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Application of CPPU on Muskmelon Alters Fruit Size and Quality

Lixia Hou[1,2] • Hui Hao[1] • Xiyan Yu[1*]

[1] State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an 271018, China

[2] Shandong Academy of Agricultural Sciences, Shandong, Jinan, 250100, China

Corresponding author: * **yuxiyan@sdau.edu.cn**

ABSTRACT

The cytokinin-active compound, N_1-(2-chloro-4-pyridyl)-N_3-phenylurea (CPPU), applied on unpollinated and pollinated ovaries, affected the fruit growth and quality of muskmelon. 10, 20 and 30 mg L^{-1} CPPU applications on the emasculated ovary on day 1 (0 day after anthesis) effectively induced fruit parthenocarpy. However, fruit growth was slightly reduced and the weights of the mature fruits were approximately 93-96% of the control fruit (seeded fruit set by hand pollination). Moreover, the contents of sugar, vitamin C and the esters volatiles in fruit were significantly lower than those of the control fruit. These results indicate that the seeds play important roles in determining fruit growth and quality of muskmelon. On the other hand, CPPU application on the pollinated fruit markedly enhanced fruit growth and improved fruit quality. Among the assayed treatments, 20 mg L^{-1} CPPU was effective for enhancing fruit growth and quality. Interestingly, we also found that CPPU applications on the pollinated fruit enhanced significantly the length and diameter of the mature fruits. Moreover, the increase in the length of the mature fruits was especially significant, resulting in elongated fruit shape of the treated fruits. Results indicate that the appropriate concentration of CPPU, when applied to pollinated fruit, is an effective technique to increase the fruit growth and quality of muskmelon.

Keywords: CPPU, growth, muskmelon fruit, parthenocarpy, quality

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is a very important horticultural crop in the world. Melon fruit, an economically important commodity, has a sweet and juicy taste and a unique aroma. The pollination of melon flowers is mainly done by insects, but the activity of such insects is low under rainy, very windy or cool conditions, hence the fruit setting is inconsistent. Therefore, hand-pollination is essential for commercial production of muskmelon. Plant growth regulators (PGRs) have been tested as alternatives to hand-pollination to reduce labor costs. Para-chlorophenoxyacetic acid (p-CPA), a synthetic auxin, is one of the most effective PGRs (Kondo and Murozono 1974), for inducing parthenocarpic fruit in melon, but not at low temperature (Hayata *et al*. 2000, 2001). N_1-(2-chloro-4-pyridyl)-N_3-phenylure (CPPU) is known to be effective for inducing parthenocarpic fruit set and enhancing fruit enlargement by stimulation of cell division and/or cell expansion in many kinds of fruits including kiwifruit (*Actinidia chinensis* Planch.) (Iwahori *et al.* 1988; Cruz-Castillo *et al.* 2002), muskmelon (*Cucumis melo* L.) (Hayata *et al.* 2000; Li *et al.* 2011), blueberry (*Vaccinium myrtillus* L.) (Willamson *et al.* 2007), and cherry (*Prunus avium* L.) (Zhang *et al.* 2011). Yu (1999) found that CPPU showed high activity in inducing parthenocarpy of Chinese white-flower gourd (*Lagenaria eucantha*) and all the parthenocarpic fruit developed to normal size. Kim *et al.* (2006) have reported that CPPU was effective for increasing fruit size of hardy kiwifruit, *Actinidia arguta* 'Mitsuko'. Antognozzi *et al.* (1996) showed that CPPU not only enhanced fruit growth but also increased sugar and starch accumulations in kiwifruit. Despite the large number of reports on the effect of CPPU on production and on fruit characteristics of many kinds of fruits, little is known about the influence of CPPU on fruit growth and quality, including aroma, of muskmelon.

The aim of this work was to study the effects of CPPU on fruit size and quality with emphasis on aroma compound, in unpollinated and pollinated ovaries of muskmelon to understand how CPPU modifies muskmelon fruit development and help to define the most appropriate cultural practices.

