בקרת גודל הגרגר והשפעתה על מופע האשכול

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דו"ח מסכם

מוגש לקרן המדען הראשי במשרד החקלאות

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Control of berry size and its effect on cluster uniformity

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הממצאים בדו"ח זה הינם תוצאות ניסויים.

הניסויים מהווים המלצות לחקלאים: **לא**

חשוב מאוד: אנו מבקשים שהדו"ח יישאר חסוי לחלוטין עד לרישום זכויות והשלמת פרסום מדעי

חתימת החוקר:

תקציר: אי אחידות בגודל הגרגרים באשכול היא בעיית איכות מרכזית בגידול ענבי מאכל, הנמצאת בתלות במספר הפרחים ואחוזי החנטה. מטרת המחקר היתה לשפר את ההבנה לגבי הבסיס לשונות בגודל הגרגר ואת השליטה בתופעה. מצאנו כי גודלו הממוצע של גרגר בקצה הסעיף עלה על זה שבתחילת הסעיף והפער בין משקל הגרגר בשני הקצוות גדל כאשר העומס גדל. חשיפה לטיפול GA העלתה באופן מובהק ודרמטי את הפרש הגודל בין קצות הסעיף וההפרש החריף עם עליית עומס הגרגרים לסעיף. לא נמצא הבדל בגודלם של שלושת גרגרי הקצה בסעיפים שנשאו 10 או 35 גרגרים, ולעומת זאת, פרחים, שחלות וגרגרים מן הקצה הרחוק של הסעיף היו גדולים באופן מובהק מאלו שבקצה הקרוב לשדרה וההבדל נובע משונות בגודל תאים. ייסוד תנאי תחרות הוגנת בין גרגרים מאותה קטגורית גודל אפשרה לגרגרים שהוגדרו כגרגרי זטרת להפוך לגרגרים גדולים וההשפעה היתה מהותית יותר ככל שתנאי התחרות ווסתו מוקדם יותר. ממצאי הנסיונות תומכים בשונות בגודל השחלות כבסיס לדיפרנציאל בין מבלעים הגורם לפתיחת פערי גודל בין חנטים באשכול עם העלייה במספר הפרחים. על פי הנחה זו חלוקה לא שוויונית של מוטמעים בין שחלות מגדלים שונים תהפוך שחלה גדולה למתחרה מוצלחת יותר עם יתרון התחלתי. ניתן לשנות גורלו של גרגר זטרת על ידי שינוי תנאי התחרות וההשפעה מהותית יותר ככל שהשינוי קרוב לתחילת התפתחותו של החנט. על בסיס ההבנות נבחן שימוש ב-ABA ונמצא כאמצעי יעיל לדילול חנטים שהוביל שיפור אחידות האשכול.

מבוא (מקוצר. סקירה מורחבת הובאה בתכנית המחקר)

בגידול ושיווק ענבי מאכל נודעת חשיבות עליונה למופע האשכול. דרישת השוק היא לאשכול מסועף בעל מבנה קוני שגרגריו גדולים, מרווחים זה מזה ואחידים בגודלם ובדרגת הבשלתם. רשתות השיווק, המשלמות מחירי פרימיום, מחמירות מאוד בדרישותיהם נוכח התחרות ההולכת וגדלה. מציאות זו מחייבת התייחסות של כל דרגי הפיתוח והייצור (מהמשביח ועד למגדל) למבנה האשכול, לצפיפותו ולאחידותו.

הארכיטקטורה של האשכול ומספר הפרחים ההתחלתי מוכתבים על ידי תהליכי ההתמיינות של פרימורדית האשכול, החלים בפקע, והשליטה בהם נעשית בעיקר באמצעות תהליך ההשבחה. לעומת זאת, צפיפות האשכול ואחידותו, אשר גם הן מושפעות ללא ספק מתכונות הזן, ניתנות לוויסות על ידי שימוש במגוון טיפולים בתפרחת ובאשכול. טיפולים ידניים אלו, **המיושמים כשגרה אצל המגדל הישראלי,** מאפשרים אמנם הגעה למוצר הסופי הנדרש **אולם מחייבים השקעה עצומה של זמן והופכים** את ענף ענבי המאכל לענף עתיר עבודת כפיים שאינה ברת החלפה ע"י מיכון

<u>צפיפות האשכול</u> מושפעת ממידת הסיעוף של האשכול, מידת התארכות השזרה, מספר הפרחים ההתחלתי, מידת דילול הפרחים, רמת החנטה וגודל הגרגר. **<u>מידת האחידות באשכול</u>** הנקבעת על פי אופי התפלגות גודל הגרגר באוכלוסיית הגרגרים על האשכול עומדת במרכז העבודה הנוכחית. אחת מבעיות האיכות המרכזיות בגידול ענבי מאכל חסרי חרצנים הינה תופעת ה"זטרת" שבה מתפתחות לצד הגרגרים הגדולים תת אוכלוסיה של גרגרים קטנים מאוד ומתקבל מופע אשכול לא אחיד. בצד השפעה של תנאי סביבה, שני הגורמים הבולטים בהשפעתם על רמת הזטרת הם הרקע הגנטי והטיפול בג'יברלין (GA) להגדלת גרגר. בזן Thompson seedless, לדוגמא, בעיית אי האחידות היא זניחה, בזנים Perllett ו- Superior יש רגישות בינונית לזטרת ואילו אשכולות הזנים Prime ו- Early sweet שהפכו בשנים האחרונות לזנים העיקריים של ענבי מאכל מבכירים הינם עתירי זטרת. כיום התנאי לשווקם של אשכולות אלו הינו טיפול "קוסמטי" עתיר עבודה במהלכו מוסרים גרגרי זטרת מהאשכול באופן ידני. טיפול זה מעלה באופן משמעותי את עלות הייצור (15-10 ימי עבודה לדונם) וחושף את האשכול המתפתח לפגיעה מיכנית ובהמשך לפגיעת פתוגנים.

המוסכמה המקובלת , המבוססת על ספרות מוגבלת ומידע מהשטח, הייתה כי בבסיס תופעת הזטרת בזנים "חסרי חרצן" עומדות שתי תופעות : 1. בתלות בתנאי סביבה ו/או טיפול GA מושרית יצירת חנטים פרתנוקרפיים. 2. בעוד שחנטים שהופרו מתפתחים כהלכה, החנטים הפרתנוקרפיים אינם מסוגלים להתפתח ויוצרים את גרגרי הזטרת. מאחר שטיפול ב-GA הוא הכרח בשגרת הגידול של ענבי מאכל חסרי חרצן, ומאחר שהוא בעל השפעה עיקרית על רמת הזטרת בכרמים מסחריים, אופיינה מערכת מבוקרת להשראת זטרת באמצעות טיפול GA, ושמשה על מנת לבחון את ההנחות המצוינות מעל ולקדם את הבנת הבסיס לאי האחידות באשכול. על בסיס התוצאות ממחקר זה (פירוט נמצא בעבודת הגמר של אורלי אורן-בהר ובדו"ח מסכם של תכנית 0675-203) הוסק כי האחידות תלויה **<u>באופן ראשוני</u>** בעומס הגרגרים על האשכול. ככלל, הוכח כי חנטה פרתנוקרפית תלוית GA אכן מתקיימת אך האופי הפרתנוקרפי אינו משפיע לרעה על יכולת התפתחותו של הגרגר הבודד. יחד עם זאת, נמצאה תלות ברורה בין מספר הפרחים על האשכול במצב טרום פריחה לדרגת אי האחידות המתקבלת בעקבות טיפול GA, וזאת כתוצאה מעלייה מובהקת באחוזי החנטה באשכול בגין השראת חנטה פרתנוקרפית. סביר להניח כי ההקצנה של אי האחידות עם העלייה במספר הפרחים הראשוני היא **בגין תחרות בין מבלעים**, והועלתה ההשערה כי אי האחידות ניתנת למיתון על ידי שינוי תנאי התחרות, או על ידי מניפולציה של הגורמים המתווכים בין תחרות על מוטמעים לתהליכי הגדילה של הגרגר. שיפור אחידות באשכול שהושג באמצעות מניפולציה של מספר הפרחים ההתחלתי תומך בהנחה . יחד עם זאת, חוקיות התחרות בין החנטים אינה ברורה. מטרת המחקר הנוכחי היא לשפר את ההבנה לגבי הבסיס לשונות בגודל הגרגר.

Results

For this final report, the significant results were processed and are presented. Several experiments which were reported in the annual reports are not detailed here. Alternatively, we included analyses that were not part of the initial plan and were launched to suggest a practical solution, based on the understanding of the limitation to affect the initial cause for variability within the cluster which appears to be variation in initial carpel size. These analyses are presented in chepter 2.

Chapter 1

1.1 Effect of flower location/position and GA3 on berry size

To further test the effects of GA, load and flower location on berry number and berry size uniformity, one shoulder per cluster was manipulated to carry 6 groups of 5 flowers and treated with GA or surfactant only (Con). Clusters were harvested 2 weeks before veraison and data were collected separately from treated shoulder (T), untreated shoulder (UT) and the rest of the cluster (Fig 1A).

The separate analyses of different parts of the clusters allowed showing that GA is mobile since application of GA to one shoulder only (GA-T) led to increased number of berries in the untreated shoulder (GA-UT) and the rest of the cluster of a GA-treated cluster, compared with similar section in control cluster. The number of berries in the rest of the cluster was 3.35 times higher in GA- treated clusters (Fig 1B), compared with the same part in control cluster. The number of berries in GA-UT shoulder was 1.8 higher than the number of berries in CON-UT shoulder (Fig 1E).

The effect of GA was analyzed initially by comparison of the recorded number of berries in the entire cluster. To minimize cluster size variability, the number of berries in each cluster was divided by the theoretical triangle occupied by the cluster (Fig 1C). According to the results, there is a significant increase in berry number in GA-treated cluster, 1.38 higher compared with the control. This increase affect size uniformity within the cluster as presented in Fig 1C. GA application led to increased number of VS berries (3.6 higher than control) and decrease in number of berries in VB and B categories (which was 1.6 and 4 folds higher in control, respectively) (Fig 1D).

Similar to the data from the entire cluster, there was a significantly higher number of berries in shoulders of GA-treated cluster, compared with relevant control (GA-T/CON-T=1.36; GA-UT/CON-UT=1.8) (Fig 1E). Moreover, the combination of manipulation and GA mobility created load difference between UT and T shoulders in both GA-treated and control clusters. Untreated shoulders carried more berries and the difference was bigger in GA- treated shoulders, compared with the control shoulders (GA-UT/GA-T=1.67; CON-UT/CON-T=1.26) (Fig 1E). The data suggests that GA application allowed set of all the flowers left on manipulated shoulder. It also suggests that GA application to the treated shoulder allowed set of higher number on non manipulated shoulders, as seen by comparison with the non- manipulated shoulder of control cluster.

