Effects of GA_3 and CPPU on Fruit Quality in Seedlessness Oval Kumquat (*Fortunella margarita* Swingle)

Nimmannoradee Promtong [1] Ching-Lung Lee [2] Yau-Shiang Yang [3]

Keywords: streptomycin sulfate, seed number, fruit size

Summary

Ten years old potted kumquat plants were sprayed 240 ppm streptomycin sulfate 7 days before flowering and then followed by GA_3 20 ppm or CPPU 10 ppm treatment 7 days after flowering. Their fruit quality were investigated in beginning of mature stage in normal and off seasons. It was found that the percentage of seedlessness fruit reached 79-85% in both seasons and significantly higher than 11-22% of untreated control. In the distribution of seed number, it was shown that 65-66% fruits with 2 and 3 seeds, 8-21% fruits with 1 and 4 seed in untreated control. Whereas only 11-21% fruits with 1 seed was found in treatments of GA_3 and CPPU in both seasons. For fruit quality, GA_3 and CPPU improved fresh weight 2.5-3 g (23-25%) and 3 - 4.1g (28-35%) respectively. GA_3 enhanced higher TSS than CPPU and untreated control in both seasons. Titrable acidity was lower in fruits of GA_3 and CPPU treatments in normal season.

Introduction

Streptomycin was successfully used for seedlessness in grape (Fukunaga and Kurooka, 1988; Pommer *et al*., 1996; Ishikawa *et al*., 1996; Tanarut *et al*., 2010) and pummelo (Kitajima *et al*., 2004). In the previous experiment (Promtong *et al*., 2014), it was found that streptomycin sulfate 240 ppm could induced 76% seedless fruits in oval kumquat, but fruit became smaller in 14%.

---

1) Graduate student in Ph.D. Program, Department of Horticulture, National Chung Hsing University.
2) Chair Professor, National Chung Hsing University.
3) Professor, Department of Horticulture, National Chung Hsing University. Corresponding author.

Application of plant growth regulators for fruit enlargement was reported by many researchers, such as application of NAA in grape (Rizk-Alla *et al*., 2011) and mandarin (Greenberg *et al.*, 2006; Stover *et al*., 2006), GA_3 in sweet cherry (Silvija *et al*., 2012) and grape (Chen and Yang, 1989; Lin *et al*., 2009; Nampila *et al*., 2010), CPPU in pear (Shargal *et al*., 2006), persimmon (Hasegawa *et al*., 1991), kiwifruit (Kim *et al*., 2006), blueberry (NeSmith, 2002)and grape (Chen and Yang, 1989; Zabadal and Bukovac, 2006; Nampila *et al*., 2010), but it was fewer reports in oval kumquat.

The purpose of this experiment tried to use GA_3 and CPPU to improve fruit size and quality in seedlessness fruits after streptomycin sulfate pretreatment and to compare the difference between normal season and off season fruits.

Material and Methods

Plant materials

This experiment was carried out at the Grape Center of Horticulture Experiment Station, National Chung Hsing University, Taichung, Taiwan. Ten-year-old healthy oval kumquat plants (grafted on sour orange) were grown in plastic containers (36 cm in diameter) with a 3 : l mixture of soil and peat moss media and strong vigor plants (110 cm in height) were selected for experiments. For normal season, shoot pruning was carried out on January 15, 2010. After having new shoots matured in late March, healthy and uniform shoots were selected for treatments. The off season experiment was conducted at Department of Horticulture in National Chung Hsing University. 11-year-old oval kumquat plants were used for experiment and shoot pruning was carried out on July 15, 2011. After new shoots matured in late August, healthy and uniform shoots were selected for treatments.

Methods of experiment

1. Streptomycin sulfate and PGRs treatments

The completely randomized design (CRD) was used in this experiment with 2 treatments and 5 replications (potted plants). Twelve shoots with 10 uniform flower buds on each plant for this experiment. Streptomycin sulfate 240 ppm was applied to flower buds on April 1 of 2010 and September 15 of 2011 (7 days before flowering) for normal season and off season, respectively. Flower buds sprayed distilled water only was used as the untreated control. Following treatment of GA_3 20 ppm or CPPU 10 ppm was carried out on April 15 and September 15 (7 days after flowering) for normal season and off season, respectively. In both seasons, young fruits were thinning to remain10 fruits in treatment of each plant on the 37th

days after flowering. All fruits were harvested 150 days after flowering.