MATERIALS AND METHODS

Plant culture

Muskmelon (*Cucumis melo* L. cv. 'Elizabeth', a hybrid with smooth yellow skin and a popular cultivar grown in Shandong province, China) was grown in a greenhouse in the experimental farm of Shandong Agricultural University in Tai'an, China from March through June 2009, with an inter-plant spacing of 50 cm and 120 cm between rows. Average day/night temperatures were about 30°C/20°C. Average daylight was about 12 h. Fertilizer was applied at two stages, a pre-plant broadcast application of 900 kg ha^{-1} of 14N-6.1P-29.9K, followed by a side dress application of 150 kg ha^{-1} N at flowering stage. Irrigation by furrows was applied as needed. Main shoots were grown upwards and topped at the 24th node. The lateral shoots between the 13th node and the 15th node of the main shoots were cut above the 2nd node. Other lateral shoots were removed. Fruits set on the 1st node of the lateral shoots were thinned 10 days after anthesis (DAA) to leave only one per plant.

Treatments

Female flowers were emasculated and covered with paper bags one day before anthesis. Thereafter, 10, 20 and 30 mg L^{-1} CPPU (Sigma Chemical Co., C 2791, USA) solutions were sprayed, respectively, to the ovaries at anthesis for inducing the parthenocarpic

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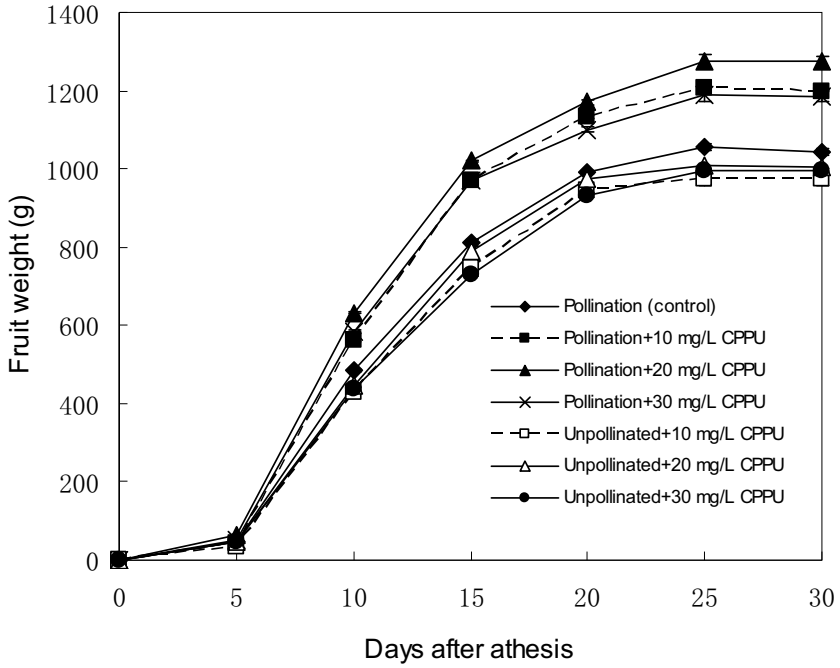

Fig. 1 Fruit weight during fruit development of the treatments and the control. The fruits were harvested at 5, 10, 15, 20 and 25 DAA and at full-slip (approximately 30 DAA). The experiments were repeated three times and ten fruit were used for each replicate. Bars indicate SES.

fruit. Female flowers were hand-pollinated, then 10, 20 and 30 mg L^{-1} CPPU solutions were sprayed to the ovaries, respectively. The control fruits were hand-pollinated at anthesis and were sprayed with water only. The paper bags were removed 3 DAA. Fruits in all plots were harvested 5, 10, 15, 20, 25 DAA, and at full-slip (approximately 30 DAA) to measure their weight and the full-slip fruits were also used to measure sugar, vitamin C and aroma volatiles.

Fruit size, weight, sugar and vitamin C contents analyses

Ten mature fruit samples from each treatment were used to measure final fruit size, weight and the contents of sugar and vitamin C. Vitamin C (ascorbic acid) was estimated by the methods of Li *et al*. (2000). Sugars were extracted by grinding flesh tissues (10 g fresh weight) in 80% ethanol, adjusted to pH 7.0 with 0.1 N NaOH, and heated for 5 min at 80°C. They were then analyzed as described by Lingle and Dunlap (1987).

Analysis of aroma volatile compounds

The extraction and analysis of the aroma volatile compounds were performed as described by Tang *et al*. (2008).