This increased load in the shoulders of the GA-treated cluster led to a big increase in number of VS berries (shot berries) on GA-T as well as GA-UT shoulders compared to the relevant control (Fig 1F; GA-T/CON-T=4.45; and GA-UT/CON-UT=3.48), Moreover, the percentage of VS berries in UT shoulder, with the higher load, was higher than that in the T shoulder, with the lower berry load (CON-UT/CON-T=1.72; GA-UT/GA-T=1.34).

In is worth noting that even on the manipulated control shoulder, not all 30 flowers set a fruit, suggesting natural thinning also in such load and GA bypass the natural thinning.

To estimate the effect of flower location on shoulder on berry size, a ratio of the average berry size in the first (F; closest to central rachis) and the last (L) group of flowers of the shoulder (see Fig 1A) was calculated (Fig 1G). In the GA-T, CON-T and CON-UT shoulders, the ratio values were 1.8±0.63, 1.6±0.74 and 2.0±0.33 accordingly, suggesting that the last berries are bigger. For GA-UT shoulders the value was 9.46±2.67, suggesting a bigger gradient between last and first groups under heavy load. Following segregation of berries in the F and L groups in T and UT shoulder by weight to Big (above 1g) and Small (below 1g) it appeared that in manipulated shoulders (T), which carry maximum 30 berries, the number of small berries in the first group was not significantly bigger than that in the last group, in both GA-treated and control. However, in the untreated shoulder, which carry higher load. the number of small berries in the first group of five berries was significantly higher than that of the last group of berries (Fig 1H)

1.2. Effect of location/position on carpel, flower and berry sizes

Flower size: To test the assumption that flower location may affect its size, analysis of the average weight of a single flower at the F and group of flowers in the inflorescence shoulder was carried out. In two separate analyses, (25-03-2014; 02-04-2014), a significant difference of 0.78 mg and 1.18 mg, respectively, was recorded between flower from F and L group, suggesting that flowers in the last group are bigger, compared to a flower in the first group (Fig. 2A-B). While flower size increased within the week between analyses (in 3.4 mg in F group and 3 mg in L group), the relative difference in size between the compared groups remains and was 18% and 16%, in the two separate analyses.

Carpel size: We similarly analyzed the effect of location on carpel size. Preliminary analysis carried during anthesis at 2013, revealed a 20% increase in carpel weight of the L group compared to the F (Data not shown). In 2014, two separate analyses (26-27 March and 30 March) were conducted, in which a significant difference of 0.6 mg (Fig. 2C) and 0.5 mg (Fig. 2D) was recorded between carpels from F and L groups, leading to 48% and 36% bigger carpels in the L group, in the two separate analyses .

Berry size: To test if such differences affect berry size, we similarly analyzed the effect of location on berry size. In 2014, two separate analyses were conducted, using GA-treated clusters. The first analysis was carried 2 weeks before varison (22-04-14) and the second at harvest (05-06-14). These analyses revealed that average berry weight in the L group is 97% (Fig. 3A) and 54% (Fig. 3B) higher, compared with that in F group.

To determine the effect of the location on berry size uniformity at harvest, the berries in each location were sorted, based on berry weight, to big (above 1g) and small (below 1g). The fraction of the small berries in the F group was 1.19 fold bigger compared with that of the L group (Fig. 3C). Accordingly, the fraction of big berries in the L group was bigger (1.35 fold) than that of the First group. Naturally, the ratio of small/big fractions was higher in the First (2.6) compared with the L group (1.6).

In 2015, analyses were repeated two weeks before harvest using GA_3 and Triton-X-100 treated clusters (CON). In both treatments, berries in the last group were bigger than berries in the first group, by 2.1 and 3.7 fold, respectively (Fig. 4A)

The effect of the treatment and location on size uniformity was documented as well. In GA treated clusters, the fraction of the small berries in the F group was 1.32 fold bigger compared with that of the L group (Fig. 4B). Accordingly, the fraction of big berries in the L group was bigger (3.9 fold) than that of the F group. Naturally, the ratio of small/big fractions was higher in the F group (12.1), when compared with the L group (2.3).

In control clusters, the fraction of the small berries in the first group was 1.3 fold bigger compared with that of the last group (Fig.4B). Accordingly, the fraction of big berries in the last group was bigger (2.2 fold) than that of the first group. The ratio of small/big fractions was higher in the first (5) compared with the last group (1.8).

It appeared that GA induced a bigger gap in size, mainly by increasing the fraction of small berries (Fig 4B).

1.3. Effect of cell number and cell area on carpel size

To verify the influence of cell number or cell area on the carpel size, histological analysis was carried out for big and small sized carpels (Big: above 1 mg, Small: below 1 mg).

In preliminary experiments with 8 carpels we recorded 5 cells/mm2 in big carpels and 5.9/$mm^{2)}$ in small carpels, suggesting that the cells in small carpels are smaller. This was supported by image- based cell area measurements which suggested that cells are 6% bigger in big carpel. Use of a cell number indicator which is a measure of cell count for the whole carpel (see Methods) suggested that there is no significant difference in the cell number between small and big carpels (Data not shown). MAYBE WILL OMIT LATER

In 2014, 20 carpels of each size were analyzed. The analysis recorded 6.4 cells /mm2 in big carpels and 7.5 cells/mm2 in small carpels, suggesting that the cell number in small carpel is 17% bigger compared to the big carpels (Fig 5A). Image- based cell area measurements suggested that big carpels were 24% bigger compared to the small carpels (Fig 5B). Use of the cell number indicator revealed no significant difference in cell number between the big and small sized carpels (Fig 5C).

1.4. Effect of cell number and cell area on berry size

The analysis described above for carpels was used to test influence of cell number and/ or cell area on the berry size. Measurements were recorded separately for hypodermis and mesocarp. While big berries were rather uniform, variation in the anatomy was observed in small berries between repeats, which reflected their developmental stage. Small berries were thus classified to 4 stages (Fig. S1B1) and stage 4 berries were used for comparison with the big berries. The other three stages resembled carpels and were ignored (Fig. S1B1-3).

In hypodermis of big berries, 1.53 cells /mm^2 were recorded while in that of small berries 2.26 cells /mm^2 were recorded, suggesting that the cells in small berries are smaller (Fig 6A). Accordingly, the cell area was 25% bigger in the big berries (Fig 6C). Similar analyses of the mesocarp revealed 0.33 cells /mm2 in big berries and 0.63 cells /mm2 in small berries (Fig 6B). Cell area was 51% bigger in mesocarp of big berries (Fig 6D).

1.5. Effect of modulation of cluster uniformity on small berry size

To evaluate the effect of competition, we intentionally induced variability by GA application two weeks before anthesis. The degree of variability in these clusters was recorded two weeks after fruit set and revealed that 21% of the berries were big (62 berries±6) while the rest were small (229 beries±35) We than manipulated clusters at three time points after fruit set (2, 4 and 6 weeks) to carry only 50 small berries (50S). In the first time point we also manipulated clusters to carry a mixture of 25 big and 25 small berries (25S/25B). Part of the clusters which were manipulated two weeks after fruit set were removed and analyzed one week later. It is important to note that berries number per cluster was mostly 50, implies that no berry shatter occurred from time of manipulation (Data not shown).

Berries were segregated to size categories using sieve and average weight, width and length of the small berries per category is presented (Fig 7). When compared to its weight at time of manipulation (S), it appeared that a small berry in a 50S cluster increased significantly in size within a week (2.4 folds). However, when accompanied by big berries in a 25S/25B cluster, the small berries did not present significant growth (Fig 7A). Similar scenario was evident based on berry width parameter (1.5 fold; Fig 7B), while the length was less informative, yet keeping similar tendency (Fig 7C).

The rest of the 50S clusters from the first modulation point and those modulated after 4 and 6 weeks were analyzed similarly at harvest. Clusters were segregated by sieve into B, S and VS size categories (there was no VB berries in these manipulated clusters which originated from 50 small berries). The results suggest that the later the manipulation, the lower percentage of the B fraction (53, 30 and 15% at 2, 4 and 6 week) and the higher percentage of S and VS berries (52, 69 and 87.5). Differentiation between S and VS berries also allowed to see that late manipulation (6 weeks) led to big increase in the VS fraction, which was 12-15% up to 4 weeks and 45.5% at 6 weeks (Fig 8A). The results suggest that removal of stronger competitors (big berries) at early stage of berry development allow about 50% of the small berries to change its fate and become a big berries, However, if such change in competition rules is delayed the small berry ability to overcome "growth inhibition" is reduced.

The effect of presence of big berries on the ability of small berry to recover following early manipulation was tested by comparing clusters with equal number of berries (total 50) and yet different size combination (50S vs 25S/25B) (Fig 8B). To follow the initially small berries development in the 25S/25B clusters we assumed that the biggest berries in amount that is half the total number on the cluster (around 25, see Methods for further explanation) represent the originally big berries. We than followed the segregation of the rest of the berries, assuming that they originated from initially small berries. According to this logic, the initially small 25 berries in 25S/25B developed into B, S and VS in the proportion of 20, 42 and 40%, respectively. On the other hand, small berries in 50S developed into B, S and VS in the proportion of 53, 40 and 12%, respectively (Fig 8B). These results suggests that removal of the big berries in 50S resulted in 2.65 fold increase in the B fraction and 3.3 fold decrease in VS fraction, if compared with the segregation of the 25 small berries which are accompanied by 25 big berries.

We therefore assume that small berries has the potential to grow if its strong competitors, the big berries, are removed. It is important to note that even within 50 small berries in 50S we see size segregation at harvest, suggesting that new competition is developed, may be related to initial flower location.

1.6. Hormonal profiling of small and big berries

Hormonal profiling of small and big berries 2 weeks after fruit set showed that level of the majority of the analyzed hormones (ABA-GE, iP, iPR, tZR, cZR, iPRMP, tZROG, DZOG, IAA-Asp, SA, IAA, DZR) was higher in small berries. However, levels of ABA, DPA, PAA and DZRMP levels were higher in the big berries.

Similar profiling of small berries from 50S and 25S/25B clusters was carried out, where the S(50S) were considered as more actively growing compared with S(25S). Thus, pattern was compared between the pair of B vs S and the pair of S(50S) vs S(25S). The data suggest few different profiles: (1) the level in B is higher than S and similarly level S(50S) is higher than in S(25S), labeled as profile A; also the level of the hormones in both S(50S) and S(25S) was higher than in S at time of modulation; (2) the level in B is lower than S and similarly level S(50S) is lower than in S(25S), labeled as profile B; (3) the level in B is lower than S but level S(50S) is higher than in S(25S), labeled as profile C.

Profile B was classified to two sub profiles. In profile B1 the level of the hormone in S(50S) was similar or higher than in S at time of modulation. In profile B2, the level of the hormone in S(50S) was significantly lower than in S at time of modulation.

