



# Article The Impact of Plant Growth Regulators and Floral Cluster Thinning on the Fruit Quality of 'Shine Muscat' Grape

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**Abstract:** Plant growth regulators (PGRs) and floral cluster thinning are commonly used to improve grape yield and quality, but their effects on different fruit quality attributes in the 'Shine Muscat' are not well understood. In this study, we investigated the impact of PGRs and floral cluster thinning on various fruit quality parameters such as the cluster weight, berry weight, diameter, shape, sugar and acid content, firmness, and residual feel of peel. Our results indicate that the gibberellic acid 3 (GA<sub>3</sub>) 25 mg/L + thidiazuron (TDZ) 5 mg/L treatment at full bloom and GA<sub>3</sub> 25 mg/L treatment at 12 days after full bloom showed the largest cluster weight, berry weight, and diameter, while the forchlorfenuron (CPPU)-treated group, with a more balanced effect on fruit skin and flesh firmness, had the highest proportion of a positive residual feel of the peel. Floral cluster thinning by 4 cm was found to be effective for promoting fruit growth and maintaining an appropriate sugar–acid ratio. These findings can be useful for grape growers and researchers in optimizing PGR and floral cluster thinning treatments to improve grape yield and quality.

Keywords: grape; shine muscat; plant growth regulators; fruit quality

# 1. Introduction

The grape (*Vitis vinifera* L.) is a perennial deciduous vine, widely cultivated worldwide as a high-value and popular fruit crop due to its distinctive appearance and flavor [1,2]. With a global production of 84.7 million tons in 2021 [3], grapes have diverse uses, including fresh consumption, wine production, and serving as a source of essential nutrients and antioxidants. The 'Shine Muscat' ('Akitsu-21' × 'Hakunan', diploid) grape variety was developed in 1988 through crossbreeding. The first selection was made in 1997, followed by the final selection and naming as 'Shine Muscat' in 2003, and registered as a variety in 2006 [4]. This variety can be harvested in mid-to-late August, which is similar to the ripening time of 'Kyoho' grapes in the Akithu region where the variety was developed. The fruit has a greenish yellow color, firm flesh, and muscat aroma, with high levels of available solids and low acidity [4]. Shine Muscat grapes are considered a premium fruit variety especially in Asian markets such as China, the Republic of Korea, Japan, and Hong Kong due to their large size, crisp and juicy texture, and high sugar content, all of which contribute to their appealing and sweet taste.

Grapes are a highly diverse fruit crop that can be classified into seeded and seedless varieties. Seedlessness can arise from the degeneration of ovules in the fruit after normal fertilization and embryonic development, in a process known as parthenocarpy [5–7]. Currently, the production of seedless grapes involves inducing parthenocarpy through the application of gibberellic acid 3 (GA<sub>3</sub>) during the flowering period, which results



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). in abnormal ovule development and stimulating fruit set by re-treating with  $GA_3$  after flowering [8–11]. One alternative to using  $GA_3$  is the application of streptomycin (SM), an aminoglycoside antibiotic produced by the Streptomyces species [12]. SM was first discovered in 1944 by S.A. Waksman et al. and has been reported to induce parthenocarpy by inhibiting cell division in the ovule, similar to GA [13].

The use of plant growth regulators is widespread to improve the quality of grape fruits. Gibberellins are a representative group of plant hormones that promote plant growth, including flowering, seed germination, stem elongation, and fruit maturation [5,6,9,10]. GA<sub>3</sub> is the most commonly used plant growth promoter [9,10,14]. Thidiazuron (TDZ) is a type of synthetic plant growth regulator that belongs to the cytokinin family of phenylureas [15–17]. When used in combination with other growth regulators, TDZ has a strong effect at low concentrations and can attain particular results in the growth and development of plants. Byun et al. (1993) found that the combination treatment of TDZ and GA<sub>3</sub> increased the fruit set and promoted fruit enlargement in 'Himrod Seedless' and 'Geobong' grape cultivars with unstable fruit sets [18]. Forchlorfenuron (CPPU) is a synthetic plant growth regulator that belongs to the cytokinin family and is often used to boost cell division and stimulate the growth and development of plants [19,20]. However, the effects of various growth regulators on 'Shine Muscat' grapes remain insufficiently researched. Therefore, farmers continue to face difficulties and further research is needed to improve the quality of production for 'Shine Muscat' grapes.

Fruit thinning is a viticultural practice performed to achieve quality uniformity in grape clusters and is used to regulate fruit quality through appropriate thinning [9,21]. According to fruit thinning experiments conducted on 'Campbell Early', although the number of particles was lower compared to the control group, the availability of solids was higher and acidity was lower, resulting in high-quality fruit production [21]. However, experiments conducted on 'Shine Muscat' showed regional variation in the degree of thinning results [9]. Therefore, further research is necessary to determine the optimal floral cluster thinning for 'Shine Muscat'.

In this study, we aimed to establish a cultivation system for producing high-quality, seedless 'Shine Muscat' grapes using a non-hormonal method of organic thinning through the application of streptomycin. We further determined the optimal treatment frequency and concentration of growth regulators such as GA<sub>3</sub>, TDZ, and CPPU. We also compared the quality of the fruit based on the length of the fruiting shoot to satisfy consumer demands.

