דו"ח מסכם לתכנית מחקר מספר 203-0943

ברור המקור לשונות בין זני גפן בתגובה לג'יברלין

Differential response of grapevine cultivars to GA-why???

צוות המחקר: **אתי אור*, אמנון ליכטר, שמעון לביא, תמר בשה, המכון למטעים, מנהל המחקר החקלאי,**

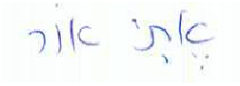
Authors: Etti Or*, Amnon Lichter, Shimon Lavee, Tamar Basha, ARO, Beit Dagan, P.O.B. 50250.:

*Corresponding author: vhettior@agri.gov.il

תקציר :השימוש בג'יברלין (GA) הוא טיפול מהותי בגפן מאכל חסרת זרעים (סטנוספרמוקרפית) ומשמש להגדלת גרגר, לריווח בין גרגרים על ידי הארכת השדרה להגברת חנטה ו/או לדילול מספר הפרחים/חנטים בזנים שונים. זנים סטנוספרמוקרפיים נבדלים ברגישותם לטיפול GA אולם הבסיס לשונות זו אינו ידוע. כמוכן לא ידוע אם רגישות זן ל-GA היא חוצת רקמות או שהינה ייחודית לרקמות מסויימות. על מנת לשפר את הבנתנו לגבי תגובת הגפן ל-GA בכלל, וגובת הענב בפרט פותח סט כלים לבחינת שונות אפשרית ברמת העברת סיגנל GA וביוסינתזה של GA והבסיס לשונות זנית נבחנה באמצעות השוואה בין שני זנים פרתנוקרפיים הנבדלים ברגישותם לטיפול GA להגדלת גרגר.

למרות שנמצאו הבדלים ברצפי החלבונים VvDELLA2 ו-VvDELLA3 בין הזנים המושווים, לא נמצאה שונות באינטראקציה בין האללים השונים לשני הרצפטורים ולחלבוני VVSLY1 במבחני Y2H ולא הושבתה יכולת פרוקם בתיווך GA, שהוראה כי עודד פרוק חלבוני VvDELLA בכל הזנים שנבחנו. על בסיס זה הונח כי השונות הבין זנית אינה נובעת משונות בפונקציה של בקרי הסיגנל כי אם שונות בכמותם. אנליזות Western תמכו בהנחה זו משום שהראו בבירור כי בזן BF ישנה צבירה לא נורמלית של שלושת חלבוני ה-DELLA. הבדל זה נראה כגורם העיקרי להבדל בתגובה בין שני הזנים המושווים- כאשר רקמות BF שהציגו רמה גבוהה של חלבוני DELLA היו רגישות יותר לטיפול GA, בהשוואה ל-SB. בנוסף לרמה הגבוהה של חלבוני DELLA, רמת תעתיקי *VvSLY1b* היתה נמוכה יותר ברקמות BF, בהשוואה ל-SB. התסריט ההיפוטטי המוצע הוא כי רמות נמוכות של *VvSLY1b* מקטינות את זמינות הקומפלקסים GID1-DELLA-SLY1 ואת יעילות הפירוק של חלבוני DELLA. יתרה מזאת, BF אף הציג רמות גבוהות של תעתיקי הרצפטורים, VvGID1s. לוגית, זן עם רמה גבוהה יותר של VvGID1 יעלה את זמינותם לקומפלקסים עם VvDELLAs ויגביר פוטנציאל תגובה ל-GA. לפיכך, קיימת אפשרות שהרמה הגבוהה של VvDELLA מבקרת במנגנון היזון חוזר את רמת הרצפטורים. בענבים מתקיים גורם שונות נוסף שעשוי להשפיע על ההבדל בתגובה ל-GA. בעוד ש-BF חסר שאריות זרע, SB מכיל עקבות זרע די גדולות שיכולות להשפיע במישרין או בעקיפין על הגברת הפעילות הביוסינתטית, עלייה ברמת GA פעיל, ירידת ברמת חלבוני DELLA עקב עידוד פירוקם ולכן ירידה ברמת התגובה ל-GA חיצוני. התסריט הכולל המוצע הוא כי הרמה הגבוהה של VvGID1A, הרמה הגבוהה של GA1 והעדר שרידי חרצן ב-BF, כולם נובעים מהרמה הגבוהה של חלבוני DELLA ב-BF, שהיא תולדה של רמות VvSLYb נמוכות.

הממצאים בדו"ח זה הינם תוצאות ניסויים. הניסויים מהווים המלצות לחקלאים: לא

אין לפרסם את הדו"ח עד להשלמת הפרסום. **חתימת החוקר:** 

NTRODUCTION

Berries of stenospermocarpic varieties carry only seed rudiments, as a result of endosperm abortion following fertilization which leads to cessation of seed development (Cheng et al. 2013). Seeds are considered the main source of GA in the berry (Lavee 1960; Kato et al. 1998; Agüero et al. 2000; Perez et al. 2000). Hence, in stenospermocarpic varieties seeds serve as the primary source of GA only prior to abortion (Conde et al. 2007), and the berries are usually small due to relatively low levels of GA (Iwahori et al. 1967). To stimulate berry development to a commercially acceptable size, stenospermocarpic varieties are routinly treated with exogenous GA, since the late 1950s,. GA application is also used routinely in the table grape industry for rachis stretching and cluster thinning, to allow proper spacing between berries, and avoid rot-based yield loss (Weaver 1958, 1965; Harrell and Williams 1987). Yet, issues of timing of application and differential sensitivity of organs and cultivars frequently result in adverse effects Pre-bloom application of GA to some stenospermocarpic cultivars causes thickening of rachis (Weaver 1961b; Agüero et al. 2000) and stem (Weaver 1958), which may increase shatter, hastens flowering, increases fruitset and results in shot berry formation (Weaver 1958). Bloom and post-bloom application may reduce fruitfulness in the succeeding season by the development of uncommitted promodia to tendrils (Srinivasan and Mullins 1981), enhance abscission probably by decreasing pollen viability (Mullins et al. 1992; Mullins 1986), and delay ripening (Weaver 1958).

Differences in varietal sensitivity to GA application have been reported in berries and other vegetative tissues/organs, including rachis and shoot (Mullins et al. 1992; Hagiwara et al. 1980; Agüero et al. 2000; Weaver 1958). For example, a single application of 57 μM GA results in 200% increase in berry size of 'Black corinth' (Weaver et al. 1962). 'Thompson seedless', the major grape cultivar in the world, requires 3-4 GA applications for a 2-fold increase in size (Dreier et al. 1998). At the end of the spectrum, application of 290 μM of GA_3 increased 'Emperatriz' berry size by only 20% (Agüero et al. 2000), while a single application of 90 μM produced similar increment in 'Superior' berries (Or, unpublished data). A single post-bloom application of 121 uM GA_3 resulted in a 24%, 50% and 30% increase in the length of internodes of 'Thompson seedless' and the lines '38-13F' and '31-123F', respectively (Hagiwara et al. 1980). A similar treatment, however, resulted in 50%, 62% and 63% increase in the weight of rachis of 'Thompson seedless', '38-13F' and '31-123F', respectively (Hagiwara et al. 1980). The basis for these differential sensitivities between organs and varieties is unknown. We previously showed that GA response in grapevine is organ specific (Acheampong et al. 2015), but it is unclear whether varietal differences in GA response is limited to certain tissue/organ type (reproductive or vegetative) or it is a whole-plant phenomenon.

From an agrotechnical perspective, the contrasting effects of GA discussed above present challenges for broad-base application in sensitive cultivars. From environmental and consumer perspective, better understanding of grapevine response to GA will help minimize the excessive use of

plant growth regulators during table grapes cultivation. Varietal differences in response to GA may result from variations in GA signaling components and/or availability of bioactive GA. Studies in model plants have shown that GA activates its response pathway by binding to the GA receptors, GID1s, which then target DELLAs, the major negative regulators of the GA-response, for degradation through binding with SLY1, GA-specific F-box proteins (Hirano et al. 2008; Sun 2010, 2011). We recently identified and characterized the major GA signaling components in grapevine (Acheampong et al. 2015). Similar to Arabidopsis (King et al. 2001; Dill et al. 2001; Cheng et al. 2004; Ariizumi and Steber 2007), the grapevine genome encodes 3 DELLA proteins (VvDELLA1, VvDELLA2 and VvDELLA3), all of which are redundantly expressed in vegetative and reproductive organs (Acheampong et al. 2015; Boss and Thomas 2002). VvDELLA1 transcripts and proteins were highest detected in internodes, rachis and tendrils, but undetected in seeds and berries. Gain-of-function mutation in this gene conferred GA-insensitive dwarf phenotype, reduced length of tendrils, and tomentosed shoot tips (Boss and Thomas 2002). Other phenotypes included short generation cycle, conversion of uncommitted meristem into inflorescence instead of tendrils, higher accumulation of GA_1 and GA_4 in organs and increased seed dormancy, but had no effect on berry size (Boss and Thomas 2002; Chaib et al. 2010). The specific function of VvDELLA2 in grapevine has not been elucidated yet, but its *in vitro* function, its ability to complement Arabidopsis mutation, and the high transcripts and proteins quantities in most organs of 'Thompson seedless' suggested a central role in regulating GA-related physiological processes in grapevine organs. Based on similar functional tests, low abundance in mature organs and higher abundance in developing tissues, it was proposed that VvDELLA3 regulate GA-mediated processes in young organs.

Two functional grapevine GID1 homologs, *VvGID1a* and *VvGID1b*, exhibited spatial and temporal expression redundancies, and were down-regulated upon GA application. Interestingly, there are also two functional VvSLY1 paralogs in grapevine, the only such multiple GA-specific F-box proteins identified in angiosperms, which exhibited inverse temporal expression profiles during organ growth and development, and were downregulated by GA (Acheampong et al. 2015).

To investigate the potential involvement of allelic variability or quantitative differences in VvDELLAs, and other GA signaling components, in difference in response to GA between tablegrape verieties, we carried a comprehensive comparative study of their sequence, their nature of interaction and their quantities in cv. Black finger (BF), considered by growers as highly sensitive to GA and cv. Spring blush (SB), considered insensitive to GA (E. Raban, personal communication). We complemented our syudy by comparisons of levels of bioactive GAs and central GA biosynthetic and degradation genes.

RESULTS

Functional analysis of VvDELLA2 in grapevine

While VvDELLA1 has been functionally-characterized and shown to mediates GA-related internode and rachis elongation, as well as fruitfulness, but not grape berry size (Boss and Thomas 2002), no functional analyses has been undertaken for VvDELLA2; even though it is the most abundant VvDELLA paralog in most organs of grapevine (Acheampong et al. 2015). To determine the functions of VvDELLA2 in grapevine, *vvdella2* transgenic grapevine plants, carrying a construct encoding gain-of-function version of VvDELLA2, along with a 2.5 kb sequence upstream of the start codon (*pVvDELLA2*::*vvdella2*) were generated. Compared to wild type, transgenic *vvdella2* lines accumulated high VvDELLA2 levels in triton-treated organs (Fig. 1A). Whereas GA treatment led to complete degradation of VvDELLA2 in control plants, similar treatment did not result in complete degradation of the mutated *vvdella2* proteins in the transgenic lines. Transgenic plants produced were characterized by reduced internode length, leaf size and tendril size (Fig. 1B). Unlike the *VvDELLA1* gain-of-function (*vvgai1*) which resulted in conversion of tendrils to inflorescence through extensive branching of uncommitted primordia (Boss and Thomas 2002), the tendrils in *vvdella2* lines were no more branched than that of wild type lines (Fig. 1C). It should be noted that present characterization was carried out on transgenic lines in their juvenile phase. Thus, the functions of this *VvDELLA* gene in reproductive organs could not be validated.

Effect of exogenous GA_3 and PAC on organs

Although available data shows varietal differences in GA response (Mullins et al. 1992; Agüero et al. 2000; Weaver 1958), it is unknown if the differences are limited to tissue/organ type or universally similar across all organs of the compared cultivars. To investigate the scope of such varietal differences in grapevines, we analyzed the response of vegetative and reproductive organs of cv. Black finger (BF) and cv. Spring blush (SB) to GA_3 application over two growing seasons. Compared to the control, GA_3 treatments resulted in 2.1- and 1.6-fold increase in internode elongation of BF and SB, respectively, while PAC treatments caused a 3.7- and 2-folds reduction in internode length of both cultivars (Fig. 2A-D). Whereas GA_3 treatment caused 5- and 1.8-fold increases in rachis lengths of BF and SB, respectively, PAC treatment resulted in approximately 2-fold reduction in both cultivars (Fig. 2E-H). GA_3 application caused a 3-fold increase in berry weight of BF, but did not significantly alter berry size of SB (Fig. 2I-L). PAC treatment led to a 2-fold decrease in berry weight of SB, but only a slight change in berry weight of BF. To verify that the effect PAC was mostly GA-related, PAC-GA treatments, involving GA_3 treatment of organs 96 h after PAC treatment, were included. Indeed, the results show that for all organs of both cultivars, the effect of PAC was either partially or fully rescued by the GA treatment.

Sources of varietal differences in response to GA

Since GA signaling regulates GA-related plant growth (Achard and Genschik 2009), it was assumed that varietal differences in response to GA_3 may, at least partly, be determined by qualitative and/or quantitative variations of the signaling components.

Allelic variations in sequences of GA signaling components

To check if the differences in GA response between BF and SB are the result allelic variation that may affect the quality of interaction and hence GA-mediated VvDELLA degradation, all alleles of VvDELLAs, VvGID1s and VvSLY1s in both BF and SB were sequenced. Functional interactions between the GA signaling components were also analyzed by Y2H assays. Nucleotide sequence analyzes showed cultivar-specific point mutations in *VvGID1a*, *VvGID1b*, *VvSLY1a* and *VvSLY1b* genes (for ref seq see Acheampong et al., 2015). Yet, these mutations did not result in changes in coded amino acids as these amino acid sequences were similar to previously sequenced genes of 'Thompson seedless' (Acheampong et al. 2015). Sequence analyses revealed that there were no cultivar-specific differences in the nucleotide or amino acid sequences of VvDELLA1. However, there were substitution and deletion in the nucleotide sequence of *VvDELLA2* of both cultivars. The substitutions occurred at positions 22 (A-G substitution), 35 (C-G substitution), 1161 (A-G substitution) and 1426 (C-T substitution). Whereas the latter two substitutions were silent and did not result in changes in amino acids, the first two resulted in $S^{8}G$ (Ser at position 8 of BF replaced by Gly in SB), and $A^{12}G$ (Ala at position 12 replaced by Gly) substitutions. Deletion of nucleotide sequence GGC (number 46-48) in *VvDELLA2* of BF, compared to SB, resulted in the in-frame deletion of Gly at position 16 (G^{16}). Two alleles of *VvDELLA3* differed between the cultivars in nucleotide substitutions G-C at positions 38 and 394, A-T at position 390, A-C at position 1092 and 1538 and T-C substitution at 1320 . While sequence variations between alleles of BF did not result in changes in amino acids, there was $S^{13}T$ (Ser-Thr) amino acid substitutions at position 13 of SB. Comparing BF ORF to ORFs of both alleles of SB, there were Ser-Thr and Glu-Gln substitutions at positions 13 ($S^{13}T$) and 132 ($E^{132}Q$), respectively .

