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### EFFECT OF GIBBERELLIN ACID TREATMENT CONCENTRATION AND TIMING ON THE FRUIT CHARACTERISTICS OF TRIPLOID GRAPE CULTIVAR, 'CHEONGYANG'

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#### Abstract

This study was designed to examine the effects of both the concentration and treatment time of gibberellin acid (GA<sub>3</sub>) on fruit quality in 'Cheongyang', a triploid grape. The first set of experiments were used to determine the optimal GA<sub>3</sub> concentration (0 (water), 25, 50, and 100 ppm) and were completed when the flowers reached full bloom, with the final fruit characteristics evaluated following their harvest. These evaluations revealed that both fruit setting and enlargement increased with increasing GA<sub>3</sub> concentration without any adverse side effects, revealing that GA<sub>3</sub> at 100 ppm was the most effective concentration for these grapes. The effect of the time of GA<sub>3</sub> treatment on fruit characteristics was evaluated by examining the major fruit indicators expressed when plants were treated with 100 ppm GA<sub>3</sub> 5 days before full bloom, at full bloom, and 5 days after full bloom. This confirmed that GA<sub>3</sub> treatment at full bloom led to the largest fruit being produced with the least deterioration in fruit quality. Thus, our data suggests that applying GA<sub>3</sub> at 100 ppm to flowers in full bloom will produce the most attractive seedless fruits in the triploid 'Cheongyang' grape cultivar.

**Cuvinte cheie:** calitate fructe, regulator de creștere a plantelor, fără semințe, *Vitis*.

**Key words:** fruit quality, plant growth regulators, seedless grapes, *Vitis*.

#### 1. Introduction

The two most important consumer traits for grapes in the Korean market are high fruit quality and seedlessness (Kim et al., 2021). These seedless grapes are produced using artificial manipulation of a small number of seeded grape cultivars including 'Shine Muscat' and 'Kyoho', which are highly responsive to plant growth regulators (Heo and Park, 2017). Producing seedless fruits from these cultivars, which are genetically programmed to produce seeds, requires treatment with specific plant growth regulators before and after the plants reach full bloom. This makes the production of these grapes difficult and reduces the ability of producers to standardize fruit quality while increasing labor demands. Thus, there is a significant demand in this market for a simpler, more reliable method of production. One of the most successful strategies for this has been the adaptation of aneuploid and triploid grape cultivars (Heo et al., 2007; Park et al., 2016; Kim et al., 2020). These grape cultivars can produce completely seedless fruits following a single treatment with a specific plant growth regulator because they have extremely low fertility (Park et al., 2015a; Heo and Park, 2017).

'Cheongyang' is a green triploid grape cultivar produced following a cross between Red Pearl and Muscat Bailey A grapes in 2009. It has excellent aroma and quality and is used as both an edible grape and in wine production. 'Cheongyang' has recently begun to be recognized for its unique fruit quality, and its cultivation area has been expanded into the northern regions of Korea. It is known that triploid grape cultivars can be easily manipulated to produce seedless fruits using only a single gibberellin acid (GA<sub>3</sub>) treatment. However, the characteristics of the fruit produced following GA<sub>3</sub> treatment can vary depending on its treatment time and concentration, which need to be optimized for this specific cultivar. Given that at the time of our submission there were no relevant studies optimizing these conditions in these grapes, we designed this study to determine both the optimal treatment concentration and the optimal treatment time to produce the best quality of seedless fruits from the triploid 'Cheongyang' grape.

## 2. Material and methods

This study was carried out sequentially over two years using 'Cheongyang' trees planted in a commercial vineyard in Hongcheon, Korea. The first set of experiments, conducted in year one, were designed to determine the optimal GA<sub>3</sub> concentration for inducing the production of seedless fruits from the 'Cheongyang' grape cultivar. This was completed by dipping the flowers in 0, 25, 50, and 100 ppm GA<sub>3</sub> at the full bloom stage, and then evaluating specific fruit characteristics following their harvest. In the second experiment, we used GA<sub>3</sub> at 100 ppm, as identified in the previous experiment, and evaluated its optimal time of treatment, treating 'Cheongyang' flowers at 5 days before full bloom, full bloom, and 5 days after full bloom before evaluating the relevant fruit characteristics and comparing the outcomes. We evaluated the fruit characteristics from each set of harvested fruit when the peel color and fruit powder were well developed. We then compared the weight and length of the clusters and berries from each group as well as the total soluble solids (TSS) and titratable acidity (TA) from their juice extracts. The weight of each cluster was investigated by randomly harvesting 5 fruits from three trees and then weighing them, while the weight of the individual berries was calculated by dividing the weight of the total cluster by the number of berries in each cluster using a digital scale. TSS evaluations were completed using the juice extracted from 10 berries from each cluster and then measured using a digital refractometer. TA was measured using an automatic titrator following the addition of 15 mL of distilled water to 5 mL of the subject fruit juice and facilitated our evaluation of the sugar content in each cluster (Heo et al., 2015). After titration with 0.1N NaOH to pH 8.2, these values were expressed as the conversion of sugar to tartaric acid.

