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Full Length Article

Exogenous Applications of Plant Growth Regulators Improve Quality of 'Fuji' Apple

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Abstract

Apple production and consumption in Asian regions are increasing with economic development and the demand for high-quality fruit. In this study, we conducted three experiments: i) GA_3 and GA_{4+7} paste treatment, ii) forchlorfenuron (CPPU) treatment with and without GA_{4+7} spraying and iii) CPPU spray treatment at different concentrations under different conditions. The GA_3 and GA_{4+7} paste treatments conducted 30 days after full bloom (DAFB) enhanced fruit growth without malformation; however, the application of this treatment was difficult. In contrast, CPPU spraying with or without GA_{4+7} enhanced fruit growth easily, but the 4 DAFB treatments induced severe fruit malformation. Among the application stages and CPPU concentrations tested, the 1 mg L^{-1} CPPU treatment on 10–20 DAFB and the 5–10 mg L^{-1} CPPU treatment on 30 DAFB produced 9–18% larger fruits without malformation than the control. The 1 mg L^{-1} CPPU treatment applied 10–20 DAFB induced a 1% decrease in the soluble solid content; whereas, 5–10 mg L^{-1} CPPU treatment application on 30 DAFB did not decrease soluble solid content. In conclusion, 1 mg L^{-1} CPPU treatment application on 10–20 DAFB or 5–10 mg L^{-1} CPPU treatment on 30 DAFB are recommended to improve the fruit size of 'Fuji' apples. © 2018 Friends Science Publishers

Keywords: Cytokinin; Fruit quality; Fruit size; GA; *Malus× domestica*

Introduction

Apple (*Malus×domestica* Borkh.) production in 2014 reached approximately 84.6 million metric tons worldwide. This is slightly less than that of bananas and similar to that of grapes and oranges; thus, apples are one of the most important fruit crops in the world (FAOSTAT, 2017). China produces almost half of these apples and exports it to countries all over the world, including India, Russia, and South East Asian countries (Mulderij, 2017). Moreover, China has a large domestic market for apples as they are consumed in the country as well (Wang *et al*., 2017). As South-East Asia is a tropical region, the conditions in these countries are not suitable for apple production. Nevertheless, apple consumption in these countries is increasing markedly, parallel to economic development (FreshPlaza, 2015). Therefore, Asian regions are potentially a huge market for apple production and consumption, by which apple industry could be developed in these regions in the near future.

In Asian countries, both premium and low-quality apple markets exist (Mulderij, 2017). The premium apple is used as a gift for special ceremonies *e.g.*, Luna New Year cerebrations between late-January and late-February. A large size, bright red skin color, and high sugar content are considered the properties of premium apples by high-income class consumers. Fruit size is one of the most important factors used to determine the value of the fruit, and larger fruits are more expensive than smaller ones (Zhang *et al*., 2005). Thus, to produce larger fruits, a range of cultivation techniques *e.g.*, appropriate pruning and fruit thinning have been developed (Westwood, 1993).

The application of plant growth regulators (PGRs) also has been used as a practical technique to increase the fruit size. For Japanese pears, the application of gibberellin A_3 (GA_3) paste to the fruit peduncle is a common cultivation technique to enhance fruit size in Japan (Ito *et al*., 2016). For apples, the spraying of gibberellin A_{4+7} (GA_{4+7}) and cytokinins (BA: benzyl adenine) is used as practical technique for enhancing fruit set and growth in Northern America; however, these treatments also enhance fruit elongation showing conical shaped fruits (Martin *et al*., 1970; Westwood, 1993; Mankotia *et al*., 2006). In Asian countries including Japan, China, and Korea, people prefer round shaped apple fruits like 'Fuji' and many apple cultivars released in these countries have a round shape. The PGRs treatment in Northern America changes the shape and external appearance of the fruits dramatically. For that reason, the treatment cannot be directly introduced to the Asian countries as a practical tool to enhance fruit development.

Gibberellin A_3, GA_{4+7} and forchlorfenuron (CPPU) are

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the potential PGRs to stimulate fruit development without fruit malformation. However, there are no reports about the suitable kinds of PGRs including the PGRs' combination, application method, their concentrations and application timing. Therefore, we intended to develop new techniques to stimulate fruit development to produce high-quality, large, and round shaped fruits, which are acceptable for the Asian market.

In this study, we conducted three experiments: i) GA_3 and GA_{4+7} paste treatment, ii) CPPU treatment with or without GA_{4+7} spraying, and iii) CPPU spraying treatment at different concentrations and under varying conditions, and determine the most suitable treatment for increasing 'Fuji' apple fruit size without malformation.

Materials and Methods

Experiment 1: GA_3 and GA_{4+7} Paste Treatments

The experiment was conducted using four10-year-old trees derived by grafting 'Fuji' apples onto a *Malus prunifolia* rootstock, trained to a leader type form (7.5 m × 3.5 m planting) and grown on an experimental farm in Hirosaki University, known as Fujisaki farm (40°39′25″N, 140°29′9″E) in 2012. For the treatments, 2.7% (w/w) GA_3 (Kyowa Hakko Bio Co. Ltd., Tokyo, Japan) and/or 1.2% (w/w) GA_{4+7} pastes (a gift from Kyowa Hakko Bio Co. Ltd.) were used. Uniformly growing fruits were randomly selected from these trees before the treatments, and 30–40 mg of a GA_3 or GA_{4+7} paste was applied to the middle portion of the pedicels. These plant growth regulators were applied to ~40 fruits per treatment on 20, 30, or 40 days after full bloom (DAFB). The control fruits were not treated and conventional horticultural practices were applied to all fruits and trees.