The strengths of interaction of these cultivar-specific alleles were quantified by β-galactosidase assays in Y2H. The results of this assay show that the varietal changes in amino acid sequences of VvDELLA2 and VvDELLA3 did not result in significant differences in strength of interaction with VvGID1s (Fig. 3A) or VvSLY1s (Fig. 3B). Similar to the recently-published data, obtained using clones of 'Thompson seedless' (Acheampong et al. 2015), both VvGID1 homologs interacted with each of the VvDELLA2 clones from both cultivars in a GA-dependent manner, while VvGID1b did not interact with any of the VvDELLA3 alleles, even in the presence of GA_3. Compared to VvSLY1a, VvSLY1b interacted stronger with all VvDELLA homologs and alleles.

We also checked whether these varietal-specific mutations in VvDELLA genes may affect stability of their transcripts *in planta*. qRT-PCR results show that there was no obvious effect of GA or

PAC treatments on the expressions of all three *VvDELLA* genes in both cultivars after 6 h and 24 h (data not shown).

Varietal differences in VvDELLA levels and in response to GA

Since both loss-of-function and gain-of-function DELLA mutants display impaired GA signaling and are defective in GA response (Dill et al. 2001; Ikeda et al. 2001; King et al. 2001; Boss and Thomas 2002), we assumed that varietal differences in VvDELLA quantities may result in differences in response to GA: a variety with higher VvDELLA quantities will exhibit greater response to GA application compared to variety with lower quantities. Accordingly, transcript and protein levels of the previously-characterized *VvDELLA*s paralogs were determined in both cultivars.

Similar to other grapevine cultivars (Boss and Thomas 2002; Acheampong et al. 2015), the *VvDELLA*s were expressed in all organs of BF and SB (Fig. 4). As previously-described for 'Thompson seedless' (Acheampong et al. 2015), *VvDELLA1* and *VvDELLA2* are the most predominant homologs in both BF and SB. Generally, *VvDELLA2* and *VvDELLA3* transcripts were higher in SB compared to BF organs of similar developmental stage. The only exceptions were in young and mature leaves where *VvDELLA3* was 1.5- and 4-folds higher in BF.

The fact that in grapevines and other model plants DELLA is mostly regulated by its protein turnover and not transcript quantities (Arana et al. 2011; Acheampong et al. 2015; Dill et al. 2004), prompted us to determine VvDELLA proteins quantities in both cultivars. Results of immunoblot analyzes, using the gene specific anti-VvDELLA polyclonal antibodies show considerably higher levels of the three VvDELLA proteins in all young organs of BF, compared to SB organs at similar developmental stage (Fig. 5).

Levels of VvDELLA1, VvDELLA2 and VvDELLA3 proteins were 20-, 4- and 38-folds higher in young internodes of BF, compared to SB (Fig. 5A), and decreased during BF internode development. In general, VvDELLA levels in mature internodes were similar in both varieties. VvDELLA1 and VvDELLA2 were 38- and 6-folds higher in young rachis of BF compared to SB, whereas VvDELLA3 was not detected in both young and mature rachis of both varieties (Fig. 5B). VvDELLA1 and VvDELLA2 protein level decreased during BF rachis maturation, but in SB the protein quantities of these genes increased, and were 2- and 22-folds higher in mature rachis of SB compared to BF.

While high levels of VvDELLA1 and VvDELLA2 proteins were detected in young leaves of BF, these proteins were not detected in young SB leaves (Fig. 5C). VvDELLA3 was 16-folds higher in young leaves of BF compared to SB. Generally, VvDELLA levels decreased as leaves of both cultivars mature. All three VvDELLA proteins were not detected in mature leaves of SB. Similar to most all organs, VvDELLA1, VvDELLA2 and VvDELLA3 were 3-, 5-, and 11-folds higher in young tendrils of BF than SB (Fig. 5D). Whereas VvDELLA1 accumulated during tendril development of both

cultivars, the quantities of VvDELLA2 and VvDELLA3 reduced. VvDELLA1 was similar in both mature tendrils of BF and SB, while VvDELLA2 was 4-folds higher in BF than SB. VvDELLA3 was not detected in mature rachis of both cultivars.

VvDELLA1 was present in substantially high level in BF carpels but was barely detected in carpels of SB (Fig. 5E). VvDELLA2 and VvDELLA3 quantities were similar in carpels of both cultivars. While VvDELLA1 was not detected in berries of SB, significantly high levels of the protein was present throughout berry development of BF. VvDELLA2 was present in both BF and SB berries but was significantly higher in BF berries at all analyzed time points (8-, 73- and 203-folds, respectively, higher in BF in berries at 0 d, 10 d, and 30 d). Interestingly, whereas VvDELLA2 protein accumulation was low and gradually decreased during SB berry development, the quantities of this protein in berries of BF peaked at 10 DAF. VvDELLA3 was detected in both cultivars at 0 d and 10 d, but was 30-folds higher in BF at 30 d increasing Similar to VvDELLA2 in BF, the levels of VvDELLA3 in berries of SB was highest at 10 d, while the levels of the protein in BF progressively increased during development of BF berries.

Based on the above, it is assumed that the higher VvDELLA protein accumulation in young organs of BF signifies greater repression of growth of organs. This repression is removed via GA-mediated proteolytic degradation of the VvDELLA, which may explain the higher response of this cultivar to GA application.

Potential sources of varietal differences in VvDELLA accumulation in BF and SB

Factors such as mRNA quantities of *GID1*s (Griffiths et al. 2006; Li et al. 2011; Willige et al. 2007) and *SLY1*s (McGinnis et al. 2003; Sasaki et al. 2003), and levels of endogenous GAs (Dill et al. 2001; Griffiths et al. 2006; King et al. 2001) result in changes in DELLA proteolysis/accumulation in different species.

Stability of VvDELLA proteins *in planta*

One potential explanation for the higher levels of DELLA proteins in BF was that GA-dependent proteolysis is completely impaired in young organs of BF. Our immunoblot analyses of young organs show that both VvDELLA1 and VvDELLA2 proteins accumulated in PAC-treated organs and were significantly reduced in response to GA (Fig. 6E). In details, while VvDELLA1 was undetected in internodes of SB, GA treatment produced a 6-fold reduction in internodes of BF. The same treatment resulted in 12- and 3-fold reduction in VvDELLA2 quantities in internodes of BF and SB, respectively. Similar GA treatments also led to significant reduction in protein levels of VvDELLA1 and VvDELLA2 in rachis and carpels of both cultivars. PAC treatment led to 4-fold increase in VvDELLA1 in SB carpels, and 2-folds increase in VvDELLA2 in both internodes and rachis of BF and in VvDELLA1 proteins of carpels. Due to a limiting amount of sampled tissues, similar *in planta* assay could not be conducted to ascertain the GA-dependent VvDELLA3 degradation.

While these results suggest that GA-dependent proteolysis of VvDELLA is functional in both varieties, they do not exclude potential differences in efficiency of such proteolysis.

Comparative spatial and temporal profiles of *VvSLY1*s transcripts in BF and SB

Since SLY1 is a central regulator of DELLA proteins degradation (McGinnis et al. 2003; Dill et al. 2004; Sun 2010; Sasaki et al. 2003), it is reasonable to assume that higher accumulation of all three VvDELLAs, detected in BF, may result from lower levels of their common regulator, *VvSLY1*, and hence lower efficiency of DELLA degradation. To explore this hypothesis, *VvSLY1* mRNA levels in different organs of both cultivars were compared (Fig. 7A and B). Indeed, young organs/tissues of SB generally presented higher *VvSLY1* transcripts than corresponding organs of BF. Compared to young organs of BF, *VvSLY1b* transcript was 3-fold higher in young internode, carpels and young berries of SB and 6-, 2- and 12-folds higher in young rachis, leaves and tendrils of SB. *VvSLY1b* in mature internodes, rachis and tendrils, was 4-, 3-, and 6-folds, respectively, higher in SB, compared to BF. *VvSLY1a* expression was, however, only marginally higher in SB carpels (1.2-folds), young rachis (1.2-folds) and berries at 0 d and 10 d (1.6- and 1.4-folds, respectively), and slightly lower in young leaves and tendrils of SB (0.2- and 0.3-folds, respectively). However, with the exception of mature tendrils, *VvSLY1a* was lower in mature organs of SB than BF, with leaves recording the highest differences of 3-folds.

Following changes during organ development within a variety, it appears that both varieties presented similar profiles of *VvSLY1a* expression - upregulated during internode and rachis development, and downregulated as leaves, tendrils and berries mature. *VvSLY1b* expression also increased during SB internode development, and decreased as SB tendrils aged. Interestingly, *VvSLY1b* expression in BF appears to be unaffected by organ growth and development, except in leaves, in contrast with *VvSLY1a*. It is worth-noting that both cultivars displayed the inverse temporal expression profiles of *VvSLY1* homologs similar to the previously-described profile of cv. Thompson seedless (Acheampong et al. 2015).

These results suggest that the observed varietal differences in VvDELLA accumulation may be resulting from varietal differences in *VvSLY1b* expression, where the lower expression in organs of BF correlates the higher VvDELLA accumulation in these organs, and the opposite for organs of SB.

comparative spatial and temporal profiles of *VvGID1*s transcripts in BF and SB

As DELLA proteins were increased in *gid1* mutants of rice and Arabidopsis (Ueguchi-Tanaka et al. 2005; Griffiths et al. 2006; Willige et al. 2007), we explored the possibility that the varietal differences in VvDELLA accumulation may result from differences in expression of *VvGID1* in the cultivars. Contrary to our expectations, we found higher levels of *VvGID1* expression in organs of BF, compared to SB (Fig. 7C-D). The only exceptions were in young tendrils, where *VvGID1a* and *VvGID1b* were 2-, and 3-folds higher in SB, respectively. For most organs, there was higher expression of *VvGID1b*, and varietal difference in expression was higher for *VvGID1b* than *VvGID1a*. In detail, *VvGID1a* (Fig. 7C) and *VvGID1b* (Fig.

7D) mRNA quantities in young internodes of BF were 2- and 20-folds higher than in SB. Similarly, *VvGID1*a and *VvGID1b* expression in rachis of BF was 2 and 10-folds, respectively, greater than in SB. Transcript levels of *VvGID1a* in young berries (10-30 DAF) of BF were at least 2-folds higher than in SB berries at similar stage, and *VvGID1b* was 6-folds higher in BF at all developmental stages of berries. In light of the results, a possibility is raised that a variety with higher *VvGID1* will have greater number of receptors for GA, more GA-VvGID1-VvDELLA complexes, greater number of VvDELLA degradation, and ultimately will exhibit greater response to GA application compared to variety with lower quantities of *VvGID1*.

Endogenous and exogenous GA regulation of VvDELLA and *VvGID1* transcripts in BF and SB

It was previously demonstrated in grapevine and other species that both exogenous and endogenous bioactive GAs regulate DELLA protein accumulation and *GID1* transcripts by negative feedback (Ueguchi-Tanaka et al. 2005; Lange et al. 2012; Acheampong et al. 2015; Voegele et al. 2011; Griffiths et al. 2006). To test if similar regulatory mechanism is evident in BF and SB, and to investigate the possible role of this mechanism in regulating the varietal differences in GA response, the levels of *VvGID1*s in GA and PAC-treated organs. In accordance with the negative feedback regulation, GA application resulted in downregulated *VvGID1* transcripts, while PAC upregulated expression of the genes in organs of both cultivars (Fig. 6A-D). Similar results were obtained in the 2011 growing season (data not shown). In line with the GA-mediated degradation of DELLA proteins, application of GA_3 resulted in significant reduction in vvDELLA1 and VvVDELLA2 levels in organs of both cultivars, while PAC led to significant accumulation of these proteins (Fig. 6).

Levels of endogenous GAs in organs of BF and SB

In general, the levels of the different bioactive GA species either decreased or remained constant as organs of both BF and SB developed.

Internodes - GA_1 was undetected in young internodes of SB and detected in young BF internodes. GA_4, however, was 3-folds higher in young internodes of SB, compared to similar organs of BF. Higher level of GA_8 (the deactivation product of $GA_{1)}$ was detected in young internodes of SB compared to BF, but there was no significant difference in quantities of GA_{34} (the deactivation product of GA_4) in young internodes of both cultivars, despite the higher GA_4 level in SB (Table 1). Similar to young internodes, GA_1 was not detected in mature internodes of SB, while BF presented high levels. While relatively high amount of GA_4 was detected in mature internodes of SB, it was not detected in similar organs of BF. GA_8 was not detected in mature internodes of both cultivars, while GA_{34} was detected in only mature internodes of SB.

Rachis – Accumulation of GA_1 in young rachises of BF was evident, accompanied by significant quantities of GA_8 (Table 1). In SB, however, both GA_1 and GA_8 were not detected in young rachis. In mature rachis both GA_1 and GA_8 were not detected in both cultivars. While both developmental stages

of rachis of SB presented GA_4, it was not detected in young or mature rachis of BF. No reliable quantification exists for GA_{34} in rachis.

Carpels and Berries - In carpels of both cultivars, GA_1 was the more abundant bioactive GA and was 2-folds higher in BF. GA_4, on the other hand, was 4-folds higher in SB (Table 2). There was, however no significant difference between the quantities of both GA_8 and GA_{34} in carpels of both cultivars. During the carpel-berry transition (fruitset), there was a significant decrease in quantities of GA_1, which was accompanied by more than 2-fold increase in GA_8 accumulation, in both cultivars. As berries of both cultivars developed, GA_1 quantities dropped to levels below detection, and this was accompanied by a corresponding decrease in GA_8. Notably, unlike berries of BF, which had no detectable quantity of GA_4, a steady level of GA_4 was recorded in the carpels and throughout berry development of SB. A convex profile of GA_{34} was recorded, which peaked at 10 DAF and dropped towards 30 DAF (Table 2).

Leaves and Tendrils - Other notable findings appeared from the bioactive GA profiling in other vegetative organs. In young leaves, GA_1 and GA_8 levels were comparable in both cultivars but GA_4 and GA_{34} levels were 2-folds higher in SB. In young tendrils, however, both GA_1 and GA_4 levels were 7-folds higher in BF. In mature tendrils, GA_1 and GA_4 were not detected in both cultivars. Deactivation products were higher in BF tendrils (Tables 1). Over all, except in leaves and berries, GA_1 were higher in young organs of BF than corresponding organs of SB. GA_4 levels, on the other hand, were considerably higher in organs of SB, apart from young tendrils.

