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# RNA-Seq reveals new DELLA targets and regulation in transgenic GA-insensitive grapevines

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## Abstract

**Background:** Gibberellins (GAs) and their regulator DELLA are involved in many aspects of plant growth and development and most of our current knowledge in the DELLA-facilitated GA signaling was obtained from the studies of annual species. To understand GA-DELLA signaling in perennial species, we created ten GA-insensitive transgenic grapevines carrying a DELLA mutant allele (*Vvgai1*) in the background of *Vitis vinifera* 'Thompson Seedless' and conducted comprehensive analysis of their RNA expression profiles in the shoot, leaf and root tissues.

**Results:** The transgenic lines showed varying degrees of dwarf stature and other typical DELLA mutant phenotypes tightly correlated with the levels of *Vvgai1* expression. A large number of differentially expressed genes (DEGs) were identified in the shoot, leaf and root tissues of the transgenic lines and these DEGs were involved in diverse biological processes; many of the DEGs showed strong tissue specificity and about 30% them carried a DELLA motif. We further discovered unexpected expression patterns of several key flowering induction genes *VvCO*, *VvCOL1* and *VvTFL1*.

**Conclusions:** Our results not only confirmed many previous DELLA study findings in annual species, but also revealed new DELLA targets and responses in grapevine, including the roles of homeodomain transcription factors as potential co-regulators with DELLA in controlling the development of grapevine which uniquely possess both vegetative and reproductive meristems at the same time. The contrasting responses of some key flowering induction pathway genes provides new insights into the divergence of GA-DELLA regulations between annual and perennial species in GA-DELLA signaling.

**Keywords:** CONSTANS, DELLA, Flowering, Grapevine, HOMEODOMAIN, Perennial, TFL1, Transgenic GA-insensitive

## Background

Gibberellins arguably has the widest role to play in the whole life cycle of a plant. Plants tightly regulate the availability of GA by coordinating the activation and deactivation of multiple biosynthesis genes of GA in different tissues at various developmental stages [1]. The flow rate and availability level of bioactive GA in various tissues are regulated by a de-repressible system in which GA interacts with its receptor *GA INSENSITIVE DWARF1* (GID1) and negative regulator, DELLA, to form a trimeric GA:GID1:-DELLA complex [2]. As a key component in the GA signaling cascade, DELLA genes, such as *Rht1* from wheat

and *GIBBERELLIN INSENSITIVE* (*GAI*) from *Arabidopsis*, have been extensively studied [3]. These genes all carry a conserved DELLA amino-acid domain in their protein sequences. A critical gain-of-function mutation in the DELLA domain prevents the formation of a stable trimeric complex with GA:GID1 required for its eventual degradation, and thus instead maintains the negative regulation of GA signaling [4]. As a result, GA-deficient plants show many abnormal developmental characteristics such as smaller darker leaves and reduced internode length [5]. In addition to basic characterization of DELLA protein genes in the GA signal transduction [6], many DELLA target genes which control development of various traits in model and annual species, have also been identified [7]. In contrast, progress in understanding DELLA regulation in perennial woody plants lags considerably behind, although

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some DELLA knowledge has been obtained from transgenic poplar research; metabolic and phenotypic changes in transgenic GA-deficient poplar [8] were found similar to their *Arabidopsis* mutant counterpart [9]. Similar to *Arabidopsis gai* mutants, transgenic poplar carrying non-degradable DELLA gene (i.e. *gai*, a mutant version of *GAI*) were dwarf but insensitive to GA-mediated restoration to wildtype [10, 11].

Grapevine (*Vitis*) is a perennial woody species with significant economic value. Impact of DELLA-mediated GA on vine growth and development was first found in a natural DELLA mutant derived from a somatic variation in the L1 meristem layer of the grape cultivar *V. vinifera* 'Pinot Meunier' [12]. The mutant vine carries a copy each of the wildtype *GIBBERELLIC ACID-INSENSITIVE1* (*VvGAI1*) and mutant (*Vvgai1*) and manifests reduced internode length and short plant stature, which is a hallmark trait of DELLA mutants in many species. The mutant vine also shows continuous flowering and bears inflorescences at most nodes after the first inflorescence appears. Apparently, both GA and DELLA proteins are involved in grape's inflorescence formation, flower induction and berry development [13].

To elucidate DELLA-mediated roles in grapevine development, we created transgenic lines in the background of a table grape cultivar, *V. vinifera* 'Thompson Seedless', carrying a grape mutant DELLA gene, noted as *Vvgai1* [14]. We analyzed the RNA-Seq profiles of shoot, leaf and root tissues of non-transgenic and representative transgenic lines and affirmed in grapevine the presence of a DELLA-centered feedback mechanism that maintains the GA homeostasis [15] and also the intricate interactions of DELLAs with numerous transcription factors controlling plant development and growth as were found in annual species [16]. We further discovered DELLA's possible roles in the induction of the anlagen, the distinct vegetative meristem for tendril/inflorescence development in grapevine [17], through coordination with meristem regulators. Moreover, we discovered unexpected expression patterns of several key flowering induction genes, including grapevine *CONSTANS* (*VvCO*), *CONSTANS 1* (*VvCO1*), and *TERMINAL FLOWER 1* (*VvTFL1 or CENTRORADIALIS*), which were in sharp contrast with what were found in annual species. These findings provide insights into how DELLA genes regulate grapevine development and growth, especially in relation to flowering, and fill some critical knowledge gaps in this important research area between annual and perennial species.

## Results

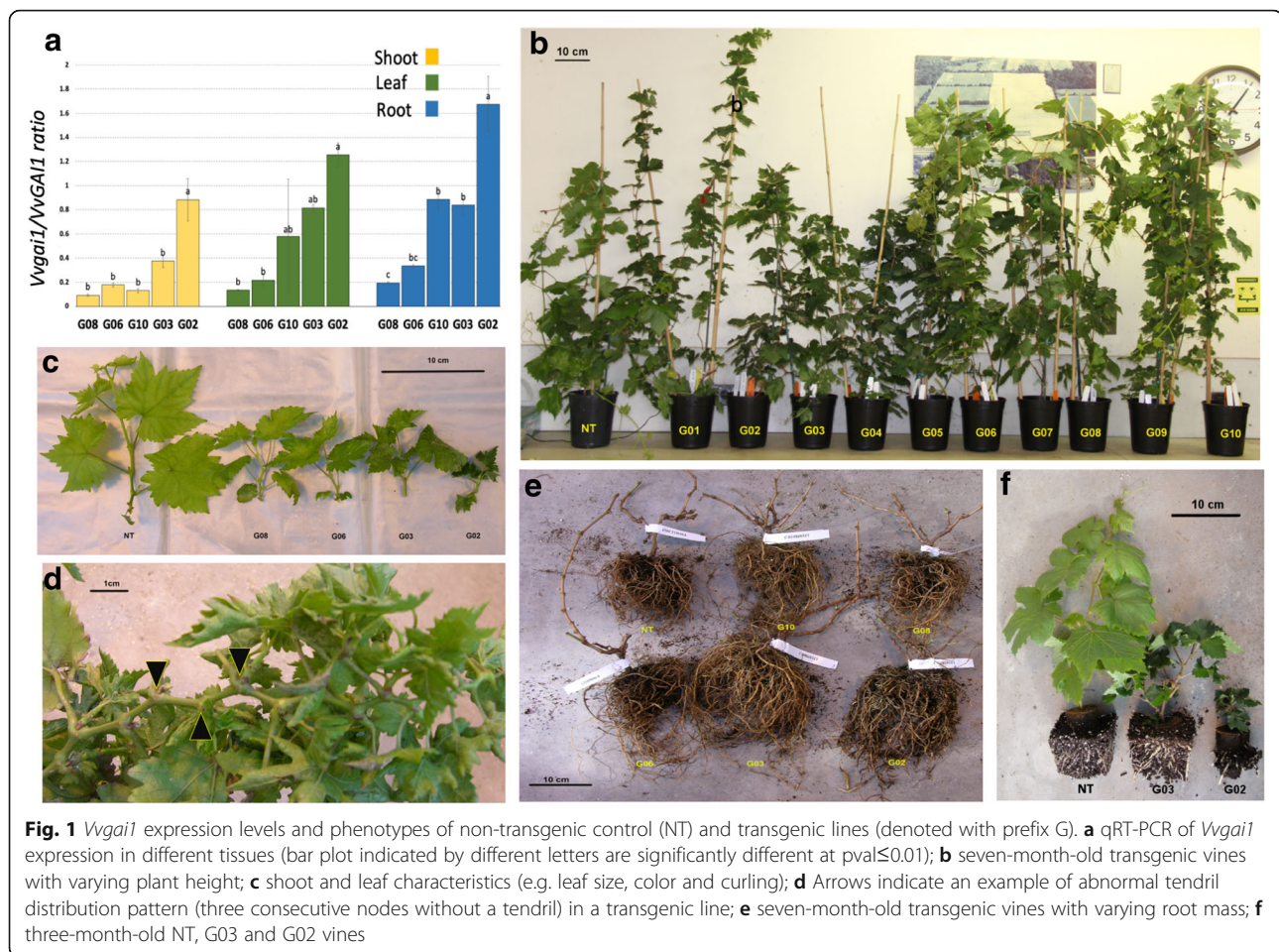### Transgenic *Vvgai1* expression and phenotypes

Ten *Vvgai1* transgenic vines were generated in this study and five representative ones covering a range of mutant phenotypic variations were chosen for RNA-Seq analysis. The internode length of four transgenic lines were significantly shorter than the NT in various degrees, with approximate three-fold reduction for the most severe transgenic line G02 (Fig. 1a and b, Table 1). More severe dwarf lines had smaller shoots and leaves that were darker and curled at the edges (Fig. 1c). The distinctive *V. vinifera* shoot patterning of two consecutive nodes with a tendril followed by node without a tendril [18] was proportionally disrupted with a higher frequency of tendril skips among the shorter dwarf lines. The tendrils also appeared progressively late among the severe dwarf vines, as late as the tenth node in G03 compared to the fifth in the NT (Fig. 1d, Table 1). No tendrils were observed for the most severe G02 line even after 1 year growth in greenhouse conditions (data not shown). More severe dwarf lines had proportionally enlarged and denser roots (Fig. 1e, f) and showed poorer root establishment.