2. Investigation of seed number

Seed number was counted from 8 fruits per treatment in each plant (totally 40 fruits) and calculated the percentage of seedless fruit and seed number distribution.

3. Fruit quality analysis

1) Fresh weight of fruit

Electronic weighing scale was used to weigh each fruit.

2) Total soluble solids (TSS) of juice

Determined by handheld tortuous account (Hand refractometer, ATAGO).

3) Titratable acid (TA) of juice

Eight fruits of same plant were mixed it's juice for TA measurement. ATAGO FS-2 was used for the measurement of acidity. Juice was diluted by adding 9 ml distilled water into 1ml sampled juice, and titrated by using 0.1N NaOH, then calculated as citric acid content.

Results

1. Normal season

Effects of GA_3 and CPPU after streptomycin sulfate pretreatment on seed number, it was found their normal and total seed number of fruit were significantly less than the untreated control, whereas more empty seed number was found in fruits of treatments. For the percentage of seedless fruit, it was shown that 79-84% was obtained in treatments of streptomycin sulfate and GA_3 or CPPU, which was significantly higher than 11% of untreated control (Table 1).

Table 1. Effects GA_3 and CPPU after streptomycin sulfate pretreatment on seed number and percentage of seedless fruit in oval kumquat in normal season.

Treatments[z]	Seed number / fruit			Seedless fruit (%)
	Normal	Empty	Total	
Untreated control	3.2a[y]	0.5b	3.7a	11.5b
SM 240 ppm→ GA_3 20 ppm	0.2b	1.1a	1.3b	84.6a
SM 240 ppm → CPPU 10 ppm	0.5b	1.9a	2.4b	79.2a

[z]Streptomycin sulfate was treated on the 7th day before flowering, GA_3 and CPPU were treated on the 7th day after flowering. Flowering time was April 8, 2010.

[y]Means within a column followed by the same letter are not significantly different by Duncan's MRT at $p \leq 0.05$.

In the distribution of seed number, result showed there were 65% fruits with 2 and 3 seeds, 21% fruits with 1 and 4 seeds in untreated control, whereas only 15-21% fruits with 1 seed and 79-84% seedless fruits was found in treatment of streptomycin and GA_3, CPPU (Fig. 1).

For effects of GA_3 and CPPU after streptomycin sulfate pretreatment on fruit quality, it was found that GA_3 and CPPU improved fruit weight 2.5 g and 3 g, respectively. It increased 23-28% in relative ratio as compared with untreated control. The total soluble solids of juice in fruits of GA_3 treatment were significantly higher than that in CPPU treatment and untreated control. For the titrable acidity, it was lower in fruits of GA_3 and CPPU treatments (Table 2).

2. Off season

Effects of GA_3 and CPPU after streptomycin sulfate pretreatment on normal seed and total seed number of fruit were similar to normal season. They were less than the untreated control, and had more empty seed in fruits. For the percentage of seedless fruit, it was found that 81-85% was also obtained in treatments of GA_3 and CPPU. Although it had higher percentage in 22% as compared with normal season in untreated control, but it showed significantly lower percentage than that of treatments (Table 3).

For the distribution of seed number in off season, it was found that 22% fruits was seedless in untreated control which was 11% higher, but 1 and 4 seeds was 13% lower than that in normal season. Fruit with 2 and 3 seeds was 66% which showed the same percentage as normal season. Whereas there was no fruit has more than 1 seed and only 15-19% fruits with 1 seed was found in treatments of GA_3 and CPPU (Fig. 2).

