2* and *pVvDELLA3::vvdella3* constructs will be used for *Agrobacterium*-mediated transformation of grape embryonic calli. In these transformants, berry-functional *vvdella* is expected to inhibit the GA-promoted berry enlargement.

The second approach will involve using artificial miR, in a miR319 precursor backbone, for the simultaneous targeted silencing of two VvDELLA genes. The constitutive silencing system clones to be used are: *35S:miR-VvDELLA1/2* (silencing VvDELLA1 and -2); *35S:miR-VvDELLA1/3* (silencing VvDELLA1 and -3); *35S:miR-VvDELLA2/3* (silencing VvDELLA2 and -3). Silencing the berry-specific VvDELLAs should produce GA-independent berry enlargement.

The evaluation of the functionality of berry-expressed DELLA genes is expected to be completed in three years.

Prior to the adoption of the above strategy we generated transgenic lines using two test constructs: *XVE:miR-VvDELLA1/2/3* (silencing all three VvDELLA genes using a miR164b

backbone), and *35S:RNAi-VvDELLA3* (to specifically silence *VvDELLA3*). Plantlets were regenerated but not yet tested for presence of the transgenes. Silencing in the *XVE:mIR-VvDELLA1/2/3* clones will be induced in specific organs/tissues by treating with 2-5 μ mole of 17- β -estradiol, whereas the *35S:RNAi-VvDELLA3* system is expected to generate constitutive silencing. Our current understanding is that the approaches described in the preceding paragraph represent a better way to approach the question under study.

Specific Aim B1: Identification of all the grapevine GA receptor genes in the cDNA database and the grape genome sequence and cloning of the full ORFs

We identified two GA receptor genes in the grape genome as described in A1 (Table 2). Full-length open reading frames for *VvGID1a* and *VvGID1b* were cloned into to GATEWAY-based pK7WG2 over-expression vector.

Table 2: Amino acid sequence homology of GID1 orthologs in *V. Vinifera* and other species

	VvGID1a	VvGID1b	AtGID1a	AtGID1b	AtGID1c
VvGID1b	79.3%				
AtGID1a	80.6%	72.2%			
AtGID1b	67.3%	76.0%	63.7%		
AtGID1c	81.7%	73.6%	85.3%	65.4%	
OsGID	60.7%	58.5%	60.7%	56.4%	61.6%

Specific Aim B2: Studies on expression patterns of all receptor genes in all organs and during berry development (+/- GA) at early stages

Consistent with the role of GA in regulating major physiological processes in higher plants, and corroborating other results, expression of the *VvGID1a* and *VvGID1b* was detected in all of the tissues analyzed, suggesting redundancy in regulating GA-responsive characters (Fig. 6). In all organs, except unfertilized and recently fertilized carpels, *VvGID1* transcript levels increase with age. This could be due to feedback regulation of *VvGID1* in response to low GA levels in these aging tissues. Unfertilized carpels and recently fertilized carpels do not follow this rule, presenting the highest level of receptors transcripts despite of GA supply from anthers.

In all organs (except leaves of ‘Black finger’), *VvGID1b* mRNA levels were higher than *VvGID1a*. It is unclear if the variations in expression levels of these paralogs are related to the functionality in the genes in these organs. This assumption, though far-fetched, cannot be discounted.

As expected, GA treatment down-regulated, while PAC treatment up-regulated the mRNA levels of both *VvGID1a* and *-b* through negative feedback (Fig. 7A & B). In all tissues/organs of both cultivars, the treatments had a greater effect on *VvGID1b* than on *VvGID1a*.

Specific Aim B3: Comparison of transcript and protein levels of the fruit expressed GA receptors in berries of seedless grape varieties differing in response to GA application.

See B2 for details.

Specific Aim B4: Functional analysis of VvGID1s by complementation of the Arabidopsis *gid1a gid1c* double mutant.

35S:VvGID1A and *35S:VvGID1B* constructs were introduced into the Arabidopsis *gid1a-2/1c-2* via *Agrobacterium*-mediated transformation. ~10 individual T3 homozygous lines per construct have been identified. Phenotyping of these lines showed full rescue of the *gid1a gid1c* mutant, indicating that both VvGID1s are functional GA receptors (Fig. 8A-C). As expected, the negative control *35S:GUS* construct did not rescue *gid1a gid1c*.