Correlation between total endogenous bioactive GAs and varietal differences in response to GA

While GA signaling is a central factor, quantities of endogenous bioactive GAs may also mediate differences in response to GA application. Data on this subject is currently limited, but the concept was supported showing that varietal difference in response of inflorescence to GA application was inversely related to the quantities of endogenous GA (Boll et al. 2009). With the exception of berries, there was however no correlation between total endogenous bioactive GAs and the varietal differences in response of organs of BF and SB.

Potential sources of varietal differences in endogenous GAs in berries

Since seed rudiments of stenospermocarpic cultivar are considered as the primary source of GA in the grape berry after endosperm abortion (Conde et al. 2007), it was envisioned that berry variations in bioactive GA content may be influenced by differences in size/presence of seed rudiments. Analyzes of 30-day old berries revealed existence of seed rudiments in berries of cv. SB, while BF berries had none (Fig. 8).

Comparative aanalyses of transcripts of central regulators of GA metabolism

To elucidate the molecular sources of the varietal differences in bioactive GA quantities, the transcript levels of grapevine 2-ODDs (*VvGA20ox*, *VvGA3ox*, *VvGA2ox* gene families) (Giacomelli et al. 2013) were quantified by qRT-PCR (Fig. 9).

Internodes - In young internodes of SB, expression of *VvGA20ox1* and *VvGA3ox1* were 2 to 3-folds higher than in young internodes of BF. In mature internodes of SB, transcript levels of *VvGA20ox5* and *VvGA2ox3* were 3- and 2-folds higher, while that of *VvGA20ox1* and *VvGA2ox8* were 2- and 20-folds lower than in mature internodes of BF.

Rachis - In young rachis of SB, *VvGA2ox6* and *VvGA3ox1* mRNA levels were 2- and 3-folds higher than in young rachis of BF. In mature rachis of SB *VvGA2ox3*, *VvGA2ox4* and *VvGA2ox6* expressions were 10-, 2- and 3-folds higher than in mature rachis of BF, while transcript of *VvGA3ox2*, *VvGA2ox1* and *VvGA2ox8* were 2-, 3-, and 5-folds higher in mature BF rachis.

Leaves - Expressions of *VvGA20ox1*, *VvGA2ox2* were 2-folds higher in young leaves of BF, while *VvGA20ox5* and *VvGA3ox2* were respectively, 5 and 3-folds higher in BF.

Carpels and Berries - In carpels, *VvGA20ox1* expression was 4-folds higher in BF compared to SB, while *VvGA2ox3* and *VvGA2ox4* were 3-folds higher in SB. Substantial quantities of *VvGA20ox5* mRNA were also detected in carpels of SB, but not detected in BF.

There were considerable varietal differences in the expressions of *VvGA20ox2*, *VvGA20ox4*, *VvGA3ox3*, *VvGA2ox2*, *VvGA2ox4* and *VvGA2ox8* in berries. Compared to BF, SB berries contained higher transcripts of *VvGA20ox2* (8-folds, 0d), *VvGA20ox4* (10-60 fold, 0-30d), and *VvGA3ox3* (which was not detected in BF at 10-30d and presented the highest expressed in SB berries, compared to all other tissues). While only one biosynthetic gene was significantly higher in BF berries (*VvGA3ox2:* 4-folds in 0-30d), two catabolic genes had higher transcript levels in this cultivar, compared to SB (*VvGA2ox1*: 2-fold in 0-30d; and *VvGA2ox8*: 6-, 3-, 2-folds in 0, 10d and 30d, respectively). Two additional catabolic genes, *VvGA2ox2* and *VvGA2ox4*, were higher in BF at 0d (3- and 1.3–fold, respectively), and then higher in SB at 10 and 30 days (30-fold, and 3-fold in SB at 10-30d, compared with BF).

Some metabolism genes displaying organ specificity were also identified in the broad spatio-temporal expression profiling (Fig. 9). *VvGA2ox8* was highest expressed in internodes, *VvGA2ox1* and *VvGA2ox5* mainly expressed in rachis, *VvGA20ox3*, *VvGA20ox7* and *VvGA2ox6* were mainly expressed in leaves, and *VvGA20ox2*, *VvGA20ox4*, *VvGA3ox3*, *VvGA2ox2* were mainly expressed in berries.

Comparative response of organs of BF and SB to application of GA_1 and GA_4

Based on the fact that different bioactive GA species appear to vary widely in their effects on different plant species, as well as mutants of the same species (Brian et al. 1962; Lange et al. 2005; Griffiths et al. 2006; Chandler et al. 2008; Lange et al. 2012), and GA_1 was identified as the more effective bioactive GA in enlarging berries of certain seedless cultivars (Weaver 1961a; Paleg et al.

1964), it is speculated that in a specific organ/tissue, the different grapevine cultivars may contain different bioactive GA species, which may also underlie varietal differences in GA response. As with Arabidopsis (Eriksson et al. 2006; Xu et al. 1997), rice (Ueguchi-Tanaka et al. 2007), and pumpkin (Lange et al. 2005), and based on lower levels of GA_4 in most organs of cv. BF, and the accompanying higher accumulation of VvDELLA proteins, it was speculated that GA_4 may be the main bioactive GA regulating VvDELLA accumulation and hence GA response in grapevine organs. To verify this, young internodes, rachises and berries of both cultivars were treated with GA_1 and GA_4. The results show that in both cultivars, application of GA_1 and GA_4 produced similar effect in all three organs (Fig. 10). This is irrespective of the fact that the endogenous levels of these GA species are markedly different in the different organs of both cultivars. Compared to controls, GA_1 and GA_4 did not significantly increase internode lengths of BF and SB (Fig. 10A-B). Both GA_1 and GA_4 produced a 3-fold increase in rachis length of BF, and slightly increased the length of SB rachis (Fig. 10C-D). While the weight of SB berries was unaffected by GA_1 and GA_4 application, BF berries were increased by 1.5-folds (Fig. 10E-F). It is worth noting that in both cultivars, the magnitude of effect of GA_3 on organ size was more pronounced that either GA_1 or GA_4, despite the similarity in response profiles.

DISCUSSION

In addition to organ/tissue-specific response to GA within a grapevine cultivar (Agüero et al. 2000; Acheampong et al. 2015), varietal-specific differences in organ response have also been reported (Weaver et al. 1962; Agüero et al. 2000; Cheng et al. 2013; Weaver 1958). Varietal differences in GA response may be complex phenomenon. Naturally, the primary potential targets for regulation of response are GA metabolism and signaling. However factors such as penetrability, cell wall composition and cell surface GA receptors cannot be discounted. The molecular mechanism regulating these response differences have neither been verified nor explored. As a first step towards understanding this phenomenon, the current comparative study focuses on the potential involvement of GA signaling and metabolism on such differential responses.

Response of cv. Black finger to GA_3 is generally higher than cv. Spring blush

Two sternospermocarpic cultivars (cv. Black Finger and cv. Spring Blush) selected initially based on growers' notes regarding their marked differences in berry response to GA, were used as a model for the current study. Here we show that the response of BF to GA_3 is higher than that of SB in all organs analyzed, suggesting that varietal-related responses to GA may be regulated by similar mechanisms in both vegetative and reproductive organs. Response to PAC was however organ-dependent; with comparable responses recorded for internodes, while responses of reproductive organs was higher for SB (Fig. 2). Similar to cv. Thompson seedless and seeded varieties (Agüero et al. 2000;

Acheampong et al. 2015), different organs exhibited different degrees of response to GA and PAC in each cultivar.

Varietal differences in GA_3 response could not be attributed to specific bioactive GA Species

Similar to Arabidopsis (Griffiths et al. 2006) and pumkins (Lange et al. 2005; Lange et al. 2012), we found high levels of GA_4 in most organs of both varieties; suggesting that it is the major bioactive GA regulating growth in grapevines. Even though specific GA species have been reported to elicit growth of specific organs in grapevine and other plant species (Paleg et al. 1964; Kato et al. 1998; Ross et al. 2000; Spielmeyer et al. 2002; Wolbang et al. 2004; Griffiths et al. 2006; Hu et al. 2008), results of on-field experiments show that both GA_1 and GA_4 affect organ growth similarly (Fig. 10), suggesting that the differential response does not involve differences in perception of a specific bioactive GA.

Varietal differences in GA_3 response was not the result of allelic variation of signaling components

When signaling components are considered as the potential source for differential response, both their availability (quantitative differences) and proper biological function (qualitative changes) have the potential to regulate the response. Quantitative and qualitative determinants to GA response have been reported in GA response mutants of model plants (Dill et al. 2001; King et al. 2001; Sasaki et al. 2003; Griffiths et al. 2006; Hirano et al. 2010; Yamamoto et al. 2010).

In the current comparative study there were no allelic differences in the ORFs of VvGID1s and VvSLY1, and Y2H assays showed that different cultivar-specific alleles of VvDELLA proteins (Fig. S1) did not differ in their interaction with VvGID1s or VvSLY1s (Fig. 3). Moreover, immunoblot analyses verified GA-dependent VvDELLA2 protein degradation. All together, the data suggest that allelic differences are limited and when present, it neither affects the nature of biological activity nor results in detectible perturbation of the degradation of VvDELLA proteins in response to GA *in plant*a (Fig. 6E).

Varietal differences in response to GA_3 may be a consequence of differences in quantity of GA signaling components

The data suggests that varietal differences in response may be regulated by factors along the GA signaling cascade. Marked differences in quantities of VvDELLA in BF and SB were recorded in young organs, which seem to be the main factor regulating the varietal differences in organs response to GA application. According to the current paradigm, application of GA releases VvDELLA-dependent repression via degradation, leading to enhanced growth of the affected organs. DELLA-

mediated varietal or ecotype differences in response to GA has also been reported in Arabidopsis where Col and *Lers* exhibit differences in male sterility in response to GA_3, and *Col-0 rga gai* loss-of-function mutants, unlike *Ler rga gai*, display complete male sterility (Plackett et al. 2014). There was no observable varietal-related trend in VvDELLA accumulation in mature organs of both cultivars. Since we did not check the GA response at this physiological state, we cannot definitively draw any conclusion between the VvDELLA protein amounts and varietal differences in response. As previously suggested for 'Thompson seedless' (Acheampong et al. 2015), it is possible that as organs mature and growth rate declines, VvDELLAs do not function on growth regulation and their quantities may not reflect the varietal differences in response of young organs.

The significant accumulation of all three VvDELLA in young BF organs could be due to (i) higher transcription, translation or post-translational modification of all three proteins (ii) lower endogenous bioactive GAs; (iii) decreased efficiency of the GA-induced proteolytic degradation mechanism of VvDELLA proteins. Expression data (Fig. 4) does not support the first assumption as *VvDELLA* transcripts in most organs appear higher in SB. Moreover, there is very little support in the literature for expression-mediated control of VvDELLA proteins. Higher or comparable levels of bioactive GAs in most BF organs, apart from leaves and berries, compared to SB rules out scenario (ii) as a probable cause. The fact that application of GA induced VvDELLA1 and VvDELLA2 degradation suggests that the VvDELLA degradation machinery is functional. Thus, the most likely cause of the high accumulation of all three VvDELLAs in young BF organs is lower efficiency of the VvDELLAs degradation, a mechanism that is expected to be shared by all three VvDELLAs. Such limited efficiency may be the result of lower quantities of a modulator required specifically for VvDELLA degradation.

The potential role of VvSLY1 as a trigger for the varietal differences

The natural suspect is VvSLY1, whose role in GA-mediated DELLA degradation and organ response was demonstrated in model plants (McGinnis et al. 2003; Sasaki et al. 2003). Compared to wild types, *sly1-10* (Arabidopsis) or *gid2* (rice) loss-of-function mutants were shown to accumulate more DELLA proteins (McGinnis et al. 2003; Sasaki et al. 2003; Dill et al. 2004). In agreement, a significantly-lower level of *VvSLY1b* transcript was recorded in BF, compared to SB. As VvSLY1b has a stronger affinity for all three VvDELLA proteins than VvSLY1a (Fig. 3), varietal differences in its expression may significantly affect the degradation efficiency of all VvDELLA. Hence, the suggested hypothetical scenario is that relatively low availability of VvSLY1b in young organs of BF results in fewer VvDELLA-VvSLY1b complexes, thus decreasing efficiency of polyubiquitination and degradation by the 26S proteasome, and increasing VvDELLA accumulation in these organs. In support of this hypothesis, Arabidopsis *sly1-d* mutants, with enhanced DELLA-SLY1 interaction than wild type, accumulated less DELLA proteins and enhanced GA signaling in *rga-Δ17* mutant lines (Dill et al. 2004). This varietal difference in *VvSLY1b* transcript could have resulted from the varietal

specific differences in the *cis* or *trans* transcription regulation element on the varietal-specific differences in the nucleotides of the putative *VvSLY1b* promoter, transcription factors or epigenetic factors. This need to be further investigated.

The consequences of higher expression of VvGID1

In addition to lower *VvSLY1b* transcript and higher VvDELLAs, BF presented higher levels of the GA receptors, *VvGID1*s. A cultivar with the greater *VvGID1* transcripts is expected to have more receptors available for the applied GA_3, and thus greater response. Hence, higher level of *VvGID1*s in all analyzed organs of BF may lead to higher response to GA. The correlation between *GID1* expression and organ response has been extensively documented. GID1- transgenic rice lines over-expressing *GID1* showed longer second leaf sheath and higher response to GA_3 application than wild type controls (Ueguchi-Tanaka et al. 2005). Additional support to the role of *VvGID1* in regulating varietal differences in response is drawn from work by Suzuki and coworkers (Suzuki et al. 2009) which attributed variations in phenotype of Arabidopsis mutants to the differential expression of Arabidopsis *GID1* homologs.

The observed varietal differences in the *VvGID1* transcripts in BF and SB could have resulted from: (i) differences in VvDELLA accumulation; (ii) differences in bioactive GA quantities. Solid support for the former can be drawn from findings in Arabidopsis (Griffiths et al. 2006; Cao et al. 2006) and rice (Ueguchi-Tanaka et al. 2008) where *GID1* transcripts were regulated by DELLA. Since GA signaling is highly conserved in higher plants (Sun 2010; Harberd et al. 2009), it is highly likely that a similar scenario may be occurring in grapevine. However, there is also support for scenario (ii) in grapevine and other model plants, as endogenous bioactive GA regulate *GID1* expression in a negative feedback fashion (Ueguchi-Tanaka et al. 2005; Acheampong et al. 2015; Griffiths et al. 2006; Li et al. 2011; Voegele et al. 2011). Principally, such level of regulation also occurs in both BF and SB (Fig. 7C, D; Table 2), but it does not seem strong enough to explain the observed varietal differences in *VvGID1* expression in the organs analyzed. Except in berries and leaves, varietal differences in endogenous GA do not correlate the observed differences in *VvGID1* transcripts and GA response, suggesting that the mechanism of regulation of varietal differences in *VvGID1* expression in most grapevine organs is not dependent on endogenous bioactive GA quantities.