As expected, *Vvgai1* expression was found in all the five transgenic lines but not in the NT using digital qRT-PCR. Among the five transgenic lines, the most severe dwarf G02 showed statistically the highest *Vvgai1* expression in all three tissues (Table 1, Fig. 1a). These results suggest that there was a positive correspondence between the relative *Vvgai1* expression levels (*Vvgai1/VvGAI1* ratios) and the observed severity of phenotypic changes among the transgenic lines.

### Differentially expressed genes associated with transgenic *Vvgai1* expression

Differential gene expression analyses in the leaf, shoot and root tissues were separately conducted for the five transgenic lines. More severe dwarf lines generally have more DEGs, although such trend was not observed in the root tissue (Fig. 2, Additional file 1: Table S1). To capture maximum numbers of DEGs associated with *Vvgai1* with the least false positives, we opted to retain only those DEGs (1.5x-fold changes at FDR ≤ 0.01) in the most severe transgenic line G02 and concurred in at least one of the four remaining transgenic lines. We also identified 25 genes originally filtered out for not having the minimum reads (i.e. 1CPM) in all the 12 libraries, but were in fact expressed only in the transgenic lines and suppressed in the NT, or vice-versa (Additional file 2: Table S2). This recovered set had several cell-wall related genes induced only in the leaf of the transgenic lines and a key flowering gene *SHORT VEGETATIVE PHASE* (*VvSVP*) highly suppressed in the root of the transgenic lines. The final numbers of DEGs for subsequent analyses were 153, 719, and 2314 for shoot, leaf and root, respectively, totaling to 2986 genes significantly differentially expressed at least once in the three sampled tissues (Fig. 2).

**Fig. 1** *Vvgai1* expression levels and phenotypes of non-transgenic control (NT) and transgenic lines (denoted with prefix G). **a** qRT-PCR of *Vvgai1* expression in different tissues (bar plot indicated by different letters are significantly different at pval≤0.01); **b** seven-month-old transgenic vines with varying plant height; **c** shoot and leaf characteristics (e.g. leaf size, color and curling); **d** Arrows indicate an example of abnormal tendril distribution pattern (three consecutive nodes without a tendril) in a transgenic line; **e** seven-month-old transgenic vines with varying root mass; **f** three-month-old NT, G03 and G02 vines

We validated the RNA-Seq data by analyzing the digital RT-PCR expression of 30 notable genes related to DELLA regulation from the same tissues. The RT-PCR and RNA-Seq datasets were in a good agreement with a general correlation coefficients (r) ranging from 0.77 to 1.0 (Additional file 3: Table S3).

## Gene ontology (GO) analysis

The annotated DEGs in the shoot and leaf tissues were comprised of about twice as many suppressed (96 for the shoot and 494 for the leaf) as induced ones (57 for the shoot and 225 for the leaf), while the root had about the same (1124 suppressed and 1190 induced) (Fig. 2).

**Table 1** Phenotypic and *Vvgai1* expression variation observed in NT and five transgenic lines (denoted with prefix G) in the study

| Genotype ID | Leaf weight (g)[4] | Internode length (cm, average of 10 nodes)[4] | Average no. of lateral branches with 5 or more nodes[4] | Average node position having first tendril[4] | Average no. of tendril per node[4] | Chlorophyll content[2] | *Vvgai1* in shoot[3] | *Vvgai1* in leaf[3] | *Vvgai1* in root[3] |
|---|---|---|---|---|---|---|---|---|---|
| G02 | 0.31* | 1.18** | 0.00 | NA[1] | NA[1] | 1.58** | 0.89[a] | 1.25[a] | 1.67[a] |
| G03 | 0.90 | 1.87** | 0.33* | 10.43** | 0.54** | 1.36* | 0.37[b] | 0.81[ab] | 0.84[b] |
| G10 | 0.71* | 2.41** | 2.33 | 5.47 | 0.66 | 1.04 | 0.13[b] | 0.59[ab] | 0.88[b] |
| G06 | 1.20 | 2.71* | 0.60** | 7.54** | 0.62** | 1.16* | 0.18[b] | 0.22[b] | 0.33[c] |
| G08 | 0.86* | 3.36 | 2.00 | 7.20* | 0.65 | 0.95 | 0.09[b] | 0.13[b] | 0.19[c] |
| NT | 1.06 | 3.67 | 5.50 | 5.86 | 0.68 | 1.00 | – | – | – |

*, ** Significantly different from NT at $p < 0.05$ and $< 0.01$, respectively
[1]No observable tendrils at the time of sampling about 5 months after potting
[2]A ratio of observed absorbance reading of a transgenic line to the NT's on the basis of a bulked sample of five mature leaves
[3]A ratio of *Vvgai1/VvGAI1* expression derived from qRT-PCR; means followed by the same letters were not significantly different at $p < 0.01$
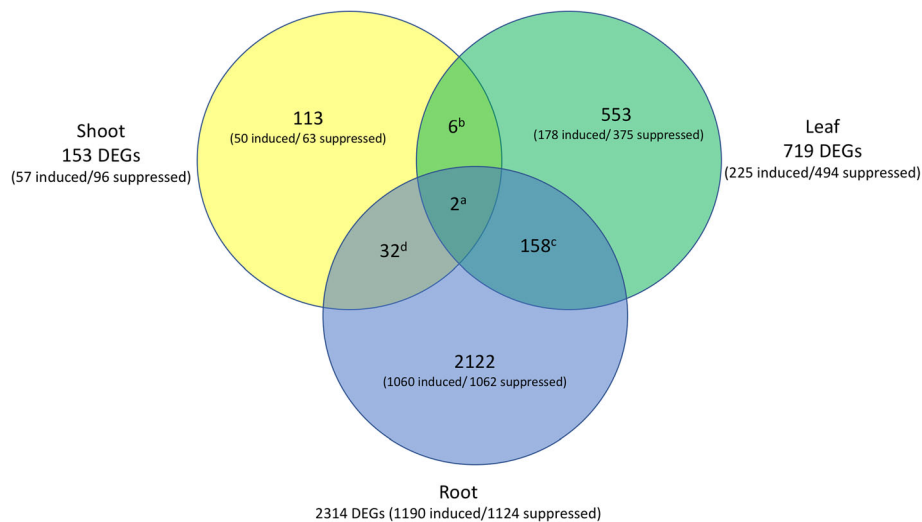[4]Measurements were taken from five biological replicates

**Fig. 2** Distribution of the 2986 DEGs (1.5x-fold changes at FDR ≤ 0.01) among the shoot, leaf and root tissues. a:2 suppressed in all tissues; b:4 suppressed in both shoot and leaf, 1 induced in both shoot and leaf, 1 induced in shoot, but suppressed in leaf; c:8 suppressed in both leaf and root, 13 induced in both leaf and root, 33 induced in leaf, but suppressed in root, 104 suppressed in leaf, but induced in root; d:17 suppressed in both shoot and root, 4 induced in both shoot and root, 2 induced in shoot, but suppressed in root, 9 suppressed in shoot, but induced in root

The enriched GO terms have large overlaps of the member genes, and a semantic clustering analysis revealed about ten common interdependent biological themes (Table 2). This is not a surprise as the arbitrary process of clustering involved a considerable number of master growth regulators with broad biological roles known to coordinate plant's morphogenetic changes and responses to stimuli, such as the transcription factors *NAM/ATAF1,2/CUC2 (NAC), WRKY, SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL), TB1-CYC-PCF (TCP)* and *THREE-AMINO-ACID-LOOP-EX-TENSION (TALE)* class of homeoproteins. There were also numerous biosynthesis and signaling genes belonging to hormones auxin, cytokinin, brassinosteroids, and ethylene.

