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INFLUENCE OF FLOWER CLUSTER THINNING AND PLANT GROWTH REGULATOR TREATMENT CONDITIONS ON THE FRUIT QUALITY, ANTIOXIDANT PROPERTIES, AND MINERAL CONTENTS OF 'CHENGHYANG' GRAPES

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Abstract

This research aimed to evaluate the impact of different treatment modalities, involving flower cluster thinning and plant growth regulators, on the fruit characteristics, antioxidant properties, and mineral composition of triploid 'Cheonghyang' grapes. The lengths of the inflorescences were adjusted to 5 cm or 6 cm immediately before full bloom. Subsequently, various plant growth regulator treatments were administered at full bloom to thinned flowers, including GA_3 at 100 ppm, GA_3 at 100 ppm combined with thidiazuron at 2.5 ppm or 5.0 ppm, and GA_3 at 100 ppm combined with CPPU at 2.5 ppm or 5.0 ppm. The treatment combining GA_3 (100 ppm) and CPPU (5.0 ppm) with 6 cm flower cluster thinning exhibited the highest cluster weight and berry firmness. These values surpass those obtained from the commonly used GA_3 (100 ppm) treatment in Korean vineyards. Although this treatment regimen showed a relative reduction in total soluble sugar content compared to the other treatments, it still resulted in berries with exceptional marketability. Furthermore, this treatment regimen displayed a superior accumulative effect on DPPH radical scavenging activity and mineral content, indicating a substantial contribution to functional enhancement. In summary, the combined application of flower cluster thinning and plant growth regulators presents a practical approach for augmenting the marketability of triploid 'Cheonghyang' grapes.

Keywords: Cytokinin. Gibberellic acid. Marketability. Seedless. *Vitis.*

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1. Introduction

According to data from the Korean Statistical Information Service, grapes are one of the most extensively cultivated fruit crops in Korea, occupying 14,655 ha by 2022. However, over the last decade, the grape cultivation area has significantly decreased. Despite the introduction of 'Shine Muscat,' which has piqued the interest of Korean consumers, the heightened focus on this variety saturated the market and led to a decline in prices. To remain competitive, some farmers sell immature 'Shine Muscat' grapes, resulting in decreased consumer satisfaction with fruit quality. Thus, reducing the reliance on 'Shine Muscat' has emerged as a critical concern for the sustainability of the Korean grape industry. In response to the growing consumer demand for high-quality seedless grapes in Korea, significant efforts have been made to develop new seedless grape cultivars that are capable of meeting this demand. This has resulted in the breeding of new seedless grape varieties with outstanding fruit quality in vineyards throughout Korea (Chung et al. 2020).

Among these cultivars, 'Cheonghyang' stands out as a triploid grape cultivar created through a cross between 'Red Pearl' and 'Muscat Bailey A.' Unlike 'Shine Muscat,' 'Cheonghyang' is capable of producing high-quality seedless berries with just a single application of a plant growth regulator (PGR) at full bloom. Additionally, it was developed in the Gangwon Province, the coldest region in Korea, and has demonstrated exceptional regional adaptability, exhibiting robust cold resistance suitable for cultivation in all regions of the country. In fact, 'Cheonghyang' is already cultivated in high-latitude regions of Korea and is renowned for its exceptional fruit quality. It is utilized in wine production as well as being enjoyed as a fresh fruit (Lee et al. 2016). In Korean vineyards, 'Cheonghyang' seedless berries are typically produced using a single application of 100 ppm gibberellic acid (GA_3).

However, there is a consensus that the cluster weight of 'Cheonghyang' is relatively small compared to other grape cultivars, necessitating improvements to expand its cultivation area. Although cluster weight is primarily influenced by genetic factors (Wang et al. 2022), it can be enhanced by controlling flower cluster length or combining GA_3 treatment with cytokinin-type PGRs (Shin et al 2019). The challenge lies in the fact that the impact of flower cluster thinning (FCT) and PGR treatment conditions on fruit characteristics appears to vary depending on the grape cultivars (Afshari-Jafarbigloo et al. 2020; Choi et al. 2023), but the extent of their effectiveness in triploid grape cultivars like 'Cheonghyang' has not been explored to date. Hence, this study aims to assess the effects of FCT and PGR treatment conditions on the fundamental fruit characteristics, antioxidant properties, and mineral contents of 'Cheonghyang' grapes.

2. Material and Methods

Preparation of plant materials

This study utilized eight eleven-year-old 'Cheonghyang' vines cultivated in a rain shelter house within the vineyard of the Gangwon State Agricultural Research and Extension Services in Korea. The vines were spaced three meters apart and trained in a T-shape. The experiment involved controlling the flower clusters on the left branches of each vine to be 5 cm long, while those on the right branches were adjusted to 6 cm long, four days prior to full bloom. The selected plant growth regulators (PGRs) for the study included GA_3 at 100 ppm, GA_3 at 100 ppm + TDZ (Thidiazuron) at 2.5 ppm, GA_3 at 100 ppm + TDZ at 5.0 ppm, GA_3 at 100 ppm + CPPU (N-(2-Chloro-4-pyridyl)-N'-phenylurea) at 2.5 ppm, and GA_3 at 100 ppm + CPPU at 5.0 ppm. At full bloom, two flower clusters from the first to fifth branches were treated successively, with each vine soaked for 10 s. Subsequently, in the field, two clusters were harvested from each of the eight vines in each treatment group (n=16). The harvest time was determined when the L* value, measured using a colorimeter on the skin on the berry at the lowest position, averaged 39. The harvested clusters were immediately transported to the plant breeding laboratory at the Gangneung-Wonju National University.