Specific Aim B5: Study the interactions between berry-expressed GID1 and DELLA by yeast two-hybrid assays in the presence or absence of GA

We checked the GA-dependent interaction of VvGID1 and VvDELLA *in vitro*. Yeast two-hybrid (Y2H) assays showed that either of VvGID1a and VvGID1b interacts with VvDELLA1 or VvDELLA2 weakly without GA. In the presence of 100 μ M GA₃, their interactions are greatly increased. Without GA, VvDELLA3 does not interact with either VvGID1s. Interestingly, in the presence of GA, VvDELLA3 interacted strongly with VvGID1a, while its interaction with VvGID1b only increased slightly (Fig. 9).

Specific Aim B6: Over-expression of a GA receptor in berry

We hypothesize that overexpression of a GA receptor may result in GA-independent DELLA degradation. If this occurred in seedless berry, it may cause an increased fruit growth without GA treatment. This hypothesis was tested first in Arabidopsis because AtGID1b has already been shown to interact with DELLAs even in the absence of GA. However, over-expression of *AtGID1b* (*GID1b-OE*) in the GA-deficient Arabidopsis mutant *ga1-3* did not rescue its dwarf phenotype, suggesting that AtGID1b is unable to interact with DELLA in the absence of GA *in planta*. The *GID1b-OE* construct has been introduced into *ms1* (*male sterile 1*) mutant by crossing. The silique length in *GID1b-OE/ms1* has no difference comparing to *ms1*, suggesting that *GID1b* over-expression alone cannot promote parthenocarpic growth in Arabidopsis (Fig. 9). In light of the above results, we did not overexpress any of the VvGID1 in grape calli.

Additional experiments not included in the proposal

1. VvSLY and VvSNE

1.1 Identification, isolation and cloning other GA signaling genes

Bioinformatic analyses of the grape cDNA (<http://compbio.dfci.harvard.edu/tgi/>) and genome sequence (<http://www.ncbi.nlm.nih.gov/>) revealed two putative homologs of F-box protein VvSLYA and VvSLYB, and one VvSNE, a redundant positive regulator of GA signaling which is functional only in the absence of SLY1.

1.2 Y2H and Y3H assays

Y2H assays showed that both VvSLY1a and VvSLY1b can interact with each of the three VvDELLAs (Fig. 11). VvSLY1b interacts with each VvDELLA more strongly than VvSLY1a. In Y3H assays, the interactions between VvSLYs and VvDELLAs were not enhanced or were only slightly increased in the presence of GA and VvGID1A (Fig. 12). This observation is surprising and is inconsistent with the current model of how GA and GID1 activate GA signaling. Future studies will be needed to examine VvSLY1 and VvDELLAs interaction *in planta*.

1.3 Complementation test

35S::VvSLY1a, *35S::VvSLY1b* and *35S::VvSNE* constructs were transformed into *sly1-10*^{+/-}. Multiple homozygous transgenic lines for each construct (in *sly1-10*) have been collected. Based on their T1, T2 plant phenotypes, VvSLYA and VvSLYB can fully rescue *sly1-10*, while

VvSNE only partially rescues *sly1-10*. We will need to perform a final phenotype analysis using representative homozygous lines.

2. Endogenous GA quantification

Our preliminary results from the endo-GA quantification (Fig. 13) suggest that GA₁ is the key bioactive GA in internodes and carpels, while GA₄ predominates in rachis, leaves and berries. Results also indicate that as organs age, the concentrations of endo-GAs decrease. A comprehensive qualitative and quantitative analysis of spatial and temporal GA levels in organs/tissues is currently underway in collaboration with Prof. Yuji Kamiya's lab (RIKEN, Japan). Results will shed more light on varietal differences in response to exo-GA application.

Near future plan

Despite the fact that the current proposal came to its official end, we will proceed in analysis of its products. In addition to the transgenic work and endo- GA quantitation, we plan to look for potential difference in alleles of each gene in varieties that exhibit differential GA response.

Different alleles of some GA signaling gene have been shown to exhibit preferences and differences in the affinities of interaction and functions. It is envisaged that differences in cultivar sensitivity to exo-GA application may also results from differences in alleles of *VvDELLA*, *VvGID1s* and *VvSLY*. To test this hypothesis, genes will be isolated from each cultivar and sequenced. If sequence results reveal consequential allelic differences, the different alleles will be tested for strength of interaction with the other members of the complex by Y2H assays.

Figure 1.

A(i)

A(ii)

A(iii)

B(i)

B(ii)

B(iii)

C(i)

C(ii)

C(iii)

Figure 2.

