

Effects of GA₃ and CPPU on Berry Size of Seedless Grapes

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Key words: Plant growth regulators, Seedless grapes, berry size

Summary

In chemical-induced 'Kyoho' seedless grapes, berry weights were increased about 30% by treatments of 12.5 ppm GA₃ or 10 ppm CPPU 12 days after full bloom. A 75% increase in berry weight was found in the treatment of 12.5 ppm GA₃ and 10 ppm CPPU applied 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom. The effect of GA₃ and CPPU on the berry sizes of 'Honey Red' was similar as 'Kyoho'. Berry size of 'Honey Red' was increased 56% by the treatment of 12.5 ppm GA₃ and 10 ppm CPPU applied 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom. The enlarged berry appeared oval-shaped in 'Kyoho' and longer oval-shaped in 'Honey Red' grapes.

In seedless cultivars, the treatment of 100 ppm GA₃ and 10 ppm CPPU applied 12 days after full bloom increased 83% of the berry size of 'Beauty Seedless' and 'Flame Seedless' grapes and a 100% increase in berry size was achieved when an additional of 25 ppm GA₃ was applied on the 25th day after full bloom. In 'Himrod' grapes, berry size was increased 133% by the treatment of 100 ppm GA₃ combined with 10 ppm CPPU applied 12 days after full bloom, however an additional treatment of 25 ppm GA₃ applied 25 days after full bloom showed no more effective. In berry shape index, it was found that enlarged berry appeared long oval-shape in 'Beauty Seedless' and round-shaped in 'Flame Seedless' and 'Himrod' grapes.

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Introduction

Seedless in grape is attracting huge interest for its better eating quality and higher returns to the farmer but seedless grapes are generally smaller in size which might represent a problem for commercialization. Hence, worldwide grapevine breeder has been focusing on the creation of new seedless cultivars with superior berry size traits. The majority of table grape plantings are seedless cultivars and there are currently more than a dozen of seedless varieties (Parry *et al.*, 2006). The seedlessness trait in most commercial cultivars is obtained from one of the three sources: 'Thompson Seedless', 'Russian Seedless', and 'Black Monukka' (Rombough, 2002). Seedless grapes is not only produced through growing seedless cultivars but also can be induced by some chemicals treatments. Some antibiotics and GA₃ were reported to show promising effects in inducing seedlessness (Kimura *et al.*, 1996; Pommer *et al.*, 1996). However, berry size is affected by both endogenous (*e.g.* nutritional and hormonal factors) and exogenous factors (*e.g.* temperature, light and water availability) (Ojeda *et al.*, 2001; Ollat *et al.*, 2002).

Plant growth regulators have been used in grapes to improve berry qualities such as size and weight. Among plant growth regulators, gibberellic acid (GA₃) has been commonly used to enhance berry size of seedless cultivars (Weaver and McCune, 1959). In addition, GA₃ when combined with cytokinin such as CPPU can act synergistically to increase the berry size in grapevines (Dokoozlian *et al.*, 1994; Ogata *et al.*, 1988). The size of chemical induced seedless berries in seeded grapes cultivars could be also increased by GA₃ and CPPU treatments (Ikeda *et al.*, 2004; Ishikawa *et al.*, 2003; Suzuki and Sukanuma, 2002). However, the desirable effect that could be obtained from the using of GA₃ and CPPU depend on several factors, including of cultivars, concentrations and the time of application.

The current study aims to study the synergistic effects of PGRs on promoting the berry size of some grape cultivars. Effects of application of GA₃ and CPPU in different stages of flower and berry development in different cultivars were investigated.

Materials and Methods

Plant materials

Field experiments were conducted at the Viticulture Research Station, National Chung Hsing University, Taichung, Taiwan in summer of 2010.