#### 2. Materials and Methods

#### 2.1. Experimental Condition of Grown Plants

The experiment was conducted during the 2018 growing season (Full bloom: 6 June 2018/Harvest: 23 September 2018) on 4-year-old 'Shine Muscat' (*Vitis vinifera* L.) grapevines, planted in a private grape orchard located in Hwangsan-ri, Goa-eup, Gumi-si, Gyeongsangbuk-do. The weather conditions during the experimental period from late May to September were as follows. The average temperatures (°C) were May 19.1, June 23.4, July 27.5, August 27.7, and September 20.3. The cumulative precipitations (mm) were May 109.1, June 150.4, July 262.8, August 258.2, and September 129.5. There was no rainfall within  $\pm 2$  days of each treatment application. A total of 24 grapevines was selected for the experiment. The distance between the plants was 2.5 m and the inter-row spacing was 4 m. The grapevines were managed following standard conventional methods: In early spring, before bud break, apply 1/4 pound of nitrogen per plant in the form of a balanced fertilizer (nitrogen: 10, phosphorus: 10, potassium 10). Repeat this application once again a month later. Additionally, apply 2–3 inches of organic matter (such as compost or well-rotted manure) around the base of the vine to help retain moisture and provide additional nutrients.

# 2.2. Streptomycin (SM) and Plant Growth Regulator (PGR) Treatments

The experiment comparing the rate of seedless induction according to SM and PGR treatment was composed of four treatments. The appropriate concentration and combination of plant growth regulators were established by referring to references [14,17]. The first group served as a control group, in which no SM treatment was applied and only GA<sub>3</sub> (12.5 mg/L) + TDZ (2.5 mg/L) were treated at full bloom. The second group was treated with SM (200 mg/L), GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) simultaneously at full bloom, while the third group was treated with SM (200 mg/L), GA<sub>3</sub> (25 mg/L) at full bloom. The fourth and last group was treated with SM (200 mg/L) at the flowering stage and then treated with GA<sub>3</sub> (25 mg/L) + CPPU (5 mg/L) at full bloom. All treatments were carried out using the dipping method with three replicates.

To compare the fruit quality by different combinations of PGR, five treatment groups were established. The first treatment group was treated with GA<sub>3</sub> (12.5 mg/L) and TDZ (2.5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) was applied 12 days after full bloom (DAFB). The second group was treated with GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) was applied 12 DAFB. The third group was treated with GA<sub>3</sub> (25 mg/L) and TDZ (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) was applied 12 DAFB. The fourth group was treated with GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) was applied 12 DAFB. The fourth group was treated with GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom, and GA<sub>3</sub> (25 mg/L) and treatments were conducted with GA<sub>3</sub> (25 mg/L) and CPPU (5 mg/L) at full bloom. All treatments were conducted with three replicates.

The detailed information about the PGR and streptomycin used in the experiment is as follows: streptomycin hydrate (NH Chemical, Seongnam, Republic of Korea), 3.1% GA<sub>3</sub> receptor (Yooill, Kim-po, Republic of Korea), 0.1% liquid CPPU (Arysta LifeScience Korea, Chungju, Republic of Korea), and 1% thidiazuron solution (Bayer CropScience Korea, Seoul, Republic of Korea). All berries across the different treatment groups were uniformly thinned to 4 cm at seven days before full bloom.

# 2.3. Floral Cluster and Berry Thinning

To compare the quality of fruit depending on the length of the floral cluster, three test groups were established with 3 cm, 4 cm, and 5 cm, and floral cluster thinning was carried out manually starting from the bottom of the floral cluster until the desired length was achieved at seven days before the full bloom. All treatments were conducted with three replicates.

# 2.4. Fruit Quality Evaluation

Four fruit clusters per treatment were randomly selected and harvested at 110 DAFB, when the fruit was considered to have reached maturation. The weight of each cluster was measured using a digital scale (Digital Scale Barquette, 5 kg; China). For berry weight measurement, a total of 80 berries was evaluated per treatment, with 20 randomly selected from each of the four fruits, using an electronic balance (CAS, RE-500; Shanghai, China). The length and width were measured from 12 selected berries per treatment, with three randomly selected berries from each of the four fruit clusters for each treatment, using Vernier calipers. The sugar and acid content was measured separately for the top, middle, and bottom parts of the fruit, with a total of 12 berries (three berries from each of the four fruit clusters) used for each part in each treatment group, using a Premium Fruit Sugar and Acid Content Measurement System (SAM-7300 PLUS; G-WON HITECH., Seoul, Republic of Korea). Sugar content was expressed in °Brix (°Bx) and acid content, in percentage (%).

To measure the firmness of the harvested fruit, a total of 12 berries (three randomly selected berries per fruit cluster) was sampled, and the firmness was measured before and after removing the skin using a texture analyzer (LLOYD TA1; USA). A flat tip with a diameter of 5 mm was used, and the maximum pressure in Newtons (N) transmitted to the plunger at a speed of 5 mm/s to a depth of 0.5 cm was measured. To investigate the effect of SM treatment and timing on the induction rate of seedlessness, a total of 16 fruit

clusters per treatment was randomly sampled, and the number of seeded fruit was counted. The seedlessness rate was calculated by dividing the number of seedless fruit by the total number of fruit in each treatment group.