Investigation of Fruit Characteristics

The effects of the FCT and PGR treatment conditions on fruit characteristics were evaluated by comparing the cluster weight, number of berries, average berry weight, number of non-commodity berries, harvest time, total soluble sugar content, titratable acidity, and berry firmness. Cluster weights were measured using an electronic scale (A&D, Tokyo, Japan). The total number of berries and non-commodity berries was visually counted. Average berry weight was calculated by dividing the total cluster weight by the total number of berries per cluster. Total soluble sugar content and titratable acidity were measured using a PAL-1 Refractometer (Atago, Tokyo, Japan) and a PAL-Easy ACID2 acidity meter (Atago), respectively, after selecting 4 four of the 16 clusters obtained for each treatment group as a repeating sample. To measure total soluble sugar content, juice from the five berries at the bottom of each cluster was extracted, squeezed, mixed thoroughly, and expressed in Brix units. Titratable acidity was determined by diluting 1 mL of the juice used to measure the total soluble sugar content with 39 mL of distilled water and expressed as malic acid percentage (%). Berry firmness was assessed using a fruit firmness meter by collecting 2 berries per cluster from the 16 clusters in each treatment group.

Investigation of antioxidant properties

The effects of FCT and PGR treatments on antioxidant properties were evaluated by measuring and comparing the total phenol content, total flavonoid content, and DPPH radical scavenging activity. For this experiment, 40 g of peel from each treatment group was randomly collected three times, dried in a hot air dryer at 60 °C for 10 d, and powdered using mortars. To measure the total phenol content, 25 µL of the solution extracted using the Folin–Ciocalteu method was mixed with 125 µL of the Folin–Ciocalteu reagent. Then, 37.5 µL of 20% sodium bicarbonate and 62.5 µL of distilled water were added. The sample was incubated at room temperature for 60 min and the absorbance was measured at 765 nm using an absorbance microplate reader (Geleta et al. 2023). Gallic acid was used as the standard, and the results were expressed as gallic acid content per weight (mg GAE/g). The aluminum chloride method was used to measure total flavonoid content with slight modifications. First, 25 µL of the extracted solution was added to a solution containing 75 µL methanol, 5 µL of 10% aluminum chloride, and 5 µL of 1 mol sodium acetate. The mixed solution was left at room temperature for 30 min and the absorbance was measured at 415 nm relative to that of the blank. Quercetin was used as the standard and the results were expressed as quercetin per dry weight (mg QE/g). The DPPH radical scavenging activity was measured according to the method described by Brand-Williams et al. (1995). Then, 100 µL of the extracted solution was mixed with 3 mL of 0.004% DPPH solution and placed in a dark room for 30 min. Subsequently, absorbance was measured at 517 nm using a spectrophotometer. Methanol and DPPH solutions of the measured extracts were used as blanks and controls, respectively. DPPH radical scavenging activity (%) was calculated using the following formula: DPPH radical scavenging activity (%) = [(control absorbance - sample absorbance) / control absorbance] × 100

Measurement of mineral content

For this experiment, 10 mL of a solution (HNO3:H2O2 solution (9:1) was added to the dried sample (0.5 g). The samples were then acid-decomposed for 30 min using a microwave digestion system. After the decomposed sample was filtered through a Whatman No. 5A filter paper (Whatman International, Maidstone, UK), its major mineral content was measured using an inductively coupled plasma spectrophotometer (PerkinElmer Co., Springfield, IL, USA).

Statistical analysis

The results of this study are presented as mean values along with their corresponding standard errors. To compare the data, we used analysis of variance (ANOVA), and for further differentiation between means, Duncan's multiple range tests were applied at a significance level of $p < 0.05$. To explore the potential relationships among the presented data, we calculated Pearson's correlation coefficients using SPSS Statistics (IBM, New York, USA).

3. Results

The impacts of FCT and PGR treatment conditions on fruit characteristics in 'Cheonghyang' grapes is summarized in Table 1. Cluster weight ranged from 162.65 to 203.08 g and was significantly influenced by both FCT and PGR treatments. Generally, the clusters exhibited greater weights when the flower cluster length was adjusted to 6 cm than when it was adjusted to 5 cm. Treatments involving GA_3 in combination with CPPU and TDZ resulted in higher cluster weights than the single GA_3 100 ppm treatment. Notably, under both 5 cm and 6 cm FCT conditions, the GA_3 100 ppm + CPPU 5.0 ppm combination consistently yielded the highest cluster weight. Berry number ranged from 48.00 to 54.31 g, with higher numbers observed in groups with 6 cm flower cluster length. Moreover, there was a significant increase in berry number in the mixed PGR treatments compared to GA_3 100 ppm treatment. Berry weight also tended to be higher in groups with larger cluster weights. However, there was no significant difference in the occurrence of non-commodity berries among treatments (Figure 1). Additionally, no cracking or softness due to berry enlargement was observed in any treatment. However, some treatments with increased cluster and berry weights compared to GA_3 100 ppm treatment experienced delayed harvest times.

Table 1. Effects of flower cluster thinning and plant growth regulator treatment on the cluster weight, number of berries, and berry weight of 'Cheonghyang' grapes.

Treatment		Cluster weight (g) [z]	Number of berries	Berry weight (g)
Flower cluster thinning	Plant growth regulator			
5cm	GA_3 100 ppm	162.65±6.43[e]	48.00±0.85[e]	3.40±0.14[b]
	GA_3 100 ppm + TDZ 2.5 ppm	167.49±4.03[e]	49.88±0.88[cde]	3.37±0.09[b]
	GA_3 100 ppm + TDZ 5.0 ppm	170.84±3.77[de]	51.25±0.79[abcd]	3.35±0.09[b]
	GA_3 100 ppm + CPPU 2.5 ppm	163.87±2.85[e]	48.94±1.27[de]	3.39±0.11[b]
	GA_3 100 ppm + CPPU 5.0 ppm	183.07±4.10[cd]	53.88±1.21[ab]	3.41±0.08[b]
6cm	GA_3 100 ppm	170.52±4.84[de]	50.86±0.98[bcde]	3.38±0.13[b]
	GA_3 100 ppm + TDZ 2.5 ppm	190.11±4.51[abc]	52.81±1.11[abc]	3.62±0.11[ab]
	GA_3 100 ppm + TDZ 5.0 ppm	198.66±4.29[ab]	53.94±0.76[ab]	3.69±0.08[ab]
	GA_3 100 ppm + CPPU 2.5 ppm	186.41±5.26[bc]	53.69±0.69[ab]	3.48±0.10[ab]
	GA_3 100 ppm + CPPU 5.0 ppm	203.08±5.50[a]	54.31±1.01[a]	3.76±0.12[a]
Flower cluster thinning (A)		***	***	*
Plant growth regulator (B)		***	***	Ns
A × B		Ns	Ns	Ns

[z] Within each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p < 0.05$). ± indicates standard error.