Statistical analysis

Significant effects of treatments were identified by analyzing data using statistical analysis system (SAS) software version 8.2 (SAS Institute, Cary, North Carolina, USA). The least significant difference (LSD) at $P \leq 0.05$ was used to distinguish significant differences between means.

RESULTS AND DISCUSSION

Fruit growth

In all the treatments, fruit growth was characterized by a sigmoid curve and most of the growth occurred within 5-20 DAA (**Fig. 1**). 10, 20 and 30 mg L^{-1} CPPU applications on the emaculated ovary on day 1 (0 DAA) effectively induced fruit parthenocarpy. However, the fruit growth was slightly reduced and the weights of the mature fruits were approximately 93-96% of the control fruit (seeded fruit set by hand pollination). Further, there were no significant differences in the length, and fruit shape index of the mature fruits between the unpollinated, CPPU-treated and the control fruits (**Table 1**). Tartarini *et al*. (1993) reported that the applications of the appropriate concentrations of CPPU on apple (*Malus pumila* Mill.) shoots and fruits enhanced fruit size and weight. Kim *et al*. (2006) found that CPPU was applied at concentrations of 5–10 mg l^{-1} at 10 days after petal fall to obtain a sufficiently enlarged fruit in *A. arguta* 'Mitsuko', without any practical adverse effects on the fruit shape and quality. Zhang *et al*. (2011) indicated that 100 ppm CPPU applied during cell division was effective at improving *P. avium* 'Bing' fruit weight by stimulating cell division. In this study, we found the weight of the mature parthenocarpic fruit induced by CPPU as being significantly less than that of the control fruit, but with small reductions in the fruit size as compared with the control fruit. This phenomenon may be caused by the induction of cell division and cell expansion of CPPU at almost normal levels despite the lack of seeds of the treated fruit.

Further, CPPU application on the pollinated fruit markedly enhanced fruit growth. Among the treatments, 20 mg L^{-1} CPPU was the most effective for enhancing fruit growth. At harvest, fruit weight was 1274 g, but the control fruit was only 1045 g (**Fig. 1; Table 1**). This result was consistent with the previous studies on apple (*Malus pumila* Mill.) (Tartarini *et al*. 1993), grape (*Vitis vinifera* L.) (Retamales *et al*. 1993), muskmelon (*Cucumis melo* L.) (Hayata *et al*. 2000), and kiwifruit (*Actinidia chinensis* Planch.) (Lawes *et al*. 1991; Costa *et al*. 1997; Kim *et al*. 2006). We also found that CPPU applications on the pollinated fruit enhanced significantly the length and diameter of the mature fruits as compared with those of the control fruits. Interestingly, the increase of length of the mature fruits was especially significant, resulting in larger fruit shape index of the treated fruits and the change of fruit shape to more elongated (**Table 1**).

There were no differences in the total seed number in the mature fruits between all the treatments and the control. But, all the seeds in the mature fruit treated by 10, 20 and 30 mg L^{-1} CPPU applications on the emaculated ovary were null, suggesting that CPPU could effectively induce muskmelon fruit parthenocarpy. This was in agreement with another study (Hayata *et al*. 2000). CPPU applications on the pollinated muskmelon fruit had almost no effects on the quality and number of seeds (**Table 1**).

Table 1 Effects of CPPU on length and diameter, shape index, weight, and seed number of mature fruit (n=10).

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit shape index	Null seeds per fruit	Full seeds per fruit	Total seeds per fruit
Pollination (control)	1045 c	13.1 c	11.9 c	1.10 c	123 c	401 a	525 a
Pollination+10 mg L^{-1} CPPU	1200 b	15.8 ab	12.7 ab	1.25 ab	145 c	367 a	513 a
Pollination+20 mg L^{-1} CPPU	1274 a	16.9 a	12.8 a	1.32 a	144 c	319 a	463 a
Pollination+30 mg L^{-1} CPPU	1187 b	14.8 b	12.5 b	1.19 bc	125 c	333 a	458 a
Unpollinated+10 mg L^{-1} CPPU	974 e	12.9 c	11.5 d	1.13 c	537 a	0 b	537 a
Unpollinated+20 mg L^{-1} CPPU	1007 d	12.9 c	11.6 d	1.11 c	559 a	0 b	559 a
Unpollinated+30 mg L^{-1} CPPU	995 de	13.0 c	11.3 d	1.15 c	442 b	0 b	442 a