1. Chemical-induced seedless grapes

Three-year-old 'Kyoho' (*Vitis vinifera* L. x *Vitis labruscana* Baily) and twenty-one-

year-old 'Honey Red' (*Vitis vinifera* L. × *Vitis labrusca* L.) grapevines grafted onto 8B Teleki rootstocks were planted at a spacing of 5.8 m x 11 m. The 'Kyoho' plants were trained to H-shaped canopies and 'Honey Red' plants were trained to X-shaped canopies respectively which were supported by a horizontal trellis at a height of 1.8 m.

Uniform inflorescences on vigorous shoots were selected and one inflorescence was kept on each shoot on the 10th day before full bloom. Inflorescence thinning was carried out on the same day and only the distal part of the inflorescence was remained with a length of ca. 3.5 cm. They were then dipped into 125 ppm streptomycin sulfate solution ($C_{21}H_{39}N_7O_{12} \cdot 1.5 H_2SO_4$, USB Corporation) on the 6th day before full bloom, followed by 12.5 ppm GA_3 at full bloom for induction of seedless berries. The percentage of induced seedless berries could be more than 95 %.

Berry thinning was carried out two times on the 10th and 30th day after full bloom and 30 berries per cluster were remained. All clusters were bagged with white paper after berry thinning.

2. Seedless grapes

Three seedless cultivars: 'Beauty Seedless' (*Vitis vinifera* L.), 'Flame Seedless' (*Vitis vinifera* L.) and 'Himrod' (*Vitis labrusca* L. x *Vitis vinifera* L.) were used in this experiment. Six-year-old vines grafted onto 8B Teleki were planted at a spacing of 2.8 m x 5 m and were trained to X-shaped canopies which were supported by a horizontal trellis at a height of 1.8 m.

Berry thinning was carried out two times on the 10th and 25th day after full bloom to maintain 75-80 berries per cluster for 'Beauty Seedless', 70-75 berries per cluster for 'Flame Seedless' and 55-60 berries per cluster for 'Himrod'. All clusters were bagged with white paper after berry thinning.

Treatments of GA_3 and CPPU

Cluster of 'Kyoho' and 'Honey Red' were treated with on GA_3 12.5 ppm or CPPU 10 ppm on the 12th day and GA_3 25 ppm on the 25th day after full bloom. In the another experiment, clusters of seedless cultivars were treated with on GA_3 100 ppm or CPPU 10 ppm on the 12th day and GA_3 25 ppm on the 25th day after full bloom.

Each treatment consisted of 10 inflorescences (clusters) as 10 replicates. Ten untreated clusters were used as the control. GA_3 (3.1%) and CPPU (Fulmet) (0.1% forchlorfenuron) were obtained from the Kyowa Hakko Kogyo Co., Ltd., Japan.

Determination of berry weigh, diameter and shape index

Clusters of 'Kyoho' and 'Honey Red' were harvested on the 90th day after full bloom, 'Beauty Seedless' and 'Flame Seedless' were harvested on the 80th day and 'Himrod' was on the 60th day after full bloom. Ten berries were randomly collected from each cluster to determine the berry weight by using an electronic balance. Longitudinal diameter (length) and transversal diameter (width) of the same berries were measured by using an electronic venire caliper (Mitutoyo factory system CD-6BS type). Berry shape index was the ratio of berry length to berry width.

Statistical analysis

Data were subjected to analysis of variance by using the Statistical Analysis System (SAS, 2007) general linear model (GLM) procedure. Significant differences among treatment means were determined by Duncan's test, at $P \leq 0.05$.

Results

1. Kyoho

Combined treatment of 12.5 ppm GA₃ and 10 ppm CPPU 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom showed the most favorable response in terms of berry weight, berry length and berry width in chemical-induced 'Kyoho' seedless grapes (Table 1). A 75% increase in berry weight was obtained by the treatment. The berry weight in treatment of GA₃ and CPPU applied alone or combined was greater than that in the control. An additional application of 25 ppm GA₃ 25 days after full bloom had an additive effect on increasing berry size. Most of enlarged berries appeared round in shape.