A scenario can be envisioned, where the high levels of GA_1, *VvGID1* transcripts in most organs of BF, and the absence of seed rudiments (seedlessness) of berries of BF are all a consequence of the low *VvSLY1* quantities, and subsequently high VvDELLA levels in organs of BF. According to this scenario, the high VvDELLA (resulting from low *VvSLY1* transcripts and thus degradation) probably enhance the expression of *VvGID1* genes and specific GA metabolism genes, (*VvGA3ox2* and *VvGA2ox8*) which favor the biosynthesis of GA_1. Elevated bioactive GA quantities resulting from high DELLA accumulation has been reported in mutants of grapevines and other species (Boss and Thomas 2002; Griffiths et al. 2006; Chandler et al. 2002; Itoh et al. 2005; Busov et al. 2006). The suggested

existence of paralog- and pathway-specificity of GA metabolism genes in grapevines is supported in our recent publication, which showed that VvGA20ox2 is functional only on non-13-hydroxylated substrates (Giacomelli et al. 2013). Along this suggested line, high GA_1 quantities in BF carpels consequently result in parthenocarpic fruit formation. Direct support for GA_1 induced parthenocarpy can be drawn from studies showing that GA_1 accumulated higher in tomato *pat-3/pat-4* parthenocarpic mutants, compared to wildtypes (Fos et al. 2001), and application of GA_1 led to induction of parthenocarpic berries in seeded grapevine cultivars (Kato et al. 1998), and resulted in parthenocarpic growth of unpollinated Madrigal tomato carpels (Fos et al. 2000). Seed rudiments are the main sources of bioactive GAs in stenospermocarpic cultivars (Conde et al. 2007). In agreement, contrary to berries of BF, the presence of seed rudiments in the berries of SB is accompanied by higher bioactive GA_4 quantities, probably resulting from the upregulation of GA biosynthetic genes, *VvGA20ox4* and *VvGA3ox3* (Fig. 9). In such a situation, the low endogenous GA, high VvDELLA accumulation and increased *VvGID1* expression synergistically enhances the response of the BF berry to GA.

FIGURE LEGENDS

Figure 1: **Functional characterization of VvDELLA2 in grapevine.** Transgenic plants were generated by *Agrobacterium*-mediated stable transformation of embryonic calli using constructs carrying VvDELLA2 gain-of-function mutation expressed under 2.5 kb of its own promoter (*vvdella2*). (**A**) Levels of VvDELLA2 proteins in GA-treated (+) and control (-) young internodes and leaves of WT and *vvdella2* transgenic lines. Control organs were treated with Triton X-100 (0.025%), while Triton X-100 (0.025%)-formulated GA_3 (121 μM) constituted GA treatment. Blots of total protein were incubated with affinity-purified, gene-specific, anti-VvDELLA polyclonal antibodies. Coomassie Brilliant Blue-stained (CBB) proteins were used as loading control. Solid black arrows show band of interest, and asterisked-bands (*) indicate non-specific proteins detected by the anti-VvDELLA antibodies. (**A**) Gross morphology of representative plants of *vvdella2* and WT; (**B**) Tendrils of Control plants and *vvdella2* transgenic grapevines. Bar: 1 cm. Pictures (**A, B**) were taken after 6 months of planting in soil-filled pots. (**C**) Average internodes, tendril, petiole and leaf lengths of WT, and transgenic grapevine lines of *vvdella2*. Parameters were measured on 6 months old pot-grown lines. The bars represent the mean ± SE of at least 8 plants.

Figure 2: Altered response of organs of cv. Black finger (BF) (A, B, E, F, I, J) and cv. Spring blush (SB) (C, D, G, H, K, L) to GA_3 and GA biosynthesis inhibitor, paclobutrazol (PAC) treatments. GA_3 and PAC (0.8 mM) were formulated in Triton X-100 (0.025%). Internodes and rachises were treated with 121 μM GA_3, while berries were treated with 90 μM GA_3. Tissues/organs were dipped or sprayed until run-off. Increase in size was monitored at specific time intervals. Young shoots and inflorescences with tightly packed flowers (stage 15, E-L 15, on the Modified Eichhorn and Lorenz system) were selected for internodes and rachis experiments, respectively. Clusters with berries of 2-3 mm diameter (E-L 27) were selected for berry experiments. (**A, C**) Gross morphology of representative internodes of BF and SB after 20 d of treatment. (**E, G**) Gross morphology of representative rachises of BF and SB after 20 d of treatment. (**I, K**) Gross morphology of representative berries of BF and SB after 30 d of treatment. Bar: 5 cm. (**B, D**) Average per cent increase in length of new internodes arising after treatment. Increase in length of internode is expressed as per cent increase of initial length, which was assumed to be 0.5 mm. (**F, H**) Average per cent increment (as a factor of pre-treatment length) in length of rachises of BF and SB. (**K, L**) Average per cent increase in berry weight relative to mean weight at time of treatment (0 d). Data points with different letters indicate significantly different values according to Tukey HSD LSMean test at $\alpha = 0.05$ and 25 measurements, except for berries with 150 measurements.

Figure 3: Different alleles of VvDELLAs isolated from cv. Black finger (BF) and cv. Spring blush (SB) interact with VvGID1s and VvSLY1s in Y2H assays. (**A**) Interaction between VvDELLAs and VvGID1s proceed in a GA-dependent manner. The addition of 100 μM GA3 to the medium enhanced GID1–DELLA interactions. (**B**) Interaction between VvDELLAs and VvSLY1s. VvDELLA3_SB1 and VvDELLA3_SB2 represent the two alleles of VvDELLA3 isolated from SB.

Figure 4: Spatial and temporal expression profiles of *VvDELLA1* (**A**), *VvDELLA2* (**B**), and *VvDELLA3* (**C**) in *V. vinifera* cv. Black finger and cv. Spring blush. Total RNA was extracted from pooled samples, and the absolute mRNA levels of each gene were determined by real-time quantitative RT-PCR (qRT-PCR) and normalized against *VvGAPDH*. To ensure accurate quantitation of transcript levels, primers of similar efficiencies were used, and calibration curves determined from known copy numbers of single plasmid containing all qRT-PCR amplicons. The bars represent the mean ± SE. of three biological repeats with two technical repeats each. In = Internodes; Ra = Rachis; Le = Leaves; Te = Tendrils; Ca = Carpels; Be = Berries; 0d = Berries sampled at 2-3 mm diameter (E-L 27); 10d = Berries sampled 10 day after E-L 27; 30d = Berries sampled 30 days after E-L 27; Y = Young; M = Mature.

Figure 5: Spatial and temporal profiles of VvDELLA proteins in cv. Black finger (BF), and cv. Spring blush (SB). Blots of total protein extracted from internodes (**A**), rachises (**B**), leaves (**C**), tendrils (**D**), carpels and berries (**E**) at different developmental stages (full description given in Materials and Methods), were incubated with affinity-purified, gene-specific, anti-VvDELLA polyclonal antibodies. Recombinant full-length proteins (R.P.) (3.75 ng each of VvDELLA1 and VvDELLA2, and 37.5 ng of VvDELLA3) were used as sizing 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 V5 and 6xHis tags on the R.P. 0 = Berries sampled at 2-3 mm diameter (E-L 27); 10 = Berries sampled 10 day after E-L 27; 30 = Berries sampled 30 days after E-L 27.

Figure 6. GA regulation of *VvGID1a* (**A, B,**) and *VvGID1b* (**C, D**) transcript and DELLA proteins in selected tissues/organs of *V. vinifera* cv. Black finger and cv. Spring blush. Organs were dipped or sprayed until run-off with a single GA3 application (G), paclobutrazol (P), or Triton X-100 (C) treatment. Tissues/organs were sampled 6 h after GA treatment and 102 h after PAC treatment (**A, C**), and 24 h and 126 h after GA and PAC treatments, respectively (**B, D**). 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 the GA treatment. The bars represent the mean ± SE of three biological repeats with two technical repeats each. (E) GA_3-induced degradation of VvDELLA1 and VvDELLA2 proteins in internodes, rachis and carpels of cv. Black finger and cv. Spring blush. Western blot analyzes of VvDELLA proteins in organs were carried out using protein-specific, affinity-purified, anti-VvDELLA polyclonal antibodies. Total proteins were extracted from organs treated with PAC (P, 0.8 mM) and GA_3 (G, 121 µM for rachis, and 90 µM for carpels). Control (C) samples were treated with Triton X-100 (0.025%). Physiological stage at which organs were treated is detailed in Materials and Methods. Recombinant full-length proteins (R.P.) (3.75 ng each of VvDELLA1 and VvDELLA2) were used as size controls. 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 7: Spatial and temporal expression profiles of *VvSLY1a* (**A**), *VvSLY1b* (**B**), *VvGID1a* (**C**) and *VvGID1b* (**D**) in *V. vinifera* cv. Black finger and cv. Spring blush. Total RNA was extracted from pooled samples, and the absolute mRNA levels of each gene were determined by real-time quantitative RT-PCR (qRT-PCR) and normalized against *VvGAPDH*. To ensure accurate quantitation of transcript levels, primers of similar efficiencies were used, and calibration curves determined from known copy numbers of single plasmid containing all qRT-PCR amplicons. The bars represent the mean ± SE. of three biological repeats with two technical repeats each. In = Internodes; Ra = Rachis; Le = Leaves; Te = Tendrils; Ca = Carpels; Be = Berries; 0d = Berries sampled at 2-3 mm diameter (E-L 27); 10d = Berries sampled 10 day after E-L 27; 30d = Berries sampled 30 days after E-L 27; Y = Young; M = Mature. Full description of experimental procedure is given in Materials and Methods.

Figure 8: Anatomy of representative berries of cv. Black finger (**A**) and cv. Spring blush (**B**), harvested at 30 d after fruitset, and showing presence or absence of seed rudiment.

Figure 9: Temporal and spatial expression profiles of GA metabolism genes in *V. vinifera* cultivars, Black finger and Spring blush. The bars represent the mean ± SE. of three biological repeats each. *VvGAPDH*, which expression is unaffected by GA was used as normalizer. In = Internodes; Ra = Rachis; Le = Leaves; Ca = Carpels; Be = Berries; 0d = Berries sampled at 2-3 mm diameter (E-L 27); 10d = Berries sampled 10 day after E-L 27; 30d = Berries sampled 30 days after E-L 27; Y = Young; M = Mature. Full description of experimental procedure is given in Materials and Methods. Y-axis is the normalized relative expression (NRE) as described by Giacomelli and coworkers (Giacomelli et al. 2013).

Figure 10: Altered response of organs of cv. Black finger (**A, C, D**) and cv. Spring blush (**B, D, F**) to GA_1 and GA_4 treatments. Both GA species (10 ppm) were formulated in Triton X-100 (0.025%). Young shoots and inflorescences with tightly packed flowers (stage 15, E-L 15, on the Modified Eichhorn and Lorenz system) were selected for internodes and rachis experiments, respectively. Clusters with berries of 2-3 mm diameter (E-L 27) were selected for berry experiments. Tissues/organs were dipped or sprayed until run-off. Increase in size was monitored at specific time intervals. (**A, B**) Lengths of new internodes arising after treating shoots. Increase in length of internode is expressed as per cent increase of initial length, which was assumed to be 0.5 mm. (**B, C**) Changes in length of treated rachises, expressed as per cent increase of initial length. (**E, F**) Per cent increase in berry weight relative to mean weight at time of treatment (0 d). Data points with different letters indicate significantly different values according to Tukey HSD LSMean test at $\alpha = 0.05$ and 25 measurements, except for berries with 150 measurements.

