Effects of Streptomycin on Pollen Germination, Ovule Development and Fruit Quality of 'Kyoho' Grape

Po-Yuan Chen[1)] Ching-Cheng Chen[2)]

Key words: Seedless, Streptomycin

Summary

In this study, we investigated the mechanism of seedlessness induced by streptomycin application in 'Kyoho' grape and the effects of GA_3 (gibberellic acid) and CPPU (fulmet) on seedless berry enlargement and fruit quality. The results suggested that the inhibition of ovule development was the major cause of seedlessness induced by streptomycin and abnormalities in pollen tube had minor effect. For the fruit quality, with increments in GA_3 or CPPU application frequency, the berry size was significantly enlarged. However, CPPU application 20 days after blooming (DAB) caused poor skin coloration, lower total soluble solids content, and higher titratable acidity. Furthermore, CPPU application during blooming and 20 DAB could induce lignification of seed trace, which lowered the seedlessness rate.

Introduction

Grape (*Vitis* spp.) is one of the most important fruit crops in the world. Grape berry can be utilized as table fruit, dried raisin, and for juice and wine-making. Due to consumer preference for the convenience of eating seedless grapes, seedlessness has become a vital trait for decades. Seedlessness is referred to as a fruit with a much-reduced number of seeds, traces of aborted seeds, or no seed (Varoquaux *et al*., 2000). Seedless fruits can be distinguished into parthenocarpy and stenospermocarpy (Varoquaux *et al*., 2000). Parthenocarpic grape develops without ovule fertilization, however, the occurrence of pollination stimulates fruit set, e.g. 'Black Corinth' (Ledbetter and Ramming, 1989). Stenospermocarpic grape requires both pollination and

1) Graduate Student, Department of Horticulture, National Chung Hsing University.
2) Assistant professor, Department of Horticulture, National Chung Hsing University.
Corresponding author.

fertilization to induce berry set and development, but the seeds are aborted during development, therefore, stenospermocarpic grape contains aborted seeds, so-called seed traces (Ledbetter and Ramming, 1989). In general, the berry size of stenospermocarpic grape is larger than the parthenocarpic grape, because of the stimulation by pollination and fertilization. In addition, the responses to berry enlargement agents, e.g. gibberellic acid (GA_3), in stenospermocarpic grapes are better than in parthenocarpic grapes. As a result, the majority of commercial seedless grapes are stenospermocarpic cultivars, e.g. 'Thompson Seedless' (Ledbetter and Ramming, 1989; Varoquaux *et al*., 2000; Rombough, 2002).

Ogasawara (1985) reported that pre-bloom application of 200 to 400 ppm streptomycin, an antibiotic fungicide, on grape inflorescence induced stable and high percentage of seedlessness. Since then, the technique of seedless grape induction by streptomycin has been well established in Japan, but the relative studies are still scarce in Taiwan.

To have a better understanding of the mechanism of seedlessness caused by streptomycin, the effects of streptomycin application on pollen germination, pollen tube growth, and ovule development were investigated in this study. In addition, the fruit quality of seedless grapes and the optimal enlargement treatment of seedless grapes were also analyzed.

Materials and Methods

Plant materials

In this study, 20-year-old 'Kyoho' grapevines (*Vitis labruscana* × *V. vinifera*) grafted on Teleki 5C rootstocks cultivated in Viticulture Research Center, College of Agriculture and Natural Resources, National Chung-Hsing University were used. The grapevines were subjected to horizontal trellis system with X-shaped pruning. The vigorous fruiting shoots were used and only one inflorescence was retained on each shoot.

Pollen germination rate and pollen tube morphology

Experiment 1.

The inflorescences were dipped in 300 ppm streptomycin solution (contained 0.05 % Tween 20) (streptomycin sulfate, USB corporation) about 7 days before blooming (2020/03/15) and no treatment was applied to the control (CK). During the blooming (2020/03/22), 5 mature un-opened flowerlets were collected from the basal, middle, and distant part of the inflorescence in each replicate, 3 replicates per treatment. A total of 15 anthers (3 anthers from each flowerlet) were put into a 1.5 mL microtube containing 1 mL Brewbaker and Kwack (B&K) medium (10% sucrose, 100 ppm boric acid (H_3BO_3), 100 ppm potassium nitrate (KNO_3), 300 ppm magnesium

sulfate ($MgSO_4 \cdot 7\ H_2O$), and 300 ppm calcium nitrate ($Ca(NO_3)2 \cdot 4\ H_2O$)) (Brewbaker and Kwack, 1963). The microtubes with anthers were vortexed until pollens were suspended evenly in the medium and 0.3 mL of the suspension was pipetted into a double concave slide followed by incubating at 25 ℃ in a dark incubator for 2 hours. The pollen germination was examined through a light microscope (Nikon/Eclipse Ci-S); 3 different regions were inspected in a replicate. A pollen was considered germinated when the length of its tube was two time longer than the grain diameter. The percentage of abnormal pollen tube was also calculated, which included abnormal callose plug, swollen wall, swollen tip, and forked tip.