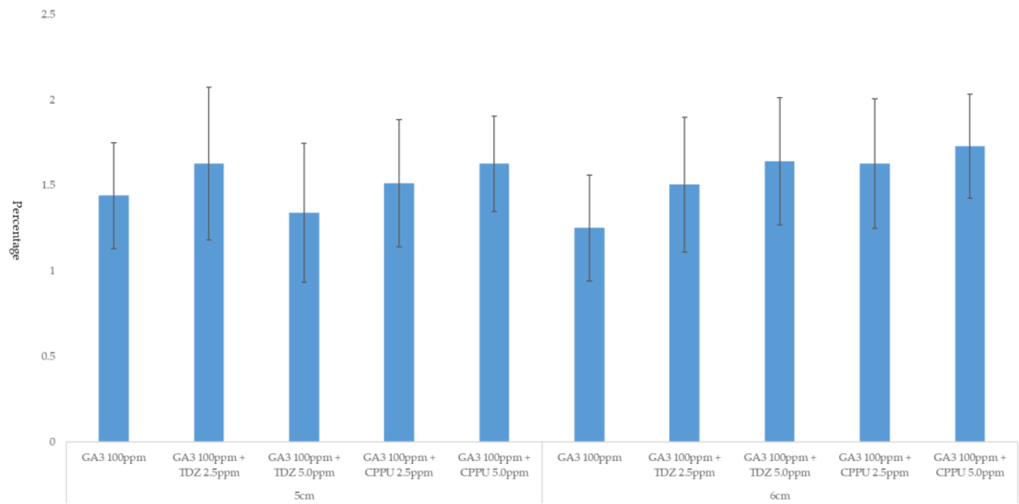

Figure 1. Effects of flower cluster thinning and plant growth regulator treatment on the occurrence rate of non-commercial berries in 'Cheonghyang' grapes. Error bar indicates the standard error of the mean.

Total soluble sugar (TSS) content ranged from 17.88 to 19.55 °Brix (Table 2). Groups with flower cluster lengths of 5 cm exhibited higher TSS values, with an average of 18.88 °Brix, compared with 18.41 °Brix in groups with 6 cm flower cluster length. Furthermore, the TSS content tended to be relatively lower in the GA_3 + TDZ and GA_3 + CPPU treatments compared than in GA_3 100 ppm treatment. Interestingly, among the mixed PGR groups, the decrease in TSS content was less pronounced in the GA_3 + CPPU group than in the GA_3 + TDZGA_3GA_3 group. Titratable acidity (TA) tended to be lower in treatments with higher TSS content while it was higher in those with lower TSS content. The TSS/TA ratio was relatively higher with 5 cm flower cluster length and GA$_3$ 100 ppm treatment. The berry firmness ranged from 12.07 to 13.15 N. FCT did not significantly influence berry firmness; however, significant differences were observed depending on the PGR conditions (Figure 2). Berry firmness tended to increase with higher PGR concentrations, with the GA$_3$ 100 ppm + CPPU 5.0 ppm treatment showing the highest firmness, being 1.1 N higher than with GA$_3$ 100 ppm treatment.

Table 2. Effects of flower cluster thinning and plant growth regulator treatment on the fruit quality of 'Cheonghyang' grapes.

Treatment		Total soluble sugar content (TSS) ^{z}	Titratible acidity (TA)	TSS/TA ratio	Harvest date
Flower cluster thinning	Plant growth regulator				
5cm	GA$_3$ 100ppm	19.18±0.09^{ab}	0.50±0.02^{c}	38.29±1.36^{a}	16th August
	GA$_3$ 100 ppm + TDZ 2.5 ppm	18.63±0.06^{cd}	0.57±0.01^{ab}	32.71±0.59^{bc}	16th August
	GA$_3$ 100 ppm + TDZ 5.0 ppm	18.25±0.10^{de}	0.59±0.01^{ab}	30.84±0.72^{bc}	16th August
	GA$_3$ 100 ppm + CPPU 2.5 ppm	19.55±0.06^{a}	0.51±0.01^{c}	38.78±0.98^{a}	16th August
	GA$_3$ 100 ppm + CPPU 5.0 ppm	18.80±0.04^{bc}	0.56±0.01^{b}	33.62±0.81^{b}	21th August
6cm	GA$_3$ 100 ppm	19.10±0.07^{b}	0.49±0.02^{c}	39.54±1.49^{a}	16th August
	GA$_3$ 100 ppm + TDZ 2.5 ppm	18.23±0.13^{de}	0.56±0.01^{ab}	32.45±0.83^{bc}	19th August
	GA$_3$ 100 ppm + TDZ 5.0 ppm	17.88±0.31^{e}	0.61±0.02^{a}	29.51±1.67^{c}	19th August
	GA$_3$ 100 ppm + CPPU 2.5 ppm	18.68±0.14^{c}	0.57±0.01^{ab}	32.69±1.02^{bc}	19th August
	GA$_3$ 100 ppm + CPPU 5.0 ppm	18.15±0.09^{e}	0.58±0.01^{ab}	31.59±0.99^{bc}	21th August
Flower cluster thinning (A)		***	ns	*	
Plant growth regulator (B)		***	***	***	
A × B		ns	ns	*	

^{z} Within each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p < 0.05$). ± indicates standard error.