Thirty fruits were randomly harvested from each treatment on November 5, 2012 and fruit weight, length, and width were measured. Soluble solids and malic acid content, fresh firmness, and skin color and water core indices were measured using 10 randomly selected fruit. The skin color index was scored on a scale of 0–4: 0=red color covering less than 30% of the fruit, 1=red color covering less than 50%, 2=red color covering more than 50%, 3=red color covering more than 80%, and 4=red color completely covering the entire peel). Firmness was measured after skin removal—to measure the firmness of the fruit alone—at two points on the equatorial area of the fruit using a penetrometer with an 11.1 mm tip (FT327; Facchinisrl, Alfonsine, Italy). Thereafter, the fruit was cut equatorially and the water core index, an important factor for Japanese consumers, was determined based on the following scale: 0 (did not appear), 1 (very little: only vascular bundles), 2 (little: around vascular bundles), 3 (middle: covered 20% of equatorial area), 4 (large: covered 50% of equatorial area) (Enseikai, 2014). In some Asian countries, especially Japan, watercore is a desirable trait in apples, as it is an indicator of full ripeness (Zupan *et al*., 2015). The apples were divided into eight pieces lengthwise and four nonadjacent pieces were squeezed with a hand juicer. The total soluble solids content of the juice was determined using a digital refractometer (N-1; Atago, Tokyo, Japan). The total titratable acidity was measured by titration with 0.1 N NaOH and represented malic acid content.

Experiment 2: Spraying Treatment of Forchlorfenuron (CPPU) 10 mg L^{-1} or CPPU 10 mg L^{-1} + GA_{4+7} 100 mg L^{-1} Solutions

This experiment was conducted in 2013 using four 'Fuji' apple trees planted in the same row as the trees used in the first experiment. Forchlorfenuron (CPPU) (Kyowa Hakko Bio Co. Ltd.) solutions (10 mg L^{-1}) with or without100 mg L^{-1} of GA_{4+7} solution (a gift from Kyowa Hakko Bio Co. Ltd.: 17.6% of GA_{4+7} solution was diluted) were used for this experiment. Approximately 200 fruit per treatment were hand-sprayed with 2.5 mL of these PGRs, on 4 and 30 DAFB. The fruits in the control were not sprayed and conventional horticultural practices were applied to all fruits and trees.

One hundred and sixty fruits were randomly harvested from each treatment on November 9, 2013 and fruit weight, length, and width were measured. Soluble solids and malic acid content, fresh firmness, water-core index, malformed fruit ratio, and skin color difference (L*, a*, b*, and hue angle) were measured using 40 randomly selected fruit. Skin color difference (L*, a*, b*, and hue angle) was measured at two points facing each other *via* the core on the equatorial area using a color difference meter (NF333; Nippon Denshoku, Tokyo, Japan), and the average values were combined into one dataset. The measurement methods of the other fruit quality parameters were the same as described earlier in the first experiment.

Experiment 3: Spraying Treatment of Different Concentration of CPPU Solutions

This experiment was conducted in 2014 using 10 'Fuji' apple trees planted on the same row as the trees used in the first two experiments and1, 5, and 10 mg L^{-1} CPPU (Kyowa Hakko Bio Co. Ltd.) solutions. Approximately 130 fruit per treatment were hand-sprayed with 2.5 mL of these CPPU solutions, on 10, 20, or 30 DAFB. The fruits in the control were not sprayed and conventional horticultural practices were applied to all fruits and trees.

One hundred fruits were randomly harvested from each treatment on November 7, 2014 and fruit weight, length, width, and malformed fruit ratio were measured. Soluble solids and malic acid content, fresh firmness, watercore index, and skin color difference (L*, a*, b*, and hue angle) were measured using 50 randomly selected fruit. The measurement methods were the same as described in the second experiment.

Statistical Analysis

The data were analyzed using Tukey-Kramer honest significant difference (HSD), two-way ANOVA, or Chi-squared tests. The JMP IN software (SAS Institute; Cary, NC, USA) was used for the first two analyses and js-STAR (KISNET; Kashiwazaki, Japan) software for the Chi-squared test. Significant differences among the treatments were determined. Unless otherwise stated, differences were considered statistically significant at $P < 0.05$.

Results

Experiment 1: Effect of GA_3 and GA_{4+7} Paste Treatments on Fruit Quality Parameters

The concentration of GA_{4+7} was less than half of GA_3. However, GA_{4+7} application had a similar effect on 'Fuji' apple fruit growth as GA_3, suggesting that GA_{4+7} increased fruit size more effectively than GA_3. The analysis of variance indicated that the application of GA_3 and GA_{4+7}, and the timing of treatments had different effects on fruit quality parameters (Table 1). Gibberellin A_3 and GA_{4+7} on 30 DAFB effectively increased fruit size resulting in heavier fresh weights, and higher fruit lengths and widths than the control (Table 1). Soluble solid content after the GA_3 treatment on 30 and 40 DAFB and the GA_{4+7} treatment on 20 DAFB were similar to the control, but the soluble solid content in the GA_3 treatment on 20 DAFB and the GA_{4+7} treatment on 30 and 40 DAFB was lower than that in the control. The GA_3 and GA_{4+7} treatments reduced malic acid content on all treatment days and the reduction was more prominent after the GA_{4+7} treatments than the GA_3 one. Flesh firmness was also reduced by the GA treatments, especially the GA_{4+7} treatments on 30 and 40 DAFB. The fruits in the GA_{4+7} treatments on 30 and 40 DAFB had a low skin color index (Table 1), which was exhibited as a poor red coloration (Fig. 1). Fruits in the GA_3 treatments on 20 and 30 DAFB had lower water core indices than those in the control. There was no notable malformation of fruits in all the treatments (data not shown) because the L/D ratio of the fruit was not severely affected by the treatments (Table 1).