2. Honey Red

A 56% increase in berry weight was obtained by the treatment of 12.5 ppm GA₃ plus 10 ppm CPPU applied 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom. The berry weight in treatments of GA₃ and CPPU applied alone or combined was greater than that in the control (Table 2). An additional application of 25 ppm GA₃ 25 days after full bloom had an additive effect on increasing berry size. The berry shape became longer in bigger berry by treatment.

Table 1. Effects of GA₃ and CPPU on berry size of chemical-induced 'Kyoho' seedless grapes

12 day after full bloom		25 days after full bloom		Berry weight (g)	Berry length (cm)	Berry width (cm)	Berry shape index (length/width)
GA ₃ 12.5 ppm	CPPU 10 ppm	GA ₃ 25 ppm					
Control				6.5f ^z (100) ^y	2.43e	2.16d	1.13d
-	+	-		8.5de (131)	2.67c	2.26c	1.18bc
+	-	-		8.4e (129)	2.57d	2.25c	1.14d
+	+	-		9.0c (138)	2.75b	2.31bc	1.19ab
-	-	+		6.9f (106)	2.52d	2.09e	1.21a
-	+	+		9.4b (145)	2.75b	2.33b	1.18bc
+	-	+		8.9cd (137)	2.80b	2.30bc	1.22a
+	+	+		11.4a (175)	2.94a	2.54a	1.16cd

z: Means separation within the same column followed by the same letter are not significantly different by Duncan's multiple range tests at $P \leq 0.05$.

y: Relative ratio to the untreated control.

Table 2. Effects of GA₃ and CPPU on berry size of chemical-induced 'Honey Red' seedless grapes

12 day after full bloom		25 days after full bloom		Berry weight (g)	Berry length (cm)	Berry width (cm)	Berry shape index (length/width)
GA ₃ 12.5 ppm	CPPU 10 ppm	GA ₃ 25 ppm					
Control				9.6e ^z (100) ^y	2.75d	2.34d	1.18bc
-	+	-		12.0c (125)	2.88c	2.55bc	1.13d
+	-	-		12.5c (130)	3.13ab	2.55bc	1.23a
+	+	-		13.6b (142)	3.12ab	2.63ab	1.19bc
-	-	+		11.0d (115)	3.09b	2.54bc	1.22ab
-	+	+		12.9bc (134)	3.03b	2.61b	1.16cd
+	-	+		12.2c (127)	3.13ab	2.51c	1.25a
+	+	+		15.0a (156)	3.38a	2.70a	1.25a

z: Means separation within the same column followed by the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

y: Relative ratio to the untreated control.

3. Beauty Seedless

Berry size parameter, in terms of berry weight, length and width, in the treatment of 100 ppm GA₃ plus 10 ppm CPPU 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom showed higher values compared with other treatments (Table 3). A 100% increase in berry weight was obtained by the treatment. All of treatments had higher average berry weight compared with the control. The berry weight in treatment of GA₃ and CPPU applied alone or combined was greater than that in the control. An additional application of 25 ppm GA₃ 25 days after full bloom had an additive effect on increasing berry size. The berry shape became longer in bigger berry by treatment.

Table 3. Effects of GA₃ and CPPU on berry size of 'Beauty Seedless' grapes

12 day after full bloom		25 days after full bloom	Berry weight (g)	Berry length (cm)	Berry width (cm)	Berry shape index (length/width)
GA ₃ 100 ppm	CPPU 10 ppm	GA ₃ 25 ppm				
Control			2.4f ^z (100) ^y	1.61f	1.49f	1.08e
+	-	-	3.6c (150)	1.95d	1.71c	1.14c
-	+	-	3.0d (125)	1.81e	1.63d	1.11d
+	+	-	4.4b (183)	2.07c	1.85a	1.12d
-	-	+	2.8e (117)	1.79e	1.57e	1.14c
+	-	+	4.4b (183)	2.13b	1.82b	1.17b
-	+	+	3.6c (150)	1.98d	1.71c	1.16b
+	+	+	4.8a (200)	2.28a	1.85a	1.23a

z: Means separation within the same column followed by the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

y: Relative ratio to the untreated control.