The data were analyzed using factorial analysis of variance. Least significant difference (LSD) at $\rho \leq 0.05$ was used to distinguish significantly different means (the letters denoting the difference at $\rho \leq 0.05$; only those parts marked with different letters significantly differ)

Table 2 Effects of CPPU on the contents of sugars and vitamin C in mesocarp of mature fruit (n=10). The data were analyzed using factorial analysis of variance. Least significant difference (LSD) at $P \leq 0.05$ was used to distinguish significantly different means (the letters denoting the difference at $P \leq 0.05$; only those parts marked with different letters significantly differ.

Treatment	Sucrose (mg g^{-1} FW)	Fructose (mg g^{-1} FW)	Glucose (mg g^{-1} FW)	Total sugar (mg g^{-1} FW)	Vitamin C (μg g^{-1})
Pollination (control)	56.53 c	18.26 c	12.23 a	87.02 c	300.47 b
Pollination+10 mg L^{-1} CPPU	59.83 b	18.75 b	12.32 a	90.91 b	308.33 b
Pollination+20 mg L^{-1} CPPU	69.6 0 a	18.78 b	12.38 a	100.77 a	334.10 a
Pollination+30 mg L^{-1} CPPU	60.33 b	54.55 a	12.76 a	92.18 b	300.10 b
Unpollinated+10 mg L^{-1} CPPU	53.10 d	18.19 c	12.38 a	83.68 d	278.97 cd
Unpollinated+20 mg L^{-1} CPPU	51.70 d	18.10 c	12.58 a	82.38 de	285.60 c
Unpollinated+30 mg L^{-1} CPPU	48.90 e	18.28 c	12.98 a	80.16 e	268.4 0 d

Sugar and vitamin C contents in fruit

CPPU applications on pollinated fruit significantly increased sucrose content in melons compared with the control, regardless of the concentration (**Table 2**). The application of 20 mg L^{-1} CPPU was the most effective treatment, sucrose content [69.60 mg g^{-1} fresh weight (FW)] being higher than that of the control (56.53 mg g^{-1} FW) by almost 1.2-fold. However, 10, 20 and 30 mg L^{-1} CPPU applications on the unpollinated fruit were not able to compensate for the lack of seeds and significantly decreased sucrose content in melons compared with the control. The fructose and glucose contents were not affected by CPPU applications on neither pollinated nor unpollinated fruit. Hence, a variation of the total sugar content among treatments was similar to that of sucrose content. Regarding vitamin C content, only 20 mg L^{-1} CPPU application on the pollinated fruit significantly increased such content in the mature fruit compared with the control, and 10 and 30 mg L^{-1} CPPU applications did not affect vitamin C content in fruit. And, similarly to the sucrose content, CPPU applications on the unpollinated fruit significantly decreased vitamin C content in melons compared with the control (**Table 2**). These results suggest strongly that the seeds play important roles in determining sugar and vitamin C contents in fruit and the appropriate concentration of CPPU application on the pollinated muskmelon fruit is an effective technique to increase sugar and vitamin C contents in fruit.

Aroma volatiles in fruit

Volatiles are considered to be one of the most important components to determine the quality level and consumer preferences of fruit (Lester 2006). In this work, the solid phase microextraction, gas chromatography-mass spectrometry (SPME GC-MS) analysis method was used to identify the volatiles present in the mature fruit. The main compounds were shown in **Table 3**. In the control mature fruit, 83.87% of the total volatiles were comprised of volatile esters, mostly acetates (80.35%), low levels of alcohols (3.5%), aldehydes (1.24%) and lactones (0.09%). These data showed that esters were the main volatiles and played the key roles in muskmelon aroma quality and these findings were in agreement with previous reports (Beaulieu and Grimm 2001; Shalit *et al*. 2001; Lamikanra and Richard 2002; Aubert and Pitrat 2006). Surprisingly, we found that CPPU applications on the pollinated fruit increased the ester relative content (In the control mature fruit was 83.87%, the mature pollinated fruits treated by 10, 20 and 30 mg L^{-1} CPPU were 86.28, 86.69 and 85.97, respectively) and changed the ester composition. The relative contents of 2,4-diacetoxypentane and phenylmethyl acetate were dramatically lower and the relative contents of methyl acetate, ethyl acetate and butyl acetate were higher than those of the control. There were no differences in the relative contents of alcohols, aldehydes and lactones between the control and the treated pollinated fruit. However, CPPU applications on the unpollinated fruit mainly altered volatiles compositions in the mature fruit compared with the control. The relative content of ester became lower but, the relative contents of alcohols, aldehydes and lactones all became higher than those of the control (**Table 3**). To our knowledge, this is the first report that CPPU applications altered the volatiles contents and composition in melon mature fruit. But, it is not clear how does CPPU application caused the volatiles changes in mature fruit in the present study.