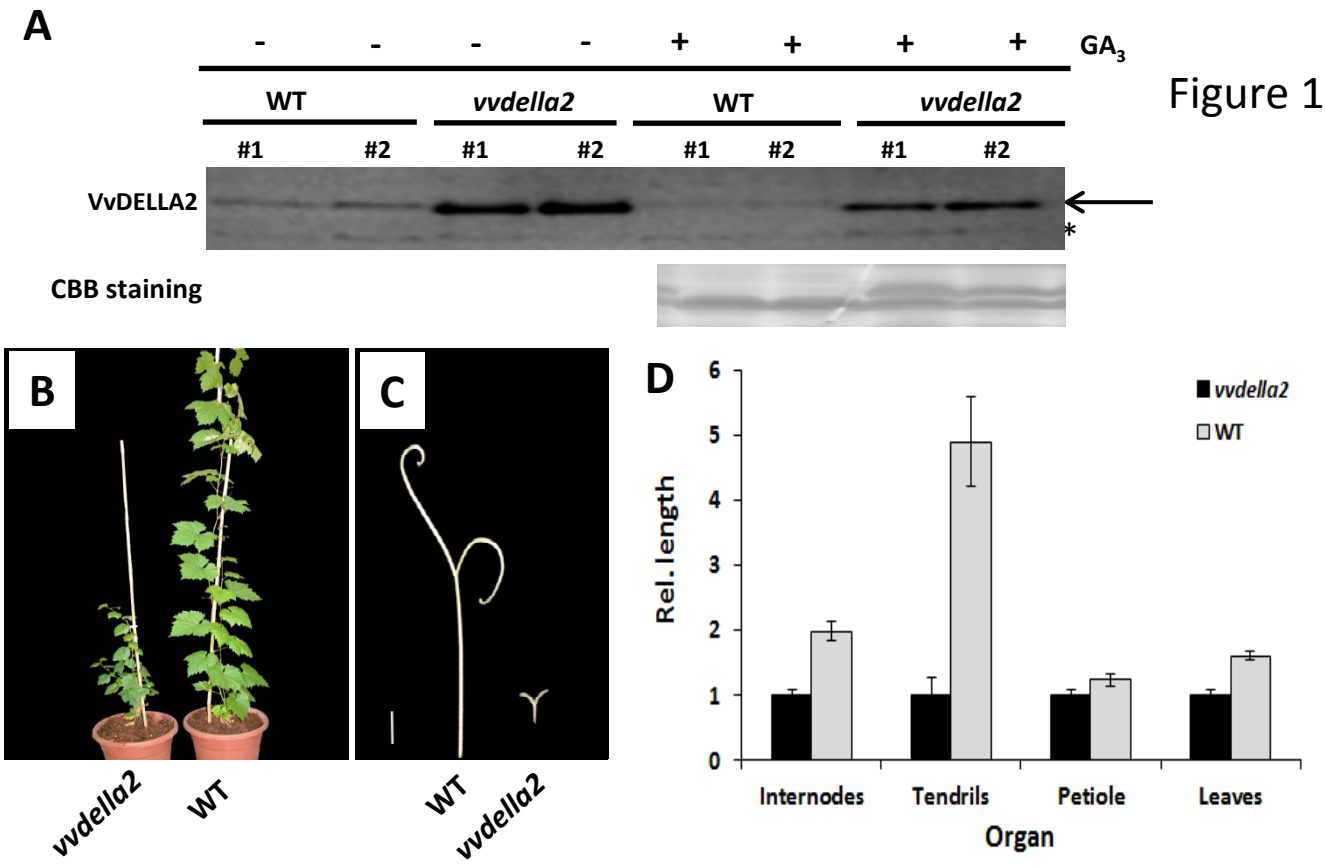


Figure 1

Figure 3

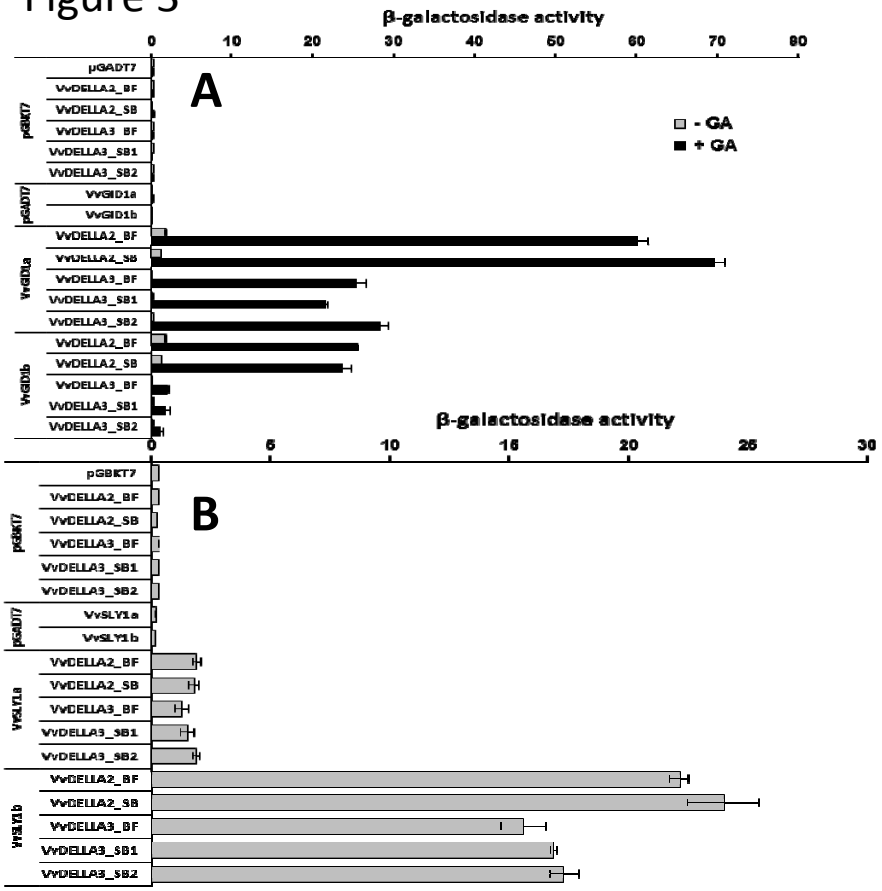
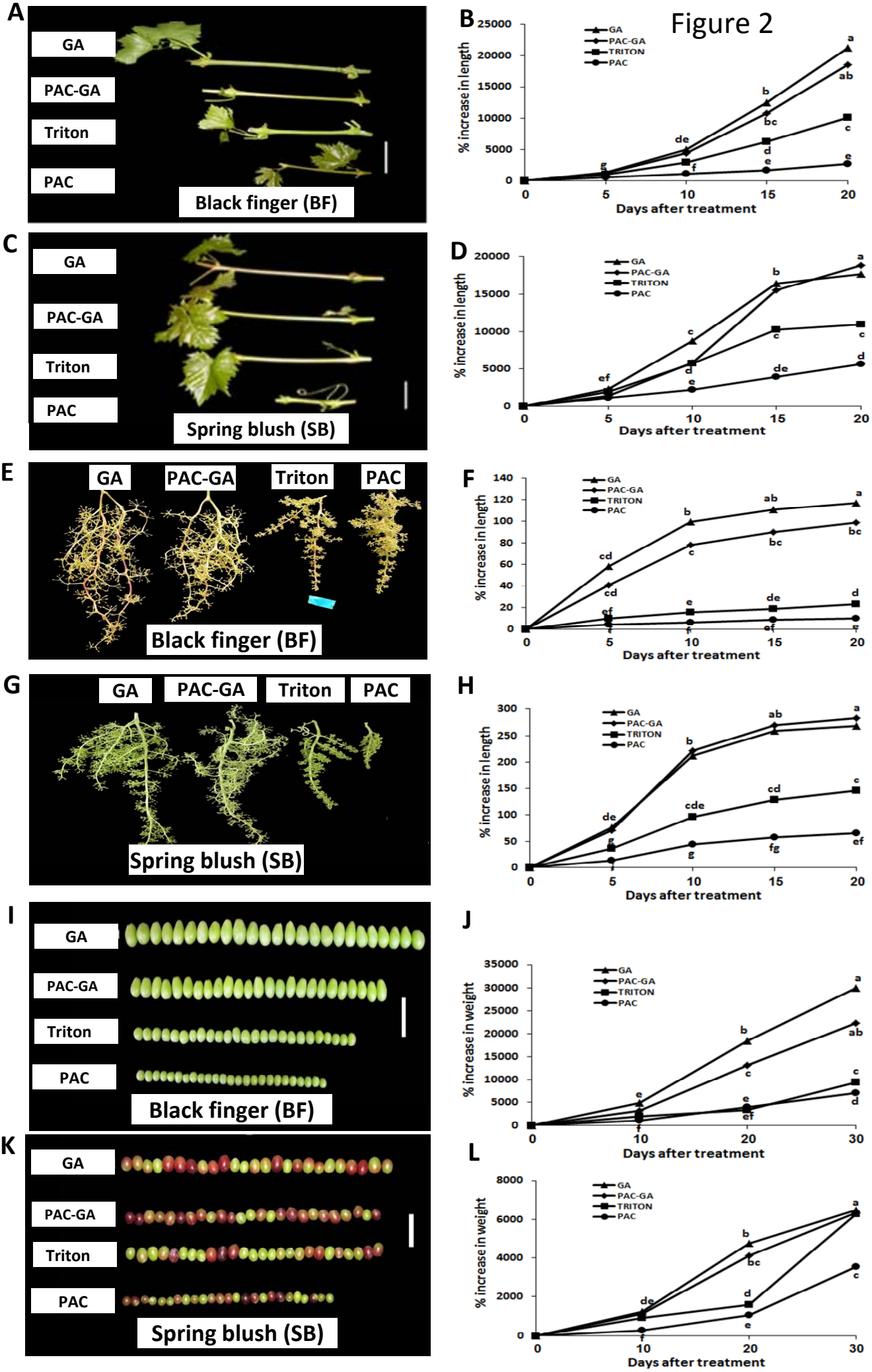


Figure 2


A
GA
PAC-GA
Triton
PAC
Black finger (BF)
B
% increase in length
Days after treatment
GA
PAC-GA
TRITON
PAC
C
Spring blush (SB)
D
E
F
G
H
I
J
% increase in weight
K
L

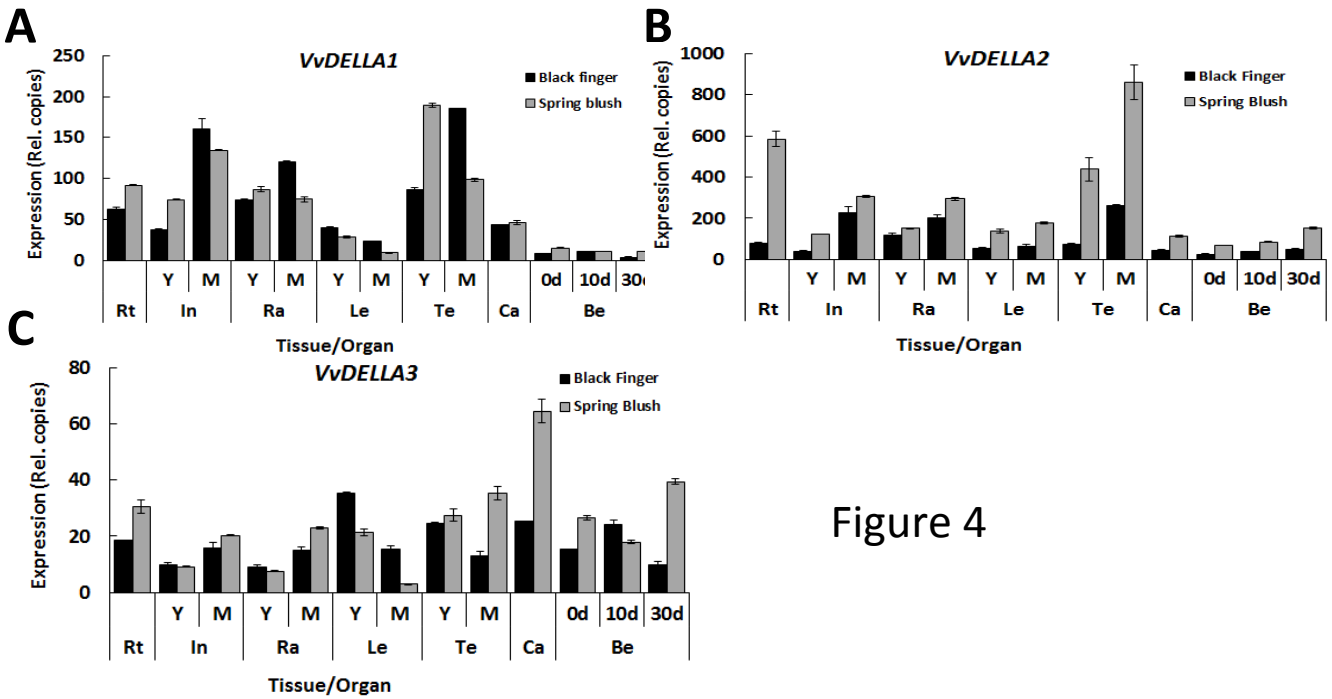


Figure 4

Figure 8

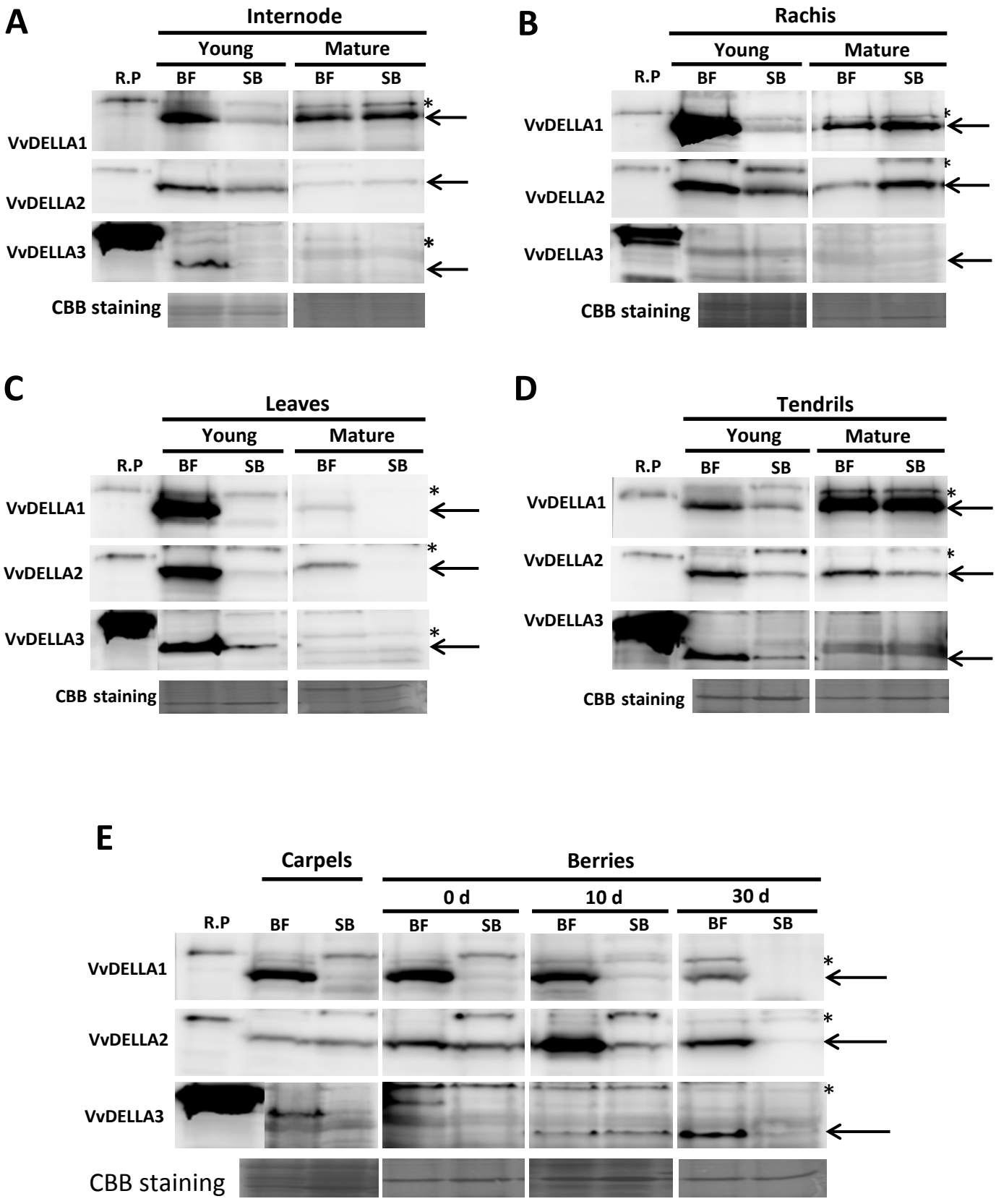

A
Internode
Young
Mature
R.P
BF
SB
BF
SB
VvDELLA1
VvDELLA2
VvDELLA3
CBB staining
B
Rachis
Young
Mature
R.P
BF
SB
BF
SB
VvDELLA1
VvDELLA2
VvDELLA3
CBB staining
C
Leaves
Young
Mature
R.P
BF
SB
BF
SB
VvDELLA1
VvDELLA2
VvDELLA3
CBB staining
D
Tendrils
Young
Mature
R.P
BF
SB
BF
SB
VvDELLA1
VvDELLA2
VvDELLA3
CBB staining
E
Carpels
Berries
0 d
10 d
30 d
R.P
BF
SB
BF
SB
BF
SB
BF
SB
VvDELLA1
VvDELLA2
VvDELLA3
CBB staining