4. Flame Seedless

Berry size of 'Flame Seedless' grapes in the treatment of 100 ppm GA₃ plus 10 ppm CPPU applied 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom showed higher values compared with other treatments (Table 4). A 100% increase in berry weight was obtained by the treatment. The berry weight in treatments of GA₃ and CPPU applied alone or

combined was also greater than that in the control. An additional application of 25 ppm GA₃ 25 days after full bloom had an additive effect on increasing berry size. The berry shapes were most significantly affected in most treatments.

Table 4. Effects of GA₃ and CPPU on berry size of 'Flame Seedless' grapes

12 day after full bloom		25 days after full bloom	Berry weight (g)	Berry length (cm)	Berry width (cm)	Berry shape index (length/width)
GA ₃ 100 ppm	CPPU 10 ppm	GA ₃ 25 ppm				
Control			2.3d ^z (100) ^y	1.54c	1.50f	1.03abc
+	-	-	3.3c (143)	1.88a	1.81c	1.04ab
-	+	-	3.4c (148)	1.71b	1.70d	1.01cd
+	+	-	4.2b (183)	1.88a	1.87b	1.01cd
-	-	+	2.9c (126)	1.72b	1.64e	1.05a
+	-	+	4.0b (174)	1.89a	1.80c	1.05a
-	+	+	3.2c (139)	1.70b	1.68de	1.01cd
+	+	+	4.6a (200)	1.93a	1.94a	0.99d

z: Means separation within the same column followed by the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

y: Relative ratio to the untreated control.

5. Himrod

Combined treatment of 12.5 ppm GA₃ and 10 ppm CPPU 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom showed the most favorable response in terms of berry weight, berry length and berry width in 'Himrod' seedless grapes (Table 5). A 140% increase in berry weight was obtained by the treatment. The berry weight in treatment of GA₃ and CPPU applied alone or combined was also greater than that in the control. Some of enlarged berries appeared round in shape.

Table 5. Effects of GA₃ and CPPU on berry size of 'Himrod' grapes

12 day after full bloom		25 days after full bloom		Berry weight (g)	Berry length (cm)	Berry width (cm)	Berry shape index (length/width)
GA ₃ 100 ppm	CPPU 10 ppm	GA ₃ 25 ppm					
Control				3.0d ^z (100) ^y	1.75c	1.60d	1.09b
+	-	-		4.7c (157)	2.09b	1.88c	1.11b
-	+	-		5.4b (180)	2.12b	2.00b	1.06c
+	+	-		7.0a (233)	2.32a	2.20a	1.05c
-	-	+		3.2d (107)	1.80c	1.63d	1.10b
+	-	+		4.8c (160)	2.12b	1.87c	1.13a
-	+	+		5.3b (177)	2.08b	1.99b	1.05c
+	+	+		7.2a (240)	2.36a	2.21a	1.07c

z : Means separation within the same column followed by the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

y: Relative ratio to the untreated control.

Discussion

In this study, the application of GA₃ and CPPU alone or combined increased the berry size in all seedless grapes. 'Kyoho' and 'Honey Red' grapes, combined treatment of 12.5 ppm GA₃ and 10 ppm CPPU 12 days after full bloom followed by 25 ppm GA₃ 25 days after full bloom showed the most favorable response in terms of berry weight, berry length and berry width. Similar results were also reported by Ikeda *et al.*, (2004) and Suzuki and Suganuma (2002). Ishikawa *et al.*, (2003) reported seedless berries of 'Fujiminori' was induced by 200 ppm streptomycin 9 days before full bloom and 2.5 ppm GA₃ at full bloom and application of 50 or 100 ppm GA₃ plus 10 ppm CPPU 11 days after full bloom increased berry weight. Han and Lee (2004) applied 25 ppm GA₃ alone or combined with 10 ppm CPPU on the 10th days after full bloom in 'Kyoho' grapes and the results indicated that combined application increased berry size more than application alone did. Suzuki and Suganuma (2002) found that berry size of 'Kyoho' grape seedless increased by a single treatment of 25 ppm GA₃ plus 5 or 10 ppm CPPU. Ikeda *et al.* (2004) sprayed 25 or 50 ppm CPPU 10 to 12 days after full bloom in 'Fujiminori' seedless grapes, it was found that the berry weight was more than 20 g, and berry quality was only slightly affected. In general combination of GA₃ and CPPU gave better results than

