Effects of Streptomycin, GA_3 and CPPU Treatments on Induction of Seedless Berry in 'Kyoho' and 'Honey Red' Grapes

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Key words: Streptomycin, Seedlessness, Grapes

Summary

Streptomycin and GA_3 were used to induce seedless berries in 'Kyoho' and 'Honey Red' grapes in this study. Inflorescences were treated with 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom and high percentage of seedless berry were induced in both summer and winter crops of 'Kyoho' and 'Honey Red' grapes. To enlarge the seedless berries, 20 ppm GA_3 alone or combined with 5 ppm CPPU were applied 12 days after full bloom. In summer crop of 'Kyoho' grapes, there was no significant difference in the berry weight between the treatment of GA_3 plus CPPU and the untreated control. However, in winter crop, the induced seedless berry was smaller than the control. The skin color value and TSS were higher in the streptomycin treated berries than those in the untreated control in both crops of 'Kyoho' grapes. In contrast, the streptomycin, GA_3 plus CPPU treated berries in both crops of 'Honey Red' grapes had lower skin color value and TSS than those in the untreated control. As a result, application of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom followed by 20 ppm GA_3 combined with 5 ppm CPPU 12 days after full bloom was efficient to produce large seedless berries comparable with seeded counterparts in 'Kyoho' grapes. For 'Honey Red' grapes, although nearly 100% seedless berry was able to be produced by the same treatment, the berry quality was slightly reduced.

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Introduction

Seedless table grapes have high demand worldwide and large berries and compact clusters are desired properties for fresh consumption in Japan (Ikeda *et al.*, 2004). Tetraploid grapes, such as 'Kyoho' and 'Fujiminori', with large berries and high total soluble solid are preferred in Japanese market (Ikeda et al., 2004). However, the seeded characteristic of those cultivars poses a barrier to some consumers' acceptance and there is a demand for seedless types (Ikeda *et al.*, 2004). To obtain a fruit without seeds is a physiological challenge. Seedless induction by gibberellins (GA_3) application before full bloom is a common practice in grapes, but this cultural method might not be able to induce complete seedlessness (Kimura *et al.*, 1996).

Ogasawara (1985) studied the use of streptomycin for induction of seedless berries in 10 seeded grape cultivars and the results showed that more than 98% seedless berries were produced in all 10 cultivars. Pommer *et al.* (1996) also successfully used streptomycin to induce seedlessness in the grape cultivar 'Rubi'. Although an inhibition on ovule development had been suggested (Kimura *et al.*, 1996), the effect of streptomycin on seed formation has not been clearly defined yet. Application of streptomycin before blooming followed by treatments of GA_3 at full boom and CPPU after full bloom in 'Fujiminori' grapes induced 100% seedless and large berries whose size were compatible with untreated seeded berries (Ikeda *et al.*, 2004). However, the application date and concentrations of GA_3 and streptomycin for inducing seedlessness in seeded grape cultivars (Fukunaga and Kurooka, 1988; Ishikawa *et al.*, 2001; Suzuki and Suganuma, 2002) and GA_3 and CPPU for enlarging berry size and improving berry quality (Ishikawa *et al.*, 2003) are crucial for successful seedless grape production.

Growth of grapevines in the tropics and subtropics is very different from that in the temperate zone because of the climate difference (Possingham, 2004). This study aimed to investigate the optimum application method of streptomycin, GA_3 and CPPU for induction of seedless berries in both summer and winter crops of 'Kyoho' and 'Honey Red' grapes in Taiwan's subtropical cultural environment.

Materials and Methods

Seventeen-year-old 'Kyoho' (*Vitis vinifera* L. x *Vitis labruscana* Bailey) and twenty four year-old 'Honey Red' (*Vitis vinifera* L. × *Vitis labrusca* L.) grapevines grown in the Viticulture Research Station, National Chung Hsing University (Taichung, Taiwan) were used in this study. Both of 'Kyoho' and 'Honey Red' grapevines, grafted on 8B Teleki rootstocks, were planted with a spacing of 5.8 x 11.6 m and trained to X-shaped canopies which were supported by a horizontal trellis at a height of 1.8 m.