## Expression profiles of key GA signaling genes

Similar to the currently accepted model in *Arabidopsis* [6], the grapevine GA signaling are comprised of three DELLA homologues of *VvDELLA1, 2* and *3*, two GA receptor GID1 of *VvGID1a* and *VvGID1b*. Grapevine, however, have two proposed F-box SLY1 genes, *VvSLY1a* and *VvSLY1b* [19]. *VvDELLA1* (synonymous to *VvGAI1*) in the NT was more abundant in the shoot and leaf (~ 50 CPM), about five folds higher than that in the root (~ 10 CPM). *VvDELLA1* in the transgenic G02 line, however, was differentially expressed with about two folds higher than the NT in the leaf and root, but not in the shoot (Fig. 3a). *VvDELLA2* had the highest expression in the root among the three NT tissues (30–240 CPM) and its expression in the root, but not in the other two tissues, was increased more than twice in the

transgenic G02 line. *VvDELLA3* was consistently expressed at low levels in all three tissues in both NT and transgenic background (~ 10 CPM). The expression profiles of *VvDELLA1* and *VvDELLA2* were validated by RT-PCR with high correlation coefficients (r = ~ 0.95, Additional file 3: Table S3). The endogenous *VvDELLAs* seemed responsive to and inducible by the transgenic *Vvgai1*.

The two homologues of GA receptor GID1 were abundantly expressed in all tissues in the NT. In the transgenic lines, *VvGID1b* showed five folds of expression increases in the leaf (~ 40 CPM to ~ 220 CPM) (Fig. 3b). The two grapevine F-box genes, *VvSLY1a* and *VvSLY1b*, consistently showed low levels of expression (< 10 CPM) in both NT and transgenic background (Fig. 3c).

Homologues of the GA biosynthesis genes *GA3 oxidase (GA3ox)* and *GA20 oxidase (GA20ox),* another group of recipients of the DELLA-controlled feedback mechanism (like *GID1*) [20], were induced in all the three sampled tissues but at different levels, seemingly preserving the observed tissue-specificities of this multi-gene family [21]. *VvGA20ox2a* showed 15-, 2- and a dramatic 50 folds of increases in the shoot, leaf and root, respectively (Fig. 3d). In a similar manner, *VvGA3ox1a* was induced by more than four folds in the leaf, and 10 folds in the root (Fig. 3e). In consonance, the GA deactivating *GA2ox* genes were generally suppressed in the transgenic background. The largest suppression was observed for *VvGA2ox1a,* which decreased by about nine folds (from 28 CPM to 3 CPM) and two folds (76 CPM to 40 CPM) in the shoot and root, respectively. *VvGA2ox8a* was also suppressed with a

**Table 2** Enriched GO terms and their average correlations (Pearson r) with *Vvgai1* expression levels in the shoot, leaf and root transcriptome

| Tissue | Class | Representative GO cluster term [a] | GO annotation | GO ID | Enrichment FDR (log10)[b] | Ave r pval (log10)[c] | Notable genes[d] |
|---|---|---|---|---|---|---|---|
| Shoot | Negatively correlated | 1 | amino sugar catabolic process | GO:0046348 | 3.4 | 5.77 | MAPK3, MATE EFFLUX, ATCHITIV, ATCHIA |
| | | 1 | cell wall macromolecule catabolic process | GO:0016998 | 3.66 | 5.65 | |
| | | 1 | hormone catabolic process | GO:0042447 | 3.01 | 3.41 | |
| | | 2 | cellular process | GO:0009987 | 4.22 | 4.58 | |
| | | 3 | response to red light | GO:0010114 | 3.6 | 5.47 | PR4, YSL3, GA2ox1, PRR7, BES1, LAC1, GRF5, DWARF4, TEMPRANILLO 2 |
| | | 3 | response to chemical | GO:0042221 | 3.05 | 4.63 | |
| | | 3 | response to abiotic stimulus | GO:0009628 | 4.46 | 4.58 | |
| | | 4 | developmental process | GO:0032502 | 3.7 | 4.85 | |
| | | 7 | floral meristem determinacy | GO:0010582 | 3.01 | 5.64 | SPLs, VvSVP, ACO4, CKX, CKX5, PRX66 |
| | | 7 | anatomical structure development | GO:0048856 | 3.49 | 5.11 | |
| Leaf | Positively correlated | 1 | phenylpropanoid metabolic process | GO:0009698 | 12.48 | 19.72 | FLS1, ATCHS, 2-ODD, UGT86A2 |
| | | 1 | aromatic compound biosynthetic process | GO:0019438 | 3.8 | 12.46 | |
| | | 10 | oxidation-reduction process | GO:0055114 | 6.01 | 15.09 | CCD1, MES10,CAT2 |
| | | 10 | secondary metabolic process | GO:0019748 | 6.07 | 13.8 | |
| | Negatively correlated | 1 | DNA replication initiation | GO:0006270 | 4.7 | 4.05 | CDC45, MCM2, GRFs, MYB59, NAC71, WRKY 57 |
| | | 1 | polysaccharide metabolic process | GO:0005976 | 2.86 | 9.22 | |
| | | 10 | lipid metabolic process | GO:0006629 | 2.74 | 9.77 | |
| | | 7 | regionalization | GO:0003002 | 4.68 | 10.59 | SHR, YABBY1, AS1, CIB4, DWARF4 |
| | | 7 | shoot system morphogenesis | GO:0010016 | 3.19 | 9.57 | |
| | | 7 | pattern specification process | GO:0007389 | 5.49 | 9.56 | |
| | | 8 | chromatin organization | GO:0006325 | 2.63 | 4.81 | CDC45, MCM2 |
| Root | Positively correlated | 1 | toxin catabolic process | GO:0009407 | 3.52 | 6.66 | SCR 21, VvDELLA1, VvDELLA2, PIF1, KNAT3, ARF16, ARF11, VvTFL1 |
| | | 1 | phenylpropanoid metabolic process | GO:0009698 | 12.48 | 5.07 | |
| | | | cellular biosynthetic process | GO:0044249 | 3.8 | 4.84 | |
| | | 1 | hydrogen peroxide catabolic process | GO:0042744 | 4.42 | 4.38 | |
| | | 3 | ethylene-activated signaling pathway | GO:0009873 | 3.96 | 6.9 | KO1, GA3ox, GA20ox1, GASA6, LRR family, UMAMIT42 |
| | | 3 | response to chemical | GO:0042221 | 19.33 | 4.93 | |
| | | 3 | response to stress | GO:0006950 | 23.53 | 4.74 | |
| | | 3 | response to abiotic stimulus | GO:0009628 | 4.01 | 4.36 | |
| | | 7 | coenzyme A metabolic process | GO:0015936 | 4.34 | 6.83 | PIF1, VvTFL1, C 4, MAP65–6, TUB8, SHR, CYP722A1, FLA4, MFS family, |
| | | 7 | cell recognition | GO:0008037 | 3.31 | 5.15 | |
| | | 7 | indole-containing compound metabolic process | GO:0042430 | 3.07 | 4.69 | |
| | | 7 | organic acid metabolic process | GO:0006082 | 10.66 | 4.44 | |
| | Negatively correlated | 1 | gene expression | GO:0010467 | 3.37 | 4.88 | CHS, LACASSE, 2-ODD, AP2/ ERF, WRKY |
| | | 1 | cellular macromolecule biosynthetic process | GO:0034645 | 4.29 | 4.78 | |
| | | 1 | cellular biosynthetic process | GO:0044249 | 3.33 | 4.64 | |
| | | 3 | gibberellic acid mediated signaling pathway | GO:0009740 | 6.55 | 8.79 | PIN3, EIN3, ARR5, ARR9, OMR1, ACS1 |
| | | 3 | ethylene-activated signaling pathway | GO:0009873 | 3.55 | 6.72 | |

**Table 2** Enriched GO terms and their average correlations (Pearson r) with *Vvgai1* expression levels in the shoot, leaf and root transcriptome *(Continued)*

| Tissue | Class | Representative GO cluster term [a] | GO annotation | GO ID | Enrichment FDR (log10)[b] | Ave r pval (log10)[c] | Notable genes[d] |
|--------|-------|-----------------------------------|---------------|-------|---------------------------|----------------------|------------------|
| | | 3 | response to red light | GO:0010114 | 5.24 | 6.31 | |
| | | 3 | response to chemical | GO:0042221 | 4.07 | 5.18 | |
| | | 3 | response to abiotic stimulus | GO:0009628 | 9.32 | 4.83 | |
| | | 7 | anatomical structure development | GO:0048856 | 4.39 | 4.46 | |
| | | 4 | developmental process | GO:0032502 | 3.96 | 4.4 | |
| | | 5 | localization | GO:0051179 | 4.49 | 3.87 | |
| | | 6 | metabolic process | GO:0008152 | 9.78 | 4.19 | |
| | | 9 | response to stimulus | GO:0050896 | 6.39 | 4.61 | |

[a]GO cluster term: 1:Aromatic compound catabolism; 2:Cellular process; 3:Detoxification of nitrogen compound; 4:Developmental process; 5:Localization; 6:Metabolism; 7:Pattern specification process; 8:Protein polymerization; 9:Response to stimulus; and 10:Secondary metabolism
[b]Enrichment pval (log$_{10}$ scale) as determined by PlantMet GenMap
[c]Average expression correlation (Pearson's r pval (log$_{10}$ scale)) of the member genes with the expression level of *Vvgail* in the enriched GO term
[d]While these genes were listed under a specific GO cluster, many of them were present in other clusters as well

decrease of about 2.5 folds (55 CPM to 20 CPM) in the leaf (Fig. 3f). These results affirm the positive feedback mechanism facilitated by DELLA to *GID1* and the biosynthesis genes *GA20ox* and *GA3ox* [15]. Interestingly in this study, we observed that such feedback signal appeared tissue-specific, i.e. different *GID1* and GA biosynthesis homologues induced in different tissues.