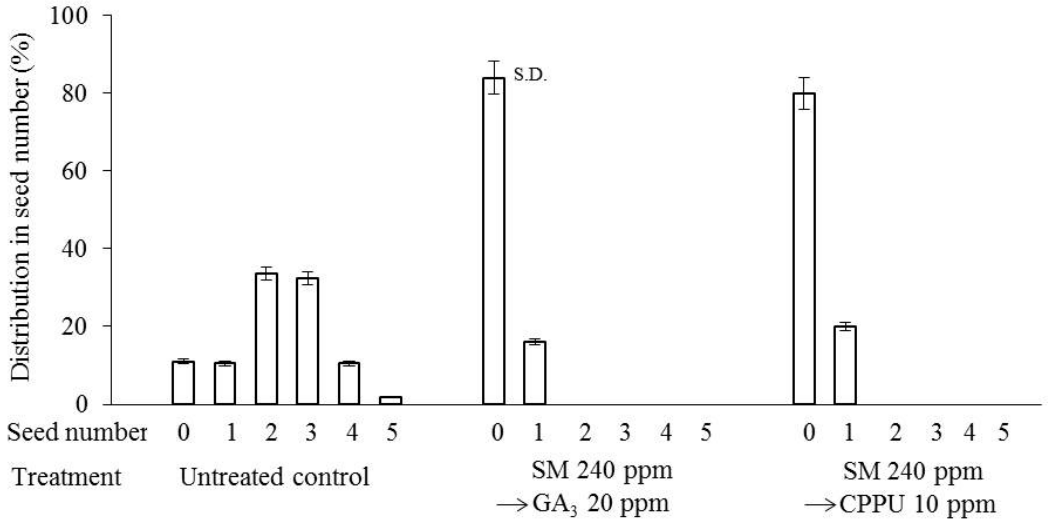

Fig. 1. Effects of GA3 and CPPU after streptomycin sulfate pretreatment on distribution of seed number in oval kumquat fruits in normal season.

Table 2. Effects of GA_3 and CPPU after streptomycin sulfate pretreatment on fruit quality of oval kumquat in normal season.

Treatments[z]	Fresh weight of fruit (g)	Total soluble solids (^oBrix)	Titratable acidity (mg/ 100 ml)
Untreated control	10.6b[y](100)[x]	7.5b	1.8a
SM 240 ppm → GA_3 20 ppm	13.1a (123)	8.2a	1.6b
SM 240 ppm → CPPU 10 ppm	13.6a (128)	7.7b	1.5b

[z]Streptomycin sulfate was treated on the 7th day before flowering, GA_3 and CPPU were treated on the 7th day after flowering. Flowering time was April 8, 2010.

[y]Means within a column followed by the same letter are not significantly different by Duncan's MRT at $p \leq 0.05$.

[x]Relative ratio to the untreated control.

Table 3. Effects GA_3 and CPPU after streptomycin sulfate pretreatment on seed number and percentage of seedless fruit in oval kumquat in off season.

Treatments[z]	Seed number / fruit			Seedless fruit
	Normal	Empty	Total	(%)
Untreated control	3.0a[y]	0.9b	3.7a	22.4b
SM 240 ppm→ GA_3 20 ppm	0.3b	1.7a	2.0b	85.0a
SM 240 ppm → CPPU 10 ppm	0.4b	1.7a	2.1b	81.0a

[z]Streptomycin sulfate was treated on the 7th day before flowering, GA_3 and CPPU were treated on the 7th day after flowering. Flowering time was September 22, 2011.

[y]Means within a column followed by the same letter are not significantly different by Duncan's MRT at $p \leq 0.05$.

For the distribution of seed number in off season, it was found that 22% fruits was seedless in untreated control which was 11% higher, but 1 and 4 seeds was 13% lower than that in normal season. Fruit with 2 and 3 seeds was 66% which showed the same percentage as normal season. Whereas there was no fruit has more than 1 seed and only 15-19% fruits with 1 seed was found in treatments of GA_3 and CPPU (Fig. 2).

For the effect of GA_3 and CPPU after streptomycin sulfate pretreatment on fruit quality, results were shown a similar trend as in normal season. GA_3 and CPPU increased fruit weight

3g and 4.1g respectively, it increased 25-35% in relative ratio as compared with untreated control. The total soluble solids of juice in GA_3 treatment was also significantly higher than that in CPPU treatment and untreated control. However, there was no significant difference in titrable acidity between GA_3, CPPU treatments and untreated control (Table 4).