Figure 5

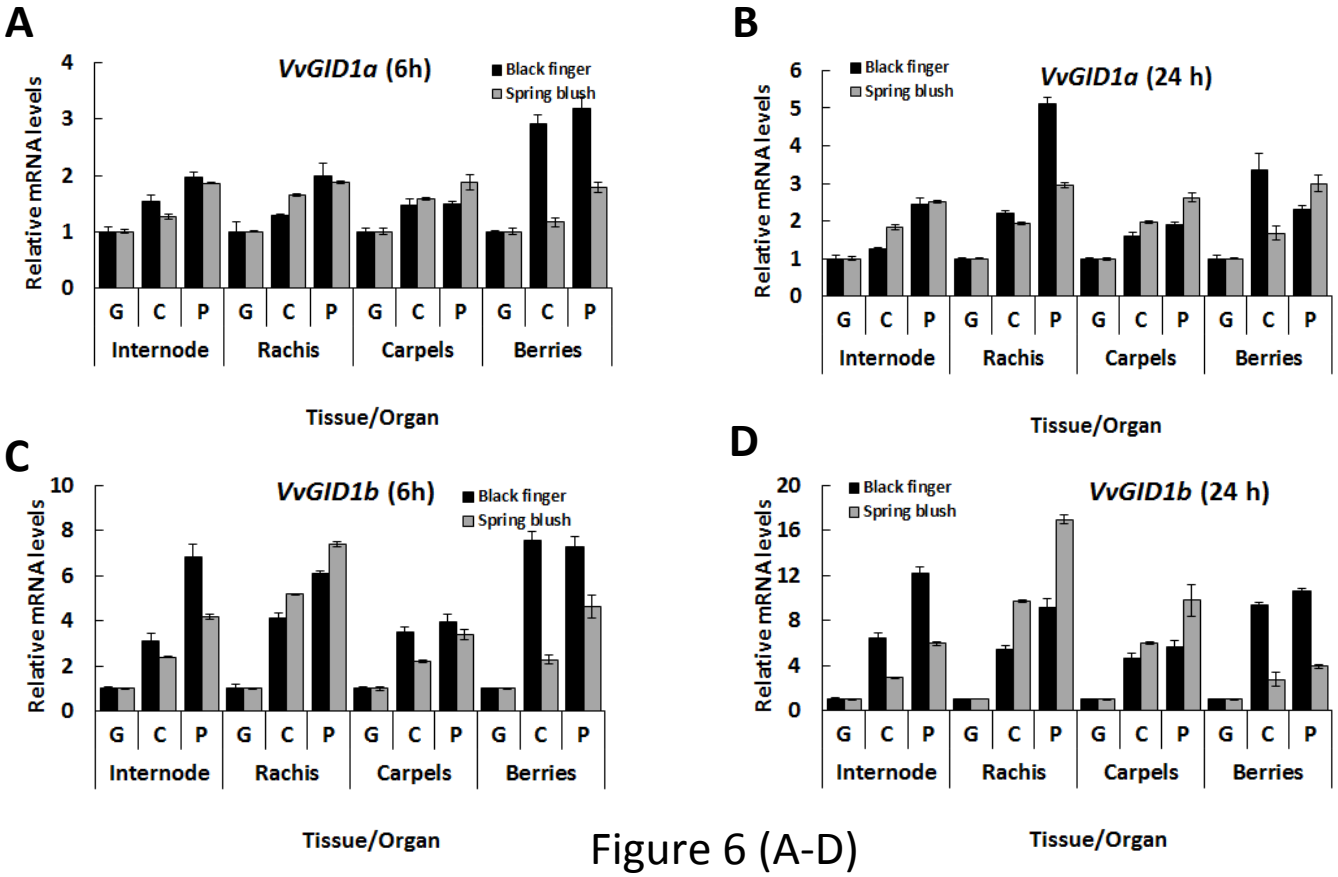


Figure 6 (A-D)

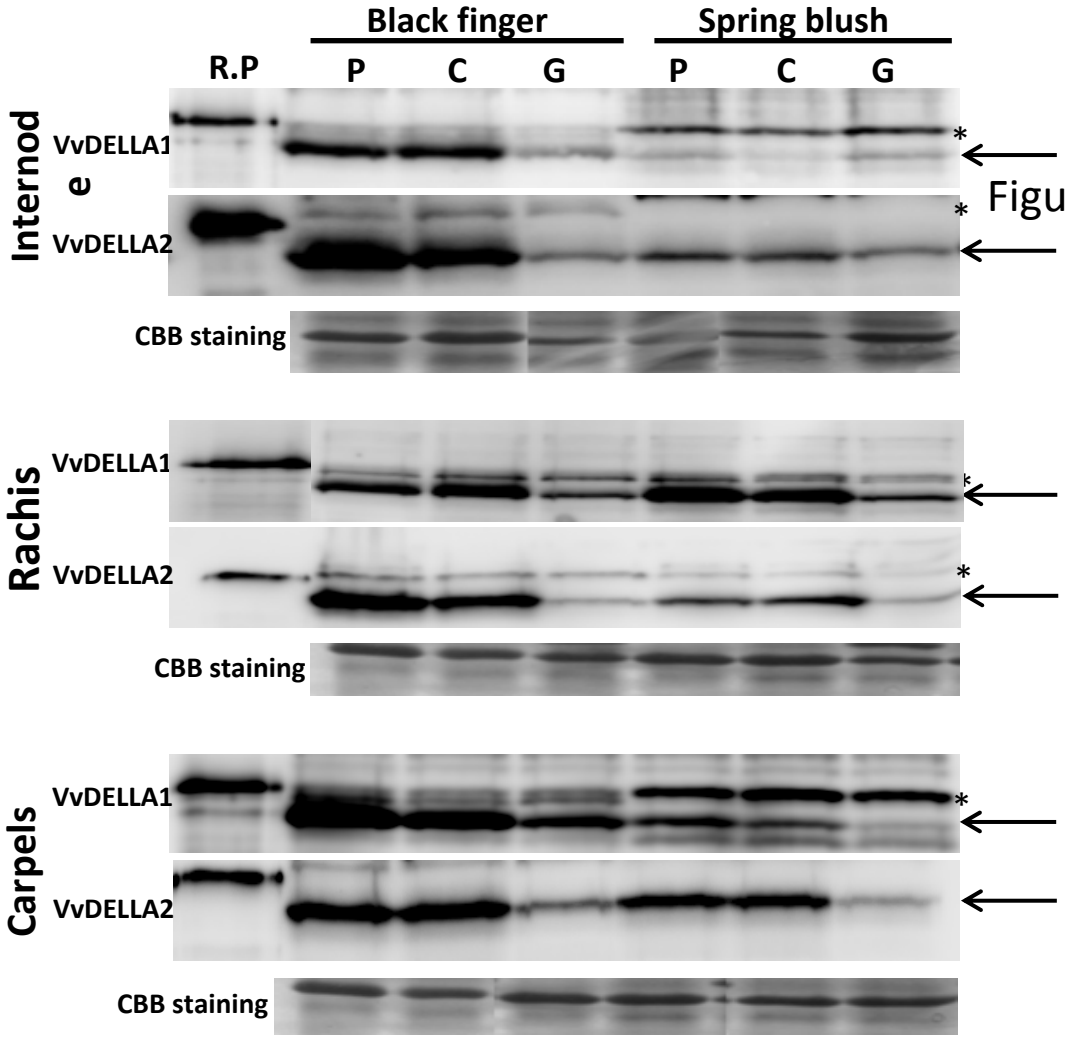


Figure 6E

Figure 7

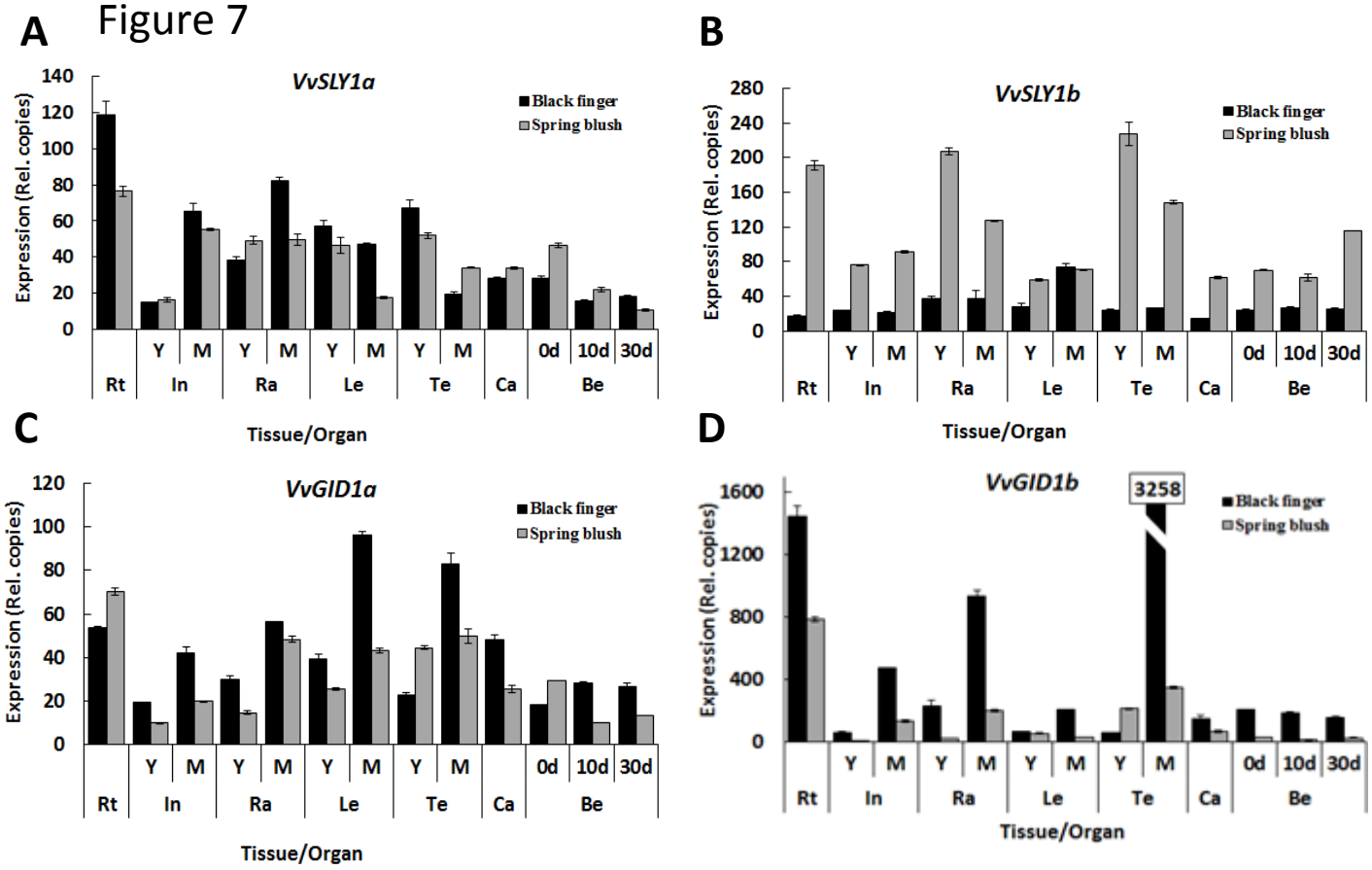


Table 1: Quantities of GA species in 13-hydroxylated (top half of Table) and non-13-hydroxylated (bottom half of Table) pathways in selected organs of *V. vinifera* cv. Black finger (BF) and cv. Spring blush (SB).

GA species	Physiological stage	Internodes		Rachis		Leaves		Tendrils	
		BF	SB	BF	SB	BF	SB	BF	SB
GA_{53}	Y	n.d.	1.7±0.5	0.8±0.2	n.d.	n.d.	n.d.	n.d.	n.d.
	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
GA_{44}	Y	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
GA_{19}	Y	14.5±1.3	29.3±1.3	4.6±1.0	7.7±1.5	24.2±1.8	53.2±1.5	29.6±3.9	2.7±0.2
	M	1.8±0.4	2.7±0.2	2.4±1.6	n.d.	n.d.	n.d.	24.4±3.5	2.9±0.0
GA_{20}	Y	n.d.	0.8±0.0	n.d.	n.d.	5.0±0.2	3.5±0.7	n.d.	n.d.
	M	n.d.	n.d.	n.d.	n.d.	n.d.	0.6±0.2	n.d.	n.d.
GA_1	**Y**	**0.8±0.2**	**n.d.**	**0.4±0.1**	**n.d.**	**2.6±0.2**	**3.1±0.2**	**3.3±0.6**	**0.5±0.0**
	M	**0.9±0.3**	**n.d.**	**n.d.**	**n.d.**	**0.5±0.1**	**0.5±0.0**	**n.d.**	**n.d.**
GA_8	Y	1.5±0.2	2.8±0.4	0.4±0.2	n.d.	n.q.	n.q.	n.q.	n.q.
	M	n.d.	n.d.	n.d.	n.d.	1.4±0.2	1.3±0.5	2.3±1.0	n.q.
GA_{12}	Y	1.5±0.2	1.5±0.6	n.d.	n.q.	0.1±0.0	n.q.	n.q.	n.d.
	M	0.1±0.0	0.3±0.0	n.d.	n.d.	n.d.	n.q.	n.d.	n.d.
GA_{24}	Y	1.9±0.2	2.2±0.3	n.d.	n.d.	1.4±0.3	n.q.	0.4±0.0	0.1±0.0
	M	0.6±0.3	n.q.	n.q.	n.d.	0.3±0.1	n.q.	n.d.	n.d.
GA_4	**Y**	**0.3±0.1**	**1.0±0.2**	**n.d.**	**0.1±0.0**	**0.9±0.2**	**1.8±0.7**	**3.0±0.0**	**0.4±0.2**
	M	**n.d.**	**0.2±0.1**	**n.d.**	**0.1±0.0**	**n.d.**	**n.q.**	**n.d.**	**0.1±0.1**
GA_{34}	Y	0.3±0.1	0.4±0.0	n.q.	n.q.	2.0±0.3	4.5±0.7	2.3±0.4	1.0±0.9
	M	n.d.	0.1±0.1	n.q.	n.q.	0.2±0.1	n.q.	n.d.	0.3±0.3

Values represent mean amounts of GA species (ng/g FW) determined in three biological replicates ± standard deviation (SD).; n.d. = undetected or could not be reliably quantified due to low abundance; n.q. = Detected, but could not be quantified due to co-migration of impurities or undetected internal standard (IS); Y = young; M = mature organs. For details of physiological stage of organs, refer to Materials and Methods.

Table 2: Quantities of GA species in 13-hydroxylated (top half of Table) and non-13-hydroxylated (bottom half of Table) pathways in carpels and berries of *V. vinifera* cv. Black finger and cv. Spring blush.