Experiment 2: Effect of Spraying Treatment of CPPU 10 mg L^{-1} Solution or CPPU 10 mg L^{-1} + GA_{4+7} 100 mg L^{-1} Solution on Fruit Quality Parameters

In this experiment, the effects of the spraying solution and the timing of each treatment were mainly visible in fruit size and shape. Irrespective of the 100 mg L^{-1} GA_{4+7} addition, 10 mg L^{-1} CPPU spraying effectively increased fruit size, that is, treated fruits were heavier and larger than the control fruits (Table 2). The most effective treatment for increasing fruit size was the 10 mg L^{-1} CPPU treatment on 4 DAFB as the fresh weight increased by~100 g per fruit. However, 10 mg L^{-1} CPPU on four DAFB treatments with and without 100 mg L^{-1} GA_{4+7} induced severe malformations of fruits at a rate of 62.5–82.5% (Table 2). In addition to longer and irregular shapes (Fig. 2), the L/D ratios of these fruit were higher than those of the control fruits and fruits treated on 30 DAFB (Table 2). The soluble solid and malic acid content, flesh firmness, and water core index did not indicate a marked effect of the treatment, although fruits in the 10 mg L^{-1} CPPU treatment on 4 and 30 DAFB had slightly lower soluble solid contents than those in the control (Table 2). Based on the values of L*, a*, b*, and hue angle there was no effect of the treatment on the red skin coloration (Table 3 and Fig. 2).

Experiment 3: Effect of Spraying Treatment of Different Concentration of CPPU Solutions on Fruit Quality Parameters

The results of Experiment 2 indicated that CPPU effectively increased fruit size, and there was no effect of the 100 mg L^{-1} GA_{4+7} addition. Moreover, CPPU spraying treatments on 4 DAFB induced severe malformations in the fruits (Table 2 and Fig. 2). Therefore, in this experiment we examined the most suitable timing for and concentration of the CPPU treatment for the improvement of fruit quality, especially size.

Earlier treatments and higher concentrations of CPPU induced more intensive fruit growth, leading to heavier, longer, and wider fruits (Table 4 and Fig. 3). The 10 mg L^{-1} treatment on 10 DAFB produced 135% heavier fruit than the control, although this treatment also induced the malformation of approximately 38% of the fruits (Table 4). However, on 20 DAFB, all concentrations of the treatment produced 108–116% heavier fruit than the control. The 5 mg L^{-1} treatment produced more malformed fruits than the control (Table 4). Furthermore, on 30 DAFB, the 5 and 10 mg L^{-1} treatments produced 114 and 118% heavier fruit, respectively, than the control. The malformed fruit ratio of these fruits was similar to that of the control (Table 4). The L/D ratio indicated that the CPPU treatment produced longer shaped fruits than the control, though significant differences were only observed in 10 mg L^{-1} treatments on 10 DAFB and 1 and 5 mg L^{-1} treatments on 20 DAFB (Table 4).

Soluble solid content was decreased by the CPPU treatment; however, the values in the 5 and 10 mg L^{-1} treatments on 30 DAFB were similar to that of the control (Table 4). Malic acid content was not affected by any of the treatments. Flesh firmness exhibited some fluctuations due to the treatments, but did not differ from the control. The watercore indices of treated fruits were lower than in the control fruits; however, they did not significantly differ except for the 5 and 10 mg L^{-1} treatments on 10 DAFB (Table 4). Fruit skin coloration was not markedly affected by the treatment, however, according to the ANOVA there were some differences among the CPPU treatments (Table 5). For instance, the 1 mg L^{-1} CPPU

Table 1: Effect of GA_3 and GA_{4+7} paste treatments on fruit quality parameters of 'Fuji' apples at harvest (November 5, 2012)

Treatment	DAFB[z]	Fresh Weight (g) (% of control)	Length (mm)	Diameter (mm)	L/D ratio	Soluble solid (°Brix)	Malic acid ($mg100\ ml^{-1}$)	Flesh firmness (N)	Skin color index	Water core index
Control		338.7 c[y] (-)	86.0 bc	89.8 c	0.96 ab	14.9 a	0.42 a	68.0 a	4.6 a	2.6 ab
2.7% GA_3	20	311.5 d (92)	85.2 c	87.4 d	0.98 a	13.3 b	0.29 bc	64.5 abc	3.6 abc	1.8 c
	30	365.5 ab (108)	88.2 ab	92.3 ab	0.96 ab	14.5 a	0.32 b	62.4 bcd	4.4 a	1.7 c
	40	336.6 c (99)	84.4 c	89.4 cd	0.94 b	14.4 a	0.31 b	66.0 ab	4.2 ab	2.7 a
1.2% GA_{4+7}	20	349.1 abc (103)	85.9 abc	90.6 bc	0.96 ab	14.6 a	0.24 c	63.5 abc	4.6 a	2.3 abc
	30	369.3 a (109)	89.3 a	93.1 a	0.96 ab	13.7 b	0.25 c	58.0 d	2.9 c	2.2 abc
	40	344.9 bc (102)	86.6 bc	90.0 c	0.95 ab	13.8 b	0.29 bc	59.4 cd	3.3 bc	1.9 bc
ANOVA[x]										
GA (A)		**	**	**	NS	**	**	**	-	-
DAFB (B)		**	*	**	NS	NS	**	**	-	-
A × B		**	NS	**	*	**	*	NS	-	-