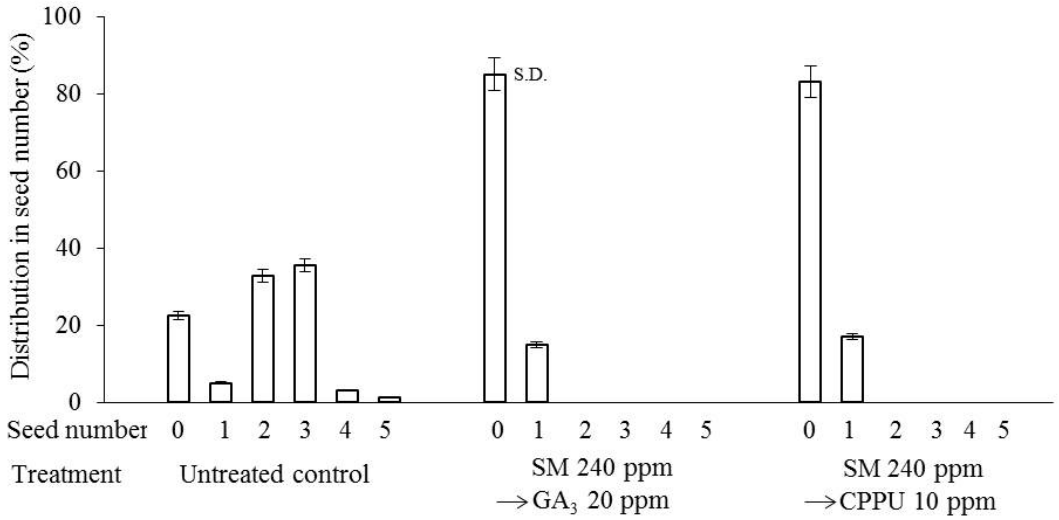

Fig. 2. Effects of GA_3 and CPPU after streptomycin sulfate pretreatment on distribution of seed number in oval kumquat fruits in off season.

Table 4. Effects of GA3 and CPPU after streptomycin sulfate pretreatment on fruit quality of oval kumquat in off season.

Treatments[z]	Fresh weight of fruit (g)	Total soluble solids (°Brix)	Titratable acidity (mg/ 100 ml)
Untreated control	11.6b[y] (100)[x]	7.6b	1.6a
SM 240 ppm → GA_3 20 ppm	14.6a (125)	8.6a	1.7a
SM 240 ppm → CPPU 10 ppm	15.7a (135)	7.7b	1.5a

[z]Streptomycin sulfate was treated on the 7th day before flowering, GA_3 and CPPU were treated on the 7th day after flowering. Flowering time was September 22, 2011.

[y]Means within a column followed by the same letter are not significantly different by Duncan's MRT at $p \leq 0.05$.

[x]Relative ratio to the untreated control.

Discussion

Streptomycin sulfate induced about 100% seedless fruit in grapes (Fukunaga and Kurooka, 1988; Pommer *et al*., 1996; Tanarut *et al*., 2010) and 'Tosa Buntan' pomelo (Kitajima *et al*., 2004). In this experiment, streptomycin sulfate pretreatment and follow applied with GA_3 or CPPU in oval kumquat resulted in producing a lower percentage of seedless fruit in 79-85%, but most of seeded fruits contained only 1 small seed. It was considered that there were high percentages in viable pollen and pollen germination after streptomycin sulfate treatment (Promtong *et al*., 2014). For the distribution of seed number in fruit, it was shown that the distribution of untreated control was different between normal season and off season (Wang and Yang, 1984). Whereas no difference was found in the distribution of seed number between GA_3 and CPPU treatment after streptomycin sulfate pretreatment in both seasons. This result implied that climatic change does not affect its distribution of seed number.

For the fruit size, it was found that fresh weight decreased 15% after streptomycin sulfate 240 ppm treatment in the previous experiment (Promtong *et al*., 2014). In this experiment, the following treatment of GA_3 and CPPU improved 23-25% and 28-35% in normal season and off season, respectively. The enhancing effect of GA_3 in cell enlargement was due to the increase in sink strength for accumulating nutrients (Lu *et al*., 1995; Taleb and Zahra, 2010; Zhenming *et al*., 2008). CPPU showed more effect in cell division and enlargement (Hamada *et al*., 2008).