Experiment 2.

The inflorescences were dipped in 300 ppm streptomycin solution about 5 days before blooming (2020/08/07), no treatment was applied to the control (CK). The flowerlets were collected at anthesis (2020/08/12) and the pollen grain suspension was made as Exp. 1. The suspension was pipetted into a 90 mm petri dish containing B&K medium with 1 % agar and the pollen grains were incubated and examined as in Exp. 1. After the examination was completed, the pollen tubes were stained with 0.1 M K_3PO_4 containing 0.1 % aniline blue and observed through a fluorescence microscope (lamp: Model C-SHG1, Nikon; filter B-2A, Nikon).

***In vivo* pollen tube observation**

The inflorescences were dipped in 300 ppm streptomycin solution (contained 0.05 % Tween 20) about 7 days before blooming (2020/03/15) (treatment before blooming, TBB) or during blooming (2020/03/22) (treatment during blooming, TDB) and no treatment was applied to the control (CK). The ovaries of each treatment were collected 3 days after blooming and fixed with 50 % EtOH in vacuum until the ovaries sank and then stored at 4 ℃ until further use. The ovaries were macerated with 2 M NaOH at 60 ℃ oven for 24 hours and washed with distilled water followed by stained with 0.1 M K_3PO_4 containing 0.1 % aniline blue for 12 hours. The ovaries were placed on a slide glass and gently crushed by a cover glass and then kept in the dark until observed by using a fluorescence microscope.

Ovule development

The inflorescences were dipped in 300 ppm streptomycin solution (contained 0.05 % Tween 20) about 7 TBB or TDB and no treatment was applied to CK. The ovaries of 2020 winter crop (2020/08) were collected during blooming, 3 days, 5days, 8 days, and 11 days after blooming. The ovaries of summer crop (2021/03-04) were collected during blooming, 4 days, 7days, and 10 days after blooming. Ovule development was examined on cross-sections of berries. The number of normally developed ovule per berry was calculated at 11days (winter crop) and 10 days (summer crop) after blooming, respectively.

Hand-pollination experiment

Bud-break of two 'Kyoho' vines was induced by hydrogen cyanamide application at one-week interval. The early-vine was a pollen donor and the later-vine was a pollen acceptor. Inflorescences and the pollens of pollen donor vine was separated into two groups, treated with 300 ppm streptomycin 7 days before blooming (ST) and non-treated (NT). During the donor vine blooming, the opened flowerlets were discarded and the anthers of unopened mature flowers were collected. The anthers were air-dried at 25 ℃ and sieved through a 210 μm pollen sieve followed by weighting and storing the pollens at 4 ℃ until further use. The same streptomycin treatments were applied to the inflorescences of pollen acceptor vine as well. The pollen acceptor inflorescences were trimmed to a distance of 3-5 cm from the inflorescence tip, followed by emasculation and bagging 1 day before blooming. During the blooming, the pollens were mixed with lycopodium powder (日本ミツワ株式会社) at ratio 1:1 and pollinated by a brush which was wetted by 10% sucrose solution, followed by bagging to prevent the flowerlets pollinated by other pollens. Berry fresh weight, seedlessness, and seeds per berry were investigated at harvest (82 days after blooming). Notably, a berry with seed trace whose length was over 2 mm and with lignified seed coat was considered as a seeded berry.

Fruit quality

The inflorescences of 'Kyoho' grapes were dipped in 300 ppm streptomycin solution at EL stage 17 (Coombe, 1995) (about 7 days or 4-7 days before blooming in summer crop or winter crop, respectively) when the flowerlets were separated. The inflorescences were trimmed to 5 cm on the distant end. After fruit set, each cluster was thinned to about 35 berries. The experimental designs were described in Table 1 (summer crop) and Table 2 (winter crop). The summer and winter crop grapes were harvested 85 days and 83 days after blooming, respectively. After harvest, fruit quality was evaluated. Seedless berries were defined as no seed or the length of seed trace was less than 2 mm; 10 berries were randomly selected for each replicate. Berry color index was measured by using a purple and black grape color chart (The Ministry of Agriculture, Forestry and Fisheries of Japan); 10 berries per replicate. Total soluble solids (TSS) was measured by a digital pocket refractometer (PAL-1, ATAGO, Japan) and titratable acidity (TA) was measured by an auto-titrator (TIM-840, Radiometer Analytical, France).

Table 1. The experimental design for 'Kyoho' summer crop in 2021.