Profile A included ABA, DPA and PAA. Profile 2a included ABA-GE an iP. Profile 2b included IAA-asp, SA, iPR, tZR, iPRMP, cZR. Profile D included IAA, DZR and DZRMP. No significant difference was detected between S(50S) and S(25S) for JA, Ja-Ilu, 9OH-ABA, BZA, OXIAA,tZRMP,cZ (data not shown).

1.7. Effect of flower load along the sholder on the development of last 3 berries

48 clusters were designed to carry 7 branches. The upper shoulder of 24 was designed to carry 10 flowers+3 last flowers while the other 24 were designed to carry 35+ last 3 flowers. All 48 designed shoulders were treated with GA as detailed in 1.1., and harvested at similar timing. The last 3 berries were sampled and analyzed as described above. No significant difference was observed between last 3 berries that originated from shoulder designed to carry additional 10 or 35 flowers (data not shown-see 1st annual report).

1.8. Can we modulate photo assimilates supply by girdling the inflorescence rachis and will it affect cluster uniformity

We initially learned that while full ring girdling of the rachis between the shoot and upper shoulder of the inflorescence had no deleterious on its development, similar treatment at fruit set led to fast degeneration of the cluster, suggesting that fruit set induce major increase in the sink power of the cluster. We than tried to ring just half circle in an attempt to analyze the effect of photo assimilate shortage on the dynamics of competition. Clusters were designed to carry 75 flowers on 5 branches (assuming that higher load will lead to shotberry formation even without manipulation of photo assimilates supply). 20 inflorescences were girdled and 20 were used as control. All 40 were treated with GA, as described above. About a week before vereiason the clusters were harvested and analyzed as described in 1.1. Based on the results, no difference in berry number (which was 68-70) or berry segregation to size categories (about 40% small) was detected. These results raise the option that such limited girdling cannot effectively limit photo assimilate supply and is the girdling strategy will not allow to study the effect of limited photo assimilates supply on uniformity. Future modulation of photosynthesis by shading may be tested as alternative.

Chapter 2

Based on the understanding of the limitation to affect the initial cause for variability within the cluster, which appears to be variation in initial carpel size, we aimed at thinning that will ease the competition and improve the ability of the initially small carpels to properly develope.Since GA is inducing fruit set, an assumption was raised that ABA may have the opposite effect which will result in thinning. In the current chapter, we tested ABA's ability to reduce berry number per cluster and improve berry size uniformity.

In summary, our analyses suggested that ABA application: (1) induces a significant reduction in berry number per cluster, in a concentration-dependent manner: 150 ppm had a mild effect on berry loss and no effect on size segregation, whereas 300 ppm had a significant effect on both; (2) improves size uniformity among berries within a cluster; (3) has no stable effect on rachis length; (4) its thinning effect depends on blooming

status of the inflorescence, with limited or no effect on flowers pre-anthesis, an effect on blooming flowers, and a very significant effect at full bloom and soon after anther drop. The response to ABA application after visual completion of bloom might supply a clear phenological marker for synchronous application.

2.1. Effect of exogenous ABA on berry total number and size uniformity

Field experiments were initially conducted in 2013 to evaluate the effect of ABA treatments (150 and 300 ppm) applied directly on inflorescences at pre-bloom, full bloom and fruit set in comparison to control inflorescences at full bloom. Analysis of the treated clusters removed from the vines 4 weeks before harvest revealed that ABA application at full bloom and fruit set leads to a significant reduction in berry number per cluster compared to controls (Figure 1A). FB-300 and FS-300 treatments resulted in a 53% and 32% decrease in berry number, respectively. PB-300 and FB-150 treatments had a milder effect on berry loss, showing a decrease of 12% and 14%, respectively (Figure 1A). Segregation of berries into two size categories, B ($\geq$13 mm in diameter) and S (<13 mm in diameter) revealed a decrease in the S fraction in ABA-treated clusters, accompanied by a parallel increase in the B fraction relative to controls (Figure 1B). More specifically, FB-300 and FS-300 treatments showed an appreciable decrease of 62% and 29% in the S fraction, respectively. However, application of a similar treatment at pre-bloom (PB-300) only slightly affected size segregation, leading to a 12% decrease in the S fraction, while the FB-150 treatment had no effect on size segregation (Figure 1B).

To further test the effect of ABA, similar analyses were carried at harvest, using clusters treated with 150 and 300 ppm at full bloom. The FB-300 treatment led to a 56% decrease in berry number per cluster, while FB-150 led to a milder decrease of 20% (Figure 1C). In agreement with the analysis carried out at the earlier developmental stage, 300 ppm ABA resulted in a 58% smaller fraction of S berries compared to controls (Figure 1D), while the 150 ppm treatment had no significant effect.

2.2. Effect of bloom stage on cluster response to ABA treatment

The above experiments suggested that ABA treatment decreases berry number, increases berry size, and improves cluster uniformity. It also indicated that these effects are concentration-dependent and may also depend on blooming status of the cluster. To

validate the influence of ABA treatment (300 ppm) and further study the potential interaction with blooming status, additional experiments were carried out in 2014 and 2015. Clusters were assigned to treatments based on careful characterization of their bloom status and removed for analysis 4 weeks before harvest. Based on the results from both 2014 (Figure 2A) and 2015 (Figure 2C), all ABA treatments decreased the number of berries per cluster. However, the degree of that effect increased as cluster blooming progressed, and was highest at fruit set. Accordingly, in 2014 (Figure 2A), the number of berries per cluster was 35%, 40%, 46% and 72% lower for PB, 50%B, FB and FS treatments, respectively, compared to controls (which were treated with surfactant only at full bloom). In 2015 (Figure 2C), the number of berries per cluster was 28%, 38%, 41%, 55% and 68% lower in pre-bloom 1 (PB1), pre-bloom 2 (PB2), 50%B, FB and FS treatments, respectively, compared to controls. The appearance of the treated and control clusters agreed well with these data, presented as clusters removed from the vines 4 weeks before harvest in 2015 (Figure 3). While control clusters were compact and carried many berries in a range of sizes (Figure 3A), clusters treated at 50% bloom (Figure 3B), full bloom (Figure 3C) and fruit set (Figure 3D) were smaller, and their berries more uniform in size and well spaced.

Size segregation of the berries showed a significant increase in the B fraction and a parallel decrease in the S fraction for the 50%B, FB and FS treatments (Figure 2B,D): in 2014, the decrease in the S fraction was 58%, 28% and 73%, respectively, relative to controls; in 2015, the respectively decreases were 39%, 49% and 63%.

A similar, albeit smaller effect of ABA on segregation in pre-bloom clusters was also evident in 2014. In 2015, however, segregation in the PB2 treatment was similar to that in the control, while that of the PB1 treatment (carried out 2 weeks before full bloom – before the beginning of any blooming in the vineyard) presented the opposite trend, with an increase in the S fraction at the expense of the B fraction.

2.3. Effect of ABA on rachis length of a cluster

To test for potential adverse effects of ABA on rachis length, this parameter was recorded in treated and control clusters (Figure 4). No clear or stable effect of ABA on rachis length was noted for any of the treatments. In 2014, ABA led to a shorter rachis in all treatments relative to controls (Figure 4B). In 2013, the rachis of PB-300- and FB-300-treated clusters was shorter, while that of FB-150- or FS-300-treated clusters was not affected relative to controls (Figure 4A). In 2015, none of the ABA treatments

led to a shorter rachis; on the contrary, all treatments except PB2 led to a longer one (Figure 4C).

2.4. Effect ABA application on a semi-commercial scale

The above experiments were carried out with individually selected clusters with identified blooming status. Toward determining the applicability of ABA, a semi-commercial treatment was designed in which 250 ppm ABA was sprayed on all the clusters across seven sections of four vines, spaced by similar sections that were treated with surfactant and served as controls. Since variability in bloom status was expected within and between sections, it was monitored for each section respectively (Figure 5B). Combined analysis of the data from all seven repeats (Figure 5, R1) revealed that (1) 22% of the clusters had not yet bloomed, 19% were at full bloom and most (58%) were at partial bloom; (2) ABA led to a 23% decrease in the number of berries per cluster. However, analysing each repeat separately, compared to its neighbouring control, revealed a more complicated situation. In repeats R1, R2 and R3, 70%, 41% and 41% of the clusters were in full bloom, respectively, and most other clusters were at partial bloom; ABA treatment led to a 55%, 62% and 14% decrease, respectively, in number of berries per cluster. In R4, R5, R6 and R7, no cluster was in full bloom, and 21%, 21%, 42% and 31% of the clusters, respectively, had not begun blooming (NB). In these repeats, the effect of ABA on decreasing berry number per cluster was limited or absent (0, 11%, 13% and 6%, respectively).

סיכום

מטרות המחקר לתקופת הדו"ח:

אי אחידות בגודל הגרגרים באשכול היא בעיית איכות מרכזית בגידול ענבי מאכל. הראנו בעבר תלות ברורה בין מספר הפרחים על האשכול במצב טרום פריחה, אחוזי החנטה ודרגת אי האחידות והועלתה ההשערה כי אי האחידות ניתנת למיתון על ידי שינוי תנאי התחרות על מבלעים. שיפור אחידות באשכול שהושג באמצעות מניפולציה של מספר הפרחים ההתחלתי תומך בהנחה ועדיין חוקיות התחרות בין החנטים אינה ברורה. מטרת המחקר העבודה הנוכחית היתה לשפר את ההבנה לגבי הבסיס לשונות בגודל הגרגר ואת השליטה בתופעה.

עיקרי התוצאות שהושגו:

גודלו הממוצע של גרגר בקצה הסעיף עלה על זה שבתחילת הסעיף והפער בין משקל הגרגר בשני הקצוות גדל כאשר העומס גדל. חשיפה לטיפול GA העלתה באופן מובהק ודרמטי את הפרש הגודל בין קצות הסעיף וההפרש החריף עם עליית עומס הגרגרים לסעיף. לא נמצא הבדל בגודלם של שלושת גרגרי הקצה בסעיפים שנשאו 10 או 35 גרגרים. לעומת זאת, פרחים, שחלות וגרגרים מן הקצה הרחוק של הסעיף היו גדולים באופן מובהק מאלו שבקצה הקרוב לשדרה ותומכם בהשערה ששונות בגודל שחלה הוא פרמטר חשוב בקביעת אי אחידות בתנאי תחרות. אנליזה מיקרוסקופית מציעה כי ההבדל נובע משונות בגודל תאים. חיגור של מחצית ההיקף לא שינה את רמת האחידות בגודל הגרגרים באשכול בהשוואה לבקורת לא מחוגרת ואנו מטילים ספק ביעילותו של חיגור זה. מאחר שחיגור מלא מנוון את האשכול בזמן חנטה לא נראה שיש כרגע כלי אמין לבירור השאלה. ייסוד תנאי תחרות הוגנת בין גרגרים מאותה קטגורית גודל אפשרה לגרגרים שהוגדרו כגרגרי זטרת להפוך לגרגרים גדולים וההשפעה היתה מהותית יותר ככל שתנאי התחרות ווסתו מוקדם יותר. אופיין פרופיל טרנסקריפטומי ופרופיל הורמונלי של חנטים קטנים וחנטים גדולים בסמוך לחנטה. על בסיס ההבנות נבחן שימוש ב-ABA ונמצא כאמצעי יעיל לדילול חנטים שהוביל שיפור אחידות האשכול.