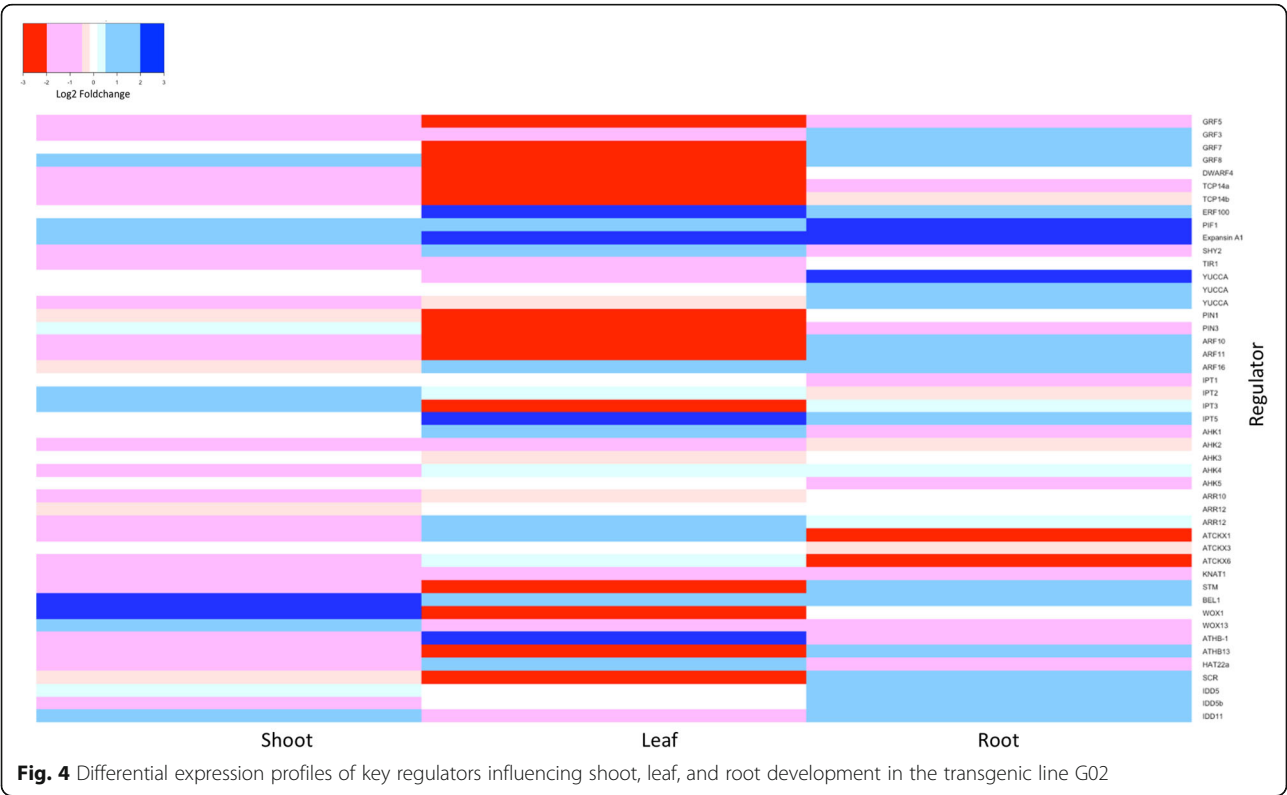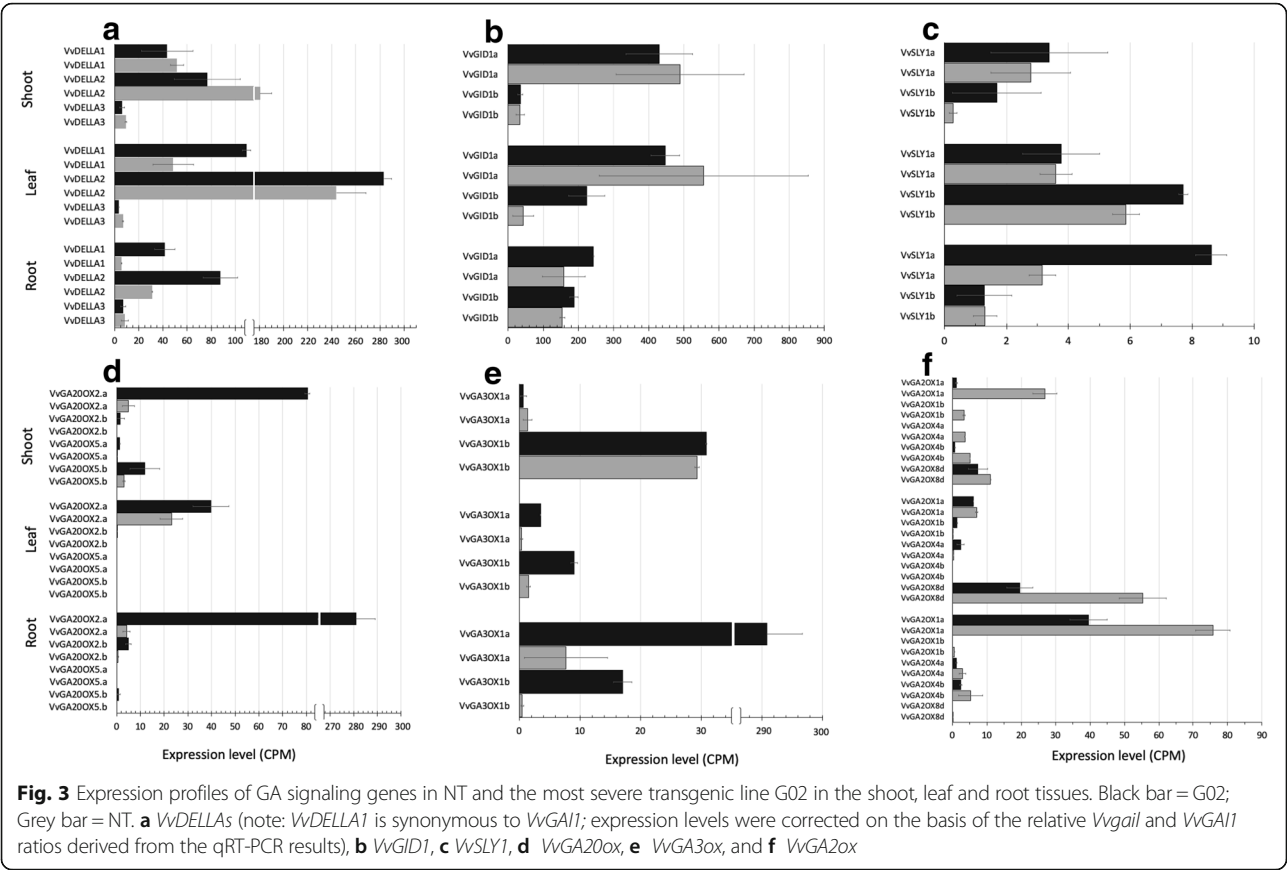## Expression profiles of some key genes influencing shoot, leaf, and root development

A number of highly responsive DEGs observed are regulators of developmental processes. Among the highly suppressed were members of the *GROWTH REGULATING FACTOR* (GRF) gene family, important regulators mostly found in intercalary meristem and leaf primordia [22]. For example, the homologue *GRF5* controlling cell number related to leaf size [23] was suppressed by about 20 folds in the leaf of the G02 line (Fig. 4). The brassinosteroid biosynthesis gene *DWARF4*, another key gene synergistically associated with auxin [24] and GA regulation [25] and whose loss-of-function mutants were dwarfs with round and dark-green leaves in shortened petioles [26], was suppressed in all three tissues, but especially in the leaf of the G02 line by nine folds (Fig. 4). The TCP transcription factor, a master regulator that influences plant height, leaf curvature [27] and tendril phylloctatic patterning in grapevine [28], was suppressed at four to nine folds in the shoot and leaf of the transgenic G02 line (Fig. 4).

Genes for light-mediated developmental processes were notably induced. *ERF105*, a gene active during intense light and freezing stress conditions [29, 30], was induced by seven and three folds in the leaf and root, respectively, and the *PHYTOCHROME INTERACTING FACTOR 1 (PIF1)*, a major gene involved in chloroplast

production through maintenance of cell number in the leaf [31], was increased by three folds in the shoot and leaf and about eight folds in the root (Fig. 4). The cell wall loosening gene *EXPANSIN A1* was also induced about two folds in the shoot and six folds in the leaf and root of the transgenic background (Fig. 4).