Treatment	7 DBB[z] 3/17	DB 3/23	10 DAB 4/2	20 DAB 4/12
CK	-	-	-	-
A	SM 300 ppm[y]	GA_3 12.5 ppm + CPPU 5 ppm	GA_3 25 ppm	-
B	SM 300 ppm	GA_3 1 ppm + CPPU 5 ppm	GA_3 15 ppm + CPPU 5 ppm	GA_3 30 ppm
C	SM 300 ppm	GA_3 1 ppm + CPPU 5 ppm	GA_3 15 ppm + CPPU 5 ppm	GA_3 30 ppm + CPPU 10 ppm
D	-	SM 300 ppm + GA_3 12.5 ppm + CPPU 5 ppm	GA_3 25 ppm	-

[z]DBB, days before blooming; DB, during blooming; DAB, days after blooming.

[y]SM, streptomycin.

Each treatment had 10 replicates.

Table 2. The experimental design for 'Kyoho' winter crop in 2021.

Treatment	4-7 DBB[z] 8/22	DB 8/29	10 DAB 9/8	20 DAB 9/18
CK	-	-	-	-
A	SM 300 ppm[y]	GA_3 12.5 ppm	GA_3 25 ppm + CPPU 5 ppm	-
B	SM 300 ppm	GA_3 1ppm	GA_3 15 ppm + CPPU 5 ppm	GA_3 30 ppm
C	SM 300 ppm	GA_3 1ppm	GA_3 15 ppm + CPPU 5 ppm	GA_3 30 ppm + CPPU 10 ppm
D	-	SM 300 ppm + GA_3 12.5 ppm	GA_3 25 ppm + CPPU 5 ppm	-

[z]DBB, days before blooming; DB, during blooming; DAB, days after blooming.

[y]SM, streptomycin.

Each treatment had 8 replicates.

Results

Pollen germination, pollen tube morphology and ovule development

Pollen germination rate was 1.5 % in streptomycin treatment which was significantly lower than the control (30.4 %) in summer crop. However, there was no significant difference between treatments in winter crop (Table 3). Pollen tube in both the control and streptomycin treatment showed some abnormity, including abnormal callose plug, swollen wall, swollen tip, and forked tip (Fig. 1). The abnormal pollen tube rates of streptomycin-treated pollens were 49.3 % and 61.7 % in summer and winter crop, respectively, which were significantly higher than the control in both crops (Table 3). Through *in vivo* pollen tube observation, it was found that pollens could germinate on the stigma in both the control and streptomycin treatment before or during blooming. Nevertheless, the majority of pollen tubes in streptomycin treatment were not able to penetrate the stigma and grow through the style (Fig. 2).

The ovules of 'Kyoho' grape rapidly developed at 8 to 11 days after blooming, however, the streptomycin treated ovules ceased to develop at 8 to 11 days after blooming (Fig. 3), which led to a significant reduction in number of normally developed ovule per berry (Table 4).

Hand-pollination experiment

The seedlessness rate of streptomycin-treated inflorescences pollinated with streptomycin-treated pollen or normal pollens was 100 % and 97.5 %, respectively. On the other hand, the seedlessness rate of normal inflorescences pollinated with streptomycin-treated pollen or normal pollens was 35 % and 16.7 %, respectively (Table 5).

Fruit quality in summer crop

All of the seedless 'Kyoho' grape berries, induced by streptomycin treatment, were significantly smaller than the seeded berries (Table 6). Among the seedless treatments, the berries treated with GA_3 or CPPU for 3 times (during blooming, 10 DAB, and 20 DAB) were significantly larger than those treated twice (during blooming and 10 DAB) (Table 6). The C treatment, 15 ppm GA_3 + 5 ppm CPPU at 10 DAB and 30 ppm GA_3 + 10 ppm CPPU at 20 DAB, showed significantly poorer peel coloration and lower TSS comparing with the seeded control (Table 6 and Fig. 4). The highest TA was also observed in the C treatment. The berries treated with 300 ppm streptomycin at 7 DBB, 12.5 ppm GA_3 + 5 ppm CPPU during blooming, and 25 ppm GA_3 at 10 DAB (treatment A) induced 94 % seedlessness (Table 6). Despite the fact that treatment B and C showed about 70 % seedlessness rate, most of the seeds in seeded berries were abnormal seeds (data not shown), the so-called floater, with empty embryo but lignified seed coat.

Fruit quality in winter crop

Seeded 'Kyoho' berries were significantly smaller than the seedless berries (Table 7). In all the seedless treatments, application of 300 ppm streptomycin at 7 DBB, 1 ppm GA_3 during

blooming, 15 ppm GA_3 + 5 ppm CPPU at 10 DAB, 30 ppm GA_3 at 20 DAB (treatment B) and 300 ppm streptomycin + 12.5 ppm GA_3 during blooming, 25 ppm GA_3 + 5 ppm CPPU at 10 DAB (treatment D), showed the highest berry weight (Table 7). Application of 30 ppm GA_3 (B) or 30 ppm GA_3 + 10 ppm CPPU at 20 DAB (C) hindered the berry peel coloration and TSS accumulation, but TA was not significantly affected, comparing with the seeded control (Table 7 and Fig. 4). Per-bloom application of 300 ppm streptomycin and 12.5 ppm GA_3 application during blooming (treatment A) induced 99 % seedlessness, which was significantly higher than treatment D (300 ppm streptomycin and 12.5 ppm GA_3 application during blooming) (Table 7).

