J. AMER. SOC. HORT. SCI. 120(6):997-1000. 1995.

Synthetic Cytokinin-1-(2=chloro=4=pyridyl)-3-phenylurea (CPPU)-Promotes Fruit Set and Induces Parthenocarpy in Watermelon

Yasuyoshi Hayata and Yoshiyuki Niimi
School of Bioresources, Hiroshima Prefectural University, Shobara, Hiroshima, 727 Japan

Naoto Iwasaki
Faculty of Agriculture, Miyazaki University Kihanadainishi Miyazaki, 880 Japan

Additional index words. growth regulators, sugar, fruit size

Abstract. **Applying a 200 ppm solution of CPPU to pollinated ovaries of watermelon *(Citrullus lunatus* Matsum) at anthesis increased fruit set from 26.9% (control) to 95%. Applying CPPU solutions to nonpollinated ovaries at anthesis induced parthenocarpy, yielding 65% and 89.5% fruit set, respectively with 20 and 200 ppm applications. However, 64% of the 20 ppm CPPU-treated parthenocarpic fruit stopped growth 10 days after treatment. Growth of CPPU-treated, pollinated, and nonpollinated fruit increased significantly compared with growth of control fruit during the first 10 days after treatment, but, except for the 20 ppm CPPU parthenocarpic fruit, growth subsequently slowed, resulting in fruit equal in size to the control by harvest. CPPU application did not affect soluble solids content of pollinated fruit, but reduced content of parthenocarpic fruit treated with 20 ppm. Fructose content was generally higher than glucose and sucrose at harvest. However, in pollinated fruit treated with 20 ppm CPPU, sucrose levels were higher than glucose and fructose. These results suggest that CPPU is practical for promoting fruit set and seedless fruit without adversely affecting fruit quality and development.**

Fruit set in watermelon is unstable at low temperatures and under cloudy or rainy weather, as the activity of flower visiting insects is sluggish and the dehiscence of anthers is hindered (Tsukahara, 1988). Artificial pollination, by hand or insects, is used to enhance fruit set, particularly in the semi-forcing culture in Japan, because of limited insect activity during winter through early spring. In spite of artificial pollination, poor fruiting due to low temperatures and cloudy or rainy weather is still a serious problem.

Several plant growth regulators have been investigated for their effects on fruit set in watermelon, including IAA (Terada and Masuda, 1940), NAA, 4-CPA (Kondou and Murozono, 1975), and BA (Takasugi, 1955; Yamamuro, 1978). Among them, BA has been most effective.

Plant growth regulators have also been used to induce artificially parthenocarpy for producing seedless watermelons. Although IAA (Miyazaki, 1965; Terada and Masuda, 1940) and NAA (Terada and Masuda, 1941) induce parthenocarpy, fruit-set percentages and fruit growth have been very low.

The plant growth regulator CPPU, a newly developed urea-derivative cytokinin, promotes grape berry growth (Nickell, 1986; Ogata et al., 1988) and prevents grape berry shatter (Tanakamaru, 1989), pear (Banno et al., 1986) and kiwifruit thickening (Iwahori et al., 1988; Ogata et al., 1988), and increases fruit set in melon (Gotou et al., 1989; Hayata et al., 1990).

In this study we examined the effects of CPPU on inducing parthenocarpy and on fruit set and development of watermelon. In addition, to evaluating CPPU as a fruit-set agent and producing seedless watermelons, soluble solids (SS) and sugar contents are examined.

Received for publication 21 Nov. 1994. Accepted for publication 15 May 1995. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Materials and Methods

Plant culture. Watermelon transplants were produced by grafting 'Asahikari' watermelon scions to bottle gourd (Kachidoki no. 2) stocks. Fifty seedlings were transplanted 80 cm apart in five beds (1.8 m wide x 8.0 m long). These beds were covered with black polyethylene mulch in a pipe frame greenhouse at Kumamoto Prefecture, Japan, on 11 Jan. 1990. Fertilization involved a preplant broadcast application of 30N-30P-40K (kg/ha) and a later sidedress application of 15.0 N kg/ha at flowering.