Statistical comparison of the fruit characteristics from each group were completed at the 95% confidence interval using an analysis of variance (ANOVA) and Duncan's multiple range test. These evaluations were completed in SPSS (Version 28, IBM, USA).

## 3. Results and discussions

Our evaluation of the differences in the fruit characteristics of grapes produced in response to different concentrations of GA<sub>3</sub> are summarized in Table 1. We confirmed that 'Cheongyang' grapes produce seedless fruits with or without GA<sub>3</sub> treatment, but GA<sub>3</sub> concentration had a significant effect on the number of seedless berries and the weight of each cluster and each berry with increasing GA<sub>3</sub> concentration producing larger fruit. There were only 7.4 berries per cluster in the control, while there were 82.3, 90.2, and 95.6 berries per cluster in the plants treated with GA<sub>3</sub> at 25 ppm, 50 ppm, and 100 ppm, respectively. The mean weight of each cluster from the untreated control was fairly low at 13.2 g, but the weight of these clusters significantly increased in response to GA<sub>3</sub>, ranging from a minimum of 210.6 g to a maximum of 273.3 g. Similarly, we confirmed that there was a significant degree of hypertrophy in the GA<sub>3</sub>-treated group when compared with that in the untreated group, which was consistent with the increases in the weight of both the clusters and berries observed in these experiments. TSS was shown to be 20.7, 22.6, and 20.8 °Bx in GA<sub>3</sub> 25, 50, and 100 ppm treatment groups, respectively, which was significantly higher than that of the control (15.7 °Bx). Conversely, TA decreased with increasing GA<sub>3</sub> concentration. Thus, we concluded that the concentration of GA<sub>3</sub> contributes to the improved fruit quality observed in these plants. Comparisons of these parameters suggest that 50 ppm GA<sub>3</sub> resulted in the best fruit quality but 100 ppm GA<sub>3</sub> was required to maximize fruit enlargement. Although the quality of the fruit produced following treatment with 100 ppm GA<sub>3</sub> was slightly lower than following 50 ppm GA<sub>3</sub> treatment, it should be noted the TSS value for these plants still greatly exceeds 18 °Bx, which is the standard for high-quality grapes in Korea. Recently, the importance of larger berries in the Korean grape market has been emphasized (Park et al., 2020), thus, we suggest that treatment with 100 ppm GA<sub>3</sub> should be adopted as the standard as it allows for the best balance of quality and size.

Table 2 summarizes the effects of treatment time on fruit quality. These evaluations revealed that the number of berries changed from 60.2, to 63.3, and then 61.3 when treated with 100 ppm GA<sub>3</sub> at 5 days before full bloom, full bloom, and 5 days after full bloom, respectively, suggesting that there was no significant difference in this parameter under these conditions. However, evaluations of cluster weight revealed that treatment timing had a significant effect on this parameter with the cluster weight changing from 218.0, to 260.2, and then 247.5 g, respectively. Similarly, the lowest berry weights were observed in the 5 days before the full bloom group (3.5 g), and the highest weights (4.1 g) were recorded for the full bloom group. Evaluations of the TSS values for these groups also revealed no significant differences with each group producing a value of 19.2, 19.0, and 18.7 °Bx, respectively. In contrast, TA was significantly different moving from 0.56%, to 0.61%, and then 0.67%, in each of these groups. This data suggests that treatment at 5 days before full bloom or at full bloom was better than treatment at 5 days after full bloom. However, the most significant fruit enlargement was observed in the group treated with GA<sub>3</sub> at full bloom,

suggesting that GA<sub>3</sub> treatment at full bloom is likely to be most effective when producing seedless fruit from 'Cheongyang' grapes.

