Combined treatment with gibberellic acid and thidiazuron improves fruit quality of ‘Red Dream’ grape cultivar

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Abstract

This study was conducted to investigate the effect of treatment conditions of plant growth regulators on the marketability of ‘Red Dream’, a triploid grape. In order to achieve the purpose of this study, gibberellic acid 3 (GA$_3$) 100 ppm single use treatment group was set as a control group. In addition, the fruit characteristics expressed when cytokinins such as thidiazuron (TDZ) or forchlorfenuron (CPPU) 2.5 ppm or 5.0 ppm mixed with GA$_3$ 100 ppm were compared with the control group. Cluster weight was commonly higher in the mixed treatment of TDZ or CPPU than in the GA$_3$ single use treatment, and it was found that the cytokinin mixed treatment could effectively induce the enlargement of ‘Red Dream’ berries. Although the harvesting period was slightly delayed compared to GA$_3$ single use treatment in the case of mixed treatment of GA$_3$ and TDZ or CPPU, there was no inhibition of coloration, soluble solid sugar content and functionality, which are important indicators of marketability. In particular, in the GA$_3$ 100 ppm + TDZ 5 ppm treatment group, where the enlargement of fruits was the largest, the occurrence rate of non-commercial berry was also found to be the lowest. Therefore, it is expected to contribute to strengthening the marketability of ‘Red Dream’ if such plant growth regulator treatment condition is applied during cultivation.

Keywords: antioxidant activity; fruit characteristics; plant growth regulator; triploid grape

Introduction

The cultivation area of grapes surveyed in Korea was 14,655 ha in 2022, which ranks 4th among all fruit crops in terms of cultivation area (Korean Statistical Agency, 2022). Hence, grapes are regarded as a very important crop in Korea (Kim et al., 2021a), but the present grape cultivation area in Korea is only 80% of what it was in the past 10 years. The competitiveness of the Korean grape industry has also been weakened. Recently, the possibility of strengthening the competitiveness of the grape industry is expected with the introduction of ‘Shine Muscat’ in Korea. ‘Shine Muscat’ is a hybrid grape cultivar with a unique muscat scent and high quality. It has the advantage of being able to produce large seedless berries when treated with a plant growth regulator (PGR) twice before and after flowering (Park et al., 2020). This has satisfied the needs of Korean consumers who value high quality and convenience, and as a result, the cultivation area of ‘Shine Muscat’ is also rapidly increasing.
The problem is that many farms are selling ‘Shine Muscat’ fruits that have not reached the optimum harvest period in order to secure price competitiveness as the competition among farms intensifies due to the increase in the cultivation area of ‘Shine Muscat’. Apart from this fact, the changing pattern of grape cultivation in Korea, such as the concentration of ‘Shine Muscat,’ can lead to a result that does not meet the characteristics of the consumer market that emphasizes diversity. If these problems intensify, the competitiveness of the Korean grape industry is highly likely to weaken again. Therefore, the introduction of various grape cultivars that can meet the needs of consumers is required in order to establish a sustainable grape industry system in Korea.

Recently, many efforts have been made to breed new grape cultivars in Korea, and various grape cultivars are being introduced to farmers (Park et al., 2016; Heo and Park, 2017; Roh et al., 2018; Kim et al., 2020; Park et al., 2022). Among them, ‘Red Dream’, which is a triploid grape registered in 2016, has the advantage of being able to produce large seedless fruits with just one-time treatment of PGR at the full bloom stage (Park et al., 2022). In addition, ‘Red Dream’ has high fruit quality and red skin that differentiates it from other cultivars, so it is expected to attract consumers’ attention. Currently, the production of seedless fruits of ‘Red Dream’ is carried out through gibberellic acid 3 (GA$_3$) at a concentration of 100 ppm. However, there is a disadvantage that the occurrence rate of non-commercial berries is too high due to berry softness and cracking, which limits the expansion of the cultivation area of ‘Red Dream’. In some crop species where a similar problem occurred to ‘Red Dream’, it was solved through a mixed treatment of GA$_3$ and cytokinin-based hormones (Joshi et al., 2018; Elmenofy et al., 2021). ‘Red Dream’ requires the treatment of PGR to produce fruits with commercial value in any case, and it can be most effectively used in the cultivation field if an appropriate PGR condition that can suppress the occurrence of non-commercial berries can be devised.