For the total soluble solids, GA_3 treatment improved 0.5-1^{o} Brix as compared with CPPU treatment and untreated control in both seasons because the sink strength of GA_3 treated fruit became higher (Sarkar and Ghosh, 2005; Taleb and Zahra, 2010). For the titrable acidity, it was no significant difference between GA_3, CPPU treatments and untreated control in off season, but the titrable acidity became lower after treatment of GA_3 and CPPU in normal season. It seemed that GA_3 and CPPU improved fruit maturity easily under warmer climate (Nkansah *et al*., 2012).

Acknowledgements

The authors would like to express thanks to Dr. Zi-Bing Huang, Department of Horticulture and Biotechnology, Chinese Culture University for suggestions. Also thanks to Lan Yan Branch Station of Hualian District Agriculture Experiment and Extension Station supported healthy seedlings.

References

Chen, B. S. and Y. S. Yang. 1989. Effect of GA_3 and KT-30 on berry growth of winter ‘Kyoho’ grapes. Horticulture NCHU. 14: 15-30.

Fukunaga, S. and H. Kurooka. 1988. Studies on seedlessness of ‘Kyoho’ grapes induced by gibberellins in combination with streptomycin. Bull. Univ. Osaka Prefect. Ser. B: Agri. Biol. 40: 1-10

Greenberg, J., I. Kaplan, M. Fainzack, Y. Egozi, and B. Giladi. 2006. Effects of auxin sprays on yield, fruit size, splitting and the incidence of creasing of ‘Nova’ mandarin. Acta Hort. 727: 249-254.

Hamada, K., K. Haegawa, and T. Ogata. 2008. Effects of CPPU and strapping on fruit size and maturity in ‘Hiratanenashi’ Japanese persimmon. J. Hort. Sci. Biol. 83:477-480.

Hasegawa K, N. Kuge, T. Mimura, and Y. Nakajima. 1991. Effects of KT-30 and GA_3 on the fruit set and the fruit growth of persimmon cvs. Saijo and Hiratanenashi. J. Japan. Soc. Hort. Sci. 60: 19-29.

Ishikawa, K. H., H. Takahashi, H. Kata, and F. Ekeda. 1996. Effect of streptomycin on seedless of ‘Fujiminori’ and ‘Kyoho’ grapes. J. Japan. Soc. Hort. Sci. 65: 240-241.

Kim, J. G., Y. Takami, T. Mizugami, K. Beppu, T. Fukuda, and I. Kataoka. 2006. CPPU application on size and quality of hardy kiwifruit. Sci. Hort.110: 219-222.

Kitajima, A., A. Yamasaki, and K. Hasegawa. 2004. Seedless fruit production in Tosa Buntan pummelo. J. Japan. Soc. Hort. Sci. 20: 469-475.

Lin, R. J., B. S. Chen, and Y. S. Yang. 2009. Effects of plant growth regulators on fruit growth and quality of ‘Kyoho’ and ‘Honey Red’ grapes. Horticulture NCHU. 34: 1-11.

Lu, J., O. Lamikanra, and S. Leong. 1995. Effects of gibberellic acid on Muscadine grape production. Proc. Fla. State Hort. Soc., 108: 360-361.

Nampila, R., B. S. Chen, C. C. Chen, and Y. S. Yang. 2010. Effects of GA_3 and CPPU on berry size of seedless grapes. Horticulture NCHU 35(3): 53-64.

NeSmith, D. S. 2002. Response of rabbiteye blueberry (*Vaccinium ashei* Reade) to the growth regulators CPPU and gibberellic acid. Hort. Sci. 37:666-668.

Nkansah, G. O., J. Ofosu- Anim, and A. Mawuli. 2012. Gibberellic acid and naphthalene acetic acid affect fruit retention, yield and quality of Keitt mangoes in Coastal Savanna ecological zone of Ghana. Amer. J. Plant Physiol. 7: 243-251.