המסקנות וההשלכות לגבי יישום המחקר והמשכו:

ממצאי הנסיונות תומכים בשונות בגודל השחלות כבסיס לדיפרנציאל בין מבלעים הגורם לפתיחת פערי גודל בין חנטים באשכול עם העלייה במספר הפרחים. חלוקה לא שוויונית של מוטמעים בין שחלות מגדלים שונים תהפוך שחלה גדולה למתחרה מוצלחת יותר עם יתרון התחלתי. שונות זו על האשכול מעניינת ומקורותיה לא נחקרו והם בעלי חשיבות מעשית שתוכל לשפר אחידות פריחה, גודל, קצב הבשלה. ניתן לשנות גורלו של גרגר זטרת על ידי שינוי תנאי התחרות וההשפעה מהותית יותר ככל שהשינוי קרוב לתחילת התפתחותו של החנט. נמצאו הבדלים מובהקים ברמת תעתיקים ומטבוליטים המציעה אפשרות של אינטראקציה של מספר הורמונים בבקרת הגודל בין גרגרים קטנים וגדולים ואנליזה זו תועמק. נמצאו גם הבדלים בולטים הקשורים למשק סוכרוז, זרחון חמצוני, חלוקת תאים ופנילפרופנואידים. פותח טיפול מעשי לשיפור האחידות באמצעות יישום ABA בין פריחה מלאה לחנטה.

האם כבר הוחל בהפצת הידע? לא.

פרסום הדו"ח: אנו מבקשים לשמור על חסיון מלא של הדו"ח עד להגנה על ממצאיו ופרסומן.

Chapter 1- Figure Legends

Fig. 1: Effect of GA, load and flower location on berry size and cluster uniformity. Two weeks before anthesis the top branch (Shoulder) of a cluster was manipulated to carry 6 groups of 5 flowers and then treated with GA_3 solution (30 ppm, 0.025% Triton-X-100) or surfactant only (Con). Clusters (25 per treatment) were harvested 2 weeks before veraison. Berries were removed and data were collected separately from treated shoulder (T), untreated shoulder (UT) and the rest of the cluster. In T and UT shoulders, berries were removed and tagged according to their location in the group closest to central rachis (First; F), the group at the edge of shoulder (Last; L) or the groups 2-5 in between F and L. Berry number and individual berry weight were recorded in F and L. For the rest of the shoulder and rest of the cluster, berries were segregated by sieve to size fractions (VB, B, S, VS). Weight and number of berries per fraction was recorded and total number of berries was calculated. Rachis and shoulder length was recorded. (A) schematic presentation of the experiment; (B) Percentage of VS berries in the rest of the cluster; (C) Number of berries/ cluster (Calculated by dividing total number of berries in a cluster by shoulder lengthX2 X rachis length); (D) Percentage of size categories out of the total number of berries per cluster. (E) Total number of berries on T and UT shoulders; (F) Percentage of VS berries on T and UT shoulders; (G) Ratio of average berry weight in Last/ First groups of T and UT shoulders in GA-treated and control clusters; (H) Percentage of small (S+VS) berries in F and L groups in T and UT shoulders in GA-treated and control clusters. Averages from 25 clusters are presented. Bars represent ±SE

Fig. 2: Effect of position across the shoulder on weight of flower and carpel at anthesis. The top branch (Shoulder) was removed from clusters which were randomly selected from different vines, and kept in humid cell. Flowers were tagged according to their location in F and L groups. Weight of single flowers was recorded. Two independent analyses were carried at 25-03-14 (A) and 02-04-14 (B). Carpel weight was similarly recorded independently at 26&27-03-14 (C) and 30-03-15 (D). Values represent an average of values from 15 and 18 shoulders for flower weight and 9 and 12 shoulders for carpel weight, respectively. Bars represent ±SE .

Fig 3: Effect of flower position across the shoulder on berry weight. Shoulders were removed from clusters treated with GA_3 two weeks before anthesis. The weight of each berry in the F and L groups was recorded. Analyses were repeated independently two weeks before veraison and at harvest. Based on records of the single berry weight, berries from each group in each cluster were classified to two final size categories (Big and Small, see Methods section). The percentage of each category of the total number of berries in the group was calculated**.** (A) Berry weight in the F and L group two weeks before veraison (22-04-14) (B) Berry weight in the F and L group at harvest (05-06-2014) (C) Size segregation of berries in F and L groups at harvest. For other

details see Fig. 2.The values represent the average of 10 and 18 shoulders at the 1st and 2nd analysis. Bars represent ±SE.

Fig 4: Effect of flower position and GA on berry weight. Experiment was carried as detailed in Fig. 3 with several modifications. Shoulders were treated with GA_3 (GA) or surfactant only (CON). GA- treated and control shoulders were harvested and analyzed 2 weeks before harvest (02-06-2015). (A) Berry weight in the F and L group in GA-treated and control shoulders; (B) size segregation of the berries in the before described F and L groups. Values represent the average weight from 20 shoulders with ± SE.

Fig 5: Cell number and cell area of carpels. Histological sections were prepared from big and small carpels as detailed in Methods section. Number of cells in a defined area (12865 μm^2 for carpels) was counted, using magnification of 40X. Outer margins of 12-15 cells were manually drawn. The scanned image was used for estimation of cell area using the IMAGE J software. Carpels cell counts were used to calculate cell number using a cell number indicator formula **(Houel et al., 2013)** (A) Cell number in big and small carpels (B) Cell area in big and small carpels (C) Cell number indicator in big and small carpels. Average of 20 carpels from each size is presented. Bars represent SE.

Fig 6: Cell number and cell area of fruitlets. Experiment was carried as detailed in Fig. 5 with several modifications. Number of cells in a defined area (12695 μm^2 for berries hypodermis; 455191 μm^2 for berry mesocarp) was counted, using magnification of 20X. (A) Cell number in hypodermis of big and small fruitlets (B) Cell number in mesocarp of big and small fruitlets (C) Cell area in hypodermis of big and small fruitlets; (D) Cell number in mesocarp of big and small fruitlets.

Fig. S1: Microscopic sections of carpels and fruitlets. (A) Representative carpel section (20X) (B) Sections of big fruitlet (1,6X and 10X) (C) Sections of small berries (20X) at different stages labeled 1-4 according to progress from carpel appearance to fruitlet appearance.

Fig 7: Effect of modulation of cluster uniformity on small berry size a week from modulation. About 2 weeks before anthesis (30-03-14), clusters were randomly selected, trimmed to carry the six uppermost branches, and the entire cluster was treated with GA as described above. Number of berries was manipulated 2, 4 and 6 weeks after fruit set (Apr 22, May 4, and May 18, 2014). At the first time point, 30 clusters were manipulated to carry only 50 small berries (50S). This treatment was repeated with 20 and 12 clusters after 4 and 6 weeks. In the first time point additional 30 clusters were manipulated to carry a mixture of 25 big berries and 25 small berries (25S/25B). At each point of design, degree of size uniformity before manipulation was estimated using size fractionation by sieve. Of the clusters manipulated after 2 weeks, 12 clusters of each treatment (50S and 25S/25B) were removed after one week and analyzed. Size of small berries at 2 weeks before manipulation is labeled as S. Size of small berries 1 week after manipulation is labeled S(50S) or S(25S). (A) Comparison of small berry

weight before and one week after manipulation at 2 weeks after fruit set. Average of 50 berries per cluster was used. (B) and (C) Comparison of small berry width and length, respectively, for samples described in A. Average of 20 berries per cluster was used. Presented values are the average of 10 clusters (S) and 30 clusters for S(50S) and S(25S). Bars represent SE.

Fig 8: **Effect of timing of modulation of cluster uniformity on small berry size at harvest.** The experiment details are in Fig. 7. At harvest (25-05-14), all the clusters from the three time points were collected. For each cluster, berries were segregated by sieve and degree of uniformity was analyzed as described above. (A) Size segregation of small berries in 50S clusters designed after 2, 4 and 6 weeks from fruit set (B) Size segregation of small berries in 50S and 25S/25B clusters designed 2 weeks after fruit set. Values represent an average of 18, 20 and 12 clusters manipulated at 2, 4 and 6 weeks from fruit set. All other details are as described in Fig. 7.

Fig 9: Hormonal profiling of small and big berries before and after modulation of cluster uniformity. For experiment details see fig. 7. Three pools of berries were sampled from non- modulated clusters at 2 weeks after fruit set and 1 week later from modulated clusters. Plant hormone quantization and data processing was carried as in Crane et al. 2011).

Chepter 2- Figure Legends

Figure 1. ABA applications reduce the number of berries and improve berry size in cv. Early Sweet clusters. Field experiment, 2013. Treatments: pre-bloom 300 ppm ABA (PB-300 ppm), full bloom 150 ppm ABA (FB-150), full bloom 300 ppm ABA (FB-300), fruit set 300 ppm ABA (FS-300), Control (CON). All solutions included Triton X-100 (0.025%) as surfactant. FB-150 and FB-300 were re-treated similarly on 2 days after first treatment. Clusters were removed for analysis about 1 month before harvest and at harvest. All berries were removed from each cluster and segregated by sieve into four size categories: very big, big, small, very small. Number of berries and total weight were recorded for each size category in each cluster. The fraction of each group out of the total number of berries per cluster was then calculated. The percent values of very big + big and very small + small subcategories were combined for each into big and small fractions, respectively. The representative fraction of a size group/treatment was calculated by averaging the relevant fraction value for each cluster subjected to the respective treatment. (A) Number of berries/cluster 4 weeks before harvest. (B) Size segregation within a cluster 4 weeks before harvest. (C) Number of

berries/cluster at harvest. (D) Size segregation within a cluster at harvest. Values are averages of the respective value in 15 clusters in each treatment ± SE.