Table 3. *In vitro* pollen germination rate of 'Kyoho' grape in 2020 summer and winter crop.

Crop	Treatment[z]	Germination rate (%)	Abnormal pollen tube rate (%)
Summer	CK	30.4 ± 17.7 a[y]	5.4 ± 0 b
	Streptomycin	1.5 ± 1.0 b	49.3 ± 22.0 a
Winter	CK	23.9 ± 14.9 a	14.4 ± 0.1 b
	Streptomycin	16.0 ± 8.2 a	61.7 ± 0.3 a

[z]300 ppm streptomycin was applied 7-10 days and 4-7 days before blooming in 2020 summer crop and winter crop, respectively.

[y]Mean ± SD, n= 3. Different letters within the same column indicate significant differences between the means by t-test (α=0.05). Percentage data were transformed by Arcsine transformation before t-test.

Table 4. Ovule development of 'Kyoho' grapes.

Crop	Treatment[z]	No. of normally developed ovule/berry
Summer	CK	1.5 ± 0.5 a[y]
	TBB	0 ± 0 b
	TDB	0 ± 0 b
Winter	CK	1.1 ± 0.6 a
	TBB	0.1 ± 0.3 b
	TDB	0.1 ± 0.3 b

[z]CK, control; TBB, streptomycin treatment about 7 days before blooming; TDB, streptomycin treatment during blooming.

[y]Mean ± SD (n= 10). Different letters within the same column indicate significant differences of the means by LSD test (α= 0.05)

Table 5. The results of hand-pollination experiment.

Treatment[z]	Seedlessness (%)	Berry weight (g)	No. of seed/berry
NT × NT	16.7 ± 17.0 b[y]	7.5 ± 1.1 a	1.10 ± 0.1 a
NT × ST	35.0 ± 12.9 b	5.8 ± 1.0 b	0.70 ± 0.1 b
ST × ST	100 ± 0 a	4.3 ± 0.2 c	0 ± 0 c
ST × NT	97.5 ± 5.0 a	4.2 ± 0.4 c	0.03 ± 0.1 c

[z]NT, non- streptomycin- treated parents; ST, streptomycin- treated parents.

[y]Mean ± SD (n= 4). Different letters within the same column indicate significant differences of the means by LSD test (α= 0.05). Percentage data were transformed by Arcsine transformation before LSD test. The mean imputation method was applied, due to the data missing in the NT × NT and the ST × ST treatments.

Table 6. Fruit qualities of 'Kyoho' grape in 2021 summer crop.

Treat-ment[z]	Berry weight (g)	Berry color index	TSS (°Brix)	TA (%)	TSS/TA ratio	Seedless-ness (%)
CK	10.6 ± 0.6 a[y]	7.7 ± 0.3 b	19.5 ± 0.4 a	0.28 ± 0.02 c	70.2 ± 6.1 a	0 ± 0 c
A	6.1 ± 0.7 c	8.2 ± 0.7 a	19.3 ± 0.7 a	0.31 ± 0.02 b	63.4 ± 7.1 b	94 ± 10 a
B	8.1 ± 1.0 b	7.8 ± 0.3 ab	18.4 ± 0.6 b	0.33 ± 0.02 b	56.7 ± 5.2 c	72 ± 17 b
C	8.7 ± 1.2 b	6.5 ± 0.7 c	18.2 ± 0.4 b	0.37 ± 0.02 a	50.1 ± 3.2 d	69 ± 17 b
D	6.2 ± 0.6 c	7.4 ± 0.6 b	19.6 ± 0.4 a	0.33 ± 0.03 b	59.2 ± 4.9 bc	87 ± 13 a

[z]CK, control; A, 300 ppm streptomycin was applied at about 7 DBB, 12.5 ppm GA_3 and 5 ppm CPPU were applied DB, 25 ppm GA_3 was applied at 10 DAB; B, 300 ppm streptomycin was applied at about 7 DBB, 1 ppm GA_3 and 5 ppm CPPU were applied DB, 15 ppm GA_3 and 5 ppm CPPU were applied at 10 DAB, 30 ppm GA_3 was applied at 20 DAB; C, 300 ppm streptomycin was applied at about 7 DBB, 1 ppm GA_3 and 5 ppm CPPU were applied DB, 15 ppm GA_3 and 5 ppm CPPU were applied at 10 DAB, 30 ppm GA_3 and 10ppm CPPU were applied at 20 DAB; D, 300 ppm streptomycin, 12.5 ppm GA_3 and 5 ppm CPPU were applied DB, 25 ppm GA_3 was applied at 10 DAB.