[z] DAFB = days after full bloom; [y] Different letters within the same column represent a significant difference based on the Tukey-Kramer's HSD test (fresh weight, length, diameter, and L/D ratio [n = 30], soluble solid and malic acid content, and flesh firmness [n = 10]) and Chi-squared test (Skin color and water core indices [n = 10]) at the 5% probability level; [x]NS, *, and ** indicate non-significant and significant differences at the 5% and 1% probability levels, respectively, according to the two-way ANOVA

Table 2: Effect of spraying forchlorfenuron (CPPU) 10 ppm or CPPU 10 ppm+ GA_{4+7} 100 ppm solutions on fruit quality parameters of 'Fuji' apples at harvest (November 9, 2013)

Treatment	DAFB[z]	Fresh weight (g) (% of control)	Length (mm)	Diameter (mm)	L/D ratio	Soluble solid (°Brix)	Malic acid ($mg\ 100\ ml^{-1}$)	Flesh firmness (N)	Water core index	Malformed fruit ratio (%)
Control		322.4 c[y] (-)	81.6 d	88.7 c	0.92 b	13.5 a	0.41 a	63.3 b	2.7 a	0.0 c
CPPU 10 ppm	4	422.4 a (131)	95.9 a	95.1 a	1.01 a	12.9 c	0.39 a	66.1 a	2.9 a	82.5 a
	30	352.4 b (109)	84.6 c	90.6 b	0.93 b	13.1 bc	0.40 a	62.7 b	2.9 a	0.0 c
CPPU 10 ppm +	4	341.5 b (106)	87.8 b	88.4 c	0.99 a	13.3 ab	0.39 a	63.9 ab	2.8 a	62.5 b
GA_{4+7} 100ppm	30	356.4 b (111)	85.3 c	90.7 b	0.94 b	13.3 ab	0.38 a	64.6 ab	2.7 a	2.5 c
ANOVA[x]										
Treatment (A)		**	**	**	NS	NS	NS	NS	-	-
DAFB (B)		**	**	**	**	NS	NS	NS	-	-
A × B		**	**	**	*	NS	NS	NS	-	-

[z] DAFB: days after full bloom: [y]Different letters within the same column represent a significant difference based on Tukey-Kramer's HSD (fresh weight, length, diameter, L/D ratio [n = 160], soluble solid and malic acid content, and flesh firmness [n = 40]) or Chi-squared tests (water core index [n = 40] and malformed fruit ratio [n = 160]) at the 5% probability level: [x]NS, *, and ** indicate non-significant and significant differences at the 5% and 1% levels, respectively, by two-way ANOVA

Table 3: Effect of spraying forchlorfenuron (CPPU) 10 pp or CPPU 10 ppm+ GA_{4+7} 100 ppm solutions on fruit skin coloration of 'Fuji' apples at harvest (November 9, 2013)

Treatment	DAFB[z]	L*	a*	b*	Hue angle
Control		45.5 a[y]	25.7 a	19.0 a	37.9 a
CPPU 10 ppm	4	44.3 a	27.1 a	19.6 a	36.8 a
	30	44.4 a	25.8 a	19.6 a	38.2 a
CPPU 10 ppm+ GA_{4+7} 100 ppm	4	45.1 a	27.0 a	19.9 a	37.5 a
	30	44.0 a	24.8 a	20.1 a	39.8 a
ANOVA[x]					
Treatment (A)		NS	NS	NS	NS
DAFB (B)		NS	NS	NS	NS
A × B		NS	NS	NS	NS

[z] DAFB: days after full bloom: [y]Different letters within the same column represent a significant difference based on the Tukey-Kramer's HSD test at the 5% probability level (n=40);[x]NS,*,and **indicate non-significant and significant differences at the 5% and 1% levels, respectively, according to the two-way ANOVA

treated fruits on each treatment day had slightly lighter skins than those in the other treatments (Table 5 and Fig. 3).

Discussion

In the Asian market, large, highly colored, and round apple fruits with high sugar content are traded for a very high price because such fruits are used as luxury gifts in Asian countries, including Japan, China, and Korea (Sansavini*et al*., 2004). In present study, we developed a practical technique for producing premium, large round shaped 'Fuji' apple fruits using plant growth regulators such as Gas and CPPU without fruit malformation.

Gibberellins are plant hormones that are mainly related to plant stem growth and are a large group of related compounds that are defined by their chemical structure (Taiz and Zeiger, 2002). In immature apple seeds, GA_3 and GA_{4+7} have been found to stimulate both cell division and cell elongation (Westwood, 1993). As a

Table 4: Effect of different concentrations of forchlorfenuron (CPPU) 10, 20 or 30 days after full bloom (DAFB) on fruit quality parameters of 'Fuji' apples at harvest (November 7, 2014)