On the 6th day before full bloom, uniform inflorescences were selected from normal vigorous shoots and only one inflorescence was kept per shoot. On the same day, inflorescences were thinned to maintain only the distal parts of the inflorescences with a length of cv. 3.5 cm. Thinned inflorescences were dipped immediately with 125 ppm streptomycin (USB Corporation) alone or combined with 12.5 ppm GA_3. The seed number per berry was determined after fruit set. Each treatment had 20 clusters. The other clusters were again treated with 20 ppm GA_3 alone or 20 ppm GA_3 combined with 5 ppm CPPU on the 12th day after full bloom. Each treatment had 20 clusters. Berry thinning was carried out twice on the 10th and 30th day respectively after full bloom and remained 30 berries per cluster. These clusters were harvested on the 90th day in 'Kyoho' and 80th day in 'Honey Red' after full bloom respectively for determination of berry quality. Skin color was measured by color charts; values 1-12, represents green to black, for 'Kyoho' and values 1-8, represents green to red, for 'Honey Red' grapes. Total soluble solids (TSS) were measured with a manual refractometer (ATAGO). Titratable acidity was determined by titrating with 0.1 N NaOH and used tartaric acid as the standard. The same experiments were conducted in both summer and winter crops.

Results

Effects of Streptomycin and GA_3 Application on Seedless Berry Induction

The percentages of seedless berry (0 seed) in the treatment of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom were 97.6% and 100% in summer crop and winter crop of Kyoho grapes, respectively (Table 1 and 2). The treatment of 12.5 ppm GA_3 alone only had 26.0% of seedless berry in summer crop and 50.2% in winter crop (Table 1 and 2). Similar results were found in Honey Red grapes which had 100% and 99.3% of seedless berries in summer crop and winter crop respectively in the treatment of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom (Table 3 and 4). The percentages of seedless berry in the treatment of 12.5 ppm GA_3 alone were 39.0% and 60.3% in summer crop and winter crop respectively which were significantly lower than those in the treatment of 125 ppm streptomycin plus 12.5 ppm GA_3 (Table 3 and 4).

Table 1. Effects of streptomycin and GA_3 application on berry seed number in summer crop of 'Kyoho' grapes.[z]

Streptomycin (ppm)	GA_3 (ppm)	Distribution of seed number per berry (%)				
		0 seed	1 seed	2 seeds	3 seeds	4 seeds
0	0	4.8c[y]	52.0a	35.1a	8.1a	0a
0	12.5	26.0b	43.0b	24.8b	6.2ab	0a
125	12.5	97.6a	2.2c	0.2c	0b	0a

[z]: Streptomycin and GA_3 were applied 6 days before full bloom.

[y]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Table 2. Effects of streptomycin and GA_3 application on berry seed number in winter crop of 'Kyoho' grapes.[z]

Streptomycin[z] (ppm)	GA_3[z] (ppm)	Distribution of seed number per berry (%)				
		0 seed	1 seed	2 seeds	3 seeds	4 seeds
0	0	1.1c[y]	48.0a	39.1a	11.8a	0a
0	12.5	50.2b	47.7b	10.9b	1.2b	0a
125	12.5	100a	0c	0c	0b	0a

[z]: Streptomycin and GA_3 were applied 6 days before full bloom.

[y]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Table 3. Effects of streptomycin and GA_3 application on berry seed number in summer crop of 'Honey Red' grapes.[z]

Streptomycin (ppm)	GA_3 (ppm)	Distribution of seed number per berry (%)				
		0 seed	1 seed	2 seeds	3 seeds	4 seeds
0	0	0c[y]	40.5a	49.7a	9.8a	0a
0	12.5	39.0b	40.8a	17.8b	2.4b	0a
125	12.5	100a	0b	0c	0b	0a

[z]: Streptomycin and GA_3 were applied 6 days before full bloom.

[y]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Table 4. Effects of streptomycin and GA_3 application on berry seed number in winter crop of 'Honey Red' grapes.[z]

Streptomycin (ppm)	GA_3 (ppm)	Distribution of seed number per berry (%)				
		0 seed	1 seed	2 seeds	3 seeds	4 seeds
0	0	12.7c[y]	42.6a	35.6a	9.0a	0a
0	12.5	60.3b	24.1b	12.9b	2.7b	0a
125	12.5	99.3a	0.5c	0.2c	0b	0a

[z]: Streptomycin and GA_3 were applied 6 days before full bloom.

[y]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Effects of GA_3 and CPPU Application on Berry Quality

In summer crop of 'Kyoho' grapes, the berry weight in the treatment of 20 ppm GA_3 plus 5 ppm CPPU applied 12 days after full bloom which followed application of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom was not significantly different from that in the untreated control (Table 5). The skin color and TSS were higher in the streptomycin treated berries than those in the untreated control and the GA_3 alone treatment (Table 5). In winter crop of 'Kyoho' grapes, the berry weights in the seedlessness induction treatments were all significantly lower than that in the untreated control (Table 6). However, the seeded berry in untreated control had lower skin color and TSS. The titratable acidity in the streptomycin treated berries was lower than that in the untreated control (Table 6).