Figure 3. DU176 recognition of His-VvDELLAs

2ng VvDELLA1 ≈ 2ng RGA

2ng VvDELLA2 ≈ 2ng RGA

30ng VvDELLA3 ≈ 1ng RGA

VvDELLA1 and VvDELLA2 are detected by DU176 at similar strength as AtRGA

DU176 detection of VvDELLA3 is ~30x weaker than AtRGA

Figure 4.

1. Protein ladder
2. VvDELLA1_DIFF
3. VvDELLA2_DIFF
4. VvDELLA3_DIFF

Figure 5. *VvDELLA1* and *VvDELLA3* partially rescue *ga1-3/rga-24*

ga1-3

ga1-3/rga-24

VvDELLA1#1-9-1

VvDELLA3#5-1-6

75d plants. Bar: 1cm

Figure 6.

Figure 7.

A (i)

A (ii)

B (i)

B (ii)

Figure 8A. 35S:VvGID1 in *Arabidopsis* *gid1a-2/1c-2*

Figure 8B. 35S:VvGID/gid1a-2/1c-2 plant height

Figure 8C. 35S:VvGID/gid1a-2/1c-2 #10 silique length

Figure 8D. 35S:VvGID/gid1a-2/1c-2 seed number in #10 silique

Figure 9. Y2H of VvGID/VvDELLA in pLexA-NLS/pACTII system, representative result of two tests

*:repeated in pGBKT7/pGADT7rec Y2H system with same result

Figure 10. Over-expression of *AtGID1b* in *ms1*

**Average length of silique #5-9
on primary stem from 6 plants**

bar: 1 cm

Figure 11. Y2H of VvSLY/VvDELLA in pLexA-NLS/pACTII system, representative result of two tests

Figure 12A. Y3H of VvSLYA/VvDELLA/VvGID1a in pGBT9/pACTII/pH3 system, representative of 3 tests

Figure 12B. Y3H of VvSLYB/VvDELLA/VvGID1a in pGBT9/pACTII/pH3 system, representative result of 2 tests

Figure 13.

(A)

(B)

Figure legends

Figure 1: Altered response to GA, GA biosynthesis inhibitor paclobutrazol (PAC) (0.8 mM) and Triton (0.025%) treatments of rachis (A), internode (B), and Berries (C) of ‘Black Finger’ (i), ‘Thompson Seedless’ (ii), and ‘Spring Blush’ (iii). For rachis and internodes, groups of 25 organs were selected from vines of uniform vigour, each treated with 121 μ M of GA₃, and 0.8 mM PAC, formulated in 0.025% Triton X100. Berry response was measured by treating, at late bloom (termed ‘110% bloom’), each group of 24 clusters, with 90 μ M GA₃, 0.8 mM PAC or water, all with 0.025% Triton X-100 as a surfactant. Samples were treated with PAC 96 h prior to other treatments to ensure complete inhibition of GA biosynthesis, and a total elimination/exhaustion of endogenous GA supplies. Length of treated rachis, new internodes arising after treating shoots, and weights of berries were monitored and data was calculated as: A) Increase in length of rachis as percent (%) of initial length, B) Increase in length of internode as percent (%) of an initial length which was assigned a value of 0.5 mm, C) Increase in the weight of the berries as percent of initial weight. Rachis and internodes length was monitored at 5 d intervals while berry weight was measured at 10 d interval. The data was analyzed by Tukey HSD LSMean Differences test (at $\alpha = 0.05$) with 25 measurements for rachis and internodes, and 150 measurements for berries sampled from eight clusters at each time point. Data points with different letters indicate significantly different values.

Figure 2: Spatial and temporal expression profiles of *VvDELLA1*, *VvDELLA2*, and *VvDELLA3* in ‘Black Finger’ (A), and ‘Spring Blush’ (B). The absolute mRNA levels of each gene were determined by real-time quantitative RT-PCR (qRT-PCR) and normalized against *VvGAPDH*. The values represent the mean $\pm$ s.e. of three biological replications with two technical repeats each. Young, Medium and Mature internodes and rachis were sampled at ‘Stage 3’ (15 cm long shoots), full bloom and at veraison, respectively. Young, Medium and Mature leaves were sampled from 1st/2nd, 6th and 12th nodes from the shoot apex, respectively. Carpels were sampled at ‘Stage 6’. Day 0, 5, 10, 15, 20 and 30 berries were sampled at 0, 5, 10, 15, 20 and 30 days after fruitset (DAF), respectively.