Plants were topped at the five-leaf stage, and the three healthiest lateral vines were allowed to grow. Treatments commenced on flowers at about the 15th node of lateral branches. Vines were topped at node 23.

Treatments. Between 15 and 25 Mar., 20 or 200 ppm solutions of CPPU (Kyowa Hakko Kogyo Co., Tokyo) with 0.1% Tween 20 as a surfactant were applied with a writing brush to ovaries of pollinated and nonpollinated flowers at anthesis. Nonpollinated female flowers were bagged from 1 day before to 3 days after anthesis. Treatment consisted of pollination (control), pollination + 20 ppm CPPU, pollination + 200 ppm CPPU, nonpollination +

Table 1. Effect of CPPU on fruit set of watermelon.

Treatmnent[z]	Treated flowers (no.)	Fruit set (no.)	Fruit set (%)
Control	26	7	26.9
	20	**18**	90.0
P C 2	2 1	20	95.2
NC **1**	17	11	65.0
NC2	19	17	89.5

[z]Control = hand pollination, PC 1 = hand pollination + CPPU 20 ppm solution, PC 2 = hand pollination + CPPU 200 ppm solution, NC 1 = nonpollination + CPPU 20 ppm solution, NC 2 = nonpollination + CPPU 200 ppm solution.

Table 2. Effect of CPPU application on weight, shape, and seed or undeveloped seed number of watermelon.

Treatment[z]	Fruit wt (kg)	Fruit diam (cm)	Fruit length (cm)	Rind width (cm)	Seeds (no.) Normal seed	Empty seedcoat	Total
Control	4.2 ± 0.7[y]	20.3	19.6	1.2 ± 0.3	336	140	476
PC 1	3.9 ± 0.6	19.3	19.0	1.0 ± 0.2	102	196	298
PC 2	4.3 ± 0.6	20.5	20.1	1.2 ± 0.2	131	297	428
NC 1	2.9 ± 0.5	18.0	17.6	1.1 ± 0.2	0	456	456
NC 2	3.9 ± 0.8	19.6	19.7	1.2 ± 0.1	0	423	423

[z]Control = hand pollination, PC 1 = hand pollination + CPPU 20 ppm solution, PC 2 = hand pollination + CPPU 200 ppm solution, NC 1 = nonpollination + CPPU 20 ppm solution, NC 2 = nonpollination + CPPU 200 ppm solution.
[y]Mean ±SD for a 10-fruit replication.

20 ppm CPPU, and nonpollination + 200 ppm CPPU. There were five plots with ten seedlings to each. Ten days after CPPU treatment, fruit set was recorded and fruit were thinned to one fruit per plant (two vines of each plant carried no fruit).

Growth and seed number measurement. Fruit length and diameter were measured with a caliper at 2-day intervals from anthesis to harvest time. Immediately after harvesting, normal seeds and empty seedcoats were counted.

SS and sugar analysis. Fruit were halved and a sample was taken from the basal, core, apical, placental, and pericarp regions and then measured with a hand refractometer (N 1; Atago Co., Tokyo).

Five grams of core tissue was prepared by cutting with a razor and crushing in a mortar. The aliquots containing the soluble sugar were passed through a membrane filter (0.45 urn), and 10 µl was injected into the high-performance liquid chromatography system (model TRI ROTAR-VI system controller, 830 RI refractive index detector, and 807-IT integrator; Japan Spectroscopic Co., Tokyo). Sugars were separated on a Shodex Sugar SC1011 column (Showadenko Co., Tokyo) at 80C with distilled water (1 ml·min^{-1}).

Experimental design. The five treatments were arranged in a randomized complete-block design with ten single-plant replications. The fruit of each plant served as a measured unit for growth, SS, and sugars. Data were expressed by mean ± SD.