In conclusion, we have demonstrated that CPPU application on the nonpollinated fruit could induced the parthenocarpy of muskmelon. But, the quality and size of the parthenocarpic fruit became worse and less than those of the seeded fruit. In contrast to the parthenocarpic fruit, CPPU applications on the pollinated fruit could improve fruit quality and increase the fruit size.

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Table 3 Effects of CPPU on the main aroma compounds in muskmelon fruits identified by GC-MS.

Volatile components[a]	**Volatile Relative Content (%)**						
	Pollination (control)	**Pollination+10 mg L^{-1} CPPU**	**Pollination+20 mg L^{-1} CPPU**	**Pollination+30 mg L^{-1} CPPU**	**Unpollinated+10 mg L^{-1} CPPU**	**Unpollinated+20 mg L^{-1} CPPU**	**Unpollinated+30 mg L^{-1} CPPU**
Methyl acetate	9.12	10.49	11.06	9.88	5.84	7.77	6.53
Ethyl acetate	2.06	8.75	8.01	9.51	3.98	2.99	2.85
Methyl butanate	1.17	Nd[b]	Nd	Nd	Nd	Nd	Nd
2-Methylpropyl acetate	1.34	1.19	1.23	1.29	1.91	0.99	1.27
Ethyl butanate	Nd	Nd	Nd	0.24	Nd	0.68	0.4
S-Methyl ethanethioic acid	5.15	5.18	4.8	4.38	1.09	Nd	0.66
Butyl acetate	3.32	5.98	6.27	6.25	1.51	2.01	2.21
3-methyl-1-butanol, acetate	5.52	6.85	7.79	6.71	4.17	5.09	4.32
Hexyl acetate	4.55	3.41	3.65	3.34	3.07	3.25	3.08
Methyl 2-(methylthio)acetate	0.56	0.14	0.72	0.08	Nd	0.18	Nd
2,4-Diacetoxypentane	6.4	1.2	1.06	0.89	0.09	0.82	0.38
Phenylmethyl acetate	31.05	27.56	27.23	28.03	25.59	28.24	26.3
2-Phenylethyl acetate	3.63	2.11	4.87	3.12	4.27	4.16	5.32
Pentanal	Nd	Nd	Nd	Nd	2.12	Nd	3.27
Hexanal	Nd	0.71	Nd	Nd	2.24	Nd	5.56
2-Hexenal	Nd	Nd	Nd	Nd	1.91	Nd	1.59
(E)-6-Nonenal	Nd	Nd	Nd	Nd	0.94	Nd	0.26
Benzaldehyde	0.91	0.48	0.47	0.35	1.07	1.24	1.78
1-Pentanol	Nd	Nd	0.09	Nd	0.22	Nd	0.96
(6Z)-Nonen-1-ol	0.29	0.31	Nd	Nd	0.59	Nd	Nd
Benzyl alcohol	1.2	0.53	0.38	0.61	0.26	0.46	0.34
1-Penten-3-one	Nd	Nd	Nd	Nd	0.25	0.32	0.72
3-Hydroxy-2-butanone	Nd	Nd	Nd	Nd	0.12	0.58	0.27

[a] Volatile compounds from freshly melons were sampled using SPME and analyzed by GC-MS as described under **Materials and Methods**. Identification was confirmed by comparison of mass spectra and retention times with those of authentic compounds analyzed under similar conditions, as indicated. [b] 'Nd' means no detected.

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