Figure 3: Recognition of His-VvDELLAs by the anti-AtRGA polyclonal antibodies, DU176. VvDELLA1 and VvDELLA2 were detected by DU176 at same strength as AtRGA, whereas detection of VvDELLA3 was 30 times weaker than AtRGA.

Figure 4: Induction/expression of differential (DIFF) proteins of VvDELLA1 (VvDELLA1_DIFF), VvDELLA2 (VvDELLA2_DIFF), and VvDELLA3 (VvDELLA3_DIFF) in *E. coli* BL21-CodonPlus (DE3) RIPL/RIL strains. Sufficient protein quantities have been generated for gene-specific, rabbit polyclonal antibody production by commercial service.

Figure 5: Functional analyses of VvDELLA1 and VvDELLA3 in *Arabidopsis gal-3/rga-24* mutant. Both genes partially rescue *gal-3/rga-24* producing semi-dwarf phenotype of wild type *Arabidopsis*.

Figure 6: Spatial and temporal expression profiles of *VvGID1a* and *VvGID1b* in ‘Black Finger’ (A), and ‘Spring Blush’ (B). The absolute mRNA levels of each gene were determined by real-time quantitative RT-PCR (qRT-PCR) and normalized against *VvGAPDH*, whose transcript levels are unaffected by GA. The values represent the mean $\pm$ s.e. of three biological replications with two technical repeats each. See Fig. 2 for detailed description of physiological state of each organ or tissue.

Figure 7: GA treatment down-regulates transcript levels of *VvGID1a* (A), and *-1b* (B) in ‘Black Finger’ (i), and ‘Spring Blush’ (ii) by feedback regulation. The absolute mRNA levels of each gene were determined by real-time quantitative RT-PCR (qRT-PCR) and normalized against *VvGAPDH*. The values represent the mean $\pm$ s.e. of three biological replications with two technical repeats each. Absolute expressions of gene, in any organs/tissues are shown relative to GA-treated values at each time point.

Figure 8: VvGID1 rescues the dwarf phenotype of *Arabidopsis gid1a-2 gid1c-2*.

- A. Gross morphology of transformants expressing *35S:VvGID1a* and *35S:VvGID1b* in a *gid1a-2 gid1c-2* mutant. Mutant was also transformed with *35S:GUS* to serve as transformation control. Phenotype of mutants and wild type (Col) are also shown.
- B. Height of plants of mutant and transformed lines. Bars represent s.e. values.

C. Length of siliques of plants of mutant and transformed lines. Bars represent s.e. values.

D. Number of seeds per 10 siliques of plants of mutant and transformed lines. Bars represent s.e. values.

Figure 9: Interaction between VvGID1 and VvDELLA (A) in a GA-dependent manner. Genes were expressed either as AD or BD fusion proteins in the pLexA-NLS/pACTII L40 Yeast-2-hybrid (Y2H) system $\pm 100 \mu\text{M}$ GA₃. The affinities of interaction between complex components was determined by β -galactosidase (β -gal) activity detected in a liquid assay with Yeast transformants (mean $\pm$ s.e.; n=3).

* assay was repeated in pGBKT7/pGADT7rec Y2H system with same result.

Figure 10: Over-expressing *AtGID1* in Arabidopsis mutant *ms-1* did not rescue the GA-deficient phenotype.

Figure 11: Interaction between VvDELLA and VvSLY1 homologs. Genes were expressed either as AD or BD fusion proteins in the pLexA-NLS/pACTII L40 Yeast-2-hybrid (Y2H) system. The affinities of interaction between complex components was determined by β -galactosidase (β -gal) activity detected in a liquid assay with Yeast transformants (mean $\pm$ s.e.; n=3). VvSLY1a (CAO71929.1); VvSLY1b (CU459218).

Figure 12: Interaction between VvSLY1a (A)/ VvSLY1b (B), VvDELLA and VvGID1 in pGBT9/pACTII/pH3 Yeast-3-hybrid (Y3H) system. The affinities of interaction between complex components was determined by β -galactosidase (β -gal) activity detected in a liquid assay with Yeast transformants (mean $\pm$ s.e.; n=3).

Figure 13: Endogenous levels of GA₁ (A) and GA₄ (B) in selected organs/tissues of 'Black finger'. Detailed description of physiological state of each organ is provided in Fig. 2. Berries were sampled 30 DAF. GA was characterized and quantified by Liquid chromatography-mass spectrometry (LC-MS). The values represent the mean $\pm$ s.d. of two technical repeats.