Results and Discussion

The effect of CPPU on fruit set of watermelon is shown in Table. 1. Because pollination occurred during a spell of rainy weather at low temperature (10 to 13C), the fruit-set percentage for control plants was low (26.9%). Treating pollinated flowers with CPPU increased fruit set to over 90%. In CPPU-treated, nonpollinated ovaries, fruit set was 65% and 89.5%, respectively, with 20 and 200 ppm treatments. Similar enhancing effects of CPPU on fruit set have been reported previously with Japanese persimmon (Hasegawa et al., 1991), melon (Gotou et al., 1989; Hayata et al., 1990), grape (Niimi et al., 1990; Tanakamaru et al., 1989), and tomato (Chin et al., 1991), so it is presumed to be effective for a wide range of plant species.

The proportion of normal seeds in control fruit was 70.5%. However, when CPPU was applied to pollinated fruit, the percentage of normal seeds decreased to 32%. CPPU applied to nonpollinated fruit induced parthenocarpy, and only small, thin, white. seed coats remained as remnants of limited ovule development (Table 2, Fig. 1).

Nitsch (1952) pointed out that seeds were necessary for fruit set and growth in many plant species. Recently, it was reported that CPPU could induce parthenocarpy in melon (Gotou et al., 1989; Tanakamaru, 1988). Our results show that CPPU treatment reduced the proportion of normal seeds in pollinated fruit, but remarkably increased the percentage of fruit set in pollinated and nonpollinated fruit. Therefore, these results suggest that the main factor in the fruit-set enhancing effect of CPPU is its parthenocarpic ability.

BA, a synthetic cytokinin, has been used extensively as fruit-set agent in melon. The role of BA in inducing set was an increase@ the ability of the young fruit to compete with the rest of the plant for assimilate (Jones, 1965) and has little parthenocarpic effect on watermelon (Ohta, 1987). Accordingly, the effect of BA on fruit set is unstable under environmental conditions unfavorable for