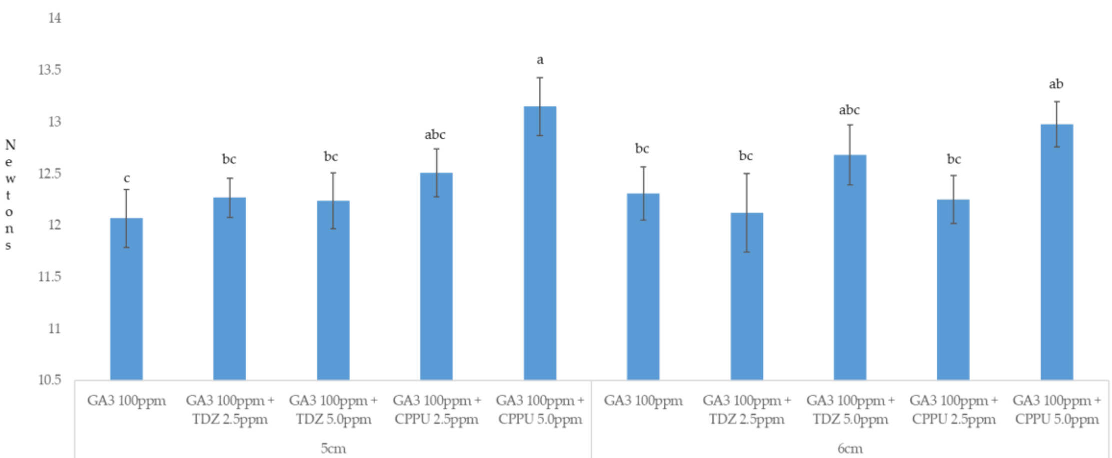

Figure 2. Effects of flower cluster thinning and plant growth regulator treatment on the berry firmness of 'Cheonghyang' grapes. Different letters are significantly different according to Duncan's multiple range test (≤ 0.05). Error bar indicates the standard error of the mean.

The total phenolic content ranged from 2.78 to 3.08 mg GAE/g, with relatively high values observed when the flower cluster length was adjusted to 6 cm (Table 3). Although no statistically significant difference was found in total phenol content across different PGR treatment conditions, GA$_3$ 100 ppm + TDZ 5.0 ppm, GA$_3$ 100 ppm + CPPU 2.5 ppm, and GA$_3$ 100 ppm + CPPU 2.5 ppm treatments consistently exhibited higher values compared to GA$_3$ 100 ppm treatment, regardless of flower cluster length. The total flavonoid content also showed relatively higher values with a 6 cm flower cluster length. Significant increases in total flavonoid content were observed with GA$_3$ 100 ppm + CPPU 5.0 ppm and GA$_3$ 100 ppm + TDZ 5.0 ppm treatments compared to GA$_3$ 100 ppm treatment. DPPH radical-scavenging activity ranged from 27.74 to 31.07%, with significant differences depending on FCT conditions. The mixed PGR treatment groups with high total phenol and flavonoid content exhibited significantly higher DPPH scavenging activity than those treated with GA_3 100 ppm treatment.

Table 3. Effects of flower cluster thinning and plant growth regulator treatment on the antioxidant properties of 'Cheonghyang' grapes.

Treatment		Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g) [z]	DPPH radical scavenging activity (%)
Flower cluster thinning	Plant growth regulator			
5cm	GA_3 100 ppm	2.84±0.08	2.34±0.04^{c}	27.74±0.65^{b}
	GA_3 100 ppm + TDZ 2.5 ppm	2.78±0.15	2.38±0.03^{bc}	28.30±0.95^{b}
	GA_3 100 ppm + TDZ 5.0 ppm	2.93+0.09	2.41±0.07^{bc}	28.92±0.28^{b}
	GA_3 100 ppm + CPPU 2.5 ppm	2.89±0.14	2.36±0.04^{c}	28.48±0.66^{b}
	GA_3 100 ppm + CPPU 5.0 ppm	2.85±0.08	2.47±0.03^{abc}	29.10±0.41^{ab}
6cm	GA_3 100 ppm	2.79±0.04	2.39±0.04^{bc}	28.07±0.57^{b}
	GA_3 100 ppm + TDZ 2.5 ppm	2.97±0.15	2.54±0.06^{ab}	29.76±0.50^{ab}
	GA_3 100 ppm + TDZ 5.0 ppm	3.08±0.12	2.61±0.06^{a}	31.07±0.72^{a}
	GA_3 100 ppm + CPPU 2.5 ppm	2.91±0.05	2.46±0.03^{abc}	29.45±0.72^{ab}
	GA_3 100 ppm + CPPU 5.0 ppm	3.00±0.07	2.50±0.05^{abc}	29.79±0.79^{b}
Flower cluster thinning (A)		ns	**	*
Plant growth regulator (B)		ns	**	*
A × B		ns	ns	Ns

[z] Within each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p < 0.05$). ± indicates standard error.

The mineral content of the grape skin, particularly potassium, ranged from 104.27 to 154.50 mg/100 g. The highest potassium content was observed with GA_3 100 ppm + CPPU 5.0 ppm treatment, regardless of the FCT conditions (Table 4). Phosphorus content ranged from 18.90 to 34.90 mg/100 g, with the highest content in GA_3 100 ppm + CPPU 5.0 ppm treatment with 5 cm FCT. Calcium content ranged from 8.70 to 13.58 mg/100 g, with the highest in GA_3 100 ppm + CPPU 5.0 ppm treatment with 6 cm FCT. Magnesium content ranged from 5.66 to 7.68 mg/100 g, with the highest in 6 cm FCT and GA_3 100 ppm + CPPU 5.0 ppm treatment.

Table 4. Effects of flower cluster thinning and plant growth regulator treatment on the mineral content of 'Cheonghyang' grapes.