DAFB[z]	CPPU (ppm)	Fresh weight (g) (% of control)	Length (mm)	Diameter (mm)	L/D ratio	Soluble solid (°Brix)	Malic acid (mg100 mL^{-1})	Flesh firmness (N)	Water core index	Malformed fruit ratio (%)
Control		337.5 e[y] (-)	82.2 e	91.0 e	0.90 c	13.7 a	0.42 a	65.8 abc	3.36 a	2.0 c
10	1	372.7 bcd (110)	86.5 bcd	94.3 bcd	0.92 bc	13.1 bcd	0.40 a	63.7 bc	3.10 abc	10.0 bc
	5	432.4 a (128)	91.3 a	99.0 a	0.92 bc	13.0 cd	0.39 a	64.0 bc	2.62 bc	12.0 bc
	10	454.1 a (135)	94.7 a	100.3 a	0.94 a	13.0 cd	0.39 a	67.5 a	2.50 c	38.0 a
20	1	368.8 cd (109)	86.0 cd	93.3 cd	0.92 b	13.1 cd	0.40 a	62.9 c	3.16 abc	10.0 bc
	5	365.8 cd (108)	86.0 cd	93.3 cd	0.92 b	13.2 bcd	0.40 a	64.4 bc	3.28 ab	26.0 ab
	10	392.1 bc (116)	87.8 c	95.8 b	0.92 bc	13.0 d	0.40 a	63.7 bc	2.98 abc	12.0 bc
30	1	342.0 de (101)	84.2 de	91.6 de	0.92 bc	13.0 d	0.39 a	64.0 bc	3.04 abc	8.4 bc
	5	385.4 bc (114)	86.3 cd	94.9 bc	0.91 bc	13.4 abc	0.41 a	64.1 bc	3.28 ab	2.0 c
	10	398.1 b (118)	86.9 cd	95.7 bc	0.91 bc	13.5 ab	0.40 a	66.4 ab	2.96 abc	10.0 bc
ANOVA[x]										
DAFB（A）		**	**	**	**	*	NS	NS	-	-
CPPU（B）		**	**	**	**	NS	NS	*	-	-
A×B		**	**	**	NS	NS	NS	NS	-	-

[z] DAFB indicated days after full bloom: [y]Different letters within the same column show a significant difference based on Tukey-Kramer's HSD (fresh weight, length, diameter L/D ratio [n = 100], soluble solid and malic acid content, and flesh firmness [n = 50]) and Chi-squared tests (water core index [n = 50] and malformed fruit ratio [n = 100]) at the 5% probability level. [x]NS, *, and ** indicate non-significant and significant differences at the 5% and 1% probability levels, respectively, according to the two-way ANOVA

Table 5: Effects of spraying different concentrations of forchlorfenuron (CPPU) 10, 20, or 30 days after full bloom (DAFB) on fruit skin coloration of 'Fuji' apples at harvest (November 7, 2014)

DAFB[z]	CPPU (ppm)	L*	a*	b*	Hue angle
Control		46.1 abc[y]	27.4 a	20.3 a	37.6 a
10	1	47.6 a	24.5 a	21.2 a	41.9 a
	5	45.7 abc	25.7 a	20.7 a	40.1 a
	10	46.7 abc	24.2 a	20.9 a	42.3 a
20	1	47.8 a	24.4 a	21.4 a	42.4 a
	5	46.1 abc	25.9 a	20.7 a	40.1 a
	10	46.7 abc	25.8 a	20.8 a	40.0 a
30	1	47.2 ab	24.4 a	21.3 a	42.5 a
	5	44.6 bc	27.3 a	20.1 a	37.4 a
	10	44.2 c	27.1 a	19.6 a	37.1 a
ANOVA[x]					
DAFB（A）		NS	NS	NS	NS
CPPU（B）		**	**	NS	*
A×B		NS	NS	NS	NS

[z] DAFB: days after full bloom. [y]Different letters within the same column represent a significant difference based on the Tukey-Kramer's HSD test at the 5% probability level (n = 50). [x]NS, *, and ** indicate non-significant and significant differences at the 5% and 1% probability levels, respectively, according to the two-way ANOVA

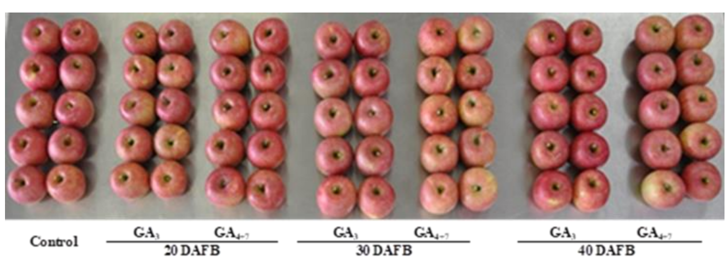

Fig. 1: Effect of GA_3 and GA_{4+7} paste treatment on fruit size and skin color of 'Fuji' apple at harvest (November 5, 2012). DAFB indicated days after full bloom.GA_3 and GA_{4+7} indicated 2.7% GA_3 and 1.2% GA_{4+7} paste treatment, respectively

practical application, a mixture of BA and GA_{4+7} can cause apple fruit elongation and accelerate the development of the crown of 'Delicious' apples (Martin *et al*., 1970; Mankotia *et al*., 2006). In this study, both GA_3 and GA_{4+7} treatments on 30 DAFB effectively increased fruit size without any malformations of the fruit shape, although there was some increase in the L/D ratio (Table 1 and Fig. 1). One of the reasons for the maintenance of the round shape might be ascribed to the application methods. We applied GA_3 or GA_{4+7} as a paste to the middle portion of the fruit pedicels, therefore the GAs were absorbed by the fruit, uniformly contributing to their normal growth. Both GA_3 and GA_{4+7} treatments on 30 DAFB, fruit indicated low malic acid content and flesh firmness (Table 1). Therefore, there is a possibility that GA treatment accelerate the fruit maturation. However, GA_{4+7} treatments on 30 DAFB indicated lower soluble solid content and poor skin coloration (Table 1). Further detailed studies are necessary to reveal the effect of GAs to the fruit maturation.

In Japan, GA_3 paste treatments 30 DAFB are commonly applied to fruit in commercial Japanese pear orchards to improve fruit size (Ito *et al*., 2016). In Korea, the GA_{4+7} paste treatment ~30 DAFB is also applied to improve fruit size of 'Niitaka' pears (Choi *et al*., 2013). Therefore, these paste treatment methods might be acceptable practical applications for apple production.