GA species	Organ	Physiological stage	Black finger	Spring blush
GA_{53}	Carpels		8.9±0.3	4.0±0.1
	Berries	0 d	0.7±0.1	0.9±0.2
		10 d	n.d.	0.4±0.0
		30 d	n.d.	n.d.
GA_{44}	Carpels		7.1±1.4	5.4±0.4
	Berries	0 d	1.2±0.2	0.3±0.0
		10 d	n.d.	n.d.
		30 d	n.d.	n.d.
GA_{19}	Carpels		39.6±6.4	54.1±2.2
	Berries	0 d	7.3±0.5	15.3±1.9
		10 d	2.0±0.2	5.7±2.0
		30 d	0.3±0.0	1.7±0.7
GA_{20}	Carpels	0 d	0.7±0.1	0.7±0.0
	Berries		3.6±0.0	3.1±0.8
		10 d	n.d.	n.d.
		30 d	n.d.	n.d.
GA_1	Carpels		**5.1±1.2**	**2.5±0.7**
	Berries	**0 d**	**0.6±0.1**	**0.8±0.2**
		10 d	**n.d.**	**n.d.**
		30 d	**n.d.**	**0.1±0.1**
GA_8	Carpels		12.4±3.8	15.7±2.5
	Berries	0 d	34.8±2.5	37.8±7.5
		10 d	3.3±0.5	2.2±0.3
		30 d	n.q.	0.4±0.1
GA_{12}	Carpels		n.q.	n.d.
	Berries	0 d	n.d.	n.d.
		10 d	n.d.	n.d.
		30 d	n.d.	n.d.
GA_{24}	Carpels		n.q.	9.9±5.6
	Berries	0 d	1.1±0.4	1.0±0.5
		10 d	0.4±0.1	0.8±0.3
		30 d	n.d.	0.1±0.0
GA_4	Carpels		**0.3±0.1**	**1.2±0.4**
	Berries	**0 d**	**n.d.**	**0.9±0.4**
		10 d	**n.d.**	**1.0±0.1**
		30 d	**n.d.**	**1.2±0.1**
GA_{34}	Carpels		0.3±0.1	0.3±0.1
	Berries	0 d	0.6 ±0.1	1.5±0.1
		10 d	n.d.	4.3±0.7
		30 d	n.d.	2.0±0.6

Values represent mean amounts of GA species (ng/g FW) determined in three biological replicates ± standard deviation (SD).; n.d. = undetected or could not be reliably quantified due to low abundance; n.q. = Detected, but could not be quantified due to co-migration of impurities or undetected internal standard (IS). Carpels were sampled at at E-L 17. Berries (0 d) were sampled at E-L 27, and subsequently at 10 (10 d) and 30 days (30 d) after the 1st sampling.

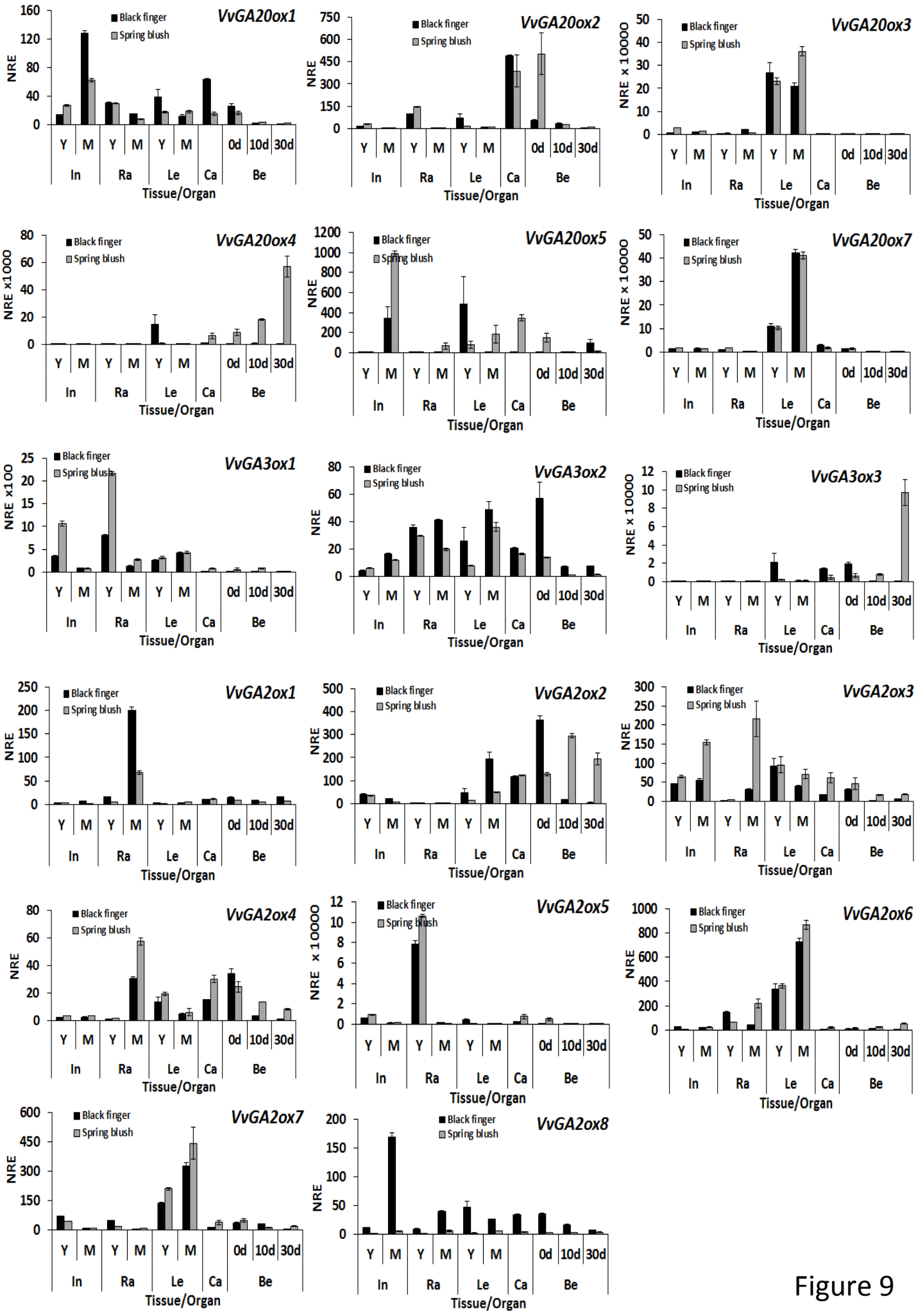

VvGA20ox1
VvGA20ox2
VvGA20ox3
VvGA20ox4
VvGA20ox5
VvGA20ox7
VvGA3ox1
VvGA3ox2
VvGA3ox3
VvGA2ox1
VvGA2ox2
VvGA2ox3
VvGA2ox4
VvGA2ox5
VvGA2ox6
VvGA2ox7
VvGA2ox8
Black finger
Spring blush
NRE
NRE x1000
NRE x 10000
NRE x100
NRE x 10000
Y M Y M Y M 0d 10d 30d
In Ra Le Ca Be
Tissue/Organ

Figure 9

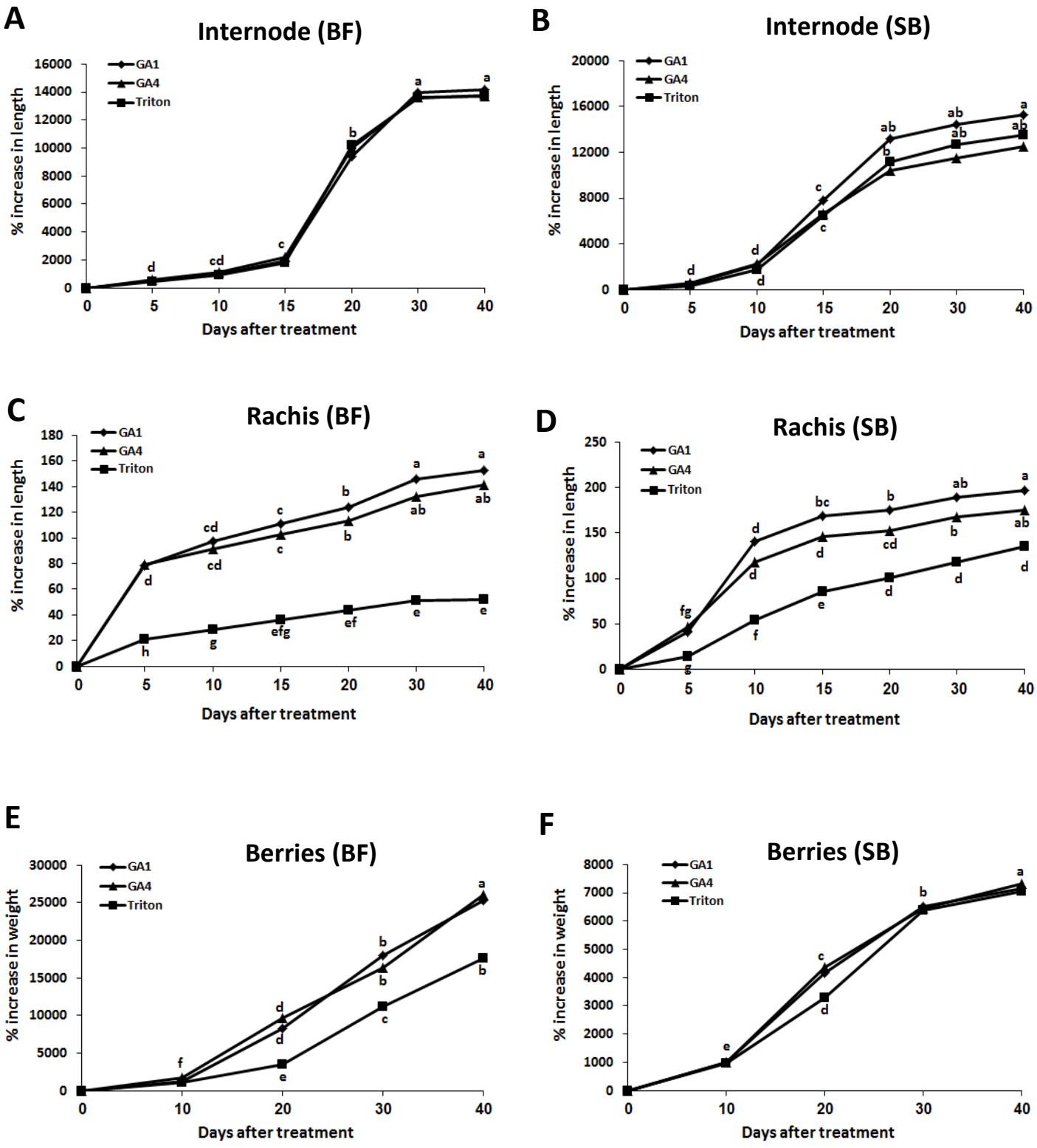

A
Internode (BF)
% increase in length
Days after treatment
GA1
GA4
Triton
B
Internode (SB)
% increase in length
Days after treatment
GA1
GA4
Triton
C
Rachis (BF)
% increase in length
Days after treatment
GA1
GA4
Triton
D
Rachis (SB)
% increase in length
Days after treatment
GA1
GA4
Triton
E
Berries (BF)
% increase in weight
Days after treatment
GA1
GA4
Triton
F
Berries (SB)
% increase in weight
Days after treatment
GA1
GA4
Triton

Figure 10

REFERENCES

Achard P, Genschik P (2009) Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. Journal of Experimental Botany 60:1085-1092

Acheampong AK, Hu J, Rotman A, Zheng C, Halaly T, Takebayashi Y, Jikumaru Y, Kamiya Y, Lichter A, Sun T-P, Or E (2015) Functional characterization and developmental expression profiling of gibberellin signalling components in *Vitis vinifera*. Journal of Experimental Botany 66 (5):1463-1476. doi:10.1093/jxb/eru504

Agüero C, Vigliocco A, Abdala G, Tizio R (2000) Effect of gibberellic acid and uniconazol on embryo abortion in the stenospermocarpic grape cultivars Emperatriz and Perlon. Plant Growth Regulation 30:9-16

Arana MV, Marín-de la Rosa N, Maloof JN, Blázquez MA, Alabadí D (2011) Circadian oscillation of gibberellin signaling in Arabidopsis. Proc Natl Acad Sci USA 108:9292-9297. doi:10.1073/pnas.1101050108

Ariizumi T, Steber CM (2007) Seed germination of GA-insensitive sleepy1 mutants does not require RGL2 protein disappearance in Arabidopsis. The Plant Cell 19:791–804

Aubert D, Chevillard M, Dorne AM, Arlaud G, Herzog M (1998) Expression patterns of GASA genes in Arabidopsis thaliana: the GASA4 gene is up-regulated by gibberellins in meristematic regions. Plant Molecular Biology 36:871-883

Boll S, Lange T, Hofmann H, Schwappach P (2009) Correspondence between gibberellin-sensitivity and pollen tube abundance in different seeded vine varieties. Mitteilungen Klosterneuburg 59:129-133

Boss PK, Thomas MR (2002) Association of dwarfism and floral induction with a grape 'green revolution' mutation. Nature 416:847-850

Brian PW, Hemming HG, Lowe D (1962) Relative activity of the gibberellins. Nature 193:946-948

Busov V, Meilan R, Pearce DW, Rood SB, Ma C, Tschaplinski TJ, Strauss SH (2006) Transgenic modification of gai or rgl1 causes dwarfing and alters gibberellins, root growth, and metabolite profiles in Populus. Planta 224:288-299

Cao D, Cheng H, Wu W, Soo HM, Peng J (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in Arabidopsis. Plant Physiology 142:509–525

Chaib J, Torregrosa L, Mackenzie D, Corena P, Bouquet A, Thomas MR (2010) The grape microvine – a model system for rapid forward and reverse genetics of grapevine. Plant Journal 62:1083-1092

Chandler PM, Harding CA, Ashton AR, Mulcair MD, Dixon NE, Mander LN (2008) Characterization of gibberellin receptor mutants of barley (Hordeum vulgare L.). Molecular Plant 1 (2):285–294

Chandler PM, Marion-Poll A, Ellis M, Gubler F (2002) Mutants at the *Slender1* Locus of Barley cv Himalaya. Molecular and Physiological Characterization. Plant Physiology 129:181-190

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reporter 11:113-116

Cheng C, Xu X, Singer SD, Li J, Zhang H, Gao M, Wang L, Song J, Wang X (2013) Effect of GA_3 Treatment on Seed Development and Seed-Related Gene Expression in Grape. PLoS ONE 8 (11):e80044. doi:10.1371/journal.pone

Cheng H, Qin L, Lee S, Fu X, Richards DE, Cao D, Luo D, Harberd NP, Peng J (2004) Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development 131:1055-1064

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Geros H (2007) Biochemical changes throughout grape berry development and fruit and wine quality. Food 1 (1):1-22

Dill A, Jung HS, Sun T (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. Proceedings of the National Academy of Sciences 98 (24):14162–14167

Dill A, Thomas SG, Hu J, Steber CM, Sun T (2004) The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. The Plant Cell 16:1392–1405

Dreier LP, Hunte JJ, Ruffner HP (1998) Invertase activity, grape berry development and cell compartmentation Plant Physiology and Biochemistry 36:865-872

Eriksson S, Bohlenius H, Moritz T, Nilsson O (2006) GA4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. The Plant Cell 18:2172-2181

Fos M, Nuez F, García-Martínez JL (2000) The gene *pat-2*, which induces natural parthenocarpy, alters the gibberellin content in unpollinated tomato ovaries. Plant Physiology 122:471-479