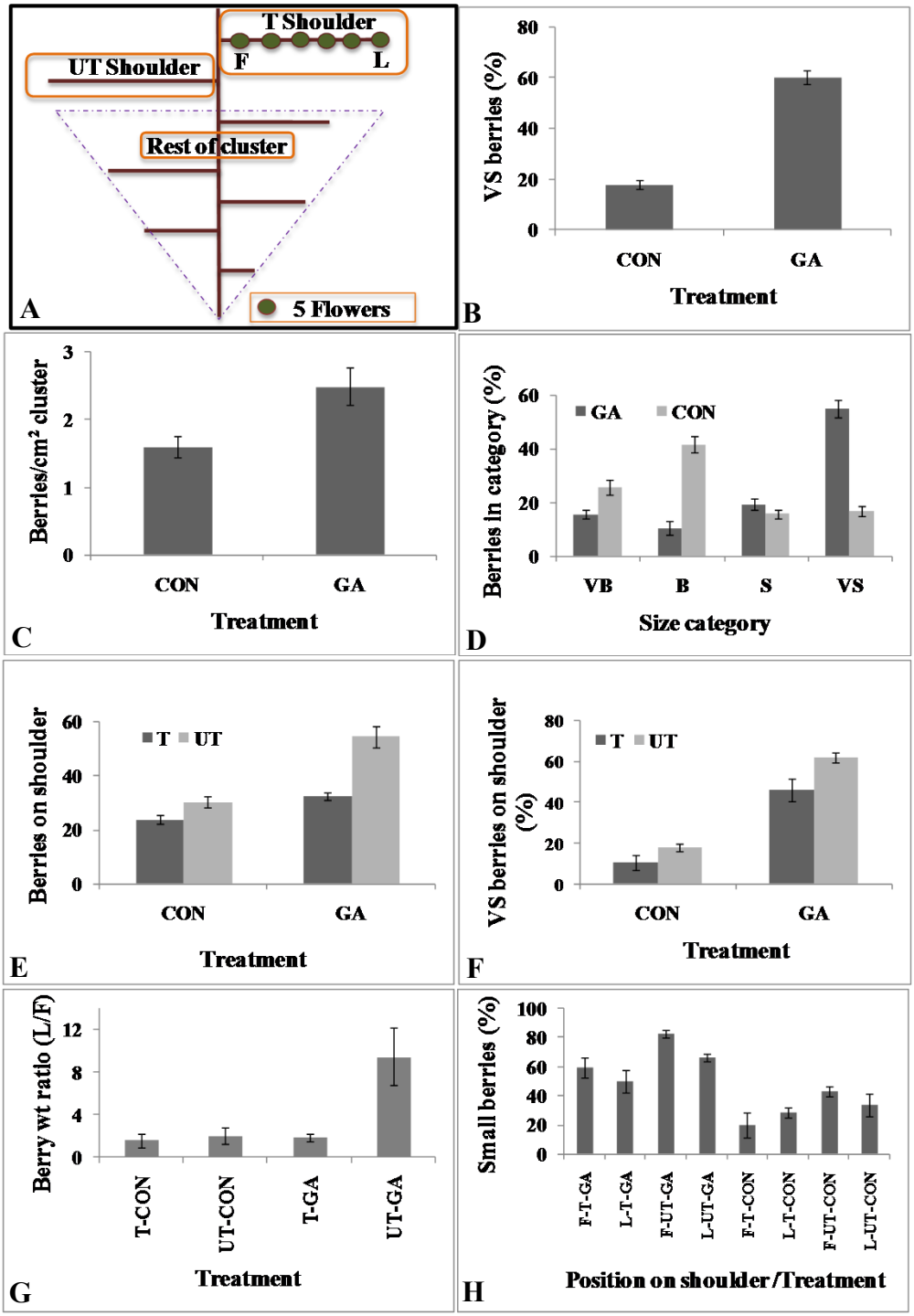
Figure 2. Effect of blooming status and bloom stage on cluster response to ABA. Two weeks before anthesis, in 2014 and 2015, 20 and 25 clusters, respectively, were assigned to treatments based on careful characterization of their bloom status and were treated with 300 ppm ABA: prebloom (PB), 50% bloom (50%B), full bloom (FB), and fruit set (FS) in 2014, and PB1 (2 weeks before full bloom), PB2 (1 week after PB1), 50%B, FB and FS in 2015. A treatment with 0.025% Triton-X-100 served as control (CON). Clusters were removed for analysis about a month before harvest. Values are averages of the respective values in 20 clusters in each treatment ± SE.

Figure 3. Appearance of clusters treated with ABA at different phenological stages. Data are presented from the experiment carried out in 2015 and shown in Figure 2. (A) Control (CON), (B) ABA applied at 50% bloom (50%B), (C) ABA applied at full bloom (FB), (D) ABA applied at fruit set (FS).

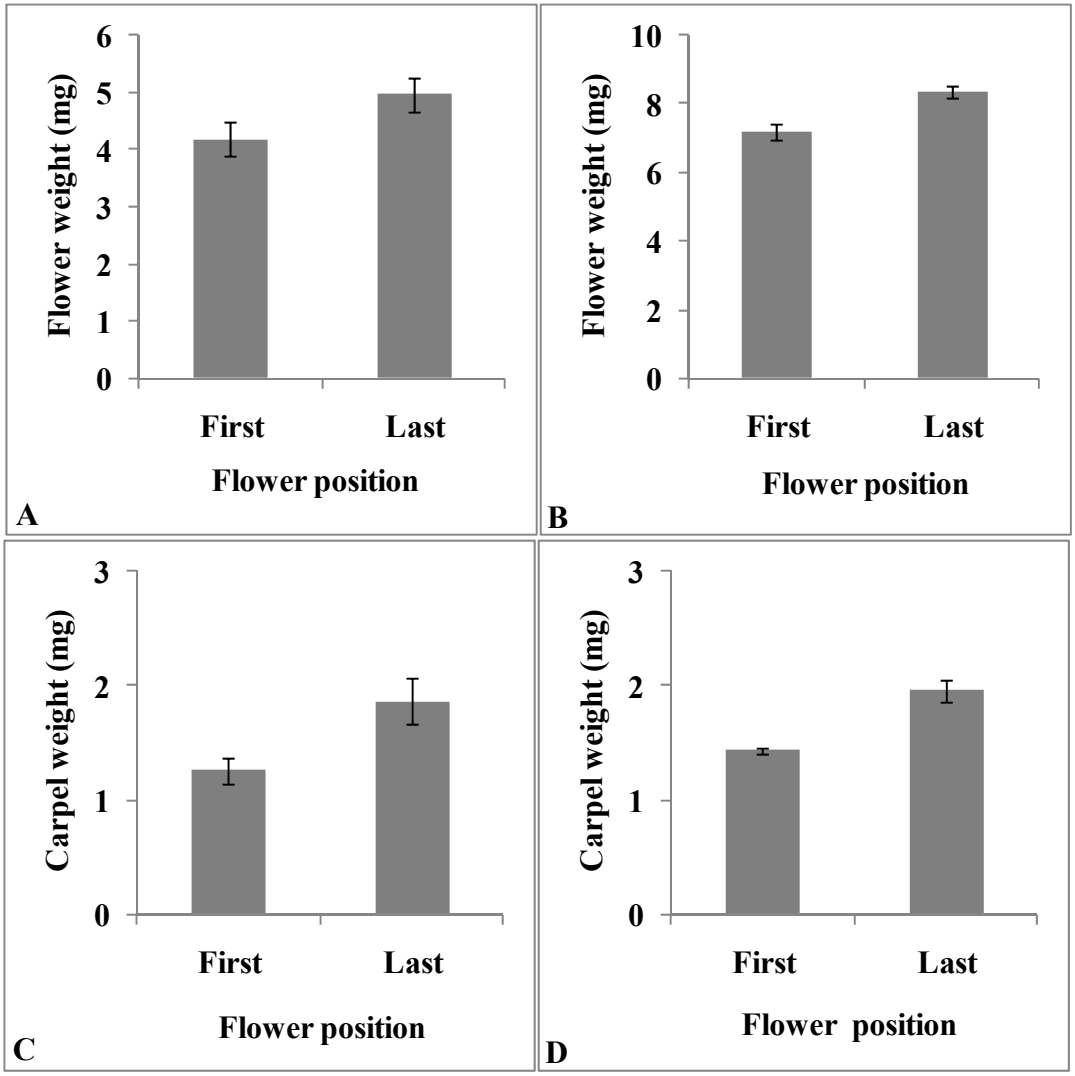
Figure 4. Effect of ABA application on rachis length. Data are presented for rachis measurements carried out on clusters treated at different phenological stages for 3 years: (A) 2013, (B) 2014, (C) 2015. All other details are as described in Figures 1 and 2.

Figure 5. Effect of semi-commercial application of ABA on cluster size is significantly affected by asynchronous blooming. A small-scale semi-commercial experiment was set up in the vineyard in 2015: 250 ppm ABA solution (ABA-250) was sprayed to full coverage on all clusters across seven sections of four vines, spaced by similar sections that were treated with surfactant and served as controls (CON). Bloom status was monitored for each section. About a week before harvest, 20 clusters were removed randomly from the two inner vines in each section and used for cluster analysis. (A) Berries/cluster. (B) Blooming status of the treated sections (NB – no bloom, ParB – partial bloom, FB – full bloom). R – repeat. Average of values from each repeat is presented.