### Auxin and cytokinin

Auxin and cytokinin related genes have extensive cross-talk with GA-DELLA regulation as master regulators for many growth and development processes in plants [32]. Auxin-related genes seemed generally active in all transgenic lines. *SHORT HYPOCOTYL 2 (SHY2)*, a negative regulator of auxin responses [33], was negatively correlated to *Vvgai1* expression in the shoot and root with a suppressed response of about two and four folds, respectively, in the transgenic line G02 (Fig. 4). Conversely, the grape homologous gene of *TRANSPORT INHIBITOR RESPONSE 1 (TIR1)*, a positive regulator of auxin [34], was only slightly induced in the shoot but increased by about two folds in the root. In conjunction, two homologues of the auxin biosynthesis gene *YUCCA* showed induced responses more prominently in the root. On the other hand, related auxin transporters and response factors exhibited various responses in the sampled tissues, reflecting the dynamic auxin flux. The grape homologue of *PIN-FORMED3 (PIN3)* was suppressed and significantly negatively correlated with *Vvgai1* in their expression in the root, which was validated by our qRT-PCR (Additional file 3: Table S3). The grape homologue of *AUXIN RESPONSE FACTOR11 (ARF11)*, one of the response factors antagonized by cytokinin when over-expressed in the root [35] was strongly induced. Interestingly, activities of cytokinin-related genes seemed unusually low in our transgenic lines. The

**Fig. 3** Expression profiles of GA signaling genes in NT and the most severe transgenic line G02 in the shoot, leaf and root tissues. Black bar = G02; Grey bar = NT. **a** *VvDELLAs* (note: *VvDELLA1* is synonymous to *VvGAI1*; expression levels were corrected on the basis of the relative *Vvgai1* and *VvGAI1* ratios derived from the qRT-PCR results), **b** *VvGID1*, **c** *VvSLY1*, **d** *VvGA20ox*, **e** *VvGA3ox*, and **f** *VvGA2ox*



**Fig. 4** Differential expression profiles of key regulators influencing shoot, leaf, and root development in the transgenic line G02

biosynthesis genes homologous to *ISOPENTENYL-TRANSFERASE2* (*IPT2*) and *IPT3* were of low expression levels (10 CPM) in the three sampled tissues. The grape *CYTOKININ OXIDASE* (*CKX*) gene family that irreversibly deactivate cytokinins [36] was suppressed in the root, although some were interestingly induced in the leaf (Fig. 4).

### Homeodomain gene family

The expressions of many homeodomain transcription factors were generally suppressed and a large number of them belonged to the family classes of *TALE, WUSCHEL RELATED HOMEOBOX* (*WOX*) and *HOMEODOMAIN-LEUCINE ZIPPER* (*HD-ZIP*) (Additional file 1: Table S1). These homeodomain transcription factors are extensively involved in regulation of meristem maintenance and plant form [37], and manifest disruption of shoot phyllotaxy [38]. *TALE* has two subfamilies, *KNOTTED1-like* (*KNOX*) and *BELLRINGER* (*BEL*). In the *KNOX* subfamily, *KNOTTED-LIKE FROM Arabidopsis thaliana1* (*KNAT1*) and *SHOOT MERISTEMLESS* (*STM*) are a homologous pair that synergistically interact with cytokinin in the shoot apical meristem (SAM) [39]. We observed that *STM* was suppressed in the shoot and leaf of the transgenic line G02; *KNAT1* was suppressed only in the shoot (Fig. 4); and *KNAT1* was not expressed in the leaf which is in agreement with its negative role in leaf primordia initiation [40]. *BEL1* (*BELLRINGER 1*), a member of the BEL subfamily which forms critical KNOX-BEL heterodimers for proper initiation and maintenance of the apical meristem [41], was induced in all three tissues in the transgenic line G02. WOX family genes are specialized transcription factors found during embryogenesis and lateral organ formation [42]. In transgenic line G02, *WOX1* gene was induced in the shoot but suppressed in the leaf, while the *WOX13* was suppressed in the root (Fig. 4). Furthermore, we found that the HD-ZIP family member genes were generally suppressed in the shoot and root tissues. HD-ZIP member genes are associated with organ and vascular development [43].
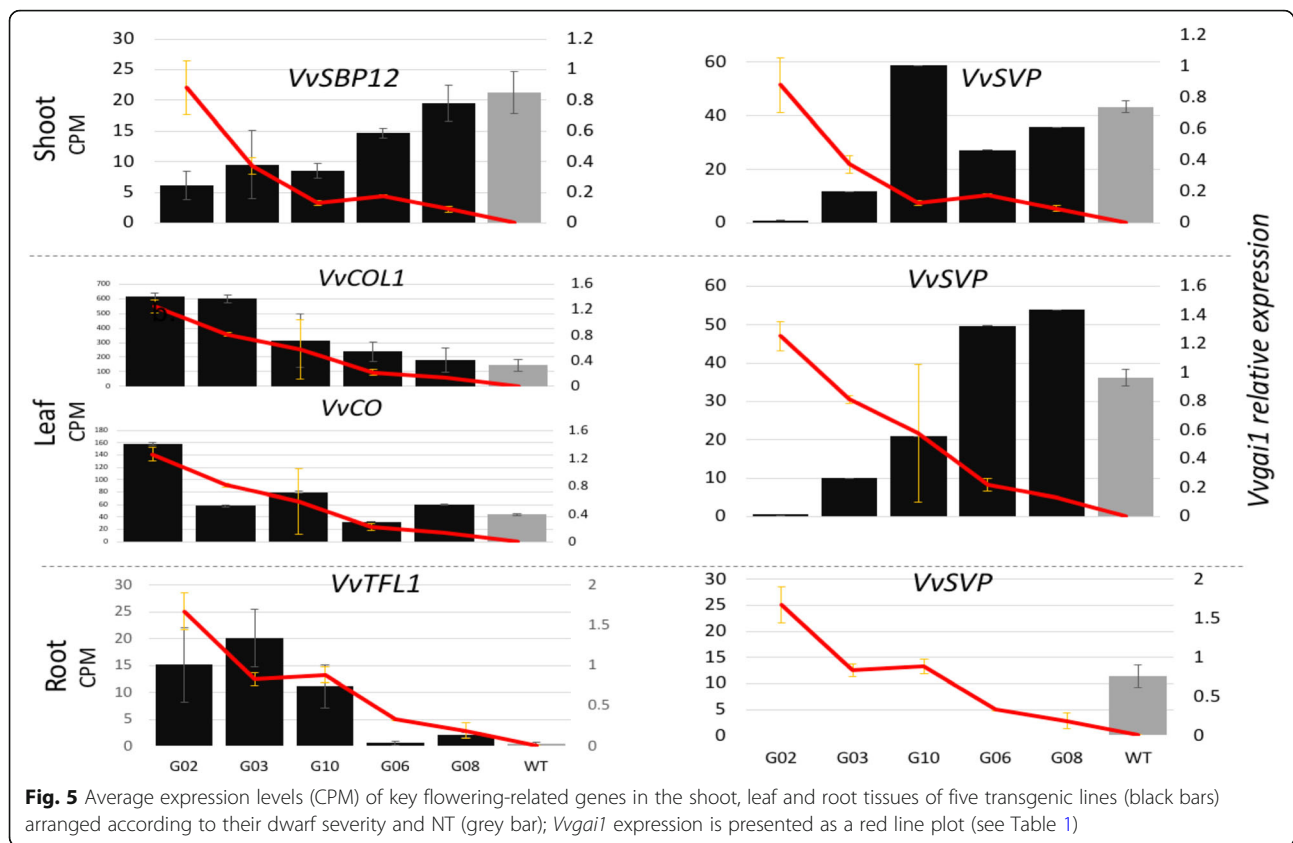
### Flower-induction pathways

Four flowering-related genes showed significant responses to *Vvgai1* expression in this study. *VvSBP12* is a member of the miR156-targeted grapevine SPL genes [44] and its homologue in *Arabidopsis*, *AtSPL3*, was shown to be a target of suppression by DELLA and a target of miRNA156/-172 phase-change regulation [45]. The *VvSBP12* was suppressed in all three tissues of the G02 line, especially in the shoot (Fig. 5). Interestingly, one of the homologues of a novel gene family associated with miRNA metabolism, HEN1 [46], showed variable response patterns among the three tissues, being induced in the shoot and leaf but strongly suppressed in the root (Additional file 1: Table S1).

On the other hand, the two *CONSTANS* homologues in grapevine, *VvCOL1* and *VvCO*, were induced in all three tissues (Fig. 5), which is in contrast in *Arabidopsis'* where they were reported as targets of suppression by DELLA [47]. Interestingly, *VvTFL1* gene, a negative regulator of meristem identity [48], was notably suppressed in the shoot but induced in the root (Fig. 5, Table 3, Additional file 1: Table S1). This intriguing expression profile was verified by the qRT-PCR results ($r = \sim 0.99$) (Additional file 3: Table S3). Furthermore, we observed that the MADS-domain-carrying *VvSVP* gene was highly suppressed in all three tissues (Fig. 5). As predicted from the suppression status of the key flowering induction genes above, we found that some of the floral meristem identity genes were not induced. Although the floral meristem identity gene *FLOWERING LOCUS T* (*FT*) had no detectable transcripts in both NT and transgenic lines, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) was expressed but not significantly differentially expressed. LFY was found expressed only in the shoot where it was likewise not differentially expressed. In grapevine, these meristem identity genes were expressed in the lateral branches after dormancy [49].

### Transcription factor motif enrichment

Transcription factor enrichment analysis among the *Vvgai1*-correlated DEGs ($r \geq abs$ (0.70)) revealed a total of 17 significantly over-represented regulatory motifs (Additional file 4: Table S4). Of these the DELLA motif was the most over-represented in all three tissues: 45% of the suppressed DEGs in the shoot (49 out of 110), 37% of the suppressed DEGs in the leaf (269 out of 725) and 34% of the induced DEGs in the root (126 out of the 371). These amounted to 395 DELLA-bearing DEGs which were about 13% of the 2986 *Vvgai1*-correlated DEGs.