However, applying the paste treatment to the pedicels as a practical tool for apple production is very inconvenient. In Japan and Korea, Japanese pears are trained to horizontal trellis systems (Tamura, 2006), thus all fruit are bearing on the same dimension and it is easy to apply the paste without use of the ladder. Conversely, apples are trained to flat open centers or central leader forms and both types of trees bear the fruits randomly within the canopy (Arakawa and Komori, 2006). Therefore, it is difficult to apply the paste on the small pedicel of apples using the ladder. For this reason, we must find other ways to apply the PGRs to each apple fruit. Machine spraying using the speed sprayer is the most effective way to apply the chemicals to the trees and fruits. However, PGRs are expensive; therefore, it is necessary to minimize spraying. Moreover, the effects of the PGRs on tree and shoot growth are unknown. To balance convenience, price, and their effect on other organs besides the fruits, we used the hand-spraying method to apply PGRs.

The CPPU is a synthetic cytokinin that promotes plant cell division and lateral growth (Matsuo *et al*., 2012). In the second experiment, we applied CPPU spraying treatments with and without GA_{4+7} and revealed that GA_{4+7} had no additional effect on size and qualities of fruit (Tables 2, 3, and Fig. 2). It is unclear why the CPPU treatment without GA_{4+7} improved fruit size more effectively than CPPU with GA_{4+7} on 4 DAFB. In Japanese pears, CPPU and GA_{4+7} treatments resulted in smaller fruits than CPPU or GA_{4+7} treatments alone (Niu *et al*., 2015). Zeng *et al*. (2016) also reported that raceme soaking treatments in 20 mg L^{-1} CPPU solution on 17 days after anthesis induced increase in endogenous GA_3 content in macadamia fruit. These results indicate the possibility that CPPU treatment can stimulate the internal GA synthesis without external GA applications. Therefore, a collapse of the fruit hormone balance might be one of the reasons that the CPPU without GA_{4+7} treatment produced larger fruit than CPPU with GA_{4+7} treatment on 4 DAFB in this study. Further research is necessary to reveal the relationship between external PGRs treatments and internal PGRs synthesis.

Irrespective of the GA_{4+7} addition, spraying CPPU on 4 DAFB induced severe malformation in fruits (Table 2), where the expansion of the calyx end and elongation of the fruit length were induced by CPPU on 4 DAFB treatments (Table 2 and Fig. 2). Sugiyama *et al*. (1993) reported 20 mg L^{-1} CPPU dipping treatment on 14 DAFB produced heavier apple fruit than control and the treated fruit indicated longer fruit shape. Mankotia *et al*. (2006) also reported 15, 20, 30 and 60 mg L^{-1} promalin (GA_{4+7}+ BA) spraying treatment on full bloom stage produced heavier apple fruit with longer calyx lobes. Fruit development consists of two processes *i.e.,* cell division and cell enlargement. The duration of cell division in fruits varies with the species, for example, in apples it occurs 4–5 weeks after anthesis. Normally, the cell division of fruit is more active in the early stage of fruit development (Westwood, 1993; Zhang *et al*., 2005). Therefore, early treatment of CPPU might induced abnormal cell division of the fruitlet and induced the fruit malformation. For 'Fuji' apple, early treatment of CPPU is not suitable to produce the round shaped fruit.

In the second experiment, treatments did not induce any adverse effect on the internal quality of the fruit, except a slight decrease in the soluble solid content with the 10 mg L^{-1} CPPU treatment (Table 2). The skin color also was not changed by the treatments (Table 3). Except the fruit malformation on 4DAFB, the CPPU spraying induced good fruit growth without any adverse effect of fruit quality parameters, whose parameters were comparable to GA paste treatments.

In the third experiment, we determined the most suitable conditions and concentrations of the CPPU spraying treatment for a practical technique to improve fruit size without undesirable effects on fruit quality parameters. We revealed that the 1 mg L^{-1} CPPU treatment on 10–20 DAFB (low concentration during early-middle stages of development) and the 5–10 mg L^{-1} CPPU treatment on 30 DAFB (middle-high concentration during late stages of development) produced 9–18% larger fruit than the control fruits and there were no malformations (Table 4 and Fig. 3). The 1 mg L^{-1} CPPU treatment on 10–20 DAFB induced a 1% decrease in the soluble solid content, whereas the 5–10 mg L^{-1} CPPU treatment on 30 DAFB did not decrease soluble solid content (Table 4). Curry and Greene (1993), Greene (2001) reported no decrease in soluble solid content due to CPPU treatments in the 'Delicious' and 'McIntosh' apple cultivars. In contrast, a delay in fruit maturation by CPPU treatments was reported in some grape cultivars, which also had low soluble solid contents, green skin colors, and high acid contents (Reynolds *et al*., 1992). Peppi and Fidelibus (2008) also reported that the effect of CPPU treatments on grape skin coloration varied among the cultivars and in 'Flame seedless' grapesit severely decreased the red coloration of the skin.