The effect of PGR on fruit characteristics in fruit crops differs not only depending on the cultivar and type of mixed hormone but also on the treatment concentration (Bons and Kaur, 2020). Therefore, sufficient prior research is needed to examine whether treatment of PGR can solve the problems occurring in ‘Red Dream’ and to find out the optimum treatment condition if it is valuable. In this study, we examined the fruit characteristics expressed in ‘Red Dream’ at harvest time after mixed treatment with different concentrations of Thidiazuron (TDZ) and Forchlorfenuron (CPPU) in GA$_3$. From this, we aimed to evaluate the effectiveness of the mixed PGR treatment and obtain information on a practically useful PGR treatment condition for ‘Red Dream’.

**Materials and Methods**

This study was conducted using 8 ten-year-old ‘Red Dream’ trees planted 3 meters apart in rows and vines at the vineyard of Gangwondo Agricultural Research and Extension Services located in Chuncheon, Korea (37°94’73.80N, 127°75’44.30E) in 2022. For this study, GA$_3$ (Yooil Company, Korea), TDZ (Daeyu Company, Korea), and CPPU (UPI, Korea) were used. The control group was treated with a single use of GA$_3$ at 100 ppm. TDZ or CPPU mixed with GA$_3$ at 2.5 ppm or 5.0 ppm was also applied to evaluate the effect of mixed PGR treatment. Each PGR condition was applied to 2 inflorescences per tree (N=16) when they reached the full bloom stage. When the optimum harvest period was reached for each treatment cluster, they were harvested in the test field and immediately transported to Gangneung-Wonju National University located in Gangneung, Gangwon-do of Korea.

The effect of PGR treatment conditions on fruit characteristics in ‘Red Dream’ was evaluated by measuring and comparing the number of berries, weight of cluster and berry, berry firmness, occurrence rate of non-commercial berries, skin color, soluble solid sugar content, titratable acidity, and functionality, which are regarded as important factors in determining the marketability of grapes (Heo and Park, 2016). Cluster weight was measured using an electronic scale (FY-3000, A&D, Japan), and berry weight was calculated by dividing the cluster weight by the number of berries per cluster. Berry firmness was measured with a fruit hardness tester.
(FHT-05, Landtek, China) after thinly removing the skin from the top of the berry and expressed in Newtons (N). The occurrence rate of non-commercial berries was investigated by calculating the ratio with the total number of berries after counting the number of cracked and softened berries for each cluster. For measurement of skin color, hunter values were measured with a colorimeter (CR-400, Konica minolta, Japan), and then L (lightness) and Hue angles, which are important in red grapes, were presented. For measurement of total soluble solid content (TSS), we extracted juice from randomly selected 10 berries per each cluster. Afterwards, it was measured with a digital refractometer (Pal-1, Atago, Japan) and expressed in Brix. Titratable acidity (TA) was measured by diluting the fruit juice used for sugar content measurement by 39 times in distilled water and then measured with a digital titratable acidity meter (Acid-2, Atago, Japan) and expressed as %.