[y]Mean ± SD, n= 10. Different letters within the same column indicate significant differences of the means by the LSD test (α= 0.05). The rate of seedlessness data were transformed by Arcsine transformation before LSD test.

Table 7. Fruit qualities of 'Kyoho' grape in 2021 winter crop.

Treat-ment[z]	Berry weight (g)	Berry color index	TSS (°Brix)	TA (%)	TSS/TA ratio	Seedless -ness (%)
CK	7.3 ± 0.7 c[y]	8.0 ± 0.6 a	20.1 ± 0.5 a	0.38 ± 0.04 a	53.3 ± 6.0 bc	0 ± 0 d[y]
A	8.5 ± 0.7 b	7.6 ± 0.8 ab	20.1 ± 0.6 a	0.35 ± 0.03 bc	58.3 ± 6.2 ab	99 ± 4 a
B	9.4 ± 1.2 a	7.0 ± 0.7 b	19.2 ± 0.8 bc	0.39 ± 0.03 a	49.2 ± 5.5 c	96 ± 5 ab
C	8.5 ± 0.6 b	5.8 ± 1.2 c	18.8 ± 1.1 c	0.37 ± 0.03 ab	50.9 ± 6.3 c	89 ± 14 bc
D	9.4 ± 0.7 a	8.1 ± 0.6 a	19.9 ± 0.5 ab	0.32 ± 0.03 c	62.9 ± 6.2 a	84 ± 14 c

[z]CK, control; A, 300 ppm streptomycin was applied at about 7 DBB, 12.5 ppm GA_3 was applied DB, 25 ppm GA_3 and 5 ppm CPPU were applied at 10 DAB; B, 300 ppm streptomycin was applied at about 7 DBB, 1 ppm GA_3 was applied DB, 15 ppm GA_3 and 5 ppm CPPU were applied at 10 DAB, 30 ppm $GA_3$was applied 20 DAB; C, 300 ppm streptomycin was applied at about 7 DBB, 1 ppm GA_3 and was applied DB, 15 ppm GA_3 and 5 ppm CPPU were applied at 10 DAB, 30 ppm GA_3 and 5 ppm CPPU were applied at 20 DAB; D, 300 ppm streptomycin and 12.5 ppm GA_3 were applied DB, 25 ppm GA_3 and 5 ppm CPPU were applied at 10 DAB.

[y]Mean ± SD, n= 8. Different letters within the same column indicate significant differences of the means by the LSD test (α= 0.05). The rate of seedlessness and shattering rate data were transformed by Arcsine transformation before LSD test.