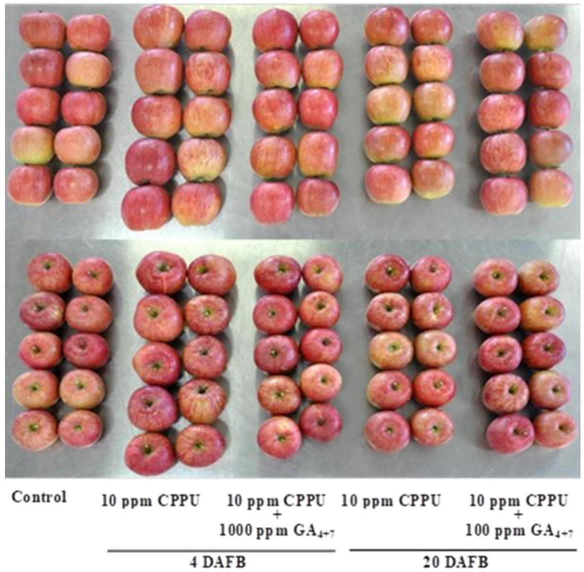

Fig. 2: Effect of spraying forchlorfenuron (CPPU) or CPPU + GA_{4+7} solution on fruit size and skin color of 'Fuji' apples at harvest (November 9, 2013). DAFB: days after full bloom

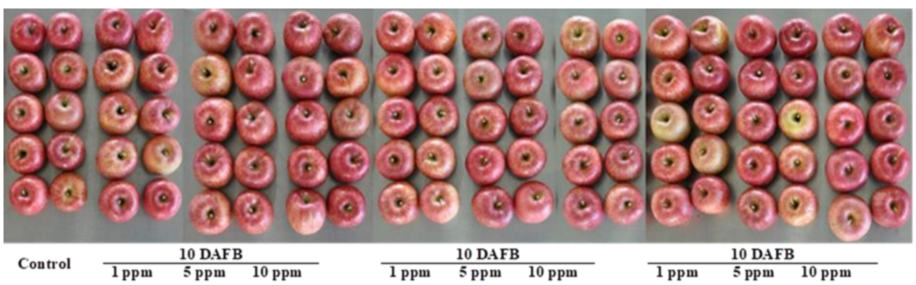

Fig. 3: Effect of spraying different concentrations of forchlorfenuron (CPPU) 10, 20 or 30 days after full bloom (DAFB) on fruit size and skin color of 'Fuji' apple at harvest (November 7, 2014)

In the present study, the red color of the 'Fuji' apple skin was not severely decreased by the CPPU treatment. Therefore, in contrast to grapes, the adverse effects of the CPPU treatment on 'Fuji' apple was limited and it stimulated fruit growth without decreasing soluble solid content (Table 4) and red skin coloration (Table 5 and Fig. 3). It is not clear why 30 DAFB treatment did not decrease the soluble solid content, which was comparable to 10–20 DAFB treatment. It is well known that Cytokinins treatment increase the sink strength and the effect is different by the cell development stage (Kuiper, 1993). Therefore, 30 DAFB treatment might induce strong sink strength of the fruit which could compensate the bigger growth of the fruit and did not decrease the soluble solid content. Further study will need to reveal the mechanisms. Moreover, additional studies that apply the CPPU treatment on other apple cultivars besides 'Fuji' are necessary because there is a possibility that its effects differ depending on the cultivars.

In recent years, fruit cracking in the stem cavity is becoming a serious problem in commercial orchards and aims to produce large 'Fuji' apples. However, in this study, there was no severe symptom of fruit cracking in the stem cavity except in the 1 mg L^{-1} CPPU treatment on 10 DAFB (Fig. 3), although fruit size increased by~9–18% (Table 4). To reduce the incidence of fruit cracking in the stem cavity, Japanese growers are using 14.7% sodium 1-naphthaleneacetate (NAA) spray on fruits on 21–28 DAFB as a practical technique (Kasai *et al*., 2011). NAA is a synthetic plant hormone in the auxin family and suppresses cell division during the developing stages of the apple fruitlet (Black *et al*., 1995; Kasai *et al*., 2011). Therefore, it can produce fruits with the same size as, but not larger than those in the control (Kasai *et al*., 2011). In this study, we did not focus on this phenomenon specifically and therefore, we did not count the number of cells in the skin and flesh of the fruits. However, Yu *et al*. (2001) reported that in *Lagenaria leucantha*, the CPPU treatment stimulated cell division and CPPU treated fruits had higher cell numbers than non-treated fruits. Kasai *et al*. (2008) found that an imbalance in cell wall extensibility between cells in the skin and flesh was one of the causes of fruit cracking in the stem cavity. Therefore, if the cell numbers of both the skin and flesh is increased by the CPPU treatment, it may reduce the development of fruit cracking in the stem cavity, because the cell wall extensibility might also be affected. Further studies are necessary to reveal the relationship between CPPU treatments and the development of fruit cracking in the stem cavity.

Conclusion

In conclusion, hand spraying treatment with the 1 mg L^{-1} CPPU on 10–20 DAFB and/or the 5–10 mg L^{-1} CPPU on 30 DAFB to the fruitlet increased the size of 'Fuji' apple without adverse effect on the other fruit quality parameters. This treatment also maintained the round fruit shape with around 9–18% increase in fruit size, which is the typical feature of 'Fuji' apple suitable for Asian market. Thus, this treatment can be used as practical method to produce larger, higher quality fruits for the Asian market.

