CPPU Promotes Growth and Invertase Activity in Seeded and Seedless Muskmelons during Early Growth Stage

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Summary
We compared the growth and invertase activity of pollinated melon (Cucumis melo L.) fruit and parthenocarpic fruit induced by CPPU [1-(2-chloro-4-pyridyl)-3-phenylurea]. CPPU –treated fruit enlarged from anthesis until the 15th day after anthesis (DAA). However, the growth of parthenocarpic fruit was slower than that of pollinated fruit during the later growth stage. Non –pollinated flowers without the CPPU treatment did not grow; they wilted within 7 DAA. Acid invertase (AI) and neutral invertase (NI) activities in the ovary rapidly decreased from 0 to 5 DAA, whereas, AI activity in the mesocarp of pollinated and/or CPPU –treated fruit dramatically increased to 5 DAA, it declined as fruit matured. AI activity in CPPU –treated fruit was higher than that of pollinated fruit without the CPPU treatment during the early stage; but between 25 and 40 DAA, the activity in CPPU –treated non –pollinated fruit was lower than that in the pollinated ones. NI activity was lower than AI activity in the mesocarp in all plots throughout fruit development. The seasonal patterns in NI activity were similar to those of AI activity. There was a close relationship between the growth rate and invertase activity in melon fruit. CPPU noticeably stimulated those activities in mesocarp tissues at anthesis and shortly thereafter.

Key Words: CPPU, fruit growth, fruit set, invertase, parthenocarpity.

Introduction
CPPU is a synthetic cytokinin which increases fruit set and growth in muskmelon. Studies have shown that CPPU promotes the growth of grape berry (Nickell, 1986; Ogata et al., 1988), pear (Banno et al., 1986), kiwifruit (Iwahori et al., 1988) and watermelon (Hayata et al., 1995). We reported that CPPU –treated, non –pollinated melon fruit not only set better but the growth of young fruit was enhanced, compared to those set by hand –pollination. However the final weight of parthenocarpic fruit was lighter than seeded ones (Hayata et al., 2000). Invertase, which hydrolyzes sucrose, often, appears to be highly active in rapidly growing fruit, where the resulting hexoses are rapidly metabolized (ap Rees, 1974; Morris and Arthur, 1984), and to be associated with a sink strength in developing fruits (Walker and Ho, 1977). In pollinated melon fruit, Iwatsubo et al. (1992) and Gao et al. (1999) observed high invertase activity in the initial growth stages when the fruit developed at a high rate, thus, establishing a close positive relationship between growth and invertase activity in melon fruit. Lee et al. (1997) reported that invertase activity in melon fruit tissues was stimulated by indole-3-acetic acid (IAA), which promotes fruit growth in many plant species (Weaver, 1972). Although CPPU affected fruit growth in non –pollinated muskmelon as cited above (Hayata et al., 2000), the effect of CPPU on the activities of sucrose –related enzymes has not been studied.

In this study, we investigated changes in invertase activity in CPPU –treated muskmelon, with or without pollination, to analyze the promotive effect of the synthetic cytokinin on seedless fruit during the early growth stage. We also examined whether treating mesocarp discs with CPPU stimulates invertases activity in vitro.

Material and Methods

1. Plant material
Seedlings (240) of muskmelon (Cucumis melo L. cv. Crest Earl’s) were transplanted in three beds in a greenhouse at the Hiroshima Prefectural University: eighty seedlings were set 45cm apart in two rows in each bed (1.2 m wide x 18 m long). Fertilizer was applied in two stages, a preplant broadcast of 500 kg - 10 a –1 of 14N –14P –36K, followed by a sidedressing of 30 kg - 10 a –1 N at anthesis. The vines (main shoots) were trained vertically and topped at the 23rd node. All lateral shoots were shortened to two nodes. The fruit were thinned to one fruit per plant at 10 DAA.
2. Treatment

Solutions of 20 mg • liter⁻¹ CPPU were applied to the ovaries of the flowers on the first node of lateral shoots between the 10–15th nodes on the main shoot at anthesis. The non-pollinated flowers were emasculated with tweezerers the day before anthesis and covered with paper bags until 3 DAA. Treatments were; (P) flowers that were pollinated by hand, (PC) flowers that were pollinated and treated with CPPU, (N) flowers that were non-pollinated, and (NC) flowers that were non-pollinated and treated with CPPU. There were 72 plants in each plot except the N plot, which had only 20 plants. Forty ovaries were sampled from each plot from anthesis to 5 DAA. Thereafter, 8 fruit from each plot were harvested every 5th day from 10 through 55 DAA. Samples were weighed immediately after harvest and subsamples of mesocarp tissues (5–10 g) were collected at 3°C and stored in sealed polyethylene bags at −80°C. Five samples of the mesocarp tissues from each plot were analyzed for enzyme activity.