To assess the extent of possible co-regulation of DELLA with other transcription factors, we examined these 395 DELLA-bearing DEGs for the abundance of other regulatory motifs. Computational motif prediction based on PlantTFDB 4.0 [50] revealed that aside from the DELLA motif, at least two of the 16 motifs discussed earlier were present in any of these DEGs (Table 3, Additional file 5: Table S5), highlighting the complex nature of interdependency among transcriptional networks involving DELLAs. The most frequent motifs co-located with DELLA were gene families of TALE, TCP, INDETERMINDATE DOMAIN (IDD), AP2/ERF, and MYELOBLASTOSIS (MYB). TALE was the most frequent motif co-located with DELLA, sharing 71% (35 out of 49), 62% (167 out of 269) and 46% (59 out of 126) of the DELLA-enriched DEGs in the shoot, leaf and root, respectively (Additional file 5: Table S5). The remaining motifs were of lower frequency and tend to be

**Fig. 5** Average expression levels (CPM) of key flowering-related genes in the shoot, leaf and root tissues of five transgenic lines (black bars) arranged according to their dwarf severity and NT (grey bar); *Vvgai1* expression is presented as a red line plot (see Table 1)

tissue-specific. The class I and II TCP motifs, for example, were found in about 4–10% of the DELLA-enriched DEGs, most of which were in the leaf. The R2R3 MYB motif, as represented by three genes (*MYB15, MYB61, MYB88*) involved in stomatal movement and lignification [51–53], was co-located with DELLA in about 4–60% of the DELLA-enriched DEGs in the shoot, 4–15% in the leaf, and 2–15% in the root. The C2H2 zinc finger motif were represented by two IDD genes (*IDD5* and *IDD11*) and co-shared with the DELLA motif in 4–8% of the DEGs in the shoot, 5–8% in the leaf, and 2–17% in the root.

The DEGs with predicted DELLA-TALE motifs include notable meristem regulator genes in varying correlation to *Vvgai1* in the respective tissues. In the shoot, they included the suppressed FT-LIKE homologue *VvTFL1* (r = − 0.73) and *VvSBP12* (r = − 0.73), *SPL13* (r = − 0.71), and *SPL9* (r = − 0.75) (Table 3). In the leaf, they included suppressed genes such as the protein-protein mediating calmudulin, *IQ-domain 5* (r = − 0.96), auxin-response factor 11 (r = − 0.93), flowering regulator *EARLY BOLTING IN SHORT DAYS* (r = − 0.82), leaf morphogenesis regulator *YABBY1* (r = − 0.92) and the homeodomain *BLH4* (r = − 0.78). In the root, they included induced genes *VvTFL1* (r = 0.76), *BEL1* (r = 0.81), an integument regulator *KANADI 4* (r = 0.84), and *IDD5* (r = 0.93) in the root (Table 3).

## Discussion

Global responses to DELLA-mediated regulation were previously reported but most were on annual model species, for a specific tissue or a specific developmental stage [21, 54, 55]. In this study, we analyzed RNA-Seq profiles in the shoot, leaf, and root of five transgenic GA-insensitive lines whose severity of mutant phenotypes corresponded well with the levels of transgene *Vvgai1* expression. By examining differential expression and correlation to *Vvgai1* in three tissues, we were able to dissect both global and tissue-specific regulation of DELLA, one of its characteristic regulatory features [56]. Our study has not only confirmed many of the previously reported GA- and DELLA-related genes and GO processes, but also found new DELLA targets and action modes, including the involvement of meristem regulators in co-regulating vine development and identification of several key flowering induction genes which responded to *Vvgai1* differently from annual species.

### DELLA's impact on the expression of GA signaling genes

In agreement with the previous report [19], we found *VvDELLA2* abundantly expressed in all three tissues of the NT, followed by *VvDELLA1*, while the expression of *VvDELLA3* was generally low. Interestingly, the endogenous *VvDELLAs* were significantly induced, but

**Table 3** Important DEGs predicted to carry both DELLA (*VvDELLA1*) and TALE (i.e. *KNAT1*) motifs and their expression correlation to *Vvgai1* in the shoot, leaf and root

| Gene ID | Arabidopsis ID | Gene Name | Correlation to *Vvgai1* | | | Annotation |
| --- | --- | --- | --- | --- | --- | --- |
| | | | Shoot | Leaf | Root | |
| GSVIVG01036145001 | AT2G27550 | VvTFL1 | −0.725*** | | 0.765*** | *CENTRORADIALIS* |
| GSVIVG01033064001 | AT5G50570 | VvSBP12 | −0.713*** | −0.844*** | | Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein |
| GSVIVG01033519001 | AT2G42200 | SPL9 | −0.749*** | −0.827*** | | squamosa promoter binding protein-like 9 |
| GSVIVG01010522001 | AT5G50570 | SPL13 | −0.73*** | | | Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein |
| GSVIVG01037598001 | AT3G22190 | IQD5 | | −0.964*** | | IQ-domain 5 |
| GSVIVG01027429001 | AT4G00820 | IQD17 | | −0.826*** | | IQ-domain 17 |
| GSVIVG01004809001 | AT4G22140 | EBS | | −0.821*** | | PHD finger family protein / bromo-adjacent homology (BAH) domain-containing protein |
| GSVIVG01001269001 | AT2G45190 | YABBY1 | | −0.923*** | | Plant-specific transcription factor YABBY family protein |
| GSVIVG01027648001 | AT2G45190 | YABBY1 | | −0.851*** | | Plant-specific transcription factor YABBY family protein |
| GSVIVG01019399001 | AT5G41410 | BEL1 | | | 0.811*** | POX (plant homeobox) family protein |
| GSVIVG01019043001 | AT2G23760 | BLH4 | | −0.781*** | 0.715*** | BEL1-like homeodomain 4 |
| GSVIVG01009589001 | AT5G42630 | KAN4 | −0.73*** | −0.916*** | 0.868*** | Homeodomain-like superfamily protein |
| GSVIVG01035391001 | AT3G13810 | AtIDD11 | | −0.8*** | 0.889*** | Indeterminate(ID)-domain 11 |
| GSVIVG01010283001 | AT2G02070 | AtIDD5 | | | 0.93*** | Indeterminate(ID)-domain 5 |
| GSVIVG01010284001 | AT2G02070 | AtIDD5 | | −0.733*** | 0.983*** | Indeterminate(ID)-domain 5 |
| GSVIVG01011412001 | AT5G15230 | GASA4 | | −0.845*** | | GAST1 protein homolog 4 |
| GSVIVG01035048001 | AT5G49300 | GATA16 | | −0.879*** | | GATA transcription factor 16 |
| GSVIVG01017011001 | AT4G32890 | GATA9 | | −0.911*** | | GATA transcription factor 9 |
| GSVIVG01019913001 | AT4G37740 | AtGRF2 | | −0.841*** | | growth-regulating factor 2 |
| GSVIVG01027535001 | AT2G36400 | GRF3 | −0.863*** | | | growth-regulating factor 3 |
| GSVIVG01038629001 | AT5G53660 | AtGRF7 | | −0.852*** | | growth-regulating factor 7 |

*$p$val ≤0.05; **$p$val ≤0.01; ***$p$val ≤0.001

varied from tissue to tissue, suggesting their regulatory interdependence and tissue-specific actions [19, 57]. Equally interesting was the observation that the *Vvgai1* (mutant DELLA) and induced endogenous DELLA transcripts in transgenic lines were not degraded as expected in a wildtype background. This was also in line with the observation of very low transcript levels of *VvSLY1a* and *VvSLY1b* (Fig. 3b), the two F-box genes that degrade DELLA through ubiquination [58]. Low *SLY1* expression was previously found associated with unusually high accumulation of *VvDELLA* and *VvGID1* transcripts [59]. We also noted that the putative DELLA-driven feedback induction of *VvGID1* [15] was tissue-specific as only one of the two *VvGID1* homologues (*VvGID1b)* exhibited a positively correlated change to *Vvgai1* and prominently in the leaf tissue. On the other hand, different homologues of GA biosynthesis components (GA20 oxidases and GA3 oxidases) were induced in the same manner as in annual [12, 21] and perennial species [10].

### DELLA and co-regulation networks in grapevine

The 2986 DEGs identified in this study represent a large number of enriched GO terms which overlapped in the three tissues in various degrees and response states, reflecting not only the breadth of GA signaling cascades [55] but also the dynamic coordination of biological functions within and between tissues [1, 60]. For example, one of the larger GO biological terms was related to meristem regulation and pattern-specification processes and largely enriched among the suppressed DEGs in the shoot and leaf. Included in the GO term were key meristem regulators of gene families such as GRFs, SPLs, SVPs, and genes such as *YABBY*, *AS1*, *STM* and *KNAT1*. In the root, however, the same GO term was enriched among the induced DEGs, including the auxin receptor *TIR1* and the major root growth and patterning regulators, the GRAS *SHORT ROOT* (*SHR*), *SCARECROW* (*SCR),* and the transcription factor *IDD5*.