In summer crop of 'Honey Red' grapes, the berry weight in the treatment of 20 ppm GA_3 plus 5 ppm CPPU applied 12 days after full bloom which followed application of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom was significantly lower than that in the untreated control (Table 7). The skin color was higher in the untreated berries than in the others and the CPPU treated berries had lower TSS (Table7). In winter crop of 'Honey Red' grapes, the berry weight in the treatment of 20 ppm GA_3 plus 5 ppm CPPU applied 12 days after full bloom which followed application of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom was significantly lower than that in the untreated control (Table 8). The skin color was not significantly different between the two treatments but the TSS and titratable acidity were higher in the untreated control (Table 8).

Table 5. Effects of streptomycin, GA_3 and CPPU application on berry quality in summer crop of 'Kyoho' grapes.

6 days before full bloom		12 days after full bloom		Berry quality			
Streptomycin (ppm)	GA_3 (ppm)	GA_3 (ppm)	CPPU (ppm)	Berry Weight (g)	Skin color (color chart value)	TSS (ºBrix)	Titratable acidity (g/100ml)
0	0	0	0	10.5a [z]	9.8bc	17.8b	0.47a
0	12.5	20	0	10.0a	9.5c	17.7b	0.46a
0	12.5	20	5	9.8ab	10.0b	17.6b	0.48a
125	12.5	20	0	8.9b	11.0a	18.9a	0.47a
125	12.5	20	5	9.2ab	10.7a	18.6a	0.49a

[z]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Table 6. Effects of streptomycin, GA_3 and CPPU application on berry quality in winter crop of 'Kyoho' grapes.

6 days before full bloom		12 days after full bloom		Berry quality			
Streptomycin (ppm)	GA_3 (ppm)	GA_3 (ppm)	CPPU (ppm)	Berry Weight (g)	Skin color (color chart value)	TSS (ºBrix)	Titratable acidity (g/100ml)
0	0	0	0	9.3a [z]	9.9b	18.4c	0.55b
0	12.5	20	0	8.6bc	10.7a	18.6bc	0.53bc
0	12.5	20	5	8.8b	10.5a	18.3c	0.66a
125	12.5	20	0	8.2d	10.8a	19.2a	0.46d
125	12.5	20	5	8.3cd	10.5a	19.1ab	0.49cd

[z]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Table 7. Effects of streptomycin, GA_3 and CPPU application on berry quality in summer crop of 'Honey Red' grapes.

6 days before full bloom		12 days after full bloom		Berry quality			
Streptomycin (ppm)	GA_3 (ppm)	GA_3 (ppm)	CPPU (ppm)	Berry Weight (g)	Skin color (color chart value)	TSS (ºBrix)	Titratable acidity (g/100ml)
0	0	0	0	10.2b[z]	5.8a	18.2a	0.59a
0	12.5	20	0	10.3b	4.5bc	18.2a	0.59a
0	12.5	20	5	11.2a	4.2c	17.7b	0.60a
125	12.5	20	0	8.8d	4.9b	18.1a	0.59a
125	12.5	20	5	9.6c	4.9b	17.2c	0.59a

[z]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Table 8. Effects of streptomycin, GA_3 and CPPU application on berry quality in winter crop of 'Honey Red' grapes.

6 days before full bloom		12 days after full bloom		Berry quality			
Streptomycin (ppm)	GA_3 (ppm)	GA_3 (ppm)	CPPU (ppm)	Berry Weight (g)	Skin color (color chart value)	TSS (ºBrix)	Titratable acidity (g/100ml)
0	0	0	0	10.0ab[z]	4.8b	18.1a	0.45bc
0	12.5	20	0	10.3a	6.0a	18.2a	0.56a
0	12.5	20	5	9.6b	6.3a	18.3a	0.48b
125	12.5	20	0	9.4bc	4.3c	17.1b	0.44c
125	12.5	20	5	8.9c	4.5bc	17.5b	0.34d

[z]: Means within the same column followed by the same letter were not significantly different by LSD test at $P \leq 0.05$.