3. Enzyme extraction and assay

All enzyme extractions were performed at 3°C. The frozen mesocarp tissues (2 g each) were homogenized with a blender (ULTRA-TURRAX T25, Janke & Kunkel GMBH Co., Staufen, Germany) in a 8 ml extraction buffer composed of 50 mM Heps-KOH (pH 7.5), 2 mM Na₂ EDTA, 10%(v/v) glycerol, 10 mM MgCl₂, and 2 mM DTT. The homogenate was filtered through four layers of cheesecloth, and then centrifuged at 15,000 × g for 10 min. The resulting supernatant was desalted by using a cellulose tubular membrane (Cellu-Sep T1; Membrane Filtration Production, Inc. USA) against distilled water for 18 to 24 hr. For the assay of acid invertase (AI: EC 3.2.1.26) activity, 100 μl extract was incubated in 50 mM sodium acetate buffer (pH 4.5) and 66 mM sucrose as a substrate in a final volume of 300 μl for 30 min at 35°C. A boiled extract was incubated under the same condition as a control. After terminating the reaction by boiling, the hexose produced, was measured by using Nelson’s reagent (Nelson, 1944) with equal amounts of glucose and fructose as an authentic standard. For neutral invertase (NI: EC 3.2.1.26), the same assay system was carried out as that for AI, except the buffer reaction mixture was 50 mM Heps-KOH buffer (pH 7.5). These procedures of AI and NI analyses are modified methods of Hubberd et al. (1989) and Lowell et al. (1989), respectively.

4. In vitro experiment of mesocarp tissue

Mesocarp cylinders of 10 mm diameter were excised from equatorial parts of non-pollinated ovaries at anthesis with a cork borer. Ten discs (3 mm thickness) prepared from the cylinder were dipped in 20 ml of 0, 0.1, 1.0, 10 or 50 mg • liter⁻¹ CPPU solutions buffered in 50 mM Pipes-NaOH (pH 6.1) for 1 hr at 25°C, and then aerated on a 1% agar medium for 23 hr at 25°C. AI and NI in the discs were then extracted and assayed by the above method.

Results and Discussion

Non-pollinated flowers without CPPU treatment did not grow, and the peel color began to turn from green to yellow at 3 DAA; they wilted within 7 DAA (Fig. 1). CPPU-treated fruits were heavier than the untreated, pollinated fruit from 0 to 15 DAA. Subsequently, the growth rate of the pollinated fruit exceeded that of CPPU-untreated non-pollinated fruit, so that their final fruit weight was less than the pollinated ones (Fig. 1). The same growth pattern was previously observed in muskmelon ‘Tokyo Earl’s 55’ (Hayata et al., 2000) and in watermelon (Hayata et al., 1995).

Invertase catalyzes the hydrolysis of sucrose to glucose and fructose and is closely related to fruit growth. Our examination of invertase activity in CPPU-treated fruit with or without pollination researched that AI in the mesocarp of both pollinated and non-pollinated fruit treated with CPPU greatly increased from 0 to 5 DAA compared to the untreated, pollinated ones. Thereafter, AI activities in the treated fruits rapidly declined as they matured. AI activity in untreated non-pollinated ovary dramatically decreased from 0 to 5 DAA, thereafter it was not detectable. From 25 to 40 DAA, AI activity in the treated, non-pollinated fruit was significantly lower than that of the seeded fruits. NI activity in the mesocarp of the untreated, pollinated, and non-pollinated fruit increased from 0 to 5 DAA compared to the untreated.

![Fig. 1. Growth curves (fresh weight) of pollinated and parthenocarpic muskmelon fruit induced by CPPU treatment. P=control, PC=pollination + 20 mg • liter⁻¹ CPPU treatment, NC=non-pollination + 20 mg • liter⁻¹ CPPU treatment, N=non-pollination. Vertical bars show standard error (n =8).](image-url)
Fig. 2. Changes in acid and neutral invertase activity of developing, pollinated and parthenocarpic muskmelon fruit induced by CPPU treatment. P=control, PC=pollination + 20 mg • liter⁻¹ CPPU treatment, NC=non-pollination + 20 mg • liter⁻¹ CPPU treatment, N=non-pollination. Vertical bars show standard error (n = 5).

Fig. 3. Enhancement of invertase activity of mesocarp discs of muskmelon at anthesis by CPPU treatment. Vertical bars show standard error (n = 10).
* Number = concn. of CPPU in mg • liter⁻¹.

not. Applications of 0.1, 1.0, 10, and 50 mg • liter⁻¹ CPPU dramatically increased NI activity; 1.0 mg • liter⁻¹ CPPU application had the highest (Fig. 3). Several studies reported that plant growth regulators; IAA (Morris and Arthur, 1986) and gibberellic acid (Estruch and Beltra'n, 1991; Miyamoto et al., 1991), regulate invertase activity. With the in vitro disc experiment, we
demonstrated that CPPU directly stimulates invertase activity in the muskmelon. These results support our proposition on mechanism of fruit growth promotion by CPPU.

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Literature Cited


CPPU はマスクメロン受粉および未受粉果実の初期生育とインペルターゼ活性を促進する

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摘 要

CPPU処理により単為結果させたメロン果実と受粉により結果させた果実の生育およびインペルターゼ活性を調査した。また、CPPUがメロン果肉部のインペルターゼ活性を高めるかどうかを in vitroで調査した。

CPPU処理はメロン果実の初期生育を促進したが、単為結果果では後期生育が鈍化し、受粉処理果実に比べ小果となった。果肉部の酸性インペルターゼ活性は全ての処理区で開花5日後が最も高く、CPPU処理区がCPPU無処理区に比べより高かった。しかし、CPPU単為結実果では生育が鈍化した開花25日から40日後における酸性インペルターゼ活性は受粉処理果実に比べ低かった。果肉部の中性インペルターゼ活性は全ての処理区で酸性インペルターゼ活性よりも低く推移したが、その消長パターンは酸性インペルターゼ活性と似たパターンとなった。果肉部ディスクへのCPPUの処理によって、処理後24時間以内にCPPUがメロン果肉部の酸性および中性インペルターゼ活性を直接的に高めることができ明らかとなった。