Misregulations of the meristem-related developmental processes in the transgenic vines was apparent all the more by the fact that numerous classes of homeodomain

transcription factors were generally in a suppressed state in the sampled tissues, including the class I KNOX transcription factors which interact with GA pathway genes in the maintenance of SAM [21, 37, 39]. In *Arabidopsis*, the class I KNOX genes *STM* and *KNAT1* critically coordinate proper SAM growth by properly localizing the hormones cytokinin and GA [39, 61, 62]. Transcriptome scans of GA-insensitive *Arabidopsis* mutants seemed to suggest that both *STM* and *KNAT1* were not particularly expressed at the early stage [15, 55, 63] as much as during the adult flowering stage [54, 64]. Our discovery of a large number of key homeodomain transcription factors among the responsive DEGs at the early growth stages of the GA-insensitive grapevines highlights the unique organ development process in a grapevine shoot – a separate vegetative and reproductive meristem (i.e. anlagen) in the same apical meristem – and the complex regulation of vegetative and reproductive phase transition it entails.

Such complex regulation was also reflected by 17 large and diverse transcription factor motifs statistically over-represented among the DEGs. Of which, DELLA was the largest, found in about 30% of the total DEGs, followed by motifs of several meristem regulators such as TALE, TCP, MYB and IDD, and stress-response regulators, WRKY, NAC and bHLH. Many of these motifs were co-located with DELLA motifs in DEGs, providing opportunities for DELLA to interact with other transcription factors to co-regulate diverse biological processes [7, 65]. Indeed, in the DELLA-motif-bearing DEGs, motifs of several important regulators were present in varying enrichment in three tissues. In the root, the motifs of AP2/ERF, MYB and IDD were relatively more enriched. The enrichment of IDD motif was notable because the IDD5 homologues were recently shown to act as a transcriptional scaffold in the competitive interaction between *SCR* and *DELLA* in mediating root growth [66]. On the other hand, in the suppressed DELLA-bearing DEGs of the shoot and leaf, the motifs of TALE, TCP and MYB were more enriched. TALE was particularly notable because it was the most frequent motif being co-present with DELLA. The putative DELLA-TALE bearing gene families included genes affecting germination (Nuclear Factors Y, IDDs), growth (GATAs), internode elongation (GRFs), shoot and leaf expansion (YABBY, BEL), as well as floral induction phase-change (SPLs, *VvTFL1*) pathways. The discoveries of these diverse transcription regulators and co-presences of their motifs with DELLA in the DEGs provide a potential explanation for how GA-DELLA signaling could impact so many different aspects of plant growth and development.

## DELLA and root growth in grapevine
One interesting phenotypic change of the transgenic vines was an enlarged root which had poor rooting

abilities (data not shown). The root tissue had the largest number of DEGs, compared with the shoot and leaf tissue in the transgenic lines. DELLA motif was statistically over-represented among the induced DEGs. Among these induced DELLA-motif-bearing DEGs were *SHR*, *SCR* and *SCL3*, which are GRAS genes involved in root maintenance and patterning [67]. In the transcriptome profiling of a transgenic GA insensitive poplar, the GRAS *SCR* was also among the genes found induced [68]. However, we observed numerous cyclin genes, D-type cyclins in particular, in a suppressed state in the leaf and root of our transgenic vines, while they were induced in transgenic poplar [68]. The D-type cyclins are mutual activators of SHR-SCR complex [69] and cytokinin [70] regulatory networks for formative cell divisions in the apical meristem, and formation of lateral organs [71]. Suppression of these cyclins in the present study may compromise their interaction with the SHR-SCR complex and cytokinin, which may explain the observed poor rooting ability in our study, in a contrast to the vigorous roots observed in transgenic poplar.

We deduce that cytokinin was diminished in our transgenic lines based on the relatively low transcript levels of its biosynthesis (i.e. *IPT2* and *IPT3*), signal reception (e.g. *AHK4*), and response regulators (type B RR) components. The enlarged roots were likely the manifestation of a very low cytokinin level in the transition zone region, where cytokinin is usually kept relatively higher than auxin to favor cell differentiation and establishment of the size homeostasis of root meristems [72]. In contrast, a root tip usually maintains a high level of auxin that favors cell division needed for maintenance of the stem cell niche, which seemed to be the case in our transgenic vines as indicated by the induced state of auxin biosynthesis (i.e. *YUCCA*), signal receptor (i.e. *TIR1*), transporter (i.e. *PINs*) and response factors (i.e. *ARFs*). Critically, we observed that *SHY2*, the key negative regulator governing auxin:cytokinin crosstalk and balance [73], was suppressed and negatively correlated to *Vvgai1* in the shoot and root. We were unable to find and verify if *SHY2* was likewise suppressed in the microarray-based study of the transgenic GA insensitive poplar [68]. However, our transgenic lines seemed chronically deficient in cytokinin and high in auxin from the expression profiles discussed above. The enlarged roots in our transgenic vines were likely due to low levels of cytokinin and thus reduced levels of cell differentiation and proper control of root meristem size. In parallel, the disrupted balance between auxin:cytokinin might result in more accumulation of auxin which can lead to poor rooting ability as previously observed in *Arabidopsis* [74].

## DELLA and grapevine flowering-induction
The induced responses of *VvCO* and *VvCOL1* to *Vvgai1* discovered in this study was in a sharp contrast to the

findings that *AtCO* was suppressed by DELLA in *Arabidopsis* [47]. Recent study showed that *VvCO* and *VvCOL1* positively regulated lateral bud induction and dormancy of grapevine; but in contrast to the *CO* and *COL1* in *Arabidopsis*, the diurnal oscillation of *VvCO* and *VvCOL1* was less affected by continuous light and had a higher amplitude that instead peaked at dawn [75]. Since it was shown that lower temperature favors tendril over inflorescence in grapevine [76] and GA signaling exhibited diurnal periodicity in *Arabidopsis* [77], GA and the photoperiod pathway genes in grapevine might have been adaptively modified to perceive warmer temperatures and light intensity instead, and less of daylength as dominant stimuli for flower induction [76].

Similar to GA-insensitive *Arabidopsis* [45], expression of several *VvSPL* genes were suppressed in the shoot and leaf of our transgenic lines. The GA-insensitive *Arabidopsis* was late-flowering because DELLA sequestered the *SPL* regulators and essentially prolonged the juvenile phase. However, the distinction between juvenile and adult phases is less defined in grapevine and obscured by the simultaneous presence of vegetative and reproductive meristems in the same shoot [78]. Recent transcriptome survey of tendril and inflorescence development revealed that *VvSPL*s were particularly expressed in the early stages of both tendril and inflorescence development [79], suggesting that the equivalent of phase-transition in grapevine takes place when the anlagen differentiates towards tendril or inflorescence formation. At least one of the *VvSPL*s, *VvSBP12,* was reported to carry the regulation motif of miRNA156/172 [44], which raises the possibility of the role of miRNAs in grapevine shoot architecture and anlagen developmental processes. *VvSBP12's* homologues in annual model plants mediate numerous phase-related developmental processes, including initiation of the first true leaves in *Arabidopsis* (*AtSPL13* [80]), grain enlargement (*OsSPL16* [81]), and ear glume development in maize (*TGA1* [82]). The suppression of *VvSPL* genes in this study might explain the observed progressive severity of tendril-less nodes correlated with high levels of *Vvgai1* expression. We noted that HEN1, a dicer-like gene involved in miRNA metabolism [46] was induced in the shoot and leaf. HEN1, however, was suppressed in the root, while another dicer homologue, CARPEL FACTORY (CAF) was not correlated to *Vvgai1*, suggesting the existence of complex interactions among DELLA, SPLs and miRNA.

We found that the gene *VvSVP,* a MADS box gene also involved in phase-transition dependent flowering in *Arabidopsis* [45, 83], was significantly suppressed in leaf and shoot tissues. The suppressed expression of *VvSVP* and *VvSBP12* in the transgenic plants was in line with the observed delayed tendril emergence and a progressive

disruption in shoot phyllotaxy. This is also supported by the fact that *VvSVP* was considered as a positive regulator of floral transition in grapevine [13, 79, 84], in contrast to being a negative regulator in *Arabidopsis* [83].

Interestingly, we also discovered an antagonistic flowering gene, *VvTFL1*, induced in the root, suppressed in the shoot and not expressed in the leaf. Since *VvTFL1* is cell mobile [85], the induced *VvTFL1* in the root could migrate and function as an antagonist of the meristem identity gene *LFY* and floral integrator *VvFT* in grapevine shoot. This finding may suggest that roots are involved in the over-all regulation of flowering in grapevine via GA-DELLA signaling cascade.

## Conclusion

Grapevine as a perennial species shared a similar DELLA-centered feedback mechanism as annual species for maintaining GA homeostasis and controlling plant development and growth through intricate interactions of DELLAs with numerous and specialized transcription factors. However, some of these interaction outcomes could be very different between perennial and annual species, as illustrated in this study that the expression behaviors of certain flowering induction and development pathway genes in the grapevine were in sharp contrast with that of annual species. It was interesting to observe that homeodomain transcription factors seemed to play important roles in coordinating vegetative and reproductive transition in grapevine in which both vegetative and reproductive meristems simultaneously co-exist in the same shoot.