The effect of PGR treatment on functionality was evaluated by measuring and comparing the total phenol content, total flavonoid content, and ferric reducing antioxidant power (FRAP). For this experiment, 40 g of grape skin were randomly sampled with three replications for each treatment group and dried in a hot air dryer at 60 °C for 10 days. The dried samples were ground using a mortar and pestle to a fine powder. From the fine powder, 0.1 g was homogenized in 7 mL of 100% methanol for each sample. Afterward, the homogenized solution was kept in a water bath (C-WBA1, Changshin Science, Korea) at 60 °C for 30 minutes. The extracted powder solution was centrifuged at 4 °C and 4500 rpm for 20 minutes (MEGA 17R, Hanil, Korea), and the supernatant was collected. The extraction was done twice, and the supernatants were combined. To measure the total phenolic content, the Folin-Ciocalteu method was used according to Geleta et al. (2023). Extracted samples of 25 µL (1 mg/mL) and 125 µL Folin-Ciocalteu reagent were mixed and then left to stand for three minutes. Afterward, 37.5 µL sodium bicarbonate (20%), and 62.5 µL distilled water were added and kept in the dark at room temperature for one hour. The absorbance of the sample was read at 765 nm using a Microreader spectrophotometer (Mobi, MicroDigital, Korea). Gallic acid (0-100 µg) was used as the standard, and the result was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW).

For the measurement of total flavonoids, the aluminum chloride colorimetric method was applied with the method of Chang et al. (2002) with slight modification. Twenty-five microliters (1 mg/mL) of the extracted samples were mixed with 75 µL methanol, 5 µL aluminum chloride (10%), and 5 µL sodium acetate (1 M). They were then left to stand for 30 minutes in the dark at room temperature, and the absorbance of the sample was read at 415 nm. Quercetin (0-250 µg) was used as the standard to construct the calibration curve. The result was expressed as mg quercetin per gram dry weight (mg QE/g DW). The ferric reducing antioxidant power (FRAP) assay was determined as described by Benzie and Strain (1996). FRAP reagent solution containing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (diluted in 40 mM HCl), and 20 mM FeCl₃, was prepared. Three milliliters of FRAP reagent solution were mixed with 50 µL of the extracted sample. The mixture of samples with FRAP reagent was incubated at 37 °C for 4 minutes, and the absorbance of the sample was read at 593 nm at the Microreader spectrophotometer. A calibration curve was constructed using different concentrations of trolox (0–100 µg), and the result was expressed as µM trolox/g DW.

Statistical comparison of fruit characteristics according to PGR treatment condition was performed using the analysis of variance and Duncan’s multiple range tests in the SPSS program (Version 28, IBM, USA).

Results and Discussion

Table 1 shows the results of cluster weight, berry number, berry weight, berry firmness, and incidence of non-commercial berries of ‘Red Dream’ according to the PGR treatment conditions. The average berry weight of ‘Red Dream’ ranged from 9.1 to 10.5 g, and the number of berries was observed between 44.1 and 49.2. The weight and number of berries were generally high in the GA₃ and CPPU or TDZ mixed treatment group, but no statistical significance was found. When the GA₃ and CPPU or TDZ were applied together, excessive berry set was observed in another grape cultivar (Kim et al., 2021b). The GA₃ and CPPU both promote fruit development and can enhance berry set, which is the process by which flowers are converted into berries. The
TDZ, on the other hand, can promote cell division and differentiation in berries, leading to the formation of more berries. When these types of PGRs are used in a mixed application, they can increase the rate of berry set and development, resulting in more fruit than usual. However, this can also lead to overcrowding and reduced fruit quality as the vine may not be able to support the growth of so many berries. This can also increase the risk of fungal diseases as the dense foliage and fruit can create a humid microclimate that is conducive to fungal growth. However, it was confirmed that such a problem did not occur even though the GA$_3$ and CPPU or TDZ combined treatment with a relatively high concentration was applied in ‘Red Dream’.