Treatment		Phosphorus [z]	Potassium	Calcium	Magnesium
Flower cluster thinning	Plant growth regulator				
5cm	GA_3 100 ppm	19.67±1.52^{d}	121.23±7.28^{bcd}	8.70±0.63^{d}	5.66±0.41^{d}
	GA_3 100 ppm + TDZ 2.5 ppm	22.37±0.32^{bcd}	118.97±3.84^{cd}	9.81±0.21^{cd}	6.03±0.21^{bcd}
	GA_3 100 ppm + TDZ 5.0 ppm	18.90±0.35^{d}	122.13±6.37^{bcd}	9.13±0.68^{d}	6.24±0.31^{bcd}
	GA_3 100 ppm + CPPU 2.5 ppm	21.40±1.95^{cd}	115.50±7.78^{cd}	10.32±0.64^{bcd}	6.32±0.51^{bcd}
	GA_3 100 ppm + CPPU 5.0 ppm	34.90±0.87^{a}	152.20±2.20^{a}	12.51±0.40^{ab}	7.01±0.14^{abc}
6cm	GA_3 100 ppm	20.97±0.52^{cd}	104.27±0.61^{d}	9.88±0.30^{cd}	5.72±0.02^{cd}
	GA_3 100 ppm + TDZ 2.5 ppm	25.37±1.87^{bc}	145.70±9.42^{a}	10.84±1.15^{bcd}	7.00±0.69^{abc}
	GA_3 100 ppm + TDZ 5.0 ppm	27.27±2.87^{b}	134.07±8.51^{abc}	11.79±0.62^{abc}	7.07±0.43^{ab}
	GA_3 100 ppm + CPPU 2.5 ppm	23.97±2.37^{bcd}	140.90±7.65^{ab}	10.32±1.04^{bcd}	6.07±0.46^{bcd}
	GA_3 100 ppm + CPPU 5.0 ppm	32.47±1.23^{a}	154.50±5.42^{a}	13.58±0.80^{a}	7.68±0.22^{a}
Flower cluster thinning (A)		*	*	*	Ns
Plant growth regulator (B)		***	***	***	**
A × B		*	*	ns	Ns

[z] Within each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p < 0.05$). ± indicates standard error.

Correlation analysis revealed positive correlations between key fruit quality parameters, antioxidant properties, and mineral contents (Table 5). However, TSS content was negatively correlated with cluster weight, berry number, and TA. Berry firmness was positively correlated with phosphorus and calcium content. Additionally, strong positive correlations were observed between total phenol content, total flavonoid content, and DPPH scavenging ability.

Table 5. Pearson's correlation coefficients among fruit-related parameters of 'Cheonghyang' grapes in response to flower cluster thinning and plant growth regulator treatment.

	CW	NB	BW	TSS	TA	TSS /TA	BF	TPC	TFC	DPPH	P	K	Ca	Mg
CW	1													
NB	.921**	1												
BW	.916**	.688*	1											
TSS	-.778**	-.724*	-.686*	1										
TA	.685*	.723*	.512	-.884**	1									
TSS /TA	-.707*	-.733*	-.541	.924**	-.990**	1								
BF	.522	0.588	.379	-0.188	0.315	-0.257	1							
TPC	.793**	.636*	.824**	-.723*	.664*	-.661*	0.277	1						
TFC	.912**	.853**	.819**	-.812**	.712*	-.731*	0.392	.825**	1					
DPPH	.897**	.836**	.810**	-.804**	.791**	-.783**	0.408	.905**	.963**	1				
P	.735*	.776**	.578	-.375	0.414	-0.411	.878**	0.352	0.605	0.545	1			
K	.811**	.823**	.663*	-.549	0.578	-0.616	0.564	0.540	.666*	.639*	.836**	1		
Ca	.823**	.790**	.735*	-.443	0.447	-0.431	.874**	0.517	.668*	.651*	.939**	.769**	1	
Mg	.856**	.773**	.814**	-.636*	0.609	-0.614	.718*	.723*	.768**	.766**	.825**	.811**	.910**	1

* and ** indicate significant correlation at $p < 0.05$ and $p < 0.01$, respectively. CW: cluster weight; NB: number of berries; BW: berry weight; TSS: total soluble sugar; TA: tritratible acidity; BF: berry firmness; TPC: total phenolic content; TFC: total flavonoid content; DPPH: DPPH radical-scavenging activity; P: phosphorus; K: potassium; C: calcium; Mg: magnesium.

4. Discussion

Our investigation demonstrated the significant influence of FCT and PGRs on fruit quality-related parameters in 'Cheonghyang' seedless grapes. FCT, which is recognized as an effective management practice, plays a pivotal role in enhancing cluster weight and berry number in grapevines (Ahmad and Zargar 2005). Grapevines have finite resources, such as carbohydrates, nutrients, and water, which are allocated to various functions, including cluster and berry development. A balanced FCT regimen enables the vine to distribute an appropriate amount of resources to each remaining berry, thereby fostering optimal berry development and larger berry weight. Nonetheless, determining the optimal FCT conditions is imperative for augmenting cluster weight and berry setting. Over-zealous flower removal during thinning can reduce the number of remaining flowers required to achieve the desired berry set. Each flower contributes to the potential berry count, and excessive removal can lead to sparse clusters with fewer berries, ultimately reducing cluster and berry weights due to the lack of potential fruit sites for resource allocation. Our findings indicate that a shorter FCT does not necessarily guarantee higher levels of clustering, berry weight, or increased berry count. Interestingly, we observed that a longer flower cluster length yielded superior outcomes for these parameters, even when employing different FCT conditions. Thus, it is reasonable to postulate that employing a longer flower cluster condition (e.g., 7 or 8 cm) than what was utilized in this study could be advantageous for enhancing cluster weight and berry number in 'Cheonghyang.'

