Effect of Streptomycin on Induction of Seedless Berries in 'Kyoho' (*Vitis vinifera* L. x *V. labruscana* Bailey) Grapes

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Summary

Streptomycin was used to induce seedless berries in 'Kyoho' (*Vitis vinifera* L. x *V. labruscana* Bailey) grapes and the results indicated that 125 ppm streptomycin applied 6 days before flowering induced 82% seedless berry and the percentage of seedlessness was increased to 94% if an additional treatment of 12.5 ppm GA_3 was applied at full bloom. Application of streptomycin 6 days and 3 days before flowering gave higher percentage of seedless berries and the concentration effect was not significant. Seedlessness was easier to be induced in distal part of clusters suggesting that thinning inflorescences to maintain only the distal part of inflorescence is necessary to have successful induction of seedlessness.

There were no significant differences between different vigorous bearing shoots. Cross pollination might occur between streptomycin treated and untreated flowers. As a result, in commercial practice, application of streptomycin and GA_3 to induce seedless grapes has to be carried out in whole vineyard and no other untreated grapevines nearby to ensure 100% seedless berry set.

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Introduction

Seedless table grapes have high demand worldwide and many grape breeders therefore focus much of their efforts on the creation of new seedless cultivars. Seedless table grapes with large berries and compact clusters are desired properties for fresh consumption (Ikeda *et al*., 2004). 'Kyoho' is a tetraploid grape cultivar with large berries and high total soluble solid (Lim *et al*., 2004). However, it has some disadvantages like vigorous vegetative growth and flower shatter and seedless shot berries. Its seeded characteristic poses a barrier to some consumers acceptance, thus there is a demand for seedless types (Ikeda *et al*., 2004). To obtain a fruit without seeds is a physiological challenge. Berry development comprises early developmental and maturation phases (Possingham, 2004). Seedless induction by gibberellins (GA_3) application before full bloom is a common practice in grapes, but this cultural method could not induce complete seedlessness (Kimura *et al*., 1996; Pommer *et al*., 1996). Because of the high dependence of induction of seedlessness on factors such as flower development stages and climatic conditions, it is difficult to reliably define the application date (Possingham, 2004). It is important to study the suitable time and concentration to use plant growth regulators such as GA_3 or streptomycin for inducing seedlessness and to increase berry size and quality (Fukunaga and Kurooka, 1988; Hiroyuki and Kenji, 2002).

Ogasawara (1986) studies the usefulness of streptomycin for the induction of seedlessness in 'Muscat Bailey A' grapes and Pommer *et al*. (1996) used streptomucin to induce seedlessness in the grape cultivar 'Rubi'. Its action was suggested to inhibit the ovule development (Kimura *et al*., 1996). However, the effects of streptomycin on the seed formation processes are not clearly defined (Kimura *et al*., 1996).

This study aimed to investigate the optimum application time and concentration of streptomycin for induction of seedlessness in 'Kyoho' grapes.

Materials and Methods

Eighteen-year-old 'Kyoho' grapevines grown in the Viticulture Research Station, National Chung Hsing University, Taichung, Taiwan were used in this study. The vines, 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 horizontal trellis at a height of 1.8 m. The following four experiments were carried out in this study.

Experiment 1. Effects of streptomycin and GA_3 on seedlessness induction

Uniform inflorescences were selected and one inflorescence was kept per shoot. Thinning of inflorescences was done 6 days before flowering and only distal part of the inflorescence was remained with a length of 3.5 cm. Thinned inflorescences were there dipped immediately with 125 ppm streptomycin sulfate (USB Corporation). Water spraying was applied for 15 minutes after streptomycin application to maintain higher humidity in the vineyard. Half of the streptomycin treated inflorescences were then treated with 12.5 ppm GA_3 at full bloom. Each treatment had 10 inflorescences as 10 replicates and 10 untreated inflorescences were used as control.

Clusters were harvested 90 days after flowering and ten berries were randomly picked from each cluster. The percentage of seedless berries, average seed number and seed-trace number per berry were recorded. Berries contained lignified seed or seeds no larger than 2.5 mm in length were classified as seedless.

Experiment 2. Effects of application time and concentration of streptomycin on induction of seedless berries in different parts of cluster

Three application dates, 6 days, 3 days and 0 days before flowering, and three concentrations, 62.5, 125 and 250 ppm, of streptomycin were treated. Whole inflorescences (without thinning) were dipped into the streptomycin solutions. At full bloom, 12.5 ppm GA_3 was applied to all streptomycin treated samples. Each treatment had 10 clusters as 10 replicates. Seedless percentage and seed number per berry were recorded at harvest, 90 days after flowering.