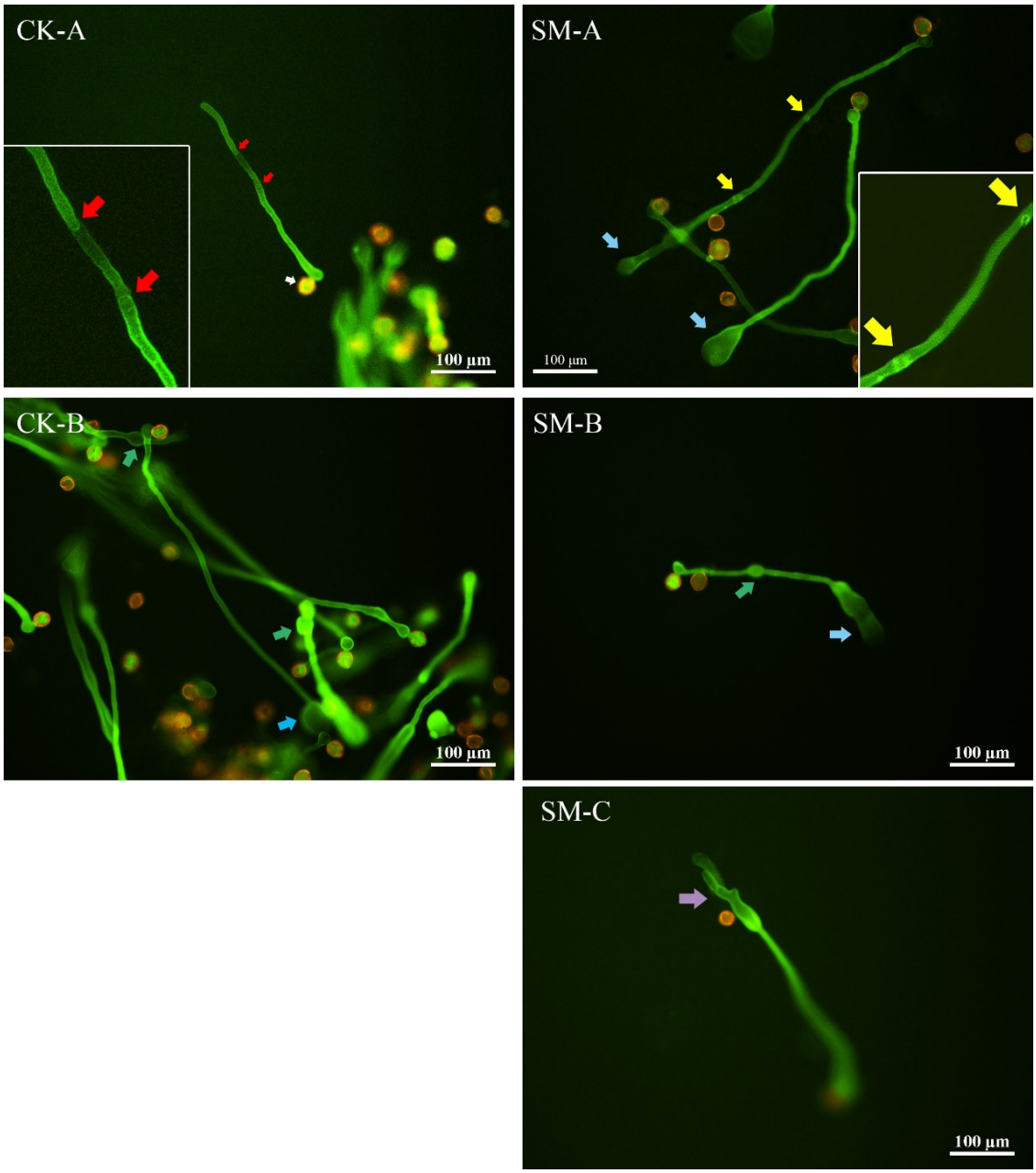

Fig. 1. Pollen tube morphology of 'Kyoho' grapes.

CK-A and CK-B, control pollen tube. SM-A, SM-B and SM-C, 300 ppm streptomycin was applied about 7 days before blooming. The red arrows indicate normal callose deposition, the white arrow indicates pollen grain, the green arrows indicate swollen pollen tube wall, the blue arrow indicates swollen pollen tube tip, the yellow arrows indicate abnormal callose plug, and the purple arrow indicate forked pollen tube tip. The experiment was conducted in August 2020.

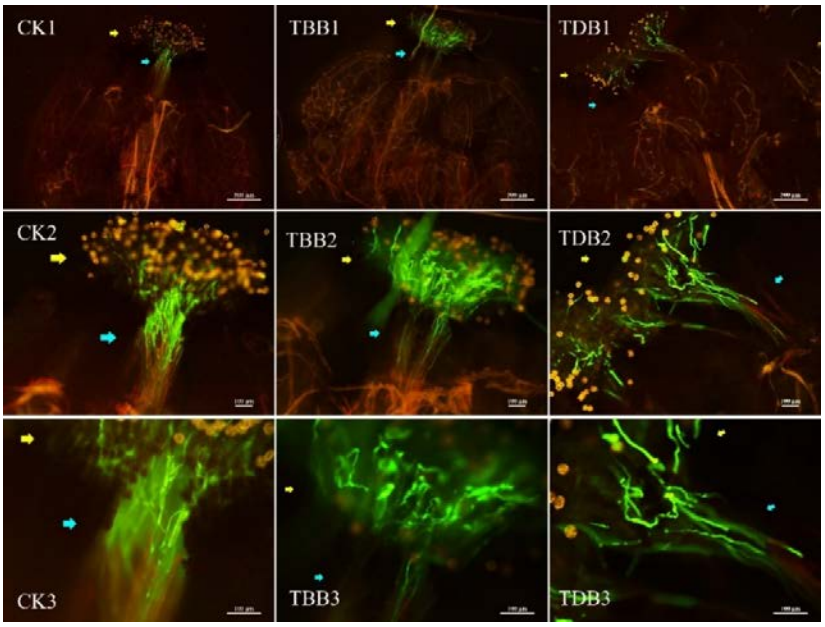

Fig. 2. Pollen tubes growth of 'Kyoho' grapes *in vivo*.

CK, control; TBB, streptomycin treatment about 7 days before blooming; TDB, streptomycin treatment during blooming. The yellow arrows indicate the stigma of the ovary, and the blue arrows indicate the style of the ovary. The yellow rounded particulates scattered on the stigma are pollen grains, and the green tubes in the style are pollen tubes. The experiment was conducted in March 2020.

Fig. 3. Ovule development of 'Kyoho' grapes.

CK, control; TBB, streptomycin treatment about 7 days before blooming; TDB, streptomycin treatment during blooming. Ovary (left), cross section of ovary (right). The red arrows indicate normal ovules and the yellow arrows indicate ovules ceased to develop. The experiment was conducted in August 2020. Bar = 1mm.