# 2.5. Sensory Evaluation of Residual Feel of Peels

To compare the residual feel of the peels among the PGR treatment groups, sensory evaluations were conducted with a panel of at least 27 to 35 participants aged 20~60 for each treatment group. They were recruited from the National Institute of Horticultural and Herbal Science. The evaluation of residual sensation was classified based on the following four criteria: (1) Strong: This category referred to the feel of the fruit peel being highly noticeable on the tongue or inside the mouth, persisting even after the fruit had been consumed. (2) Mild: This category described the sensation of the peel being present but not overly noticeable or distracting. (3) Minimal: This category referred to the feel of the fruit peel being barely noticeable or not noticeable at all. (4) None: This category described the absence of any residual feel of the peel in the mouth. If the residual feel of the peel was classified as strong or mild, it was considered a negative feel, whereas if it was evaluated as minimal or none, it was considered a positive feel.

### 2.6. Statistical Analysis

The statistical analysis of the collected data was performed using the SPSS program (ver. IBM SPSS Statistics 23; SPSS Inc. Armonk, NY, USA: IBM Corp.: Armonk, NY, USA) and the significance between treatments was analyzed using Duncan's multiple range test (p = 0.05). The correlation analysis of the fruit qualities according to the PGR treatments was conducted using Pearson's correlation coefficient (r) in the corrplot package in R version 4.2.1 [22]. In cases where the p-value of the correlation was higher than 0.01, we denoted the correlation as blank and did not display it in the figure.

#### 3. Results

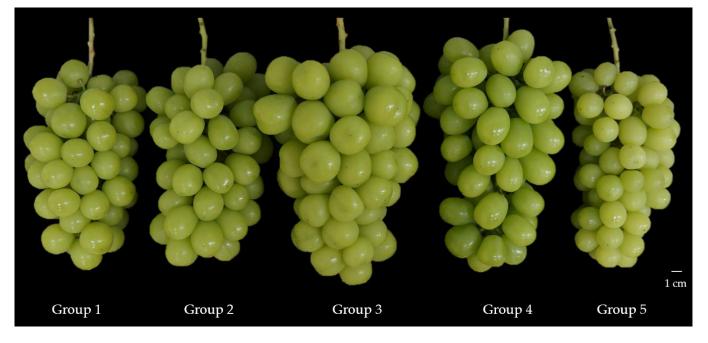
# 3.1. The Impact of Streptomycin (SM) and Plant Growth Regulator (PGR) Treatment on Seedless Induction

The impact of SM and PGR treatment on seedless induction was investigated and the results are presented in Table 1.

	Seedless Rate			
Group	7 Days Before Full Bloom	Full Bloom	(%)	
1	-	GA <sub>3</sub> 12.5 + TDZ 2.5	90.1	
2	-	SM 200 + GA <sub>3</sub> 25 + CPPU 5	100.0	
3	-	SM 200 + GA <sub>3</sub> 25 + TDZ 2.5	99.7	
4	SM 200	GA <sub>3</sub> 25 + CPPU 5	100.0	

**Table 1.** Effect of streptomycin (SM), gibberellic acid 3 (GA<sub>3</sub>), thidiazuron (TDZ), and forchlorfenuron (CPPU) application on the induction of seedlessness in the 'Shine Muscat' grape.

The numbers that appear with the PGR are the concentrations and their unit is mg/L. From now on, we will omit these units in this manuscript. The group treated with only  $GA_3$  12.5 + TDZ 2.5 at full bloom without SM treatment showed a 90% rate of seedless induction. On the other hand, the group treated with SM 200 +  $GA_3$  25 + CPPU 5 at full bloom showed a 100% rate of seedless induction and the group treated with SM 200 +  $GA_3$  25 + TDZ 2.5 at full bloom showed a 99% rate of seedless induction. The last group, which was treated with SM 200 at seven days before full bloom and with  $GA_3$  25 and CPPU 5 at full bloom also showed a 100% rate of seedless induction.



3.2. The Effect of Applying Plant Growth Regulators (PGRs) on the Quality of Fruits

An experiment was conducted to compare the effects of various PGR combinations on fruit quality among five groups (Figure 1, Table 2).

**Figure 1.** The shape of 'Shine Muscat' grapes at harvest according to PGR treatment. The combinations of PGR treatments applied at full bloom (F) and 12 days after full bloom (DAFB) are as follows. Group 1: F: GA<sub>3</sub> 12.5 + TDZ 2.5, 12 DAFB: GA<sub>3</sub> 25. Group 2: F: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: GA<sub>3</sub> 25. Group 3: F: GA<sub>3</sub> 25 + TDZ 5, 12 DAFB: GA<sub>3</sub> 25. Group 4: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: untreated. The numbers that appear with the PGR are concentrations and their unit is mg/L. Abbreviations: gibberellic acid 3 (GA<sub>3</sub>), thidiazuron (TDZ), forchlorfenuron (CPPU).