Discussion

For seedless berries induction in 'Kyoho' and 'Honey Red' grapevines in both summer and winter crops in Taiwan, application of 125 ppm streptomycin plus 12.5 ppm GA_3 6 days before full bloom was an efficient treatment (Table 1, 2, 3 & 4). However, application of 12.5 ppm GA_3 alone only produced 26% and 39% seedless berries in summer crop and 50.2% and 60.3% seedless berries in winter crops in 'Kyoho' and 'Honey Red' grapevines, respectively (Table 1, 2, 3 & 4). The results suggest that streptomycin must have to be added to the treatment solution with GA_3 to insure high percentage of seedless berry induction. Similar results were also found in 'Kyoho' grape in Japan (Fukunaga and Kurooka, 1988).

Application of CPPU after berry set can enlarge berry size of seeded and seedless grape cultivars (Ben-Arie *et al.*, 1997; Zabadal and Bukovac, 2006). Treatment of GA and CPPU applied after berry set significantly enhanced berry weight of 'Fujiminori' (Ishikawa *et al.*, 2003; Ikeda *et al.*, 2004) and 'Kyoho' grapes (Suzuki and Suganuma, 2002; Han and Lee, 2004). In the current study, the berry weight in the treatment of GA_3 plus CPPU in summer crop of 'Kyoho' grapes was not significantly different from that in the untreated control (Table 5). However, the berry weight in the GA_3 alone treatment was significantly lower than that in the control (Table 5), indicating that CPPU application is helpful for large seedless berry production. In winter crop of 'Kyoho' grapes, 20 ppm GA_3 alone or mixed with 5 ppm CPPU applied 12 days after full bloom produced smaller berries than the control (Table 5), suggesting that higher concentrations of GA_3 and CPPU are probably needed because winter crops produced in Taiwan are generally smaller than the summer crops due to lower temperature during berry growth stage.

The skin color and TSS were higher in the streptomycin induced seedless berries than those in the untreated seeded control in both crops of 'Kyoho' grapes (Table 5 & 6). Similar result was also found in 'Pione' grape (Asano et al., 2001). Nevertheless, streptomycin induced seedless berries of 'Honey Red' grapes which were enlarged by GA_3 and CPPU treatment had lower skin color value and TSS than the untreated seeded control in both crops (Table 7 & 8). Ripening and maturation of grape berries might be delayed by CPPU treatment (Ben-Arie et al., 1997). It seems that the effect of CPPU on delaying fruit ripening and maturation was more significant in 'Honey-Red' grape than in 'Kyoho' grape. The tatratable acidity was affected by the streptomycin and CPPU treatments in summer crops of both cultivars (Table 5 & 7) but slightly reduced in winter crops (Table 6 & 8) which was uncoupled with the differences in skin color and TSS.

Taken together, to use streptomycin, GA_3 and CPPU to produce large seedless berries comparable with seeded counterparts in 'Kyoho' grapes, application of 125 ppm streptomycin plus 12.5 GA_3 6 days before full bloom followed by 20 ppm GA_3 combined with 5 ppm CPPU 12 days after full bloom should be an efficient method. For 'Honey Red' grapes, although nearly 100% seedless berry could be produced by the same treatment, the berry quality might be slightly reduced.

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鏈黴素、GA3 與 CPPU 處理對'巨峰'及'蜜紅'葡萄無子化及果實品質之影響

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關鍵字：鏈黴素、無子化、葡萄

摘要：本研究利用鏈黴素及 GA_3 誘導'巨峰'及'蜜紅'葡萄無子化。於滿花前 6 天以 125 ppm 鏈黴素及 12.5 ppm GA_3 處理'巨峰'及'蜜紅'葡萄花穗，均可獲得高無子率之夏果及冬果。於滿花後 12 天施用 20 ppm GA_3 或加施 5 ppm CPPU 以促進經無子化處理之果實肥大。結果顯示無子化果粒經 GA_3 加 CPPU 處理之'巨峰'夏果果粒重與有子之對照組差異不顯著，但冬果果粒重則顯著小於對照組。經鏈黴素誘導之'巨峰'無子果果色值及可溶性固形物均顯著高於有子之對照組。然而，鏈黴素誘導之'蜜紅'無子果實經 GA_3 及 CPPU 催大後，其果色值及可溶性固形物均顯著低於有子之對照組。因此，於滿花前 6 天以 125 ppm 鏈黴素加 12.5 ppm GA_3 處理'巨峰'葡萄花穗，並於滿花後 12 天施用 20 ppm GA_3 加 5 ppm CPPU 處理果穗可有效生產與有子果粒大小相當之無子果。相同處理雖然也可以有效的誘導'蜜紅'葡萄無子化，但果實品質則略低於有子果。

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