The factors determining the marketability of grapes include the appearance of the berry setting, cluster weight, and fruit quality. As grapes have dozens of berries in each cluster, berry setting and size affect both shape and productivity. We confirmed that both the number of berries and their weight per cluster significantly increased in response to GA<sub>3</sub> treatment, which agreed with a previous report describing this treatment protocol in a different triploid grape cultivar (Park et al., 2015b). However, the number of berries per setting and their enlargement was more limited when the plants were treated with lower concentrations of GA<sub>3</sub>. It should be noted that the growth response of each grape to GA<sub>3</sub> is closely associated with their genetics (Heo and Park, 2016), and thus we suggest that the reduction in these effects at 25 and 50 ppm GA<sub>3</sub> was likely a result of incomplete activation of the relevant pathways in these grapes. This was further supported by the fact that we observed the maximum berry setting and hypertrophy in the 100 ppm GA<sub>3</sub> treatment group. In addition, these groups presented without any unwanted side effects. These results suggest that this concentration induces optimal cell division and proliferation in 'Cheongyang' plants. In addition, we confirmed that the effects of the timing of GA<sub>3</sub> treatment on fruit enlargement were most obvious when treatment coincided with the full bloom stage of the growth cycle. Previous data has established that treatment timing is critical for GA<sub>3</sub> when it is being used for the production of seedless fruits as differences in its timing can greatly affect fruit development. It is widely agreed that it is more efficient to apply GA<sub>3</sub> after fruit setting because cell expansion is greatly promoted. This is exemplified by the fact that in seeded grapes the vines are treated with GA<sub>3</sub> two weeks before full bloom, to induce fruit setting without fertilization (parthenocarpy) via its inhibition of the pollen tube, and then treated again for fruit enlargement two weeks after full bloom. However, unlike seeded grapes, triploid grapes can proceed through their normal germination cycle and still produce seedless progeny. However, it remains important to treat these plants at the most effective time to facilitate both fruit setting and enlargement in order to produce commercially viable seedless fruits using only one GA<sub>3</sub> treatment. Thus, we propose treating these grapes at full bloom as this provides the most beneficial effect in 'Cheongyang' grapes. However, explaining the exact reason for this remains difficult and the underlying mechanisms regulating this process require additional evaluation.

Taste is also an important factor in fruit competitiveness and is mainly determined by the content of TSS and TA in each berry (Piazzolla et al., 2016). TSS and TA generally present with a contradictory trend; if TSS content is increased, TA content is generally decreased. This was also true for our data. Traditionally, high TSS content is known to help make fruits more desirable (Crisosto and Crisosto, 2005; Tian et al., 2020) and as such is often considered the most important factor in fruit evaluation. Anjum et al. (2019) and Kaplan et al. (2017) reported that TSS content changed in response to changes in the concentration and timing of GA<sub>3</sub> treatment, but the response of specific grape cultivars to GA<sub>3</sub> treatment can be very different. Here, we revealed that TSS increased with GA<sub>3</sub> treatment and was greatest at 50 ppm GA<sub>3</sub>. However, when the treatment time was changed, there was no significant difference in TSS. Thus, we suggest that this key indicator of fruit quality was more affected by the treatment concentration than the treatment time in this cultivar. This suggested that GA<sub>3</sub> concentration is more important for the regulation of sugar accumulation in Cheongyang fruit than timing. We also propose that the differences between the 50 ppm and 100 ppm GA<sub>3</sub> treatment groups were also likely the result in differences in the osmotic force needed for the increased cellular expansion associated with the increased GA<sub>3</sub> concentration and related changes in the expression of those genes necessary for sugar accumulation in these grapes. However, given the nature of this study it is necessary to investigate these mechanisms through additional studies in the future. Omics based network evaluations using samples in which the phenotypic differences are significant have successfully revealed some of the underlying mechanisms regulating specific phenotypic differences (Zhang et al., 2018) and additional studies using samples from conditions that induced differences in berry size and quality are expected to play a major role in elucidating the genetic factors controlling the phenotype of these plants, and may reveal several new factors for consideration in the breeding and cultivation in grapes.

#### 4. Conclusions

Our study shows that you can easily produce seedless fruits from triploid 'Cheongyang' grapes via the single application of GA<sub>3</sub> but berry enlargement and quality may differ between concentrations and treatment times for these plants. Improvements in both berry size and characteristics remains one of the major focus areas in grape cultivar production and further evaluation of the underlying factors determining these characteristics should help to expand this field.

However, given the fact that there are now several triploid grape cultivars available for cultivation in Korea, our determination of the optimal concentration and treatment timing to produce good quality, seedless fruits may help to advance the adoption of these other strains as well.

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## Tables

**Table 1. Effect of GA3 concentration on the fruit characteristics of 'Cheonghyang' grapes**

GA3 Concentration (ppm)	Cluster weight (g) <sup>z</sup>	Berry setting (No)	Berry weight (g)	Total soluble solids (Bx)	Titrateable acidity (%)	Seedlessness (%)
0	13.3d	7.4c	1.8b	15.7c	0.8a	100a
25	210.6c	82.3b	2.6ab	20.7b	0.5b	100a
50	256.7b	90.2ab	2.8a	22.6a	0.5b	100a
100	272.3a	95.6a	2.8a	20.8b	0.4c	100a

<sup>z</sup>Means followed by the same letter are not significantly different at p = 0.05.

**Table 2. Effect of GA3 treatment time on the fruit characteristics of 'Cheongyang' grapes**

Concentration (ppm)	Cluster weight (g) <sup>z</sup>	Berry setting (No)	Berry weight (g)	Total soluble solids (Bx)	Titrateable acidity (%)	Seedlessness (%)
5 days before full bloom	218.0c	60.2a	3.6b	19.5a	0.56b	100a
Full bloom	260.2a	63.3a	4.1a	19.0a	0.61ab	100a
5 days after full bloom	247.5b	61.3a	4.0a	18.7a	0.67a	100a

<sup>z</sup>Means followed by the same letter are not significantly different at p = 0.05.