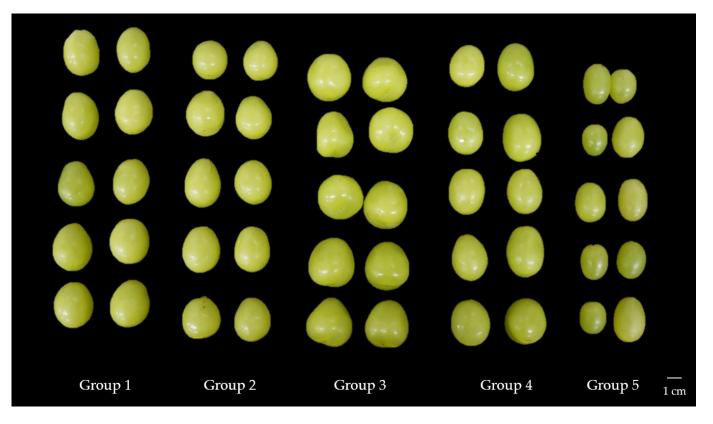
**Table 2.** Effects of gibberellic acid 3 (GA<sub>3</sub>), thidiazuron (TDZ), and forchlorfenuron (CPPU) application on the fruit qualities of seedless 'Shine Muscat' grape at harvest.

	Treatment (mg/I	.)	Cluster	Down	Dama	D	
Group	Full Bloom	12 Days after Full Bloom	Weight (g)	Berry Weight (g)	Berry Length (mm)	Berry Diameter (mm)	L/D Ratio
1	GA <sub>3</sub> 12.5 + TDZ 2.5	GA <sub>3</sub> 25	708.67 bc	12.48 c	28.43 b	26.64 c	1.07 a
2	GA <sub>3</sub> 25 + CPPU 5	GA <sub>3</sub> 25	648.67 b	11.10 b	30.16 c	24.23 b	1.25 b
3	GA <sub>3</sub> 25 + TDZ 5	GA <sub>3</sub> 25	931.67 d	15.44 d	31.73 d	28.80 d	1.11 a
4	GA <sub>3</sub> 25 + CPPU 5	GA <sub>3</sub> 25+ CPPU 5	722.25 с	13.00 c	32.32 d	26.07 c	1.24 b
5	GA <sub>3</sub> 25 + CPPU 5	-	510.33 a	8.4 a	25.88 a	21.53 a	1.21 b

Different letters within columns indicate significant differences based on Duncan's multiple range test ( $p \le 0.05$ ).

The third group treated with GA<sub>3</sub> 25 + TDZ 5 at full bloom and GA<sub>3</sub> 25 treatment 12 days before full bloom showed the largest cluster weight (931.67 g), berry weight (15.44 g), and berry diameter (28.80 mm). In contrast, the fifth group treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom without a second treatment showed the smallest values in all aspects of cluster weight (510.33 g), berry weight (8.4 g), berry length (25.88 mm), and

berry diameter (21.53 mm) compared to all other groups. The fruit shape was evaluated by examining the length (L) and diameter (D) of the berry and determining the L/D ratio (Figure 2).



**Figure 2.** The shape of 'Shine Muscat' grape berries at harvest according to the PGR treatment. The combinations of PGR treatments applied at full bloom (F) and 12 days after full bloom (DAFB) are as follows. Group 1: F: GA<sub>3</sub> 12.5 + TDZ 2.5, 12 DAFB: GA<sub>3</sub> 25. Group 2: F: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: GA<sub>3</sub> 25. Group 3: F: GA<sub>3</sub> 25 + TDZ 5, 12 DAFB: GA<sub>3</sub> 25. Group 4: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: GA<sub>3</sub> 25 + CPPU 5. Group 5: F: GA<sub>3</sub> 25 + CPPU 5, 12 DAFB: untreated. The numbers that appear with the PGR are the concentrations and their unit is mg/L. Abbreviations: gibberellic acid (GA<sub>3</sub>), thidiazuron (TDZ), forchlorfenuron (CPPU).

The berries appeared round when treated with TDZ (1.07: treated with GA<sub>3</sub> 12.5 + TDZ 2.5 at full bloom and with GA<sub>3</sub> 25 at 12 DAFB; 1.11: treated with GA<sub>3</sub> 25 + TDZ 5 at full bloom and GA<sub>3</sub> 25 at 12 DAFB) and elliptical when treated with CPPU (1.25: treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom and with GA<sub>3</sub> 25 at 12 DAFB; 1.24: treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom and GA<sub>3</sub> 25 + CPPU 5 at 12 DAFB; 1.21: treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom and GA<sub>3</sub> 25 + CPPU 5 at 12 DAFB; 1.21: treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom.

The sugar content of the fruits was measured by dividing it into the top, middle, and bottom parts of the fruit clusters (Table 3).

	Treatment (mg/L)			SSC (°Brix)			Acidity (%)			SSC/Acidity		
Group	Full Bloom	12 Days after Full Bloom	Тор	Middle	Bottom	Тор	Middle	Bottom	Тор	Middle	Bottom	
1	GA <sub>3</sub> 12.5 + TDZ 2.5	GA <sub>3</sub> 25	18.3 d	18.1 d	18.1 d	0.49 b	0.48 a	0.48 ab	37.6 d	37.6 c	37.8 bc	
2	GA <sub>3</sub> 25 + CPPU 5	GA <sub>3</sub> 25	17.1 c	16.6 bc	16.7 c	0.46 b	0.44 a	0.43 ab	31.7 bc	36.8 bc	38.8 c	
3	GA <sub>3</sub> 25 + TDZ 5	GA <sub>3</sub> 25	15.9 b	15.7 b	15.4 b	0.45 a	0.44 a	0.45 a	35.3 cd	35.2 c	34.3 bc	
4	GA <sub>3</sub> 25 + CPPU 5	GA <sub>3</sub> 25+ CPPU 5	16.9 bc	16.8 c	16.6 c	0.53 c	0.53 b	0.52 b	31.9 b	31.8 b	32.2 b	
5	GA <sub>3</sub> 25 + CPPU 5	-	13.0 a	13.0 a	13.2 a	0.61 d	0.62 c	0.61 c	21.7 a	21.3 a	22.3 a	