In our study, a specific combination of cytokinin-related PGR treatment with GA_3 significantly increased the cluster weight, berry weight, and berry number, irrespective of the FCT condition. TDZ has been effectively used to enhance berry sets by facilitating the development of ovaries into grape berries (Kok and Bal 2016). TDZ is also known to positively influence the cluster and berry weight by augmenting the cell size and number of cells in the berry (Nisler 2018). Similar to TDZ, CPPU increases fruit set by stimulating cell division in developing ovaries (Bian et al. 2022). CPPU is recognized for its capacity to augment fruit size

(Pujari et al. 2021), as it spurs cell division and enlargement, resulting in heavier berries. Optimal combinations of mixed PGRs can be highly effective in yielding larger and more abundant berries than single GA_3 treatments (Lee and Heo 2023). In our study, the most substantial increases in cluster weight and berry number were observed in GA_3 100 ppm + CPPU 5.0 ppm treatment group. These findings suggest that this particular combination may have stimulated cell division and elongation in berries most effectively among the PGR conditions employed in this study.

In our investigation, we observed that treatment groups with an FCT of 6 cm tended to display a lower TSS content and higher TA than those with 5 cm FCT. These variations seemed to be influenced by differences in berry weight. Larger berries generally possess a higher water content relative to their total volume than smaller berries (Remberg et al. 2010). Because soluble solids are dissolved in water, the concentration of these compounds appears lower in larger berries owing to the dilution effect. Conversely, an increase in the total soluble solids content led to a higher concentration of sugars relative to the remaining acids in the berry, potentially resulting in reduced acidity. These findings suggest that enhanced berry quality can be achieved through 5 cm FCT combined with GA_3 at 100 ppm or a lower dosage of mixed PGR. It is important to note that in Korea, grapes with sugar content higher than 18 °Brix are typically considered top-quality products. In our study, all treatments, except for GA_3 at 100 ppm + TDZ at 5.0 ppm with a 6 cm FCT, surpassed this quality threshold, indicating their competitiveness in terms of fruit quality in the market. Moreover, berry firmness in the GA_3 at 100 ppm + CPPU at 5.0 ppm treatment exhibited a significant increase compared to other treatments in ‘Cheonghyang’ grapes. GA_3 can enhance berry firmness because larger cells are less susceptible to collapse or loss of turgidity. In addition, CPPU can lead to an increase in cell number and a reduction in ethylene production, both of which are associated with berry softening (Jung et al. 2015). ‘Cheonghyang’ grapes displayed a more pronounced berry firmness response to the combination of GA_3 and CPPU. This synergistic effect on berry firmness is likely to occur at higher concentrations (5.0 ppm), where these PGRs act in concert to strengthen and maintain the integrity of the cell walls in berries, resulting in increased firmness.

This study revealed significant disparities in the antioxidant properties linked to FCT, corroborating the findings of Liu et al. (2021). These differences likely stem from the fact that FCT conditions directly influence crucial quality indicators, such as cluster and berry weights, subsequently affecting antioxidant properties. Moreover, it has been documented that PGRs can influence antioxidant properties in grapes as well (Wang et al. 2023).

Our study confirmed that specific conditions of PGR treatment positively affected the phenolic and flavonoid levels. Two potential explanations have been explored. First, different accumulation patterns within the berries may arise due to the application of different types of PGRs. Research has demonstrated that cytokinins can increase the synthesis of polyphenols in grapes by regulating the expression of the genes involved in their synthesis (Tyagi et al. 2022). Similarly, gibberellin can contribute to the increased expression of genes involved in flavonoid synthesis (Jadhav et al. 2020). These results suggest that a synergistic effect may occur, enhancing the production of both polyphenols and flavonoids when an appropriate concentration of cytokinin and gibberellin is applied together compared to gibberellin alone. Second, variations in total polyphenol and flavonoid contents based on treatment conditions could be influenced by ripening time and skin thickness. The application of GA_3 and CPPU to ‘Cheonghyang’ grapes led to a delay in harvest time compared with other treatments, indicating that these PGRs can extend the ripening period. Delayed ripening allows fruits to remain on the vine for a longer duration, during which the fruit skin, one of the primary sites for phenolic and flavonoid compound accumulation, has more time for the production and accumulation of these compounds (Navarro et al. 2015). Although the study did not investigate how PGR conditions specifically affect changes within skin tissue, it can be assumed that larger berries may have thicker skin than smaller ones. This aligns with a report indicating that skin thickness increases with berry weight, owing to the influence of PGR (Jáuregui-Riquelme et al. 2017). An increase in skin thickness may provide more tissue for the storage of phenolic and flavonoid compounds, resulting in higher concentrations and greater accumulation of these compounds. PGR treatment is crucial for producing commercially valuable triploid grape cultivars. Recently, consumers have placed greater importance on the functional substance content when purchasing grapes. To maximize the effect of PGR treatment on triploid grapes in the future, it will be valuable to understand the specific mechanisms directly related to flavonoid and phenol content. Consumption of fruits rich in antioxidants can offer an overall improve health (Akbari et al. 2022).

In this study, we measured the antioxidant scavenging ability of DPPH, a stable free radical compound commonly used in laboratory experiments, to assess the antioxidant properties of substances (Sihag et al. 2022). A higher DPPG-scavenging percentage indicates that the substance readily donates electrons or hydrogen atoms, showing that they can effectively neutralize radicals and prevent oxidative damage (Gulcin and Alwasel 2023). Consequently, fruits with higher DPPH radical-scavenging abilities are often considered more health-promoting because they contain higher concentrations of these beneficial compounds (Thakur et al. 2023). Interestingly, we discovered a significant correlation between DPPH radical-scavenging ability and the total phenolic and flavonoid contents. Similar findings have been reported for apples (Geleta and Heo 2023), where phenolic and flavonoid compounds are significant contributors to their antioxidant capacities. Furthermore, it is important to note that the ability of a plant to scavenge DPPH radicals is directly linked to the presence and concentration of antioxidants within the plant (Calvo et al. 2022). Antioxidants in plants include a wide range of compounds, including vitamins and tannins, as well as phenolic and flavonoid compounds (Ayub et al. 2023). This observation about DPPH scavenging suggests that PGRs likely influence the accumulation of phenols and flavonoids, as well as other bioactive molecules. Therefore, it is necessary to evaluate how other health-promoting substances present in grapes accumulate and determine their contents in future research.