## Methods

### Expression cassette and transformation

A binary vector construct, named as pVv::VvGAI$^{L38H}$, was used in this study, containing a modified 5.1 kb-long fragment of the grape *VvGAI* genomic sequence cloned from *V. vinifera* 'Pinot Noir'. The modified *VvGAI* genomic sequence has a 2171-bp sequence upstream of the ATG start codon, a 1773-bp coding sequence with an introduced point mutation, which results in replacing a leucine amino acid (L) by a histidine (H) at the amino acid position 38 in the DELLA domain of the encoded GAI protein, and a 1157-bp sequence downstream of the stop codon. Other detail information about the construct was previously reported [14].

The Ralph M. Parsons Plant Transformation Facility at the University of California, Davis (https://ptf.ucdavis.edu/services) generated the transgenic grapevines through *Agrobacterium*-based transformation in the *V. vinifera* 'Thompson Seedless' background for this study. Ten transgenic *Vvgai1* vines were generated and five of the representative ones were used in this study after

preliminary evaluation (Table 1). A non-transgenic *V. vinifera* 'Thompson Seedless' was provided as a control.

## Propagation of transgenic grapevines and phenotyping

Freshly received transgenic grapevines were potted in 4-in. pots using Promix Biofungicide media (Premiere Horticulture, Canada). Green stem cuttings from individual transgenic lines were used to generate multiple clones for subsequent experiments. Vines were maintained in 1-gal pots in a greenhouse by applying routine vine management practices.

Phenotypic traits, such as plant height, internode length and tendril distribution pattern were observed from five biological replicates of the transgenic lines and NT. In the case of leaf weight, the measurements were from 5 to 10 pooled leaves in each biological replicate. Similarly, total chlorophyll content was assessed from pooled leaf discs of five expanded leaves of each vine following an acetone extraction method [86]. Root characteristics were assessed from depotted pots after 5-month of growth in the greenhouse.

## RNA-Seq library preparation and sequence reads processing

All tissues for RNA-Seq profiling were collected from 5-month-old vine clones of individual transgenic lines. Duplicated samples from at least two clonal vines were collected for each transgenic line and the non-transgenic control. Three types of tissues were taken. The shoot tissue sample contained 1-3 cm shoot tips of main branches, comprised of the apical region and two young, unfolding leaves. The leaf tissue sample was pooled 5th and 6th expanded leaves from the top and the root was the pool of several 3 cm-clippings of roots from depotted plants. All samples were taken fresh, flash frozen and stored at − 80 °C until further processing.

RNA extraction and RNA-Seq library preparation were performed as previously described [87]. RNA-Seq libraries were multiplexed for paired-end 2 × 100-bp (root and shoot tissue samples) or single-end 100-bp (leaf tissue samples) sequencing using Illumina HiSeq 2000 at the Cornell University Biotechnology Resource Center, Ithaca, NY.

Sequence reads of each RNA-Seq library were pre-processed using Trimmomatic (Illumina, San Diego, CA, USA) and FastQC. The artifact-free sequences were then individually aligned to the *Vitis* reference genome (12X *V. vinifera*, Phytozome ver. 12) using Tophat2, following the workflow for splice-aware RNAseq alignment (http://ccb.jhu.edu/software/tophat/manual.shtml).

## Digital RT-PCR validation

Thirty genes were selected for digital RT-PCR verification of the RNA-Seq gene expression. cDNAs were synthesized from the same mRNAs processed for RNA-Seq

libraries. The Qiagen multiplex PCR plus kit was used for the 1st round of amplification of 150–200 bp fragments in individual genes. For each tissue sample, four multiplex reactions were performed to cover 40 different regions in 30 genes. For the 2nd round PCR amplification, the Illumina Nextera dual-indexing primer system was used to barcode each individual sample and the PCR reaction was purified by AmpureXP and eluted in 10 µl water. Equal amount of DNA from individual libraries was pooled together for Illumina HighSeq sequencing (2 × 150 paired end).

Three sequence fragments each with an allele-specific SNP were amplified to confidently distinguish the transgenic *GAI* allele (*Vvgai1*, [14]) from non-transgenic one (*VvGAI1*, GSVIVG01011710001 [19]) in the 'Thompson Seedless' background. The ratios of *Vvgai1*/*VvGAI1* in the three regions were then averaged and the mean ratio was used to reflect the relative qRT-PCR expression levels of the transgene *Vvgai1* in a tissue. The mean ratios derived from the qRT-PCR data were further used for a correlation analysis of the RNA-Seq expression levels of individual endogenous genes with that of *Vvgai1* across different transgenic vines.

## Differential expression analysis

For each tissue, we constructed and analyzed 12 RNA-Seq library samples: five transgenic lines and one non-transgenic Thompson Seedless, each with two biological replicates. The shoot library samples were single-end sequenced while the other two tissues were paired-end sequenced.

The abundance of each read was determined by HTseq [88] and differential expression analysis was done using EdgeR [89], following the analysis workflow described in our previous work [28]. To focus on the genes which were most likely affected by *Vvgai1*, we included in the further analysis only those differentially expressed genes (1.5x-fold changes at FDR ≤ 0.01) identified in the strongest transgenic line G02 and also in at least one other transgenic line when compared with the non-transgenic control (NT). We used count per million mapped reads (CPM) as the normalized expression unit in assessing the expression level of a gene.

## Functional analysis

Gene Ontology (GO) analyses were done for the DEGs correlated to *Vvgai1* (r > = 0.50) in individual tissues using Plant MetGenMAP [90]. To simplify pattern interpretation, the resulting lists of enriched GO terms (FDR ≤ 0.10) were clustered into similar functions using REVIGO [91]. To assess the relative responses of endogenous genes to the transgenic *Vvgai1* expression, pairwise correlation was computed between the expression variation of each DEG among the transgenic lines

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with the relative expression level of *Vvgai1* (ratio of *Vvgail1*/*VvGAI1*) in each tissue. The average correlation indices of the genes in a particular enriched biological GO term was then taken as the relative gauge of association to *Vvgai1*. Transcription factor enrichment and putative pairwise regulator-target interactions were facilitated by using PlantTFDB 4.0 and PlantRegMap [50]. Gene annotation, cross-referencing, and sequence homology analyses were conducted using TAIR [92], Phytozome 12 [93], PlantTFDB 4.0 and PlantMetGenMap [90]. Whenever possible, we used the gene names reported in relevant grapevine research. Otherwise we followed the nomenclature indicated in the Genoscope's 12X *V. vinifera* genome database (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) in conjunction with the TAIR database (https://www.arabidopsis.org/). For providing further clarity and avoiding potential confusion, whenever appropriate we added a prefix "*Vv*" to a gene name referred in the main text as presented in Additional file 1: Table S1.

## Additional files

**Additional file 1: Table S1.** A list of DEGs and their expression correlation to Vvgai1 in the shoot, leaf and root tissue. (XLSX 530 kb)

**Additional file 2: Table S2.** A summary of the uniquely induced and suppressed genes (XLSX 11 kb)

**Additional file 3: Table S3.** Pearson correlation (r) of standardized RNA-Seq and digital qRT-PCR expressions for a selection of 30 genes in the transgenic lines. (XLSX 11 kb)

**Additional file 4: Table S4.** Transcription factor motifs significantly over-represented among the DEGs correlated to Vvgai1 (r ≥ 0.70) in the shoot, leaf and root tissues. (XLSX 13 kb)

**Additional file 5: Table S5.** A list of the 395 DELLA-bearing DEGs with co-presences of other TF motifs predicted. (XLSX 67 kb)

### Abbreviations
ARF: Auxin Response Factor; BEL: Bellringer; CAF: Carpel factory; CKX: Cytokinin oxidase; CO: Constans; CPM: Count per million reads aligned; FT: Flowering locus 1; GA: Gibberellic acid; Ga20ox: GA20 oxidase; Ga3ox: GA3 oxidase; GAI: Gibberellic acid-insensitive; GATA: GATA-binding; GID1: GA insensitive dwarf1; GO: Gene Ontology; GRF: Growth regulating factor; HD-ZIP: Homeodomain-leucine zipper; IDD: Indeterminate domain; IPT: Isopentenyltransferase; KNAT1: Knotted-like from *Arabidopsis thaliana*1; KNOX: Knotted1-like; MYB: Myeloblastosis; NAC: Nam/ataf1,2/cuc2; NT: Non-transgenic; PIN: Pin-formed; SAM: Shoot apical meristem; SCR: Scarecrow; SHR: Short root; SHY2: Short hypocotyl 2; SOC1: Suppressor of overexpression of constans 1; SPL: Squamosa-promoter binding protein-like; STM: Shoot meristemless; SVP: Short vegetative phase; TALE: Three-amino-acid-loop-extension; TCP: Tb1-Cyc-Pcf; TFL1: Terminal flower 1 or Centroradialis; TGA1: Teosinte glume architecture; TIR1: Transport inhibitor response1; Vvgai1: Grapevine gibberellic acid-insensitive1; WOX: Wuschelrelated homeobox

### Authors' contributions
GYZ conceived the project. YY and JA did the laboratory work and data analysis, respectively. JA, YY, QS and GZ wrote the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate
Not applicable.

### Consent for publication
All the authors consent to the publication of this manuscript.

### Competing interests
The authors declare that they have no competing interests.

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