**Table 3.** Effects of gibberellic acid 3 (GA<sub>3</sub>), thidiazuron (TDZ), and forchlorfenuron (CPPU) application on the fruit sweetness (SSC) and acidity of the seedless 'Shine Muscat' grape at harvest.

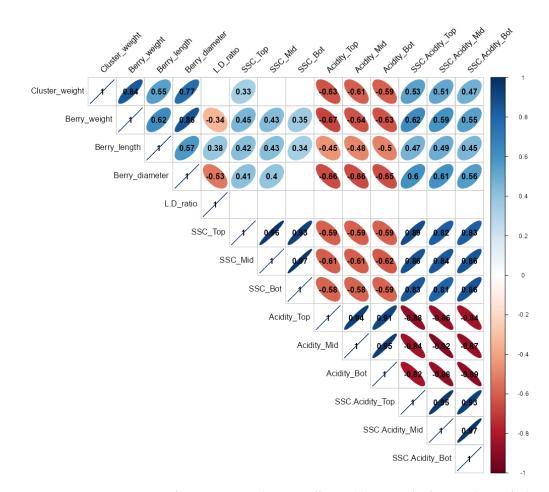
Different letters within columns indicate significant difference based on Duncan's multiple range test ( $p \le 0.05$ ).

Group 1 (treated with GA<sub>3</sub> 12.5 + TDZ 2.5 at full bloom and treated with GA<sub>3</sub> 25 at 12 DAFB) had the highest sugar content in all parts (top:  $18.3 \pm 1.3$ , middle:  $18.1 \pm 1.1$ , bottom: 18.1  $\pm$  1.2), while group 5 (treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom) had the lowest sugar content in all parts (top:  $13.0 \pm 0.8$ , middle:  $13.0 \pm 1.2$ , bottom:  $13.2 \pm 1.4$ ). The acidity showed differences depending on the fruit part, with the lowest acidity (0.45%)in the top part of the berries from group 3 (treated with  $GA_3 25 + TDZ 5$  at full bloom, and GA<sub>3</sub> 25 treatment at 12 DAFB), while group 2 (treated with GA<sub>3</sub> 25 + CPPU 5 at full bloom, and  $GA_3$  25 treatment at 12 DAFB) and group 3 showed the same level (0.44%) of acidity in the middle part, and group 2 showed the lowest acidity in the bottom part (0.43%). In contrast, the fifth group showed the highest acidity in all parts of the fruit (top: 0.61%, middle: 0.62%, bottom: 0.61%). When comparing the ratio of sweetness to acidity, group 1 and 3, which were treated with TDZ, showed a relatively higher SSC/acidity ratio in the top (group 1:  $37.6 \pm 4.1$ , group 3:  $35.3 \pm 4.1$ ) and middle (group 1:  $37.6 \pm 3.5$ ) parts compared to other groups, while group 2, treated with CPPU, showed the highest SSC/acidity ratio in the bottom (38.8  $\pm$  2.6). In contrast, group 5 showed the lowest SSC/acidity ratio in all parts (top: 21.7  $\pm$  3.1, middle: 21.3  $\pm$  3.6, bottom: 22.3  $\pm$  4.5).

# 3.3. Correlations between Fruit Qualities of 'Shine Muscat' by Plant Growth Regulator Treatment

To investigate the correlation between the results of the differences in fruit quality characteristics according to the PGR treatment, a correlation analysis was conducted using Pearson's correlation coefficient (r) in Figure 3.

The external traits such as cluster weight, berry weight, berry length, and berry diameter showed positive correlations ( $0.55 \sim 0.86$ ) with each other. In addition, these external traits showed positive correlations with SSC ( $0.33 \sim 0.45$ ) and the SSC/acidity ratio ( $0.45 \sim 0.62$ ) and negative correlations with acidity ( $-0.45 \sim -0.67$ ). The correlation analysis among the internal fruit quality characteristics, such as sugar content and acidity, showed that SSC, which represents sweetness, had a negative correlation with acidity ( $-0.58 \sim -0.62$ ) in all parts of the fruit, while it had a high positive correlation with the SSC/acidity ratio ( $0.81 \sim 0.89$ ).



**Figure 3.** Heatmap matrix of Pearson's correlation coefficient (*r*) among the fruit qualities of 'Shine Muscat' according to plant growth regulator treatments. (n = 60/12 samples  $\times$  5 treatment).

# 3.4. Effect of Plant Growth Regulator (PGR) Treatment on Fruit Firmness and Sensory Evaluation for Residual Feel of Peels

The firmness of the fruit skin and flesh was measured for each treatment group, and the results are presented in Table 4.