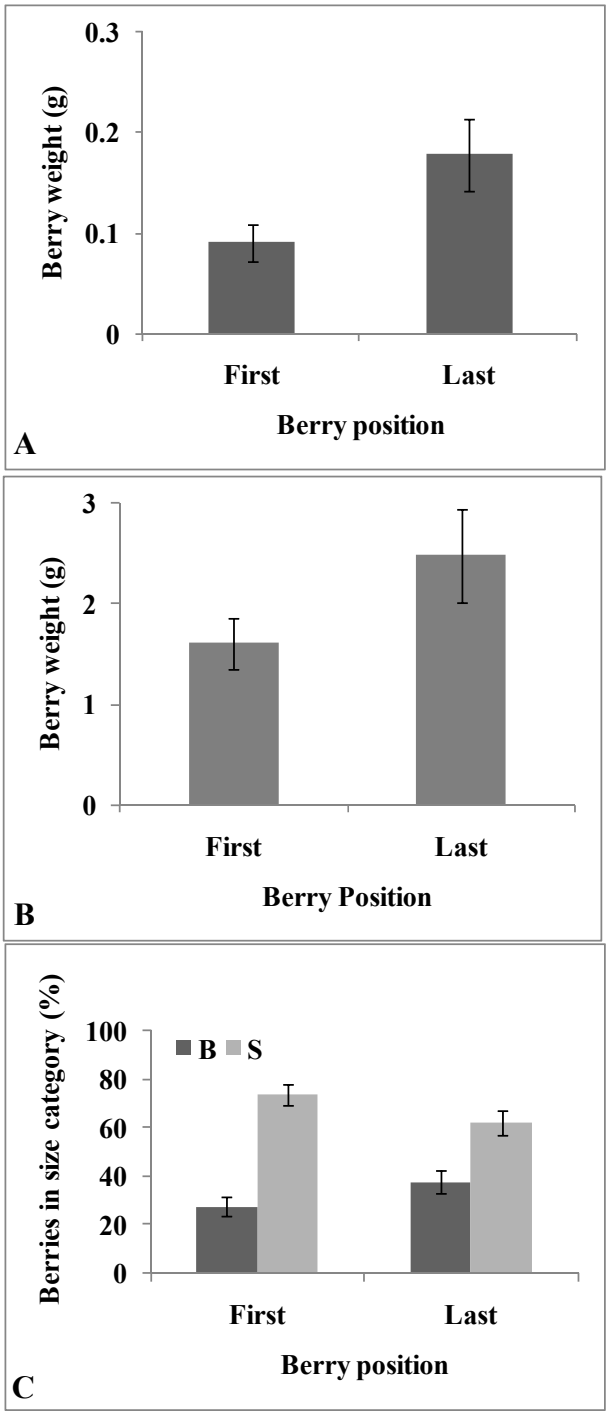
Chapter 1-Figure 1



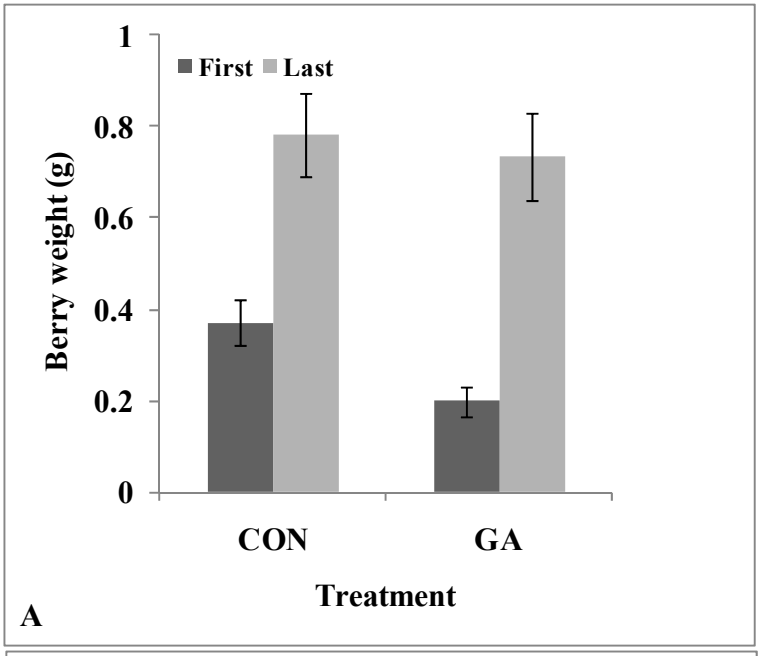
Chapter 1-Figure 2

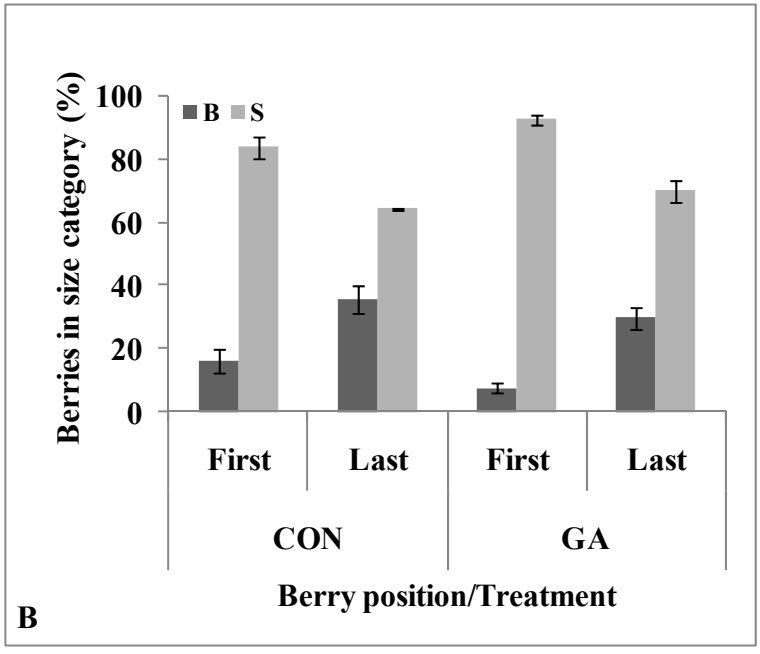


Chapter 1-Figure 3

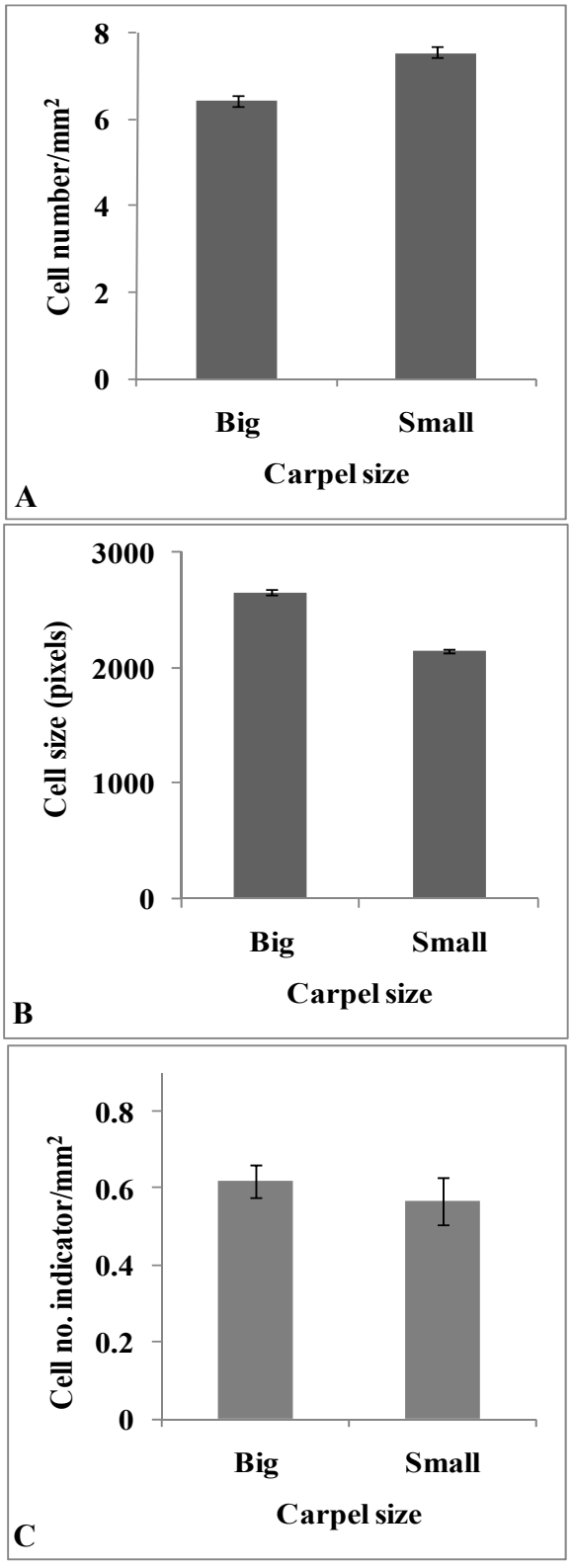


Chapter 1-Figure 4

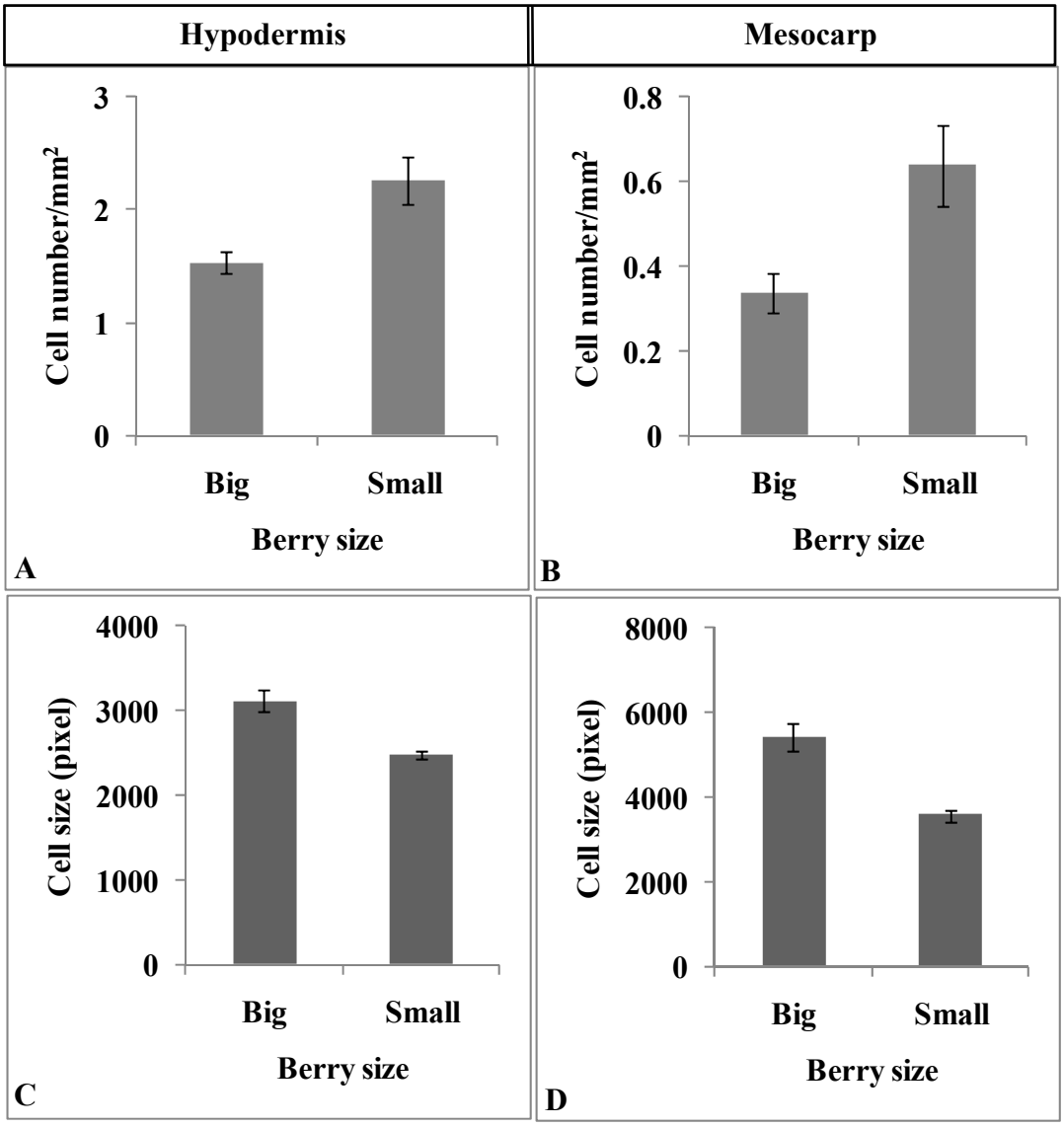