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References

Arakawa, O. and S. Komori, 2006. Apple. *In*: *Horticulture in Japan,* pp: 34–42. The Japanese Society for Horticultural Science (ed.). Nakanishi Printing, Hokkaido, Japan

Black, B.L., M.J. Bukovac and J. Hull, 1995. Effect of spray volume and time of NAA application on fruit size and cropping of Redchief 'Delicious' apple. *Sci. Hortic.*, 64: 253–264

Choi, J.J., J.H. Choi, J.H. Han, H.S. Choi and S.K. Jung, 2013. Postharvest behavior of 'Niitaka' pear fruit as affected by GA pasting. *J. Food Agric. Environ.*, 11: 530–533

Curry, E.A. and D.W. Greene, 1993. CPPU influences fruit quality, fruit set, return bloom, and preharvest drop of apples. *HortScience*, 28: 115–119

Enseikai, 2014. *Guidelines for Apple Production in Aomori Prefecture*. Enseikai, Japan

FAOSTAT, 2017. *Food and agriculture organization of the United Nations.* <http://www.fao.org/faostat/en/#data>

FreshPlaza, 2015. *Global Apple Consumption Grows.* http://www.freshplaza.com/article/149489/Global-apple-consumption-grows

Greene, D.W., 2001. CPPU influences fruit quality and fruit abscission of 'McIntosh' apples. *HortScience*, 36: 1292–1295

Ito, A., D. Sakamoto, A. Itai, T. Nishijima, N. Oyama-Okubo, Y. Nakamura, T. Moriguchi and I. Nakajima, 2016. Effects of GA_{3+4} and GA_{4+7} application either alone or combined with Prohexadione-Ca on fruit development of Japanese pear 'Kosui'. *Hortic. J.*, 85: 201–208

Kasai, S., S. Kudo and O. Arakawa, 2011. Reduction of fruit cracking by application of NAA in 'Fuji' apples. *Hortic. Res.*, 10: 69–74

Kasai, S., H. Hayama, Y. Kashimura, S. Kudo and Y. Osanai, 2008. Relationship between fruit cracking and expression of expansin gene *MdEXPA3* in 'Fuji' apples (*Malus domestica* Borkh.). *Sci. Hortic.*, 116: 194–198

Kuiper, D., 1993. Sink strength: established and regulated by plant growth regulators. *Plant Cell Environ.*, 16: 1025–1026

Mankotia, M.S., P.S. Chauhan and A. Sud, 2006. Role of Promalin (GA_{4+7}+BA) application on fruiting and quality of Delicious apples. *In: Temperate Horticulture Current Scenario,* pp: 249–253. Kishore, D.K., S.K. Sharma and K.K. Pramanick (eds.). New India Publishing Agency, New Delhi, India

Martin, G.C., D.S. Brown and N.M. Nelson, 1970. Apple shape changing possible with cytokinin and gibberellin sprays. *Calif. Agric.*, 24: 14

Matsuo, S., K. Kikuchi, M. Fukuda, I. Honda and S. Imanishi, 2012. Roles and regulation of cytokinins in tomato fruit development, *J. Exp. Bot.*, 63: 5569–5579

Mulderij, 2017. *Overview Global Apple Market*. http://www.freshplaza.com/article/173636/OVERVIEW-GLOBAL-APPLE-MARKET

Niu, Q., T. Wang, J. Li, Q. Yang, M. Qian and Y. Teng, 2015. Effects of exogenous GA_{4+7} and N-(2-chloro-4-pyridyl)-N'-phenylurea on induced parthenocarpy and fruit quality in *Pyrus pyrifolia* 'Cuiguan'. *Plant Growth Regul*., 76: 251–258

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

Reynolds, A.G., D.A. Wardle, C. Zurowski and N.E. Looney, 1992. Phenylureas CPPU and thiadiazuron affect yield components, fruit composition, and storage potential of four seedless grape selections. *J. Amer. Soc. Hortic. Sci.*, 117: 85–89

Sansavini, S., F. Donati, F. Costa and S. Tartarini, 2004. Advances in apple breeding for enhanced fruit quality and resistance to biotic stresses: new varieties for the European market. *J. Fruit Ornam. Plant Res.*, 12: 13–52

Sugiyama, N., S. Sansavini, D. Neri and S. Tartarini, 1993. Effect of CPPU on fruit and shoot growth of apple. *J. Hortic. Sci*., 68: 673–677

Taiz, L. and E. Zeiger, 2002. *Plant Physiology*, Third edition. Sinauer Associates, Sunderland, Massachusetts, USA

Tamura, F., 2006. 1. Japanese pear. *In: Horticulture in Japan 2006.* pp: 50–57. The Japanese Society for Horticultural Science (ed.). Nakanishi Printing, Hokkaido, Japan

Wang, N., J. Wolf and F. Zhang, 2017. Towards sustainable intensification of apple production in China –Yield gaps and nutrient use efficiency in apple farming systems. *J. Integr. Agric*., 15: 716–725

Westwood, M.N., 1993. *Temperate-zone Pomology*. Or. Timber Press, Portland, Oregon, USA

Yu, J.Q., Y. Li, Y.R. Qian and Z.J. Zhu, 2001. Cell division and cell enlargement in fruit of *Lagenaria leucantha* as influenced by pollination and plant growth substances. *Plant Growth Regul.*, 33: 117–122

Zeng, H., W. Yang, C. Lu, W. Lin, M. Zou, H. Zhang, J. Wan and X. Huang, 2016. Effect of CPPU on carbohydrate and endogenous hormone levels in young macadamia fruit. *PLoS One*, 11: e0158705.

Zhang, C., K. Tanabe, F. Tamura, K. Matsumoto and A. Yoshida, 2005. ^{13}C-photosynthate accumulation in Japanese pear fruit during the period of rapid fruit growth is limited by the sink strength of fruit rather than by the transport capacity of the pedicel. *J. Exp. Bot.*, 56: 2713–2719

Zupan, A., M. Mikulic-Petkovsek, F. Stampar and R. Veberic, 2015. Sugar and phenol content in apple with or without watercore. *J. Sci. Food Agric.*, 96: 2845–2850

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