**Table 4.** Comparison of berry firmness and sensory evaluation of the fruit according to the growth regulator treatment in the 'Shine Muscat' grape.

Treatment (mg/L)		Skin -	Flesh	FDBSF a	Residual Feel of Peel (# of Penal)				Negative	Positive Feel <sup>c</sup>	
Group	Full Bloom	12 Days after Full Bloom	Firmness(N)	Firmness (N)	(N)	Strong	Mild	Minimal	None	Feel <sup>b</sup> (%)	(%)
1	GA <sub>3</sub> 12.5 + TDZ 2.5	GA3 25	6.1 a	3.3 ab	2.8 a	1	9	17	0	37.0	63.0
2	GA <sub>3</sub> 25 + CPPU 5	GA <sub>3</sub> 25	5.9 a	3.8 b	2.1 a	0	5	21	9	14.3	85.7
3	GA <sub>3</sub> 25 + TDZ 5	GA3 25	8.5 b	2.7 a	5.8 b	6	10	15	1	50.0	50.0
4	GA <sub>3</sub> 25 + CPPU 5	GA <sub>3</sub> 25+ CPPU 5	5.2 a	2.9 a	2.3 a	6	12	17	0	51.4	48.6
5	GA <sub>3</sub> 25 + CPPU 5	-	6.1 a	2.9 a	3.2 a	19	10	1	2	90.6	9.4

Different letters within columns indicate significant differences based on Duncan's multiple range test ( $p \le 0.05$ ). <sup>a</sup> Firmness difference between skin and flesh (FDBSF). <sup>b</sup> (Strong + Mild)/total × 100. <sup>c</sup> (Minimal + None)/total × 100.

Group 3 had the highest fruit skin firmness (8.5 N), but the lowest fruit flesh firmness (2.7 N), resulting in the largest difference in firmness between the skin and flesh (5.8 N). There was no statistically significant difference in the fruit skin and flesh firmness among

the other treatment groups. Sensory evaluation was also performed to assess the residual feel of the peels (Table 4). The fifth group had the highest proportion of negative feel (90.6%). In contrast, the 2nd group, which had the smallest difference in firmness between the peel and flesh, had a high proportion of minimal and no feels towards the peel, with 85.7% of the panelists having positive responses.

# 3.5. Effect of Floral Cluster Thinning on Fruit Qualities

To evaluate the impact of floral cluster thinning on fruit quality, treatments were conducted one week prior to full bloom for three different lengths: 3 cm, 4 cm, and 5 cm (Figure 4, Table 5).



**Figure 4.** The shape of 'Shine Muscat' grapes at harvest according to different degrees of thinning to 3 cm, 4 cm, and 5 cm. Thinning was performed seven days before the full bloom.

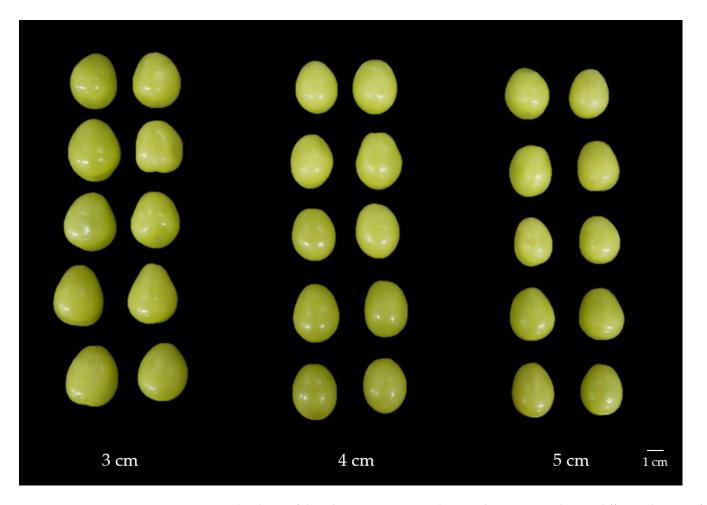
Group <sup>a</sup>	Thinning Degree	Cluster Weight (g)	Berry Weight (g)	Berry Number (ea)	Berry Length (mm)	Berry Diameter (mm)	L/D Ratio
1	3 cm	629.1 a	12.8 b	48.8 a	30.7 a	26.4 c	1.16 a
2	4 cm	743.5 b	13.1 b	56.9 b	30.6 a	25.7 b	1.19 ab
3	5 cm	713.8 b	11.3 a	60.6 c	30.3 a	24.5 a	1.23 b

**Table 5.** Effects of different lengths of floral cluster thinning by hand on the berry development of the 'Shine Muscat' grape at harvest time.

Different letters within columns indicate significant differences based on Duncan's multiple range test ( $p \le 0.05$ ). a Floral cluster thinning was conducted 7 days before full bloom.

The group thinned to 4 cm displayed the highest cluster weight (743.5 g) and berry weight (13.1 g), while the group thinned to 5 cm had the highest number of berries (60.6 ea). Although berry length was consistent across all groups, the 3 cm group had the longest berry diameter (26.4 mm). The ratio of length-to-diameter was found to be the smallest in

the 3 cm group (1.16) and the largest in the 5 cm group (1.23). Furthermore, the sweetness, acidity, and sugar–acid ratio were measured in different parts of the fruit to assess overall fruit quality (Figure 5, Table 6).