**Table 1. Effects of plant growth regulator treatment on fruit characteristics of ‘Red Dream’**

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Cluster weight (g)</th>
<th>Berry number</th>
<th>Berry weight (g)</th>
<th>Berry firmness (N)</th>
<th>Incidence of non-commercial berries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA$_3$ 100 ppm</td>
<td>418.15±27.64ab</td>
<td>44.12±3.49a</td>
<td>9.68±0.56a</td>
<td>6.28±0.14b</td>
<td>19.73±2.72a</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + CPPU 2.5 ppm</td>
<td>447.77±25.90ab</td>
<td>49.25±1.22a</td>
<td>9.12±0.54a</td>
<td>6.38±0.17ab</td>
<td>19.89±3.88a</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + CPPU 5.0 ppm</td>
<td>458.07±36.73ab</td>
<td>46.62±3.17a</td>
<td>10.02±0.30a</td>
<td>6.34±0.15b</td>
<td>15.97±3.48ab</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + TDZ 2.5 ppm</td>
<td>456.33±21.24ab</td>
<td>46.25±2.19a</td>
<td>9.95±0.46a</td>
<td>6.49±0.13b</td>
<td>14.28±3.09ab</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + TDZ 5.0 ppm</td>
<td>494.71±29.21a</td>
<td>48.00±4.34a</td>
<td>10.54±0.45a</td>
<td>6.55±0.11a</td>
<td>12.32±2.53b</td>
</tr>
</tbody>
</table>

Different letters indicate the significant differences between the mean at p < 0.05. The same letters denote no significant difference at p < 0.05. ± indicates standard error.

Mean cluster weight ranged from 418.1 to 494.7 g. It was found that cluster weight significantly increased in the GA$_3$ and CPPU or TDZ mixed treatment group compared to the GA$_3$ single use treatment group, which was presumed to be a result of the slight increase in the number and weight of berries. Similar to cluster weight, berry firmness was higher in the GA$_3$ and CPPU or TDZ combination treatment groups than in the GA$_3$ single use treatment, regardless of the type and concentration. In particular, the highest cluster weight and berry firmness were observed in the GA$_3$ 100 ppm + TDZ 5.0 ppm treatment group. It has been reported that the CPPU induces enhanced cell durability and redirects the plant’s energy and resources toward the development of berries, while the TDZ promotes cell division and elongation by inducing cell proliferation (Nisler, 2018; Rojas et al., 2021). Accordingly, larger berry firmness or size is frequently induced in grapes, as in the case of ‘Red Dream’ when the GA$_3$ and CPPU or TDZ are treated together (El-Abbasy et al., 2015). However, the GA$_3$ and CPPU combined treatment was more effective than GA$_3$ and TDZ mixed treatment for increasing berry weight and firmness in ‘Shine Muscat’, unlike our results (Wang et al., 2019). These results indicate that the activity of mixed treatment with the GA$_3$ and CPPU or TDZ can differ depending on the grape cultivar. It is assumed that the effect on berry weight and firmness was more effective in the high-concentration the GA$_3$ and TDZ mixed treatment group because the activity of TDZ in berry cells of ‘Red Dream’ could be higher than that of CPPU.

The incidence rate of non-commercial berries was generally reduced in the GA$_3$ and CPPU or TDZ mixed treatments compared to the GA$_3$ single use treatment group (Table 1). The lowest occurrence rate of non-commercial berries was observed in the GA$_3$ 100 ppm + TDZ 5.0 ppm treatment group, which had the highest berry firmness by reducing berry softening and cracking. Retamales et al. (1994) and Stern et al. (2013) also found that berry firmness was increased due to intracellular structural changes that increased the thickness of the cell wall, which led to a decrease in the incidence of non-commercial berries when GA$_3$ and cytokinin-based PGRs were treated together. These results suggest that the combined treatment with GA$_3$ and TDZ in ‘Red Dream’ inhibited the occurrence of softening and cracking berries by inducing an increase in berry
firmness. The use of PGRs like GA₃, CPPU, and TDZ can promote cell division and expansion, leading to larger and firmer berries (Wang et al., 2020; Rojas et al., 2021). The combination of these PGRs can have a synergistic effect on increasing berry size and firmness, thereby reducing the incidence of non-commercial berries. Additionally, firmer berries may also have a longer shelf life and better transportation properties, which can improve the overall quality of the grape crop. Therefore, it seems necessary to obtain information on what kind of changes PGR treatment induces in the cell structure of ‘Red Dream’ berries in the future. The effect of PGR treatment on the coloration of ‘Red Dream’ is shown in Table 2. In this experiment, it was confirmed that the L and Hue angle values, which are important in red grape cultivars, did not show statistical significance in all PGR treatment groups. However, it was observed that the harvest period was delayed by about 3 to 7 days as the coloring was delayed in the GA₃ and CPPU or TDZ mixed treatment group in this experiment.