GA₃ and CPPU applied alone for berry size improvement, because combined application of CPPU and GA₃ had synergistic effect on berry enlargement (Dokoozlian *et al.*, 1994; Ogata *et al.*, 1988).

In seedless cultivars, examined in this study treatments of CPPU and GA₃ generally increased all the variables related to berry size. Diaz and Maldonado (1992) found that different CPPU treatments applied to different seedless cultivars, including 'Flame Seedless', did not alter their shape. It was apparent from these results that CPPU generally increased grape berry weight, but specific effects on berry morphology probably vary depending on the grapevine cultivar and the berry physiology and environmental conditions at the time of application. Ben-Arie *et al.* (1997) sprayed 25 ppm GA₃ or 10 ppm CPPU at fruit set in 'Perlette', 'Superior' and 'Thompson Seedless'. GA₃ application alone and combination with 10 ppm CPPU had similar effect on increasing berry size. Dokoozlian *et al.*, (1994) observed an increase in berry size in CPPU treated 'Thompson Seedless' and the maturity was delayed. Combination of GA₃ and CPPU application increased berry weight, berry length and berry diameter in 'Perlette', 'Superior' and 'Thompson Seedless' grapes (Ben-Arie *et al.*, 1997). Optimal GA₃ concentration varies with cultivars and environment and can be established between 75 and 650 ppm (Dass and Radhawa, 1972; Bhujbal and Chaudhari, 1973; Korkas *et al.*, 1999). GA₃ applied in 'Emperatriz' seedless grapes in onset of cell enlargement stage increased berry fruit weight (Casanova *et al.*, 2009).

Application of GA₃ and CPPU can increase berry size in all the cultivars tested in the current study, however the total soluble solids were reduced and titratable acids were increased due to probably a delay of berry maturation ripening.

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GA₃ 及 CPPU 對無子葡萄果粒大小之影響

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關鍵字：植物生長調節劑、無子葡萄、果粒大小

摘要：本研究目的為探討 GA₃ 及 CPPU 處理對其無子葡萄果實大小之影響。

利用藥劑誘導'巨峰'葡萄無子化後，在花後 12 日單以 GA₃ 12.5 ppm 或 CPPU 10 ppm 處理皆可增加果粒大小約 30%。同樣處理若在花後 25 日再以 GA₃ 25 ppm 追加處理，則可增加果粒大小達 75%。在'蜜紅'無子葡萄上，亦呈現類似'巨峰'的效果，花後 12 日以 GA₃ 混合 CPPU 及花後 25 日再以 GA₃ 處理之果粒大小可增大約 56%。由果形指數可知，'巨峰'因處理變愈大的果粒會趨於圓形，而'蜜紅'則趨於橢圓形。

在無子葡萄品種方面，花後 12 日處理 GA₃ 100 ppm 混合 CPPU 10 ppm 可有效地促進'Beauty Seedless' 及 'Flame Seedless'果粒大小達 83%。若在花後 25 日追加處理 GA₃ 25ppm 則更為有效，可增大 100%。在'Hirmod'花後 12 日之混合處理可增大果粒 133%，但花後 25 日之 GA₃ 追加處理則效果不明顯。在果粒形狀方面，'Beauty Seedless'會因 GA₃ 及 CPPU 之處理使果形趨於橢圓形，而'Flame Seedless'及'Hirmod'則趨於圓形。

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