**Figure 5.** The shape of the 'Shine Muscat' grape berry at harvest according to different degrees of thinning to 3 cm, 4 cm, and 5 cm. Thinning was performed seven days before the full bloom.

**Table 6.** Comparison of soluble solid content (SSC) among the fruits from different lengths of floral cluster thinning by hand.

Group <sup>a</sup>	Thinning Degree -		SSC (° Brix)			Acidity (%)		SSC/Acidity		
	Degree -	Тор	Middle	Bottom	Тор	Middle	Bottom	Тор	Middle	Bottom
1	3 cm	16.6 a	16.5 a	16.4 a	0.48 a	0.48 ab	0.48 b	34.4 ab	34.2 ab	34.6 a
2	4 cm	17.2 a	17.0 a	16.9 a	0.46 a	0.47 a	0.45 a	37.0 b	36.4 b	37.3 b
3	5 cm	16.8 a	16.4 a	16.6 a	0.51 b	0.50 b	0.50 c	32.8 a	32.9 a	33.0 a

Different letters within columns indicate significant differences based on Duncan's multiple range test ( $p \le 0.05$ ). a Floral cluster thinning was conducted 7 days before full bloom.

There were no significant differences in sweetness among the treatments. However, when thinned to 5 cm, acidity was observed to be highest in all parts, resulting in a lower sugar–acid ratio. Conversely, when thinned to 4 cm, the sugar–acid ratio was found to be the highest.

# 4. Discussion

# 4.1. Rate of Seedless Induction

In this study, we investigated the effect of SM and PGRs on the rate of seedless induction in 'Shine Muscat' grapes. The results that SM and PGR affect seedless induction in grapes are consistent with previous reports [9,18,20,23]. Our results show that the combination of SM with PGR treatments significantly increased the rate of seedless induction (Table 1). Specifically, the group treated with SM  $200 + GA_3 25 + CPPU 5$  at full bloom had the highest (100%) rate of seedless induction, indicating a synergistic effect of SM in this treatment regimen. Additionally, the group treated with SM 200 +  $GA_3$  25 + TDZ 2.5 at full bloom also showed a high (99.1%) rate of seedless induction, suggesting the potential effectiveness of this combination. Interestingly, our findings also suggest that the timing of SM application may be an important factor in maximizing seedless induction. The group treated with SM 200 seven days before full bloom and with GA<sub>3</sub> 25 and CPPU 5 at full bloom showed a 100% rate of seedless induction. These results imply that there is no problem in producing seedless grapes even when SM and PGR are mixed and applied at full bloom, which is different from the previous study that found that the production of seedless grapes required separate treatments of SM at seven days before full bloom and PGR at full bloom [9]. While our study provides valuable insights into the potential use of SM in combination with PGRs for seedless grape production, further research is needed to elucidate the most effective combination or mechanisms underlying the observed synergistic effects and to optimize treatment protocols for commercial production.

# 4.2. Effects of Plant Growth Regulator Treatments on Fruit Quality

The use of PGR is a very common practice among grape growers, as PGRs have been shown to induce changes in the metabolic processes of plants and have demonstrated their effectiveness in various studies, including studies focused on improving fruit quality in various grape cultivars such as 'Kyoho' [14,18], 'Sovereign Coronation' [23], 'Flame Seedless' [24], and more recently, 'Shine Muscat' [9,10]. The present study investigated the effect of PGRs on 'Shine Muscat' grape fruit quality. We compared five groups treated with different PGR combinations and measured various parameters related to fruit quality (Tables 2–4). The third group, treated with GA<sub>3</sub> 25 + TDZ 5 at full bloom and GA<sub>3</sub> 25 at 12 DAFB, showed the largest cluster weight, berry weight, and berry diameter (Table 2). It has been reported that fruit size increases when GA<sub>3</sub> is applied at a concentration of 25 rather than 12.5 for both 'Kyoho' [14] and 'Shine Muscat' [9], which is consistent with our findings. Although there are reports that fruit size increases as the concentration of CPPU increases for 'Flame Seedless' [24] and 'Sovereign Coronation' as well as 'Simone' [23], our experiments, as well as those conducted by Shin 2019 et al., suggest that in the case of 'Shine Muscat', GA<sub>3</sub> + TDZ is more effective than GA<sub>3</sub> + CPPU in enhancing fruit size [9].

Grape PGR treatment experiments are usually designed to be performed twice, once at full bloom and again two weeks after full bloom [9,14]. This practice has been additionally validated through this experiment. The group treated with  $GA_3 25 + CPPU 5$  at full bloom without a second treatment showed the smallest values in all aspects of cluster weight, berry weight, berry length, and berry diameter, emphasizing the importance of a secondary treatment to ensure appropriate fruit size growth (Table 2).