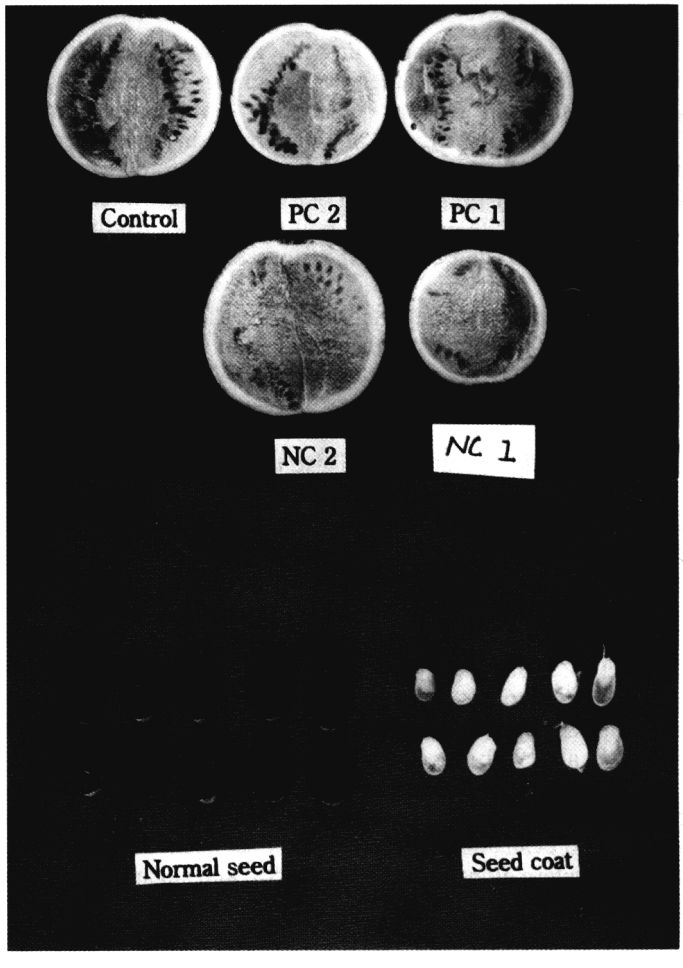

Fig. 1. Normal fruit and seeds compared to parthenocarpic fruit and partially developed seed coats induced by CPPU. Control = hand pollination, PC 1 = hand pollination + CPPU 20 ppm solution, PC 2 = hand pollination + CPPU 200 ppm solution, NC 1 = nonpollination + CPPU 20 ppm solution, NC 2 = nonpollination + CPPU 200 ppm solution.

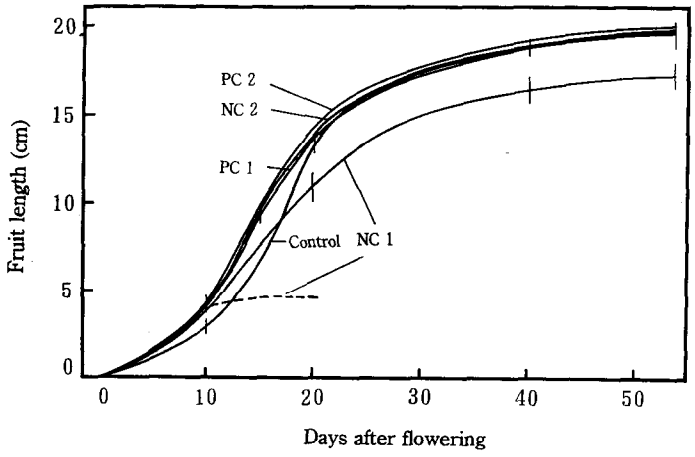

Fig. 2. Effect of CPPU application on growth of fruit in watermelon. Control = hand pollination, PC 1 = hand pollination + CPPU 20 ppm solution, PC 2 = hand pollination + CPPU 200 ppm solution, NC 1 = nonpollination + CPPU 20 ppm solution, NC 2 = nonpollination + CPPU 200 ppm solution. Bars are SDs for ten fruit replication.

pollination or fertilization (Ohta, 1987). Compared to BA, CPPU seems to have a much more consistent and stronger effect on growth and fruit set, probably due to its capacity to induce parthenocarpy.

CPPU enhanced the first growth stage of watermelons (Fig. 2). However, between 10 and 20 days past anthesis, the growth rate of control fruit exceeded most CPPU-treated fruit, so that final fruit size was similar among most treatments. The exception was nonpollinated fruit with 20 ppm CPPU, 64% of which completely stopped growth 10 days after treatment.

In grapes (Nickell, 1986; Ogata et al., 1988) and kiwifruit (Iwahori et al., 1988; Ogata et al., 1988), CPPU stimulated marked increases of fresh fruit weight. In watermelon, it is conceivable that higher concentrations of CPPU may enhance fruit growth after the mid-growth stages. Nitsch (1952) suggested that fruit size and shape are closely correlated with seed number and seed distribution in many fruit. Kurata (1983) described that the number of seeds is related to the development of watermelons. Therefore, it is also possible that the reduction of normal seeds in the CPPU-treated fruit to less than half the number of seeds in the fruit of the control, is one of the factors responsible for the decreased growth rate of the fruit observed after about 15 days in the CPPU treatment plots.

SS from apical, core, and placental regions of fruit with either artificial pollination or 200 ppm CPPU treatments averaged 9.7 to 10.0 %, which was higher than basal (9.4) and pericarp (9.1) regions (Table 3). CPPU treatment did not affect SS content except in 20 ppm treatments, where SS content decreased compared to other treatments. In melon, Tanakamaru (1989) reported that CPPU decreased SS content of fruit without artificial pollination. In watermelon, the decrease of SS content was hardly observed when the fruit were produced parthenocarpically by 200 ppm CPPU treatments compared to control fruit. Miyazaki (1965) reported that SS content of parthenocarpic fruit induced by IAA treatments did not differ to one pollinated artificially; nevertheless, the parthenocarpic fruit induced by IAA treatments were very small and deformed.