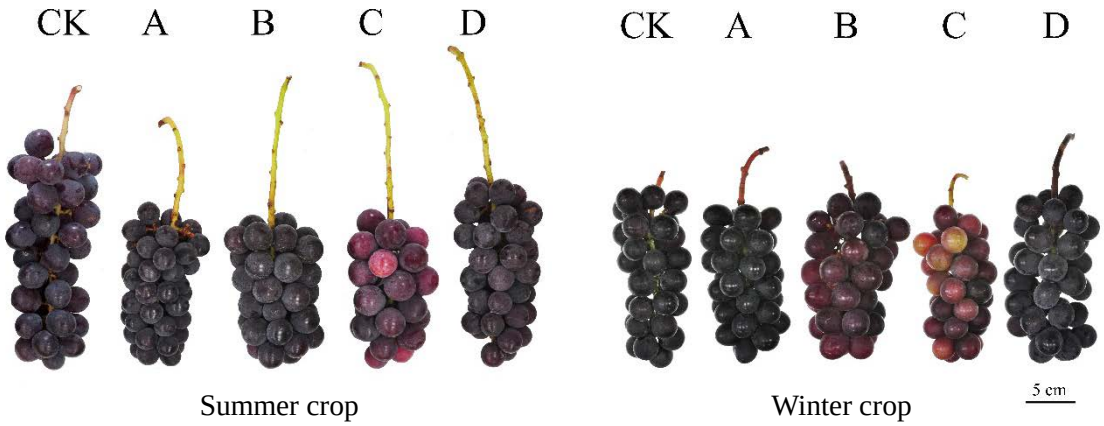

Fig. 4 Fruit clusters of 'Kyoho' grape in 2021 summer crop and winter crop.

Discussion

Streptomycin treatment at 7 days before blooming decreased pollen germination rate in summer crop, but not in winter crop, which indicated that the effect of streptomycin on pollen germination was not the same in different seasons. Nevertheless, streptomycin application caused a high proportion of abnormal pollen tube development *in vivo* and *in vitro* in both summer and winter crop. Therefore, abnormality of pollen tubes led to poor ovule fertilization could be a possible factor of seedlessness induced by streptomycin application. Previous literatures also reported that poor pollen germination, down-sizing pollen grains, and abnormal pollen tube development can be induced by streptomycin application (Ogasawara, 1985; Thanarut and Yang, 2014). However, Kimura *et al.* (1996) referred that streptomycin application significantly induced 'Muscat Bailey A' grape seedlessness, but did not affect pollen germination rate. The ovules of seeded 'Kyoho' berry started to rapidly develop 5-8 days after blooming; on the contrary, the ovules of streptomycin-treated berry ceased to develop. The results of hand pollination experiment demonstrated that streptomycin-treated inflorescences had significantly higher seedlessness than non-treated ones. Baba *et al.* (2008) reported that 200 ppm streptomycin application on 'Fujiminori' grape inflorescences about 2 weeks before blooming retarded egg apparatus and embryo sac development, while inhibition of pollen germination was less significant. The optimal timing of streptomycin application should be 3 to 1 weeks before full blooming when embryo sacs was developing; hence streptomycin application may cause a certain degree of damage to the developing embryo sacs, which gave rise to abortion of the embryo in the following development (Baba *et al.*, 2008). Taken together, inhibition of grape ovule development could be the major factor of seedlessness induced by streptomycin application.

Berry size, as an important index for high quality table grape, can be significantly affected by the number of seeds per berry (Walker *et al*., 2005). Seedless grape berry lacks stimulation from plant hormones produced by seeds, thus berry enlargement is subjected to be retarded. Consequently, exogenous application of plant growth regulators has become a necessary manipulation for the seedless grape production. Bordelon and Moore (1994) reported that GA inhibited grape seed development, instead CPPU promoted seed and seed trace development of stenospermocarpic grape. In the aspects of berry size, the responsivity of grape to CPPU application during blooming and 20 DAB were not as high as application at 10 DAB. Zabadal and Bukovac (2006) reported that CPPU application significantly induced berry enlargement when berry's diameters were 4-7 mm. The result may be associated with berry mesocarp cell division, which speeds up at fruit set and slows down 3 weeks after blooming (Coombe and McCarthy, 2000). Pérez *et al*. (2000) reported that GA_3 was the most efficient plant growth regulator for grape berry enlargement. Moreover, application with GA_3 and CPPU mixture induced greater berry weight than application with GA_3 or CPPU alone (Dokoolian *et al*., 1994). Previous studies revealed that with an increce in the number of GA_3 and CPPU applications, higher berry size, higher TA and lower TSS were observed (Ben-Tal, 1989; Peppi and Fidelibus, 2008), which are similar to our findings. To sum up, for the purpose of grape berry enlargement, our study suggested that application of GA_3 and CPPU mixture at 10 DAB will be an essential operation, and at 20 DAB, application of GA_3 alone should be enough.