This experiment underscores the significant impact of controlling the FCT and employing PGRs on the accumulation of mineral components. Mineral content increased notably in groups with larger berry weights and slightly delayed ripening periods. This observation suggests that extended maturation or thicker grape skins associated with larger berries, as previously proposed regarding antioxidant properties, may benefit the ripening process, which involves mineral transformation. Minerals, such as magnesium, phosphorus, potassium, and calcium, play pivotal roles in human health. Magnesium is involved in over 300 enzymatic reactions in the body, supports cardiovascular functions, and regulates blood sugar levels (Franken et al. 2022). Phosphorus is crucial for cellular energy production and various biochemical processes and is essential for bone and tooth formation (Kolodiazhnyi 2021). Potassium is vital for maintaining proper muscle and nerve function and aids in preventing muscle cramps and maintaining cardiovascular health (Zoroddu et al. 2019). Calcium is critical for preventing osteoporosis and other bone-related issues (Chauhan 2022). These findings strongly suggest that the conditions created by PGRs that lead to higher mineral levels have positive implications for overall human health. Additionally, calcium and potassium contents are closely linked to increased berry firmness. The role of potassium in regulating turgor pressure in plant cells can help maintain fruit firmness (da Silva et al. 2023). Calcium affects the structural integrity of plant cell walls and contributes to fruit firmness by regulating the cell turgor pressure (Huang et al. 2023). These results indicate that the observed effect in this study may be attributed to the mixed treatment with CPPU. Improving berry firmness offers several advantages, including reducing the risk of berries cracking during cultivation, minimizing damage during distribution, and extending the shelf-life of grapes. Overall, a combined treatment involving GA_3 at 100 ppm and CPPU at 5.0 ppm with 6 cm FCT is expected to ensure high berry weight, enhance antioxidant properties, and maintain mineral content while keeping 'Cheonghyang.' Grape cluseters marketable.

5. Conclusions

Our study highlights the effectiveness of FCT and PGRs in enhancing cluster weight and enriching bioactive compound contents in 'Cheonghyang' grapes while maintaining fruit marketability. These findings offer crucial insights into optimizing the cultivation practices of 'Cheonghyang' seedless grapes. However, it is essential to recognize that specific outcomes pertaining to fruit quality are influenced by the unique conditions of PGR treatment and FCT. With the increasing cultivation of various seedless grapecultivars in Korea, further research is warranted to identify the optimal treatment conditions for PGRs and FCT to ensure their commercial viability. Therefore, future studies should prioritize maximizing commercial marketability through tailored FCT and PGR treatments across grape cultivars.

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References

AFSHARI-JAFARBIGLOO, H., et al. Cluster and berry characteristics of grapevine (*Vitis vinifera* L.) as influenced by thinning agents and gibberellic acid applications. *International Journal of Horticultural Science and Technology* 2020, **7**, 377-385. https://doi.org/10.22059/ijhst.2020.201718.108.

AHMAD, M. F. and ZARGAR, G. H. Effect of trunk girdling, flower thinning, GA_3 and ethephon application on quality characteristics in grape cv. Perlette under temperate Kashmir valley conditions. *Indian Journal of Horticulture*. 2005, **62**, 285-287.

AKBARI, B., et al. The role of plant derived natural antioxidants in reduction of oxidative stress. *BioFactors.* 2022, **48**, 611-633. https://doi.org/10.1002/biof.1831.

ALI, M. A., et al. Effect of foliar spray of sitofex, moringa leaves extract and some nutrients on productivity and fruit quality of "Thompson seedless" grapevine. *Journal of the Advances in Agricultural Researches*. 2020, **25**, 112-129.

AYUB, H., et al. A comprehensive review on the availability of bioactive compounds, phytochemicals, and antioxidant potential of plum (*Prunus Domestica*). *International Journal of Food Properties*. 2023, **26**, 2388-2406. https://doi.org/10.1080/10942912.2023.2249254.

BIAN, L., et al. Genome-wide characterization of cytokinin response regulator in grape and expression analyses during berry set process. *Russian Journal of Plant Physiology*. 2022, **69**, 46. https://doi.org/10.1134/S1021443722030049.

BRAND-WILLIAMS, W., et al. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*. 1995, **28**, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5.

CALVO, M. M., et al. Antioxidant, antihypertensive, hypoglycaemic and nootropic activity of a polyphenolic extract from the halophyte ice plant (*Mesembryanthemum crystallinum*). *Foods.* 2022, **11**, 1581. https://doi.org/10.3390/foods11111581.

CHAUHAN, R. C. Calcium as a boon or bane for athlete: A review. *Asian Journal of Research in Marketing*. 2022, **11**, 1-8. http://dx.doi.org/10.5958/2277-6621.2022.00003.2

CHOI, S., et al. The impact of plant growth regulators and floral cluster thinning on the fruit quality of 'Shine Muscat' grape. *Horticulturae*. 2023, **9**, 392. https://doi.org/10.3390/horticulturae9030392.

CHUNG, K. H., et al. The History, Current Status and Future Prospects of Fruit Breeding in Korea. *Korean Journal of Breeding Science*. 2020, **52**, 144–160. https://doi.org/10.9787/KJBS.2020.52.S.144.

DA SILVA, A. P. G, et al. Cell wall and oxidative metabolisms of ripening 'Paluma' guava under potassium fertilization. *Journal of Agriculture and Food Research*. 2023, **12**, 100568. https://doi.org/10.1016/j.jafr.2023.100568.

FRANKEN, G. A. C., et al. Structural and functional comparison of magnesium transporters throughout evolution. *Cellular and Molecular Life Sciences*. 2022, **79**, 418. https://doi.org/10.1007/s00018-022-04442-8.