It is presumed that more time is required for natural coloring with the mixed application of the GA₃ and CPPU or TDZ in ‘Red Dream’. Although they can be effective when used together in a mixed application, they can delay the harvest time of grapes. When used together, these compounds can result in larger fruit size than usual but still immature in terms of sugar content and flavor development. This is because the growth rate of the fruit is increased, but the maturation and ripening process is not necessarily accelerated. Additionally, the CPPU and TDZ can both delay the ripening process of grapes by suppressing the production of ethylene, which is a hormone that plays a crucial role in fruit ripening (Carvajal-Millan et al., 2001; Jung et al., 2015). In ‘Flame seedless’, coloration was greatly inhibited when cytokinin-type hormones were treated, which not only delayed the harvest period but also interfered with the production of marketable berries (Peppi and Fidelibus, 2008). In ‘Red Dream’, the GA₃ and CPPU or TDZ mixed treatment only caused a delay in the harvest time, but there was no difference in the final coloration. Hence, it was estimated that the GA₃ and CPPU or TDZ mixed treatment had no negative effect on the coloration of ‘Red Dream’.

### Table 2. Effects of plant growth regulator treatment on coloration and harvest time of ‘Red Dream’

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>L</th>
<th>Hue angle</th>
<th>Harvest time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GA₃ 100 ppm</strong></td>
<td>34.24±0.35a</td>
<td>41.29±3.55a</td>
<td>September 19</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + CPPU 2.5 ppm</strong></td>
<td>34.71±0.34a</td>
<td>42.68±3.54a</td>
<td>September 22</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + CPPU 5.0 ppm</strong></td>
<td>34.47±0.44a</td>
<td>41.76±3.45a</td>
<td>September 22</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + TDZ 2.5 ppm</strong></td>
<td>35.37±0.43a</td>
<td>42.85±1.08a</td>
<td>September 22</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + TDZ 5.0 ppm</strong></td>
<td>34.88±0.48a</td>
<td>42.34±2.69a</td>
<td>September 26</td>
</tr>
</tbody>
</table>

The same letters denote no significant difference at p < 0.05. ± indicates standard error.

### Table 3. Effects of plant growth regulator treatment on total soluble solid content and titratable acidity of ‘Red Dream’

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Total soluble solid content (TSS)</th>
<th>Titratable acidity (TA)</th>
<th>TSS/TA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GA₃ 100 ppm</strong></td>
<td>17.55±0.23ab</td>
<td>0.44±0.01b</td>
<td>40.16±0.60a</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + CPPU 2.5 ppm</strong></td>
<td>17.07±0.19b</td>
<td>0.43±0.00b</td>
<td>40.18±0.38a</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + CPPU 5.0 ppm</strong></td>
<td>18.65±0.35a</td>
<td>0.48±0.01a</td>
<td>39.16±0.95a</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + TDZ 2.5 ppm</strong></td>
<td>16.77±0.17b</td>
<td>0.42±0.01b</td>
<td>39.84±0.86a</td>
</tr>
<tr>
<td><strong>GA₃ 100 ppm + TDZ 5.0 ppm</strong></td>
<td>17.87±0.28ab</td>
<td>0.44±0.00b</td>
<td>40.62±0.54a</td>
</tr>
</tbody>
</table>