Fructose, glucose, and sucrose were the main sugars in the fruit, and fructose content was higher than glucose and sucrose at harvest (Table 4), as reported by Elmstrom et al. (198 1). As with SS, there was no consistent relationship between sugar content and CPPU application (Table 3).

In previous studies on the production of seedless watermelons by hormonal agents IAA, NAA, and 4-CPA, treatments resulted in low fruit-set percentages and a tendency to produce only thick rind

Table 3. Soluble solids contents of five regions in watermelons treated with CPPU.

	Fruit soluble solids (%)					
Treatment[z]	Basal region	Core region	Apical region	Placental region	Pericarp region	Avg
Control	9.6 ± 1.2[y]	10.3 ± 1.0	9.5 ± 1.7	10.2 ± 0.8	9.3 ± 0.8	9.8 ± 0.4
PC 1	9.2 ± 0.4	10.2 ± 0.4	9.9 ± 0.4	9.9 ± 0.4	9.5 ± 0.8	9.7 ± 0.4
PC 2	9.6 ± 0.6	9.7 ± 0.5	9.9 ± 0.8	10.2 ± 0.5	9.5 ± 0.5	9.8 ± 0.3
NC 1	8.5 ± 0.5	9.8 ± 0.8	8.6 ± 0.4	9.4 ± 0.5	7.6 ± 0.5	8.8 ± 0.9
NC 2	10.0 ± 0.4	10.6 ± 0.3	10.6 ± 0.9	10.3 ± 0.5	9.4 ± 0.4	10.2 ± 0.5
Average	9.4 ± 0.6	10.1 ± 0.4	9.7 ± 0.7	10.0 ± 0.4	9.1 ± 0.8	9.7 ± 0.4

[z]Control = hand pollination, PC 1= hand pollination + CPPU 20 ppm solution, PC 2 = hand pollination + CPPU 200 ppm solution, NC 1 = nonpollination + CPPU 20 ppm solution, NC 2 = nonpollination + CPPU 200 ppm solution.
[y]Mean ±SD for a 10-fruit replication.

Table 4. Effect of CPPU application on sugar content of watermelon.

	Sugar content of core region (%)			
Treatment[z]	Glucose	Fructose	Sucrose	Total
Control	2.8 ± 1.0[y]	4.1 ± 1.0	2.0 ± 1.8	8.8
PC 1	1.6 ± 0.4	3.4 ± 0.5	3.9 ± 1.0	8.9
PC 2	3.0 ± 0.3	4.7 ± 0.3	1.3 ± 0.6	9.0
NC 1	2.5 ± 1.5	4.2 ± 0.5	2.2 ± 1.8	9.0
NC 2	2.3 ± 0.8	3.9 ± 0.6	3.6 ± 1.8	9.7
Average	2.4 ± 0.6	4.1 ± 0.5	2.6 ± 1.2	9.1

[z]Control = hand pollination, PC 1= hand pollination + CPPU 20 ppm solution, PC 2 = hand pollination + CPPU 200 ppm solution, NC 1 = nonpollination + CPPU 20 ppm solution, NC 2 = nonpollination + CPPU 200 ppm solution.
[y]Mean ±SD for a lo-fruit replication.

and deformed fruit. Therefore, they have not been put to practical use. In contrast, CPPU treatments of nonpollinated watermelon flowers produced seedless fruit, and 200 ppm CPPU did not adversely affect fruit size, SS, sugars, or rind thickness and may have practical application as a result.

Literature Cited

Banno, K., S. Hayashi, and K. Tanabe. 1986. Effect of KT-30 spray on the growth and quality of pear fruit. Abstr. Jpn. Soc. Hort. Sci. Autumn Meeting 55:521-522.