GELETA, B. T. and Heo, J. Y. Variation of fruit characteristics and antioxidant properties among ten apple cultivars grown in two different high-latitude regions of South Korea. *Erwerbs-Obstbau*. 2023, **65**, 2165-2174. https://doi.org/10.1007/s10341-023-00934-4.

GELETA, B. T., et al. Antioxidant activity and mineral content in unripe fruits of 10 apple cultivars growing in the northern part of Korea. *Horticulturae.* 2023, **9**, 114. https://doi.org/10.3390/horticulturae9010114.

GULCIN, I. and ALWASEL, S. H. DPPH radical scavenging assay. *Processes*. 2023, **11**, 2248. https://doi.org/10.3390/pr11082248

HUANG, W., et al. The calcium-mediated homogalacturonan pectin complexation in cell walls contributes the firmness increase in loquat fruit during postharvest storage. *Journal of Advanced Research*. 2023, **49,** 47-62. https://doi.org/10.1016/j.jare.2022.09.009.

JADHAV, U., et al. Gibberellic acid induces unique molecular responses in 'Thompson Seedless' grapes as revealed by non-targeted metabolomics. *Journal of Plant Growth Regulation*. 2020, **40**, 293-304. https://doi.org/10.1007/s00344-020-10102-7

JÁUREGUI-RIQUELME, F., et al. Pre-anthesis CPPU treatment modifies quality and susceptibility to post-harvest berry cracking of *Vitis vinifera* cv. 'Thompson seedless'. *Journal of Plant Growth Regulation*. 2017, **36**, 413-423. https://doi.org/10.1007/s00344-016-9649-3

JUNG, M. H., et al. Characteristics of the fruits and flesh softening delay induced by GA_3 and Thidiazuron (TDZ) treatment in 'Heukboseok' grape. *Horticultural Science and Technology*. 2015, **33**, 186-195. https://doi.org/10.7235/hort.2015.14100

KOK, D. and BAL, E. Seedless berry growth and bioactive compounds of *cv.* 'Recel Uzümü' (*V. Vinifera* L.) as affected by application doses and times of pre-harvest thidiazuron. *Erwerbs-Obstbau*. 2016, **58**, 253-258. https://doi.org/10.1007/s10341-016-0286-6

KOLODIAZHNYI, O. I. Phosphorus compounds of natural origin: prebiotic, stereochemistry, application. *Symmetry*.2021, **13**, 889. https://doi.org/10.3390/sym13050889.

LEE, H. Y., et al. Comparison of quality characteristics of 'Cheonghyang' wine fermented with different commercial yeasts. *Journal of the East Asian Society of Dietary Life. 2016,* **26**, 543-549. https://doi.org/10.17495/easdl.2016.12.26.6.543

LEE, S. Y. and HEO, J. Y. Combined treatment with gibberellic acid and thidiazuron improves fruit quality of 'Red Dream' grape cultivar. *Notulae Scientia Biologicae.* 2023, **15**, 11499. https://doi.org/10.55779/nsb15211499

LIU, M., et al. Effects of flower cluster tip removal on phenolics and antioxidant activity of grape berries and wines. *American Journal of Enology*. 2021, **72**, 298-306. https://doi.org/10.5344/ajev.2021.20072

NAVARRO, J. M., et al. Influence of deficit irrigation timing on the fruit quality of grapefruit (*Citrus paradisi* Mac.). *Food Chemistry*. 2015, **175**, 329-336. https://doi.org/10.1016/j.foodchem.2014.11.152

NISLER, J. 2018. TDZ: mode of action, use and potential in agriculture. Thidiazuron: from urea derivative to plant growth regulator; AHMAD, N., FAISAL, M., Eds.; Springer: Singapore 37-59. https://doi.org/10.1007/978-981-10-8004-3_2

PUJARI, C. V., et al. Improving fruit set in custard apple (*Annona squamosa* L) by using growth regulators. *International Journal* of *Current Microbiology*. 2021, **10**, 237-242. and *Applied Sciences*. https://doi.org/10.20546/ijcmas.2021.1002.029

REMBERG, S. F., et al. Influence of postflowering temperature on fruit size and chemical composition of Glen Ample raspberry (*Rubus idaeus* L.). *Journal of Agricultural* and *Food Chemistry. 2010,* **58**, 9120-9128. https://doi.org/10.1021/jf101736q

SHIN, H. W., et al. Effects of plant growth regulators and floral cluster thinning on fruit quality of 'Shine Muscat' grape. *Horticultural Science and Technology*. 2019, **37**, 678-686. https://doi.org/10.7235/HORT.20190068

SIHAG, S., et al. Antioxidant properties and free radicals scavenging activities of pomegranate (*Punica granat*um L.) peels: An in-vitro study. *Biocatalysis and Agricultural Biotechnology*. 2022, **42**, 102368. https://doi.org/10.1016/j.bcab.2022.102368

THAKUR, M., et al. Nutritional and health-promoting effects of lichens used in food applications. *Current Nutrition Reports*. 2023, **12**, 555–566. https://doi.org/10.1007/s13668-023-00489-6

TYAGI, K., et al. Effects of gibberellin and cytokinin on phenolic and volatile composition of Sangiovese grapes. *Scientia Horticulturae*. 2022, **295**, 110860. https://doi.org/10.1016/j.scienta.2021.110860

WANG, L., et al. Regulation of anthocyanin and sugar accumulation in grape berry through carbon limitation and exogenous ABA application. *Food Research International*. 2022, **160**, 111478. https://doi.org/10.1016/j.foodres.2022.111478

WANG, L., et al. Advances of anthocyanin synthesis regulated by plant growth regulators in fruit trees. *Scientia Horticulturae*. 2023, **307**, 111476. https://doi.org/10.1016/j.scienta.2022.111476

ZORODDU, M. A., et al. The essential metals for humans: A brief overview. *Journal* of *Inorganic Biochemistry.* 2019, **195**, 120-129. https://doi.org/10.1016/j.jinorgbio.2019.03.013