Grape peel coloration is regulated by genes, temperature, orchard management, and plant growth regulators. In this study, the data showed that GA_3 + CPPU application at 20 DAB not only hindered berry coloration, but also caused heterogeneous coloration within a cluster. On the other hand, GA_3 + CPPU application at 10 DAB did not affect berry coloration. Previous literatures suggested that CPPU application inhibited grape peel anthocyanin accumulation, leading to poor coloration (Dokoozlian *et al*., 2000; Peppi and Fidelibus, 2008). Tyagi *et al*. (2021) demonstrated that GA_3 application did not affect grape peel coloration, nevertheless, CPPU or CPPU + GA_3 application significantly reduced peel anthocyanin content by downregulating expression of *MYB14*, *MYB15*, phenylpropanoid-related genes, and ripening-related genes. CPPU application at 20 DAB should be avoided to prevent from poor coloration occurrence.

References

Baba, T., K. Ishikawa, and F. Ikeda. 2008. Streptomycin inhibits embryo sac development in grape 'Fujiminori'. J. Agric. Sci. Tokyo Univ. Agric. 53: 139-143.

Ben-Tal, Y. 1990. Effects of gibberellin treatments on ripening and berry drop from 'Thompson

Seedless' grapes. Am. J. Enol. Vitic. 41: 142-146.

Brewbaker, J. L. and B. H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50: 859-865.

Coombe, B. G. and M. G. McCarthy. 2000. Dynamics of grape berry growth and physiology of ripening. Aust. J. Grape Wine Res. 6: 131-135.

Dokoozlian, N., M. M. Moriyama, and N. C. Ebisuda. 1994. Forchlorfenthuron (CPPU) increases the berry size and delays the maturity of 'Thompson Seedless' table grapes. In: Proc. Intl. Symp. Table Grape Prod. pp. 63-68.

Dokoozlian, N., N. Ebisuda, S. Hammamoto, and A. Macias. 2000. Influence of CPPU on the growth and composition of several table grape cultivars. Res. Rpt. Calif. Table Grape Comm. 31.

Kimura, P. H., G. Okamoto, and K. Hirano. 1996. Effects of gibberellic acid and streptomycin on pollen germination and ovule and seed development in 'Muscat Bailey A'. Am. J. Enol. Vitic. 47: 152-156.

Ledbetter, C. A. and D. W. Ramming. 1989. Seedlessness in grapes. Hortic. Rev. 11: 159-184.

Ogasawara, S. 1985. Establishment of seedless berry production technique of grapes by use of streptomycin preparation. I. Induction of parthenocarpy in grape by application of SM. Bul. Fruit Tree Expt. Sta. Hiroshima Prefect. 11: 39-49.

Peppi, M. C. and M. W. Fidelibus. 2008. Effects of forchlorfenuron and abscisic acid on the quality of 'Flame Seedless' grapes. HortScience 43: 173-176.

Pérez, F. J., C. Viani, and J. Retamales. 2000. Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. Am. J. Enol. Vitic. 51: 315-318.

Rombough, L. 2002. The grape grower: A guide to organic viticulture. Chelsea Green Publishing, Vermont.

Thanarut, C. and Y. S. Yang. 2014. Studies on the mechanism of seedless and seedlessness in 'Kyoho' (*Vitis vinifera* L. × *Vitis labruscana* Bailey) grapes induced by streptomycin. Acta Hortic. 1059: 205-212.

Varoquaux, F., R. Blanvillain, M. Delseny, and P. Gallois. 2000. Less is better: new approaches for seedless fruit production. Trends Biotechnol. 18: 233-242.

Walker, R. R., D. H. Blackmore, P. R. Clingeleffer, G. H. Kerridge, E. H. Rühl, and P. R. Nicholas. 2005. 'Shiraz' berry size in relation to seed number and implications for juice and wine composition. Aust. J. Grape Wine Res. 11: 2-8.

Zabadal, T. J. and M. J. Bukovac. 2006. Effect of CPPU on fruit development of selected seedless and seeded grape cultivars. HortScience 41: 154-157.

鏈黴素處理對'巨峰'葡萄花粉萌芽、胚珠發育與果實品質之影響

陳 柏 源[1)]　　陳 京 城[2)]

關鍵字：葡萄、鏈黴素、果實品質

摘要：本研究探討施用鏈黴素誘導'巨峰'葡萄無子化之機制，並分析無子'巨峰'葡萄經激勃酸(GA_3)及福芬素(CPPU)誘導果實肥大後品質之改變。結果顯示，胚珠發育受阻是鏈黴素誘導無子化的主要原因，而花粉管發育異常則是次要因素。經 GA_3 或 CPPU 處理之無子果實，隨著施用次數增加，果實重量也隨之上升。但花後 20 天施用 CPPU 卻會促使果皮轉色不良、總可溶性固形物下降與酸度上升。此外，開花期或花後 20 天施用 CPPU 亦導致種痕木質化之現象，降低無子率。

1) 國立中興大學園藝學系碩士班研究生。

2) 國立中興大學園藝學系助理教授，通訊作者。

本研究承蒙行政院農業委員會農糧署 110 農科-4.1.3-糧-Z1 (2)計畫補助部分經費，謹致謝忱。