Pommer, C. C., E. J. Pires, M. M. Terra, and I. R. S. Passor. 1996. Streptomycin induced seedlessness in the grape cultivar Rubi (Italia Red). Amer. J. Enol. Vitic.47: 340-342.

Promtong, N., C. L. Lee, and Y. S. Yang. 2014. Effects of streptomycin sulfate on seedlessness

in oval kumquat (*Fotunella margarita* Swingle). Horticulture NCHU 39(2): 1-12.

Rizk-Alla, M. S., M. A. Abd El-Wahab, and O. M. Fekry. 2011. Application of GA_3 and NAA as a means for improving yield, fruit quality and storability of cv. Black Monukka grape. Nature and Sci. 9:1-19.

Sakar, S. and B. Ghosh. 2005. GA_3 application increased fruit weight, volume and length of fruit and also increased soluble sugar content in mango cv. Amrapali. Environ. Ecol. 23: 379-380.

Shargal, A., S. Golobovich, Z. Yablovich, L. A. Shlizerman, R. A. Stern, G. Grafi, S. Lev-Yadun, and M. A. Flaishman. 2006. Synthetic cytokinins extend the phase of division of parenchyma cells in developing pear (*Pyrus communis* L.) fruits. J. Hort. Sci. Biotech. 81: 915-920.

Silvija, Z., C. Zlatko, and J. Tomislav. 2012. Size and weight of sweet cherry (*Prunus avium* L. 'Regina') fruit treated with 3,5,6-TPA and GA_3. Agri. Conspectus Sci. 77: 45-47.

Stover, E., S. Ciliento, M. Myers, and B. Boman. 2006. Fruit size and yield of mandarins as influenced by spray volume and surfactant use in NAA thinning. Hort. Sci. 41: 1435-1439.

Taleb, R. and A. Zahra. 2010. Berry size of Thompson Seedless as influenced by the application of gibberellic acid and cane girdling. Pak. J. Bot. 42:1755-1760.

Tanarut, C., C. C. Chen, and Y. S. Yang. 2010. Effects of streptomycin, GA_3 and CPPU treatments on induction of seedless berry in 'Kyoho' and 'Honey Red' grapes. Horticulture NCHU 35(3): 1-11.

Wang, N. L. and Y. S. Yang. 1984. Seed number of summer and winter 'Kyoho' grapes. Horticulture NCHU 9: 17-22.

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

Zhenming, N., X. Xuefeng, W. Yi, L. Tianzhong, K. Jin, and H. Zhenhai. 2008. Effects of leaf applied potassium, gibberellin and source-sink ratio on potassium absorption and distribution in grape fruits. Sci. Hort. 115: 164-167.

GA_3 及 CPPU 對無子長實金柑 (*Fortunella margarita* Swingle)果實品質之影響

石 巧 月[1)] 李 金 龍[2)] 楊 耀 祥[3)]

關鍵字：鏈黴素、種子數、果實大小

摘要：本研究以 10 年生盆栽長實金柑植株為試驗材料，分別在正產期及非正產期之開花前 7 日先以鏈黴素 (streptomycin sulfate) 240ppm 噴施花蕾誘導無子化，再於花後 7 日以 $GA_3$20ppm 或 CPPU10ppm 處理幼果後，在果實開始成熟時調查其對果實品質之影響。

由試驗之結果發現，兩產期經鏈黴素及 GA_3 或 CPPU 處理者的無子率可達 79-85%，顯著高於未處理對照組的 11-22%。在種子數的分布率，可知未處理的對照組 65-66%的果實含有 2 個及 3 個種子，8-21%含有 1 個及 4 個種子。相對地在 GA_3 或 CPPU 處理者則僅有 11-21%的果實含有 1 個種子，其他為無子。在果實的品質，GA_3 促進果實的鮮重增加 2.5-3g(23-25%)，CPPU 則促進 3-4.1g(28-35%)。GA_3 在兩產期皆增加果實糖度，GA_3 及 CPPU 在正產期會促使果實酸度降低。

1) 國立中興大學園藝學系博士班研究生。
2) 國立中興大學講座教授。
3) 國立中興大學園藝學系教授，通訊作者。