Different letters indicate the significant differences between the mean at p < 0.05. ± indicates standard error.
Table 3 shows the effect of the GA$_3$ and CPPU or TDZ mixed treatment on TSS and TA. TA and TSS ranged from 0.42 to 0.48 °Brix and 16.8 to 18.7 %, respectively. TA was found to be significantly higher only in the GA$_3$ 100 ppm + CPPU 5.0 ppm treatment group compared to other treatments. There was a significant difference in TSS depending on the treatment group. The GA$_3$ 100 ppm + CPPU 2.5 ppm and the GA$_3$ 100 ppm + TDZ 2.5 ppm showed significantly lower values in TSS, but other treatment groups had higher values than the GA$_3$ single use treatment group. Similarly, it has been reported that TSS is affected by PGR treatment combination and condition (Shin et al., 2019; Wang et al., 2020; Tyagi et al., 2021). This is presumed to be because sugar uptake and transport in grape berries based on treatment conditions could be regulated differently. However, it should be noted that the TSS/TA ratio has a decisive effect on consumer preference for grapes (Park et al., 2015). Grapes with a high TSS/TA ratio are considered sweeter and more flavorful, while grapes with a low ratio may be tart or bland. The TSS/TA ratio can also affect the storage and shelf life of grapes (Shahkoomahally et al., 2021). Grapes with a high TSS/TA ratio tend to have a longer shelf life, as the high sugar content can help preserve the fruit and prevent spoilage. On the other hand, grapes with a low TSS/TA ratio may spoil more quickly, as the low sugar content can provide a favorable environment for microorganisms. Interestingly, the TSS/TA ratio ranged from 39.2 to 40.6, and there was no statistical significance in all treatment groups. Hence, it was evaluated that the PGR treatment conditions used for this study would not have a significant difference in the berry quality perceived by consumers.

Table 4. Effects of plant growth regulator treatment on functionality of ‘Red Dream’

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Total phenol content (mg GAE/g dry weight)</th>
<th>Total flavonoid content (mg QE/g dry weight)</th>
<th>Ferric reducing antioxidant power (µM trolox/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA$_3$ 100 ppm</td>
<td>5.64±0.17b</td>
<td>3.88±0.07bc</td>
<td>154.55±4.31b</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + CPPU 2.5 ppm</td>
<td>6.19±0.09a</td>
<td>4.09±0.02a</td>
<td>153.72±5.24b</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + CPPU 5.0 ppm</td>
<td>5.98±0.24ab</td>
<td>3.98±0.06ab</td>
<td>161.61±7.93ab</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + TDZ 2.5 ppm</td>
<td>6.11±0.06ab</td>
<td>3.81±0.05bc</td>
<td>177.35±8.34a</td>
</tr>
<tr>
<td>GA$_3$ 100 ppm + TDZ 5.0 ppm</td>
<td>5.71±0.12ab</td>
<td>3.72±0.09c</td>
<td>158.83±3.19ab</td>
</tr>
</tbody>
</table>

Different letters indicate the significant differences between the mean at p < 0.05. ± indicates standard error.