Fos M, Proaño K, Nuez F, García-Martínez JL (2001) Role of gibberellins in parthenocarpic fruit development induced by the genetic system pat-3/pat-4 in tomato. Physiologia Plantarum 111 (4):545-550

Giacomelli L, Rota-Stabelli O, Masuero D, Acheampong AK, Moretto M, Caputi L, Vrhovsek U, Moser C (2013) Gibberellin metabolism in Vitis vinifera L. during bloom and fruit-set: functional characterization and evolution of grapevine gibberellin oxidases. Journal of Experimental Botany 64 (14):4403-4419

Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ, Gong F, Phillips AL, Hedden P, Sun T, Thomas SG (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. The Plant Cell 18:3399–3414

Hagiwara K, Ryugo K, Olmo HP (1980) Comparison between responsiveness of selected grape clones to gibberellin applications and their endogenous levels in breaking buds and maturing berries. American Journal of Enology and Viticulture 31:309-313

Harberd NP, Belfield E, Yasumura Y (2009) The Angiosperm Gibberellin-GID1-DELLA Growth Regulatory Mechanism: How an" Inhibitor of an Inhibitor" Enables Flexible Response to Fluctuating Environments. The Plant Cell 21:1328–1339

Harrell DC, Williams LE (1987) The influence of girdling and gibberellic acid application at fruitset on Ruby Seedless and Thompson Seedless grapes. American Journal of Enology and Viticulture 38:83-88

Hirano K, Asano K, Tsuji H, Kawamura M, Mori H, Kitano H, Ueguchi-Tanaka M, Matsuoka M (2010) Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. The Plant Cell 22 (8):2680-2696

Hirano K, Ueguchi-Tanaka M, Matsuoka M (2008) GID1-mediated gibberellin signaling in plants. Trends in Plant Science 13:192-199

Ho SN, Hunt HD, Horton RM, Pullena JK, Pease JR (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene 77:51-59

Hu J, Mitchum MG, Barnaby N, Ayele BT, Ogawa M, Nam E, Lai WC, Hanada A, Alonso JM, Ecker JR, Swain SM, Yamaguchi S, Kamiya Y, Sun T (2008) Potential sites of bioactive gibberellin production during reproductive growth in Arabidopsis. The Plant Cell 20:320-336

Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J (2001) Slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the SLR1 gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. The Plant Cell 13:999–1010

Itoh H, Sasaki A, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Hasegawa Y, Minami E, Ashikari M, Matsuoka M (2005) Dissection of the phosphorylation of rice DELLA protein, SLENDER RICE1. Plant and Cell Physiology 46 (8):1392-1399

Iwahori S, Weaver RJ, Pool RM (1967) Gibberellin-like Activity in Berries of Seeded and Seedless Tokay Grapes. Plant Physiology 43:333-337

Kato K, Ohara H, Takahashi E, Matsui H, Nakayama M Endogenous gibberellin-induced parthenocarpy in grape berries. In: XXV International Horticultural Congress, Part 4: Culture Techniques with Special Emphasis on Environmental Implications, Brussels, Belgium, 1998. ISHS, pp 69-74

King KE, Moritz T, Harberd NP (2001) Gibberellins are not required for normal stem growth in Arabidopsis thaliana in the absence of GAI and RGA. Genetics 159:767–776

Lange MJP, Knop N, Lange T (2012) Stamen-derived bioactive gibberellin is essential for male flower development of Cucurbita maxima L. Journal of Experimental Botany 63:2681-2691. doi:doi:10.1093/jxb/err44

Lange T, Kappler J, Fischer A, Frisse A, Padeffke T, Schmidtke S, Lange MJP (2005) Gibberellin Biosynthesis in Developing Pumpkin Seedlings. Plant Physiology 139:213-223

Lavee S (1960) Effect of Gibberellic Acid on Seeded Grapes. Nature 185:395

Li H, Wang Y, Li X, Gao Y, Wang Z, Zhao Y, Wang M (2011) A GA-insensitive dwarf mutant of Brassica napus L. correlated with mutation in pyrimidine box in the promoter of GID1. Molecular Biology Reports 38:191-197

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun T, Steber CM (2003) The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. The Plant Cell 15 (5):1120–1130

Mullins MG (1986) Hormonal regulation of flowering and fruit set in the grapevine. Acta Horticulturae 179:309-316

Mullins MG, Bouquet A, Williams LE (1992) Biology of the grapevine. Cambridge Univ Press,

Paleg L, Aspinall D, Coombe B, Nicholls P (1964) Physiological effects of gibberellic acid. VI. Other gibberellins in three test systems. Plant Physiology 39:286-290

Perez FJ, Viani C, Retamales J (2000) Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. American Journal of Enology and Viticulture 51:315-318

Plackett ARG, Ferguson AC, Powers SJ, Wanchoo-Kohli A, Phillips AL, Wilson ZA, Hedden P, Thomas SG (2014) DELLA activity is required for successful pollen development in the Columbia ecotype of Arabidopsis. New Phytologist 201 (3):825-836

Ross JJ, O'Neill DP, Smith JJ, Kerckhoffs LH, Elliott RC (2000) Evidence that auxin promotes gibberellin A_1 biosynthesis in pea. The Plant Journal 21:547-552

Russell JR, Hosein F, Johnson E, Waugh R, Powell W (1992) Genetic differentiation of cocoa (Theobroma cacao L.) populations revealed by RAPD analyis. Molecular Ecology 2:89-97

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong DH, An G, Kitano H, Ashikari M, Matsuoka M (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299:1896-1898

5

Spielmeyer W, Ellis M, Chandler PM (2002) Semidwarf (sd-1), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc Natl Acad Sci USA 99:9043-9048

Srinivasan C, Mullins MG (1981) Physiology of flowering in the grapevine - a review. American Journal of Enology and Viticulture 32:47-63

Sun T (2010) Gibberellin-GID1-DELLA: A Pivotal Regulatory Module for Plant Growth and Development. Plant Physiology 154:567-570

Sun T (2011) The Molecular Mechanism and Evolution of the GA-GID1-DELLA Signaling Module in Plants. Current Biology 21 (9):R338-R345

Suzuki H, Park S, Okubo K, Kitamura J, Ueguchi-Tanaka M, Iuchi S, Katoh E, Kobayashi M, Yamaguchi I, Matsuoka M (2009) Differential expression and affinities of Arabidopsis gibberellin receptors can explain variation in phenotypes of multiple knock-out mutants. The Plant Journal 60:48-55

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow T, Hsing Y, Kitano H, Yamaguchi I, Matsuoka M (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437:693-698

Ueguchi-Tanaka M, Hirano K, Hasegawa Y, Kitano H, Matsuoka M (2008) Release of the repressive activity of rice DELLA protein SLR1 by gibberellin does not require SLR1 degradation in the gid2 mutant. The Plant Cell 20:2437–2446

Ueguchi-Tanaka M, Nakajima M, Katoh E, Ohmiya H, Asano K, Saji S, Hongyu X, Ashikari M, Kitano H, Yamaguchi I (2007) Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. The Plant Cell 19:2140–2155

Voegele A, Linkies A, Müller K, Leubner-Metzger G (2011) Members of the gibberellin receptor gene family GID1 (GIBBERELLIN INSENSITIVE DWARF1) play distinct roles during Lepidium sativum and Arabidopsis thaliana seed germination. Journal of Experimental Botany 62:5131-5147. doi:doi:10.1093/jxb/err214

Weaver RJ (1958) Effect of gibberellic acid on fruit set and berry enlargement in seedless grapes of *Vitis vinifera*. Nature 181:851-852

Weaver RJ (1961a) Growth of grapes in relation to gibberellin. Adv in Chem 28:89-108

Weaver RJ (1961b) Growth of Grapes in Relation to Gibberellin. In: GIBBERELLINS, vol 28. Advances in Chemistry. American Chemical Society, pp 89-108. doi:doi:10.1021/ba-1961-0028.ch010

Weaver RJ (1965) Relation of Ringing to Gibberellin-like Activity in Berries of Vitis vinifera L. Nature 206:318-319

Weaver RJ, McCune SB, Hale CR (1962) Effect of plant regulators on set and berry development in certain seedless and seeded varieties of *Vitis vinifera* L. . Vitis 3:84-96

Willige BC, Ghosh S, Nill C, Zourelidou M, Dohmann E, Maier A, Schwechheimer C (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. The Plant Cell 19:1209–1220

Wolbang CM, Chandler PM, Smith JJ, Ross JJ (2004) Auxin from the Developing Inflorescence Is Required for the Biosynthesis of Active Gibberellins in Barley Stems. Plant Physiology 134:769-776

Xu YL, Gage DA, Zeevaart JAD (1997) Gibberellins and stem growth in Arabidopsis thaliana. Effects of photoperiod on expression of the GA_4 and GA_5 loci. Plant Physiology 114:1471-1476

Yamamoto Y, Hirai T, Yamamoto E, Kawamura M, Sato T, Kitano H, Matsuoka M, Ueguchi-Tanaka M (2010) A rice *gid1* suppressor mutant reveals that Gibberellin is not always required for interaction between its receptor, GID1, and DELLA proteins. The Plant Cell 22:3589-3602. doi:doi/10.1105/tpc.110.074542

Zhang SC, Wang XJ (2008) Expression pattern of GASA, downstream genes of DELLA, in Arabidopsis. Chinese Science Bulletin 53:3839-3846

סיכום עם שאלות מנחות

מטרות המחקר לתקופת הדו"ח : זנים סטנוספרמוקרפיים נבדלים ברגישותם לטיפול GA אולם הבסיס לשונות זו אינו ידוע. כמוכן לא ידוע אם רגישות זן ל-GA היא חוצת רקמות או שהינה ייחודית לרקמות מסוימות. על מנת לשפר את הבנתנו לגבי תגובת הגפן ל-GA בכלל, ותגובת הענב בפרט המטרות היו (1) פיתוח סט כלים לבחינת שונות אפשרית ברמת העברת סיגנל GA וביוסינתזה של GA (2) בחינת הבסיס לשונות זנית באמצעות השוואה בין שני זני מאכל "חסרי חרצן" הנבדלים ברגישותם לטיפול GA להגדלת גרגר- בלק פינגר (BF) וספרינג בלאש (.SB) .

עיקרי התוצאות שהושגו בתקופה אליה מתייחס הדו"ח: נמצא כי חנטי SB ופרקי סולטנינה אינם רגישים כלל ל-GA ורגישים מאוד ל-PAC וזאת בנבדל מהתנהגות אברים אחרים באותם זנים. מכאן עולה כי אין שימור גורף של רגישות ל-GA באברים שונים של אותו זן. רגישות BF ל-GA היא הגבוהה משלושת הזנים ברקמות שונות ורגישותו ל-PAC הנמוכה ביותר, ומכאן נראה כי תתכן רגישות "חוצת אברים" בזנים מסויימים. רגישות השדרה ל-GA גבוהה מזו של המפרק ורגישותה ל-PAC נמוכה יותר, בהשוואה להתנהגות הבקורת, ומכך ניתן אולי להניח כי יש שימור של הבדל ברגישות ל-GA בין רקמות מסוימות .

זוהו ושובטו הרצפטורים ובקרי סיגנל GA ופרוקו- -DELLA ו SLY. תפקודם אושר invivo ו-invitro. יוצרו נוגדנים המזהים ספציפית את שלושת חלבוני DELLA . נמצאה שונות בין BF לשני הזנים האחרים בקצה האמינו טרמינלי של מסגרות הקריאה המקדדות לחלבוני VvDELLA2ו-VvDELLA3, בסמוך לאתר DELLA אך לא נמצאה שונות באינטראקציה בין האללים השונים לשני הרצפטורים ולחלבוני VVSLY1 במבחני Y2H ולא הושבתה יכולת פרוקם בתיווך GA, שעודד פרוק חלבוני VvDELLA בכל הזנים ורב האברים שנבחנו. . על בסיס זה הונח כי השונות הבין זנית אינה נובעת משונות בפונקציה של בקרי הסיגנל כי אם שונות בכמותם. הוראה כי רמת חלבוני DELLA גבוהה יותר דווקא ברקמות צעירות שרמות ה-GA בהם גבוהה יותר ונמצא כי בזן BF ישנה צבירה לא נורמאלית של שלושת חלבוני ה-DELLA ברקמות הצעירות. בניגוד לצפוי. בנוסף לרמה הגבוהה של חלבוני DELLA, רמת תעתיקי *VvSLY1b* המווסת פרוק DELLA היתה נמוכה יותר ורמת הרצפטור VvGID1b גבוהה יותר ברקמות BF, בהשוואה ל-SB. תועדו רמות GA באברים שונים, שלבים שונים וזנים שונים. נמצא גם דיפרנציאל בתגובת חלבוני DELLA ל-PAC ברקמות שונות שעשויה לנבוע משינויים בתכולת GA אנדוגני. בגרגרים מתקיים גורם שונות נוסף שעשוי להשפיע על ההבדל בתגובה ל-GA. בעוד ש-BF חסר שאריות זרע, SB מכיל עקבות זרע די גדולות שיכולות להשפיע במישרין או בעקיפין על הגברת הפעילות הביוסינתטית

המסקנות המדעיות: על בסיס מה שנלמד עד כה ניתן להעריך בזהירות המתבקשת כי (1) ככלל, אין שימור גורף של רגישות ל-GA באברים שונים של אותו זן בכל הזנים, אולם יש זנים בהם שימור כזה מתקיים (2) יש שימור של הבדל ברגישות ל-GA בין רקמות מסוימות בכל הזנים (3) ככלל נראה כי גם רמות GA אנדוגניות באיבר מסוים וגם שינויים ביעילות מערכת הובלת הסיגנל גורמים לשונות ברגישות. הצבירה הבולטת של שלושת חלבוני ה-DELLA ב-BF נראית כגורם העיקרי להבדל בתגובה בין שני הזנים המושווים- כאשר רקמות BF שהציגו רמה גבוהה של חלבוני DELLA היו רגישות יותר לטיפול GA, בהשוואה ל-SB. התסריט ההיפוטטי המוצע הוא כי רמות נמוכות של *VvSLY1b* ב-BF מקטינות את זמינות הקומפלקסים GID1-DELLA-SLY1 ואת יעילות הפירוק של חלבוני DELLA. יתרה מזאת, BF אף הציג רמות גבוהות של תעתיקי הרצפטורים, VvGID1s. לוגית, זן עם רמה גבוהה יותר של VvGID1 יעלה את זמינותם לקומפלקסים עם VvDELLAs ויגביר פוטנציאל תגובה ל-GA. לפיכך, קיימת אפשרות שהרמה הגבוהה של VvDELLA מבקרת במנגנון היזון חוזר את רמת הרצפטורים.

הבעיות שנותרו לפתרון והתייחסות המשך המחקר לגביהן. ההמשך המתבקש הוא איתור הבסיס להבדל בביטוי *VvSLY1b*.

האם כבר הוחל בהפצת הידע? כן-בהרצאות בימי עיון מגדלים ובכנסים בינלאומיים.

פרסום הדו"ח: חסוי עד לפרסום.