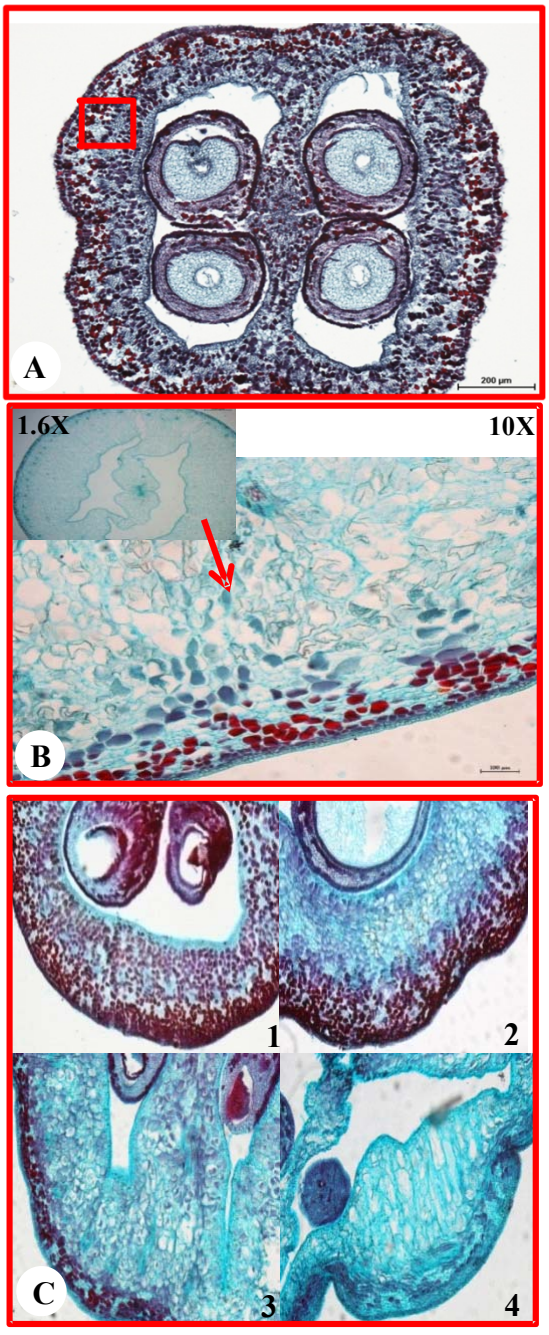
Chapter1-Figure 5



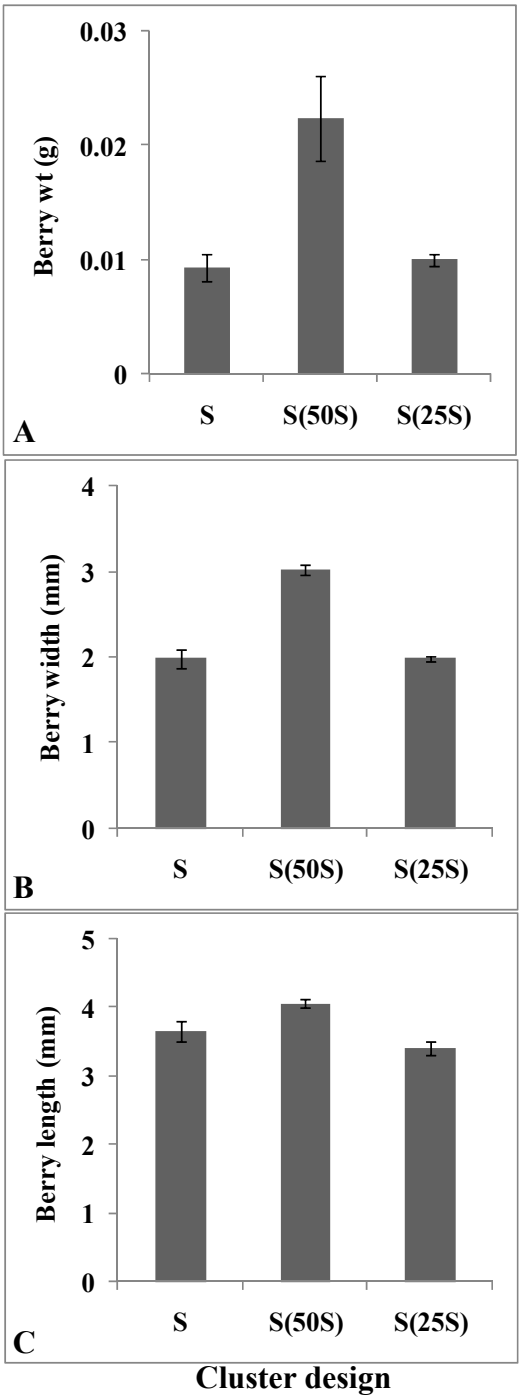
Chapter 1-Figure 6



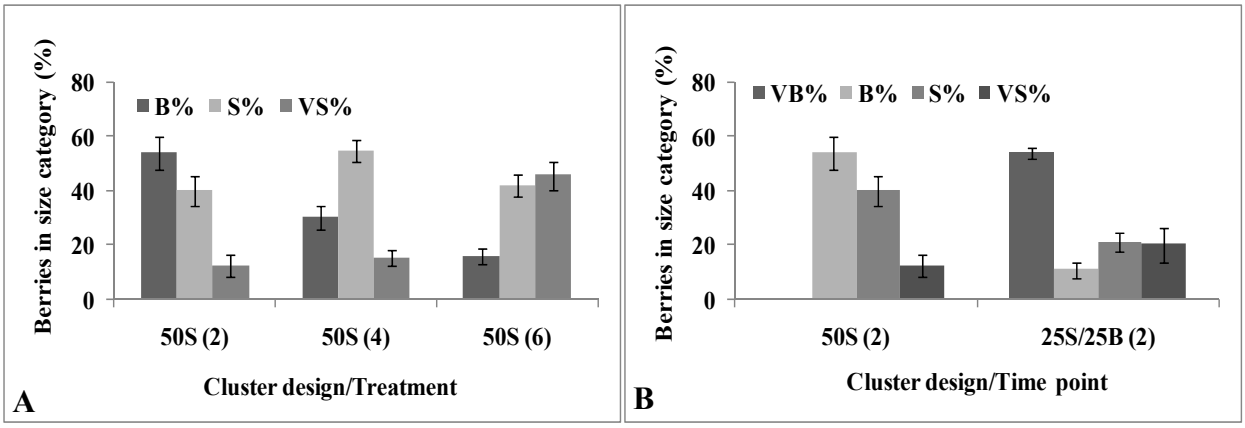
Chapter1- Fig S1



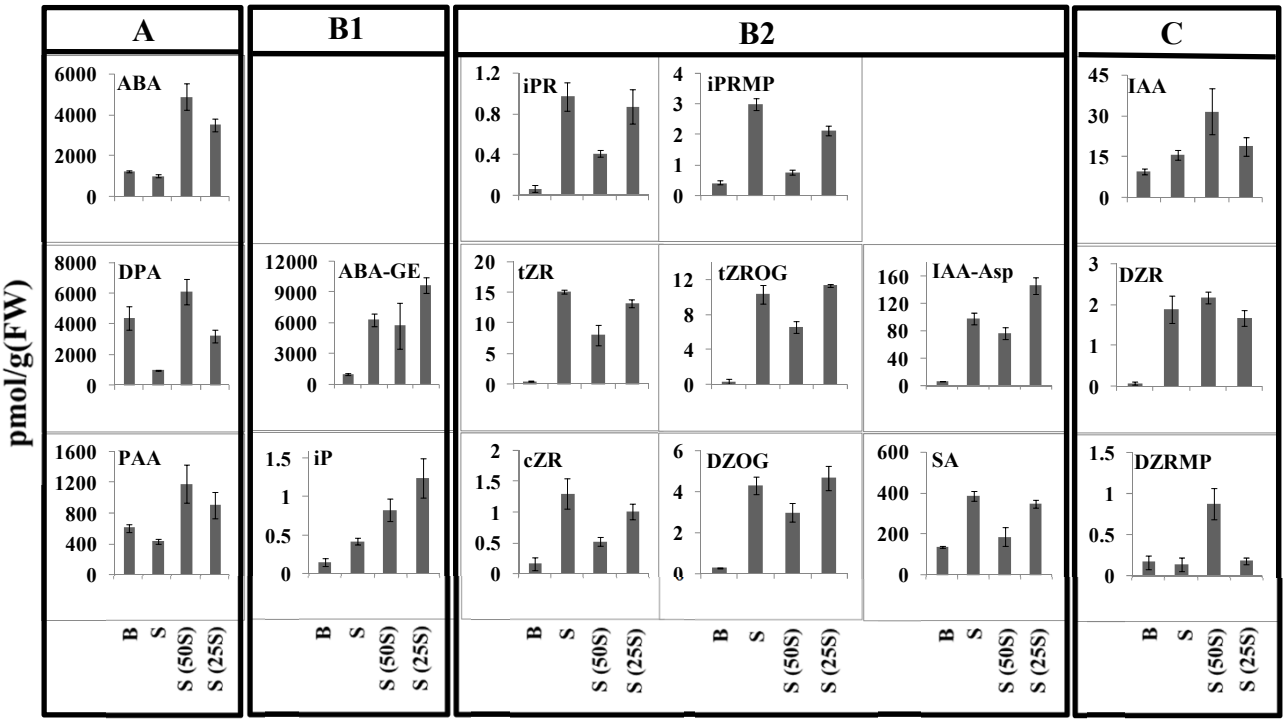
Chapter 1-Figure 7



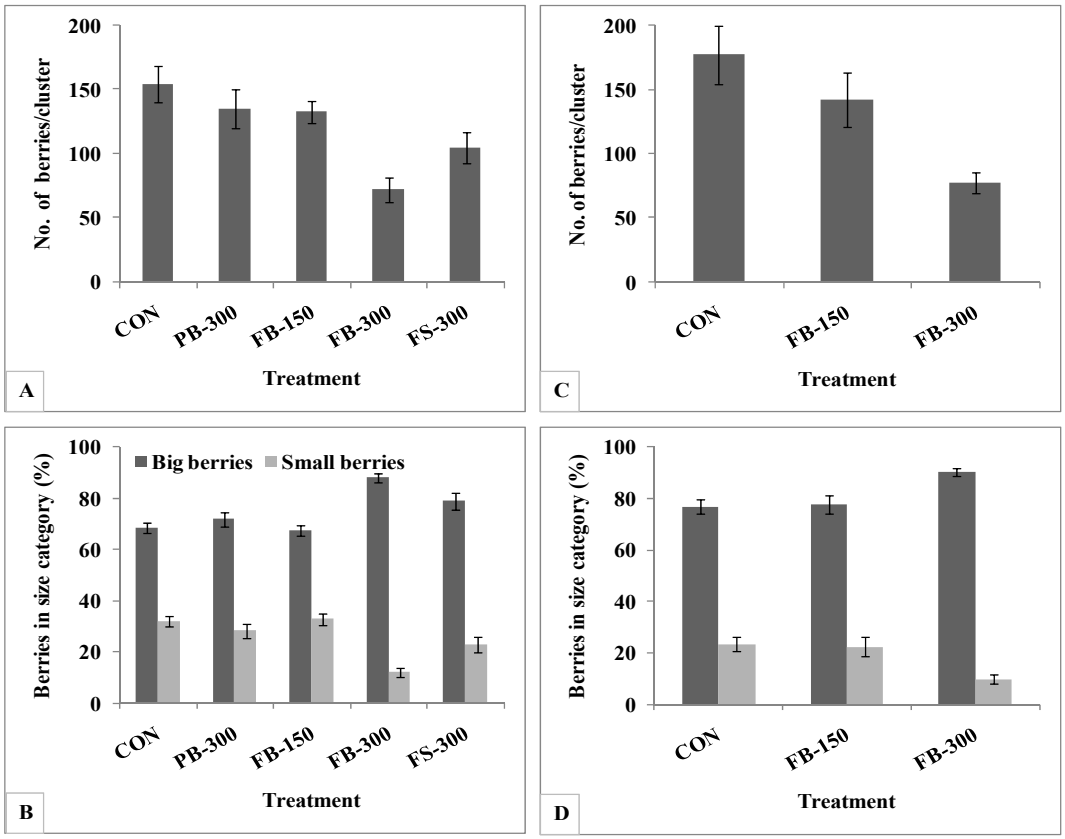
Chapter 1-Figure 8



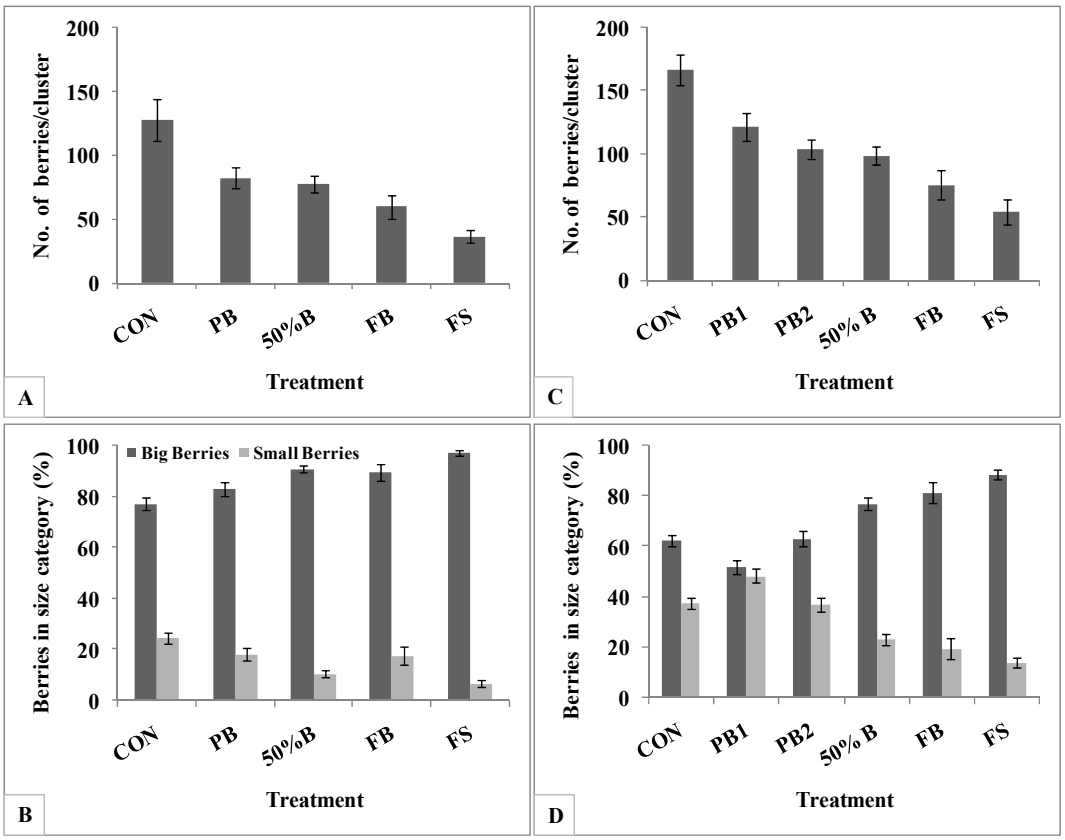