Chin, L., Y. Hayata, Y. Niimi, and R. Isoda. 1991. Effect of Tomatotone and Flumett application on the growth and quality of tomato fruit. Abstr. Jpn. Soc. Hort. Sci. Spring Meeting 60:276-277.

Elmstrom, G.W. and P.L. Davis. 1981. Sugars in developing and mature fruits of several watermelon cultivars. J. Amer. Soc. Hort. Sci. 106:330-333.

Gotou, T., K. Kamata, U. Yoshida, and M. Yukinaga. 1989. Growth and ripening of parthenocarpic melon fruit induced by authentic cytokinin 4PU-30. Abstr. Jpn. Soc. Hort. Sci. Spring Meeting 58:326-327.

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

Hayata, Y., Y. Niimi, R. Isoda, and K. Makita. 1990. Effect of cytokinin on the fruit setting and sugar accumulation of melon fruit. Abstr. Jpn. Soc. Hort. Sci. Spring Meeting 59:178-179.

Iwahori, S., S. Tominaga, and T. Yamasaki. 1988. Stimulation of fruit growth kiwi fruit, *Actinidia chinensis* Planch., by N-(2-chloro-4-pyridyl)-N'-phenylurea, a diphenylurea-derivative-cytokinin. Scientia Hort. 35: 109-l 15.

Jones, CM. 1965. Effect of benzyladenine on fruit set in muskmelon. Proc. Amer. Soc. Hort. Sci. 87:335-340.

Kondou, Y., and M. Murozono. 1975. Study of artificial fruit setting of watermelon. Abstr. Jpn. Soc. Hort. Sci. Autumn Meeting 44:178-179.

Kurata, H. 1983. Physiology and ecology of fruit-set in water melon, p. 57-58. In: The system of agricultural technology, melon and watermelon. Soc. Cult. Agr. Vill. Press, Tokyo

Miyazaki, H. 1965. Study of parthenocarpy of watermelon. I. Effect of plant hormone on parthenocarpy of watermelon. Bul. Miyazaki Agr. Coll. 1:3-8.

Nickell, L.G. 1986. The effect of N-(2-chloro-4-pyridyl)-N-phenylurea on grapes and other crops. Proc. Plant Growth Regulat. Soc. Amer. 13:236-241.

Niimi, Y., Y. Dohi, Y. Makita, Y. Hayata, and R. Isoda. 1990. Effects of plant regulators on the growth of fruit. Study of Hiroshima Agr. 26: 19-30.

Nitsch, J.P. 1952. Plant hormones in the development of fruits. Quarterly Rev. Biol. 27:33-57.

Ogata, R., T. Saito, and K. Oshima. 1988. Effect of N-phenyl-N'-(4-pyridyl) urea (4-pu) on fruit size: Apple, Japanese pear, grapevine, and kiwi fruit. Acta Hort. 239:395-398

Ohta, Y. 1987. Regulation plant growth and development by using plant hormones. Soc. Cult. Agr. Vill. Press Tokyo. p. 120.

Tanakamaru, K. 1989. Development and characteristic of New plant growth regulator [Fulmett solution]. Agr. Chem. 36:40-46.

Takasugi, K. 1955. Culture methods of seedless watermelon. Agr. Hort. 30:559-564.

Terada, T. and K. Masuda. 1940. Parthenocarpy of watermelon by hetero auxin. Agr. Hort. 15:458468.

Terada, T. and K. Masuda. 1941. Parthenocarpy of watermelon by single or complex application of plant hormones. Agr. Hort. 15:458-468

Tsukahara, A. 1988. Semi-forcing culture in watermelon, p. 194-196. In: The system of agricultural technology, melon and watermelon. Soc. Cult. Agr. Vill. Press, Tokyo.

Yamamuro, K. 1978. Effect of growth regulators on fruit setting of watermelon. Bul. Ibaraki Hort. Expt. Sta. 7: 1-15.