Experiment 3. Effects of streptomycin on seedlessness induction in different vigour of bearing shoots

Uniform inflorescences from different lengths of bearing shoots, 50-60 cm, 40-50 cm and 30-40 cm were selected 6 days before flowering. Thinning of inflorescences and treatments of streptomycin and gibberellins were carried out as in experiment 1. Each treatment had 10 clusters as 10 replicates. Seedless percentage and seed number per berry were recorded at harvest, 90 days after flowering.

Experiment 4. Effects of inflorescences bagging after treated with streptomycin on seedlessness induction

Inflorescences were selected, thinned and treated with streptomycin as in experiment 1 and GA_3 was applied at full bloom. All inflorescences were bagged with white paper bags 2 days

before flowering to prevent cross-pollination. Each treatment had ten inflorescences as 10 replicates. Seedless percentage and seed number per berry were recorded at harvest, 90 day after flowering.

Results

1. Effects of streptomycin and GA_3 on seedlessness induction

Seedless percentage in the treatment of streptomycin plus GA_3 was 94% which was significantly higher than streptomycin alone (82%) and the control (1%) (Table 1). The seed number per berry was reduced by streptomycin treatment. Application of GA_3 at full bloom (0.1 seed/berry) did not lead to a significant difference in seed number per berry compared to the treatment of streptomycin alone (0.2 seed/berry), but significantly lower than the control of 1.8 seed/berry.

Zero seed trace percentage was found to be significantly linked to streptomycin and GA_3 treatments. Streptomycin plus GA_3 treatment resulted in a 75 % of zero seed-trace which was not significantly different to the 69.9% in streptomycin alone treatment, but significant higher than the control (Table 1).

Table 1. Effects of streptomycin on seedlessness induction and seed number in 'Kyoho' grape berries[z].

Streptomycin (ppm)	GA_3 (ppm)	Seedless percentage (%)	Zero seed-trace percentage (%)	Seed number per berry
0	0	1.0c[y]	0b	1.8a
125	0	82.0b	69.9a	0.2b
125	12.5	94.0a	75.0a	0.1b

[z] Streptomycin was applied 6 days before flowering and GA_3 was applied at flowering.

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

2. Effects of application time and concentration of streptomycin on induction of seedless berries in different parts of clusters

The dates of application (6, 3 and 0 day before flowering) and concentrations of streptomycin (62.5, 125 and 250 ppm) were investigated to determine their influence on seedlessness induction. Berries with higher levels of seedlessness were from inflorescences treated with streptomycin of all concentration on 6 and 3 days before flowering (Table 2). Berries in distal part of clusters had higher percentage of seedlessness. The differences in seedless percentage between different concentration treatments applied at the same date were not significant. In addition, berries from inflorescences treated with streptomycin of 62.5, 125 and 250 ppm on 6 and 3 days before flowering had lower seed number, 0.1~0.6 seed per berry, compared to 1.5~ 1.6 seed per berry in the control (Table 2).

Table 2. Effects of application time and concentration of streptomycin on induction of seedless berries in different parts of cluster in 'Kyoho' grapes[z].

Application time (days before flowering)	Streptomycin (ppm)	Seedless percentage (%)			Seed number per berry		
		Basal cluster	Middle cluster	Distal cluster	Basal cluster	Middle part cluster	Distal cluster
6	62.5	96.0a[y]A[x]	96.9aA	97.9abA	0.2dA	0.2deA	0.2cdA
	125	91.4aB	90.8aB	99.2aA	0.6cdA	0.6bcdA	0.1dB
	250	95.3aA	97.3aA	99.0aA	0.6bcdA	0.3edAB	0.1dB
3	62.5	92.9aA	90.2aA	98.9aA	0.3dAB	0.5cdeA	0.1dAB
	125	97.6aA	92.3aA	97.0abA	0.2dA	0.3deA	0.2cdA
	250	88.2aA	92.1aA	94.9abA	0.5dA	0.4deA	0.2cdA
0	62.5	71.0bA	75.1bcA	83.0dA	1.0abA	0.9bA	0.6bA
	125	78.9bB	81.1cAB	91.8bcdA	1.0abA	0.6bcdAB	0.5bB
	250	75.1bB	86.3abA	86.3cdA	0.95bcA	0.8bcA	0.6bA
Control	0	0cA	0.9dA	4.1eA	1.5aA	1.5aA	1.6aA

[z] All inflorescences, except control, were treated with 12.5 ppm GA_3 at full bloom.

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

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

3. Effects of streptomycin on seedlessness induction in different lengths of bearing shoots

There were no significant differences between treatment (Table3). The seedless berries was 99.2% in shoots 50-60 cm and 100% in shoots 40-50 cm and 30-40 cm.