Chapter1-Figure 9



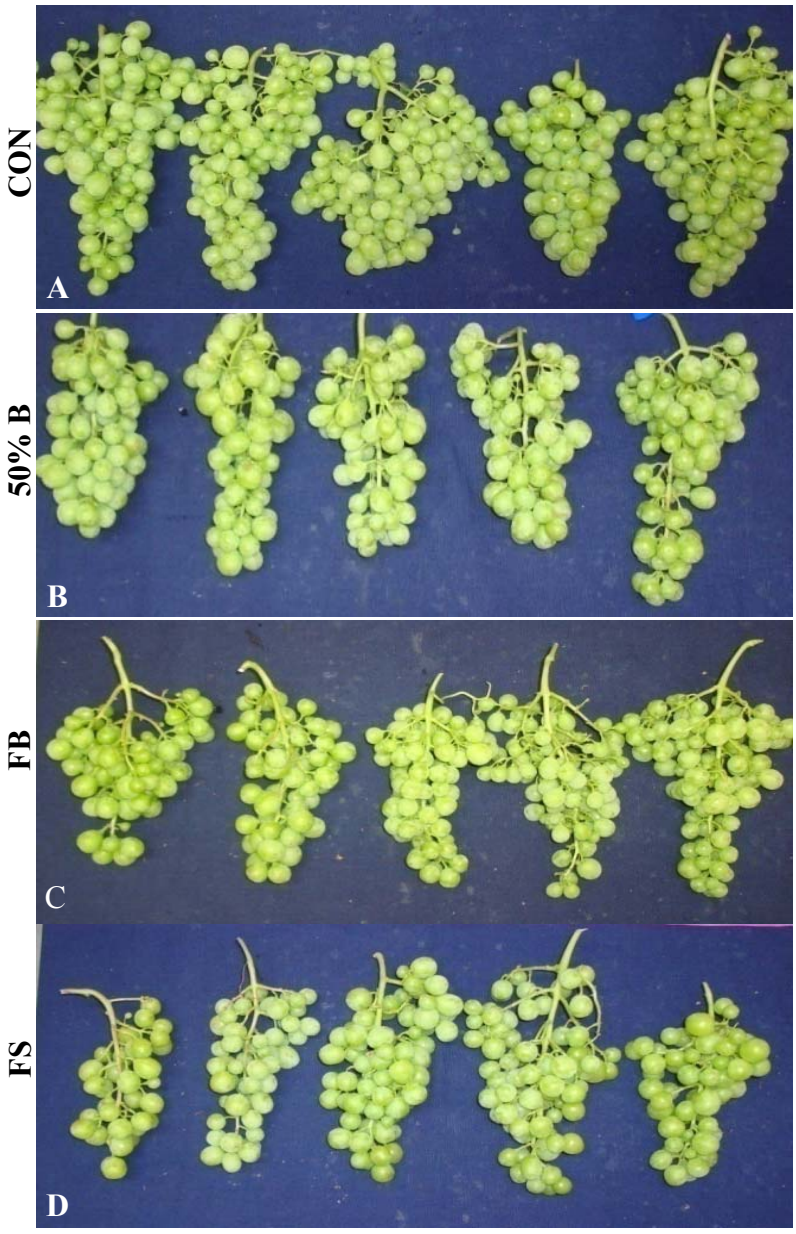
Chapter2-Figure 1



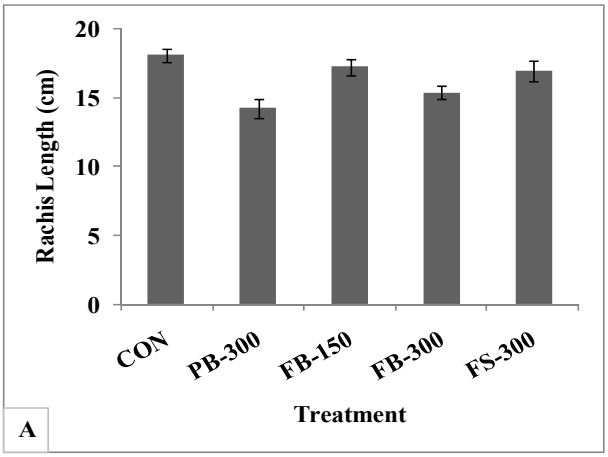
Chapter 2-Figure 2

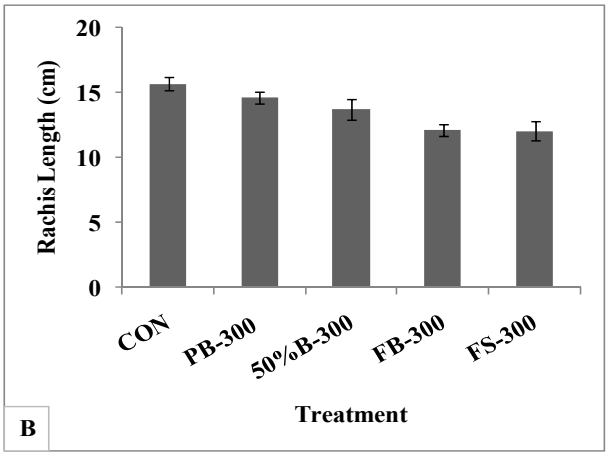


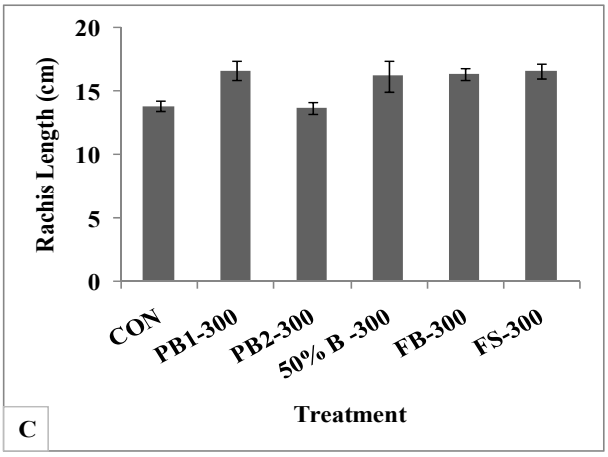
Chapter 2-Figure 3



Chapter 2-Figure 4







Chapter 2-Figure 5