We also evaluated the fruit shape based on the length and diameter of the berry and found that the berry appeared round when treated with TDZ and elliptical when treated with CPPU (Table 2), which is consistent with the previous study [9]. The result that the shape of the fruit can be induced to a certain shape depending on whether TDZ or CPPU is applied is an important finding in that fruit shape also has a significant impact on consumer preference [25,26]. The sugar content of the fruit varied depending on the PGR treatment, with the highest sugar content observed in group 1 treated with GA<sub>3</sub> 12.5 + TDZ 2.5 at full bloom and treated with GA<sub>3</sub> 25 at 12 DAFB in all parts of the fruit cluster (Table 3). These results are consistent with those of Lee's study, in which treatment with GA<sub>3</sub> at 12.5 produced higher sugar content than treatment with GA<sub>3</sub> 25 [14]. On the other hand, in their

experiment conducted on 'Shine Muscat', the sugar content was only similar to our results in one of the two locations where the experiment was conducted [9]. It is noteworthy that this location also had a fruit cluster weight similar to our experimental results. This treatment also showed a consistently high level of the SSC/acidity ratio, making it suitable for growing varieties with high sugar and high SSC/acidity ratios [27]. Such results were also consistent with the experimental results on 'Kyoho' [14]. Although the sugar content of the third group treated with TDZ was the fourth highest among all treatments, its sugaracid ratio was in the middle position among all treatment groups. On the other hand, there were differences in the sugar-acid ratios of the CPPU treatment groups depending on the fruit parts, and the fifth group had a low sugar content, high acidity, and very low sugar-acid ratio, indicating the need for additional considerations when applying CPPU treatment. Based on the correlation analysis of 14 fruit characteristics according to PGR treatment investigated in this experiment (Figure 3), the positive correlation between berry weight and berry diameter/length, as well as the negative correlation between sugar content and acidity, were consistent with previous results [28–30]. The relationship between berry weight and acidity has been studied in other experiments, and various correlations have been reported, such as a larger fruit size being associated with a higher acidity in 'Cabernet Sauvignon' [31] or cases where the correlation was not statistically significant [29,30]. However, our experiment revealed a strong negative correlation between berry weight and acidity. Furthermore, the negative relationship between berry weight and the L/D ratio was revealed in this study, which highlights the need for attention during 'Shine Muscat' cultivation.

# 4.3. Firmness Measurement and Sensory Evaluation

The results of this study indicate that the application of plant growth regulators (PGRs) has a significant effect on the firmness [24] of fruit skin and flesh, as well as on the residual feel of the peel (Table 4). The third group showed the highest firmness of fruit skin but the lowest firmness of fruit flesh. As a result of the sensory evaluation, the second group, which had the smallest difference in firmness between fruit skin and flesh, had the highest (85.7%) proportion of a positive residual feel of the peel (Table 4). This suggests that PGR treatments that have a more balanced effect on fruit skin and flesh firmness may result in a better sensory quality for the consumer. The effect of fruit qualities such as sweetness on consumer preferences has been investigated by sensory panels [32,33], but this is the first evaluation of the sensory perception of residual peel feel.

Overall, these findings suggest that the application of PGRs may have both positive and negative effects on fruit quality. While some treatments may lead to improvements in fruit firmness, others may have a negative impact on the sensory quality of the fruit. Further research is needed to identify the specific PGR combinations that can optimize both the physical and sensory quality of fruits. Additionally, the findings of this study may have practical implications for the postharvest handling and storage of fruits treated with PGRs.

#### 4.4. Effect of Floral Cluster Thinning on Fruit Qualities

Cluster thinning has been shown to reduce competition for resources among developing fruits, with studies indicating that grape cluster thinning can affect both the quantity of phenolic compounds and antioxidants, as well as basic quality factors [9,32,34]. The present study investigated the effect of floral cluster thinning on various fruit quality attributes. Our results revealed that the group thinned to 4 cm had the highest cluster weight and berry weight, indicating that 4 cm thinning is effective for promoting fruit growth. On the other hand, thinning to 5 cm resulted in the highest number of berries. However, it is important to note that increasing the number of berries resulted in a smaller berry size (11.3 g), which can ultimately affect a fruit's quality. The sugar and acid content also showed differences depending on the thinning method, and the largest difference was observed in the sugar–acid ratio. The highest ratio was observed in the group thinned to 4 cm, while the lowest ratio was observed in the group thinned to 5 cm. Overall, our findings indicate that the timing of floral cluster thinning can have a significant impact on fruit quality attributes such as the cluster weight, berry weight, number of berries, berry diameter, shape, acidity and sugar–acid ratio. These results may be useful for growers in determining the optimal degree of floral cluster thinning based on their specific objectives, whether they be promoting fruit growth, increasing yield, or improving fruit quality. In the previous experiment, the fruit size was largest when thinned to 5 cm, and the sweetness varied depending on the region, making it difficult to conclude which treatment is most suitable [9]. We suggest that the 4 cm thinning treatment may be optimal for maximizing cluster and berry weight, while maintaining an appropriate sugar–acid ratio.

# 5. Conclusions

Based on the results of our study, we can conclude that plant growth regulators (PGRs) and floral cluster thinning have significant effects on various fruit quality attributes in grapevines. The use of PGRs such as  $GA_3 + TDZ$  treatment can increase fruit size. On the other hand, the  $GA_3$  25 + CPPU 5 treatment showed the highest positive response in the residual feel of the peel. Furthermore, we revealed that PGR treatment conducted at least two times is needed to achieve appropriate fruit growth. Thinning floral clusters to 4 cm may be optimal for maximizing cluster and berry weight, while maintaining an appropriate sugar–acid ratio. Our findings provide useful information for grapevine growers and researchers to optimize PGR and thinning treatments for improving fruit quality.

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