Table 3. Effects of bearing shoot length on streptomycin induced seedlessness in 'Kyoho' grape berries[z].

Length of bearing shoot	Seedless percentage (%)	Seed number per berry
50-60 cm	99.2a	0.01a
40-50 cm	100a	0a
30-40 cm	100a	0a

[z] Inflorescences were treated with 125 ppm streptomycin 6 days before flowering and 12.5 ppm GA_3 at full bloom.

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

4. Effects of inflorescences bagging after treated with streptomycin on seedlessness induction

Berries from bagged inflorescences after treated with streptomycin were induced to 100% seedlessness and was significantly higher than non-bagged inflorescences which was only 72% (Table 4). The percentage of berries with zero seed-trace in bagged cluster was 93% and the non-bagged clusters was only 66.5%. The seed number per berry was 0.6 in non-bagged treatment which was significantly higher than the bagged treatment.

Table 4. Effects of inflorescences bagging on streptomycin induced seedlessness in 'Kyoho' grape berries[z].

Inflorescence bagging	Seedless percentage (%)	Zero seed-trace percentage (%)	Seed number per berry
Yes	100a[y]	93.0a	0b
No	72b	66.5b	0.6a

[z] Inflorescences were treated with streptomycin 6 days before flowering and bagged 2 day before flowering.

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

Discussion

Among the three dates of streptomycin application, 6 and 3 days before flowering treatments induced higher percentage of seedless berries in all parts of clusters than the 0 days before flowering treatment (Table 2). The results suggested that some flowers had already completed pollination and fertilization at full bloom. Therefore, application of streptomycin should not be later than 3 days before flowering to insure the efficiency of seedless berries induction. The difference in seedless berries induction between different concentrations of streptomycin at each application date was not significantly different (Table 2). As a result, to use a concentration of streptomycin between 125 and 250 ppm should be high enough to obtain high percentage of seedless berries. However, the seedless percentage was not 100%. Fukunaga and Kurooka (1988) reported that application of 200 or 400 ppm streptomycin combined with 20 ppm GA_3 at anthesis in 'Kyoho' grapes resulted in 100% seedless berry set. Therefore, higer concentration of streptomycin in combination with GA_3 is required if 100% seedlessness is desired.

According to the report by Ishikawa *et al*. (2001), the induction rates of seedless berries in tetraploid grapes, such as 'Kyoho' and 'Fujiminori', by streptomycin spraying differed with applied shoot lengths. The longer the shoots at the treatment application, the higher the rates of seedlessness induced (Ishikawa *et al*., 2003). However, an additional application of 12.5 ppm GA_3 at flowering overcame the shoot length effect. The current study had also shown that 'Kyoho' grapes can be successfully induced by streptomycin to produce seedless grapes and induction efficiency was not influenced by lengths of bearing shoots (Table 3) in Taiwan’s sub-tropical environment.

Bagging inflorescences before blooming after treated with 125 ppm streptomycin 6 days before flowering induced complete seedlessness and significantly different from non-bagging inflorescences (Table4). Grape flowers are cleistogamous but cross-bollination might happen in open vineyards. Bagging inflorescences before blooming could prevent streptomycin treated flowers pollinated by pollens from untreated flowers nearby. Therefore, in commercial practice, application of streptomycin and GA_3 to induce seedless berries needs to be carried out in whole vineyard and there are no other untreated grapevines nearby to ensure 100% seedless berry set.

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鏈黴素對巨峰葡萄無子化之影響

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關鍵字：葡萄、無子化、鏈黴素

摘要：利用鏈黴素誘導`巨峰'葡萄(*Vitis vinifera* L. x *V. labruscana* Bailey cv. Kyoho)無子化，結果顯示 125ppm 鏈黴素於滿花前 6 天施用可產生 82%無子率，而如果於滿花時再施用 12.5ppm GA_3則可使無子率增加至 94%。於滿花前 6 天或 3 天施用鏈黴素比滿花日才施用，可產生較高之無子率，但不同施用濃度間並無顯著差異。花穗末端之小花比花穗中段及基部之小花較容易被誘導成無子果實，因此，處理時只有保留花穗末端小花可以提高無子果之誘導率。

不同枝條強度間之無子果誘導率並無顯著差異。有經鏈黴素處理與未經處理之花穗可能有互相授粉之情形發生，因此，在經濟栽培利用鏈黴素及 GA_3來誘導葡萄無子化時，必須全園施用，並且附近沒有未處理之葡萄植株，以確保能獲得100%之無子率。

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