The effect of PGR treatment on the functionality of ‘Red Dream’ is shown in Table 4. Consumers are increasingly interested in purchasing foods that offer health benefits beyond basic nutrition, and functional foods such as grapes can provide added value to the market. In addition, functional compounds can improve the sensory characteristics of the fruit, such as flavor and color, which can also enhance marketability. Therefore, understanding and improving the functionality of grapes through practices such as PGR treatments can help to increase their marketability and ultimately benefit both producers and consumers. The total phenol content (TPC) was found to be significantly higher in the GA$_3$ and CPPU or TDZ mixed treatment group than in the GA$_3$ single use treatment group. The total flavonoid content was 4.09 mg QE/g in the GA$_3$ 100 ppm + CPPU 2.5 ppm treatment group and 3.98 mg QE/g in the GA$_3$ 100 ppm + CPPU 5.0 ppm treatment group, which was significantly higher than that of the GA$_3$ single use treatment group with a value of 3.88 mg QE/g. However, it was found that there was no statistical difference in the GA$_3$ and TDZ mixed treatment compared to the GA$_3$ single treatment. FRAP ranged from 153.7 to 177.35 µM trolox/g in the GA$_3$ and CPPU or TDZ mixed treatment group and was higher overall compared to the GA$_3$ single application treatment group. As a result, it was confirmed that the content related to functionality was generally higher in the GA$_3$ and CPPU or TDZ mixed treatments than in the GA$_3$ single use treatment. It is presumed that the content of
the functional substance would have increased as the activity of the cell increased. As we found, it has been reported that the mixed treatment of GA$_3$ and TDZ in 'Pione' significantly increased TPC compared to the single treatment of GA$_3$ (Lee et al., 2003). Antioxidants are compounds that help to protect cells from damage caused by free radicals, which are unstable molecules that can damage DNA and other molecules in the body (Martemucci et al., 2022). Grapes are known as a rich source of antioxidants, including polyphenols such as resveratrol and flavonoids (Wijekoon et al., 2022; Zhou et al., 2022). Cytokinin has been shown to increase the synthesis of polyphenols in grapes, which can contribute to higher antioxidant activity (Tyagi et al., 2022). Cytokinin can also help to regulate the expression of genes that are involved in the synthesis of antioxidants, leading to higher levels of these compounds in the fruit. Gibberellin, on the other hand, has been shown to increase the expression of genes that are involved in the synthesis of flavonoids, which can also contribute to higher antioxidant activity in grapes (Jadhav et al., 2020). When cytokinin and gibberellin are applied together, they can work synergistically to enhance the production of both polyphenols and flavonoids, leading to higher antioxidant activity compared to gibberellin alone. This is because the two hormones can target different pathways involved in antioxidant synthesis and work together to increase the overall output of these compounds. Apart from this fact, Kaplan et al. (2019) reported that when PGRs are treated at inappropriate concentrations, useful functional substances such as flavonoids were not accumulated properly. Although only three functionality related parameters were measured in this experiment, similar or higher values were observed in all parameters when the results of the GA$_3$ and CPPU or TDZ combined treatment groups were compared with the GA$_3$ single use treatment group. This result strongly indicates that the functionality was also not impaired by the GA$_3$ and CPPU or TDZ mixed treatment in 'Red Dream'.

Overall, the combined treatment of GA$_3$ and CPPU or TDZ in 'Red Dream' is effectively applied in suppressing the incidence of non-commercial berries and increasing cluster enlargement without significantly impairing fruit quality compared to the GA$_3$ single use treatment. In particular, the GA$_3$ 100 ppm + TDZ 5.0 ppm treatment group was confirmed to have the highest cluster weight and berry firmness. In addition, the incidence rate of non-commercial berry was also reduced by 37.6% compared to the GA$_3$ single use treatment. Hence, it was estimated to be the most effective treatment condition to supplement the cultivation problems of 'Red Dream' among PGR treatment combinations used in this experiment.

**Conclusions**

In this study, we investigated the effect of various PGR combinations on the fruit quality of newly bred 'Red Dream' grape. The results showed that the GA$_3$ and CPPU or TDZ mixed treatment resulted in significantly higher cluster weight and lower incidence rate of non-commercial berries compared to the GA$_3$ single use treatment group. The functionality was also higher overall in the mixed treatment group. These findings suggest that the mixed treatment of GA$_3$ and CPPU or TDZ can enhance the marketability of 'Red Dream' grape, possibly by increasing the activity of cells and the synthesis of antioxidants such as polyphenols and flavonoids. However, it is important to note that inappropriate concentrations of PGRs can negatively affect the TSS. Our result suggests that the mixed treatment of GA$_3$ and CPPU or TDZ can be an effective strategy for enhancing the marketability of 'Red Dream' grape.

**Authors’ Contributions**

SYL: Conceptualization; Data curation; Investigation; Methodology; Software; Writing - original draft. JYH: Formal analysis; Methodology; Supervision; Writing - review and editing.

Both authors read and approved the final manuscript.
**Ethical approval** (for researches involving animals or humans)

Not applicable.

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**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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