Journal of Plant Physiology 168 (2011) 920–926

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Chlorophenoxyacetic acid and chloropyridylphenylurea accelerate translocation of photoassimilates to parthenocarpic and seeded fruits of muskmelon (*Cucumis melo*)

Xin-Xian Li [a], Fumiyuki Kobayashi [b], Hiromi Ikeura [c], Yasuyoshi Hayata [c,*]

[a] *Fruit Processing Research Center, AOHATA Corporation, Takehara, Hiroshima 729-2392, Japan*
[b] *Faculty of Applied Life Science, Nippon Veterinary and Life Science University, Musashino, Tokyo 180-0022, Japan*
[c] *School of Agriculture, Meiji University, Kawasaki, Kanagawa 214-8571, Japan*

ARTICLE INFO

Article history:
Received 24 June 2010
Received in revised form
24 November 2010
Accepted 25 November 2010

Keywords:
1-(2-Chloro-4-pyridyl)-3-phenylurea
^{14}C-photoassimilate
Muskmelon
para-chlorophenoxyacetic acid
Seed

ABSTRACT

We compared the effect of *p*-chlorophenoxyacetic acid (*p*-CPA) and 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) on parthenocarpic and seeded muskmelon (*Cucumis melo*) fruits in regards to fruit development and the transport of photoassimilates from leaves exposed to $^{14}CO_2$ to the developing fruits. Ten days after anthesis (DAA), the fresh weight, total ^{14}C-radioactivity and contents of ^{14}C-sucrose and ^{14}C-fructose were higher in the CPPU-induced parthenocarpic fruits than in seeded fruits. However, at 35 DAA, fresh weight and sucrose content in mesocarp, placenta and empty seeds of the parthenocarpic fruits were lower than in seeded fruits. Also, total ^{14}C-radioactivity and ^{14}C-sugar content of the parthenocarpic fruits were lower as well as the translocation rate of ^{14}C-photoassimilates into these fruits. Application of *p*-CPA to the parthenocarpic fruits at 10 and 25 DAA increased fresh weight and sugar content. Moreover, these treatments elevated the total ^{14}C-radioactivity, ^{14}C-sucrose content and the translocation rate of ^{14}C-photoassimilates. The ^{14}C-radioactivity along the translocation pathway from leaf to petiole, stem, lateral shoot and peduncle showed a declining pattern but dramatically increased again in the fruits. These results suggest that the fruit's sink strength was regulated by the seed and enhanced by the application of p-CPA.

Introduction

Size and sweetness are primary factors determining the quality of muskmelon fruit. Studies have shown that parthenocarpic muskmelon fruit induced by the application of the synthetic cytokinin 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) showed enhanced growth in the early development stage, but that growth and sucrose accumulation of the fruit declined during later developmental stages (Hayata et al., 2000, 2002a; Li et al., 2000). Therefore, seeds are considered to play an important role in fruit growth and sucrose accumulation. Moreover, studies have demonstrated that the application of CPPU induces fruit growth (Lewis et al., 1996; Yu, 1999; Cruz-Castillo et al., 2002). It is well-known that the hormone appears to be involved in the growth and quality of fruit (Coombe, 1976; Crane, 1964). In a previous study investigating endogenous indoleacetic acid (IAA) and abscisic acid (ABA) in ovaries and fruits of muskmelon during early development, we found that pollination and CPPU treatment increased endogenous IAA content and decreased endogenous ABA content, both of which promote the setting and growth of the fruit (Hayata et al., 2002b). We also reported that the application of CPPU and the synthetic auxin *p*-chlorophenoxyacetic acid (*p*-CPA) to a developing parthenocarpic melon fruit induced by CPPU increased the size and sucrose content of the fruit (Li et al., 2000, 2002a); the sucrose content in particular rose when *p*-CPA was applied to parthenocarpic fruit between 5 and 25 days after anthesis (DAA). The application of *p*-CPA increased the levels of IAA in mesocarp, placenta, and empty seeds of the parthenocarpic fruits 5 days after treatment, although the levels remained lower than those observed in artificially pollinated fruits (Li et al., 2002a).

Hubbard et al. (1989) reported that sucrose phosphate synthase (SPS) was a key enzyme in sucrose accumulation in melon fruit. More recent research indicated that the level of sucrose synthase (SS) activity was also closely associated with the rate of sucrose accumulation (Hayata et al., 2001b). When we investigated the direct response of excised mesocarp discs of the muskmelon fruit

Abbreviations: ABA, abscisic acid; CPPU, 1-(2-chloro-4-pyridyl)-3-phenylurea; *p*-CPA, *p*-chlorophenoxyacetic acid; DAA, days after anthesis; IAA, indoleacetic acid; PAR, photosynthetically active radiation; SPS, sucrose phosphate synthase; SS, sucrose synthase.

* Corresponding author. Tel.: +81 44 934 7812; fax: +81 44 934 7812.
E-mail address: yhayata@isc.meiji.ac.jp (Y. Hayata).

0176-1617/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2010.11.005

at different growth stages to CPPU and *p*-CPA treatments, we found that in the early growth stages CPPU and *p*-CPA promoted invertase and SPS activities, and also that *p*-CPA promoted SS activity at various developmental stages (Li et al., 2002b).

The photoassimilate imported into muskmelon fruit is closely related to the growth and sugar accumulation of the fruit (ap Rees, 1974; Daie, 1985; Gillaspy et al., 1993; Ho, 1988; ap Rees and Hill, 1994). It has been reported that stachyose and raffinose represent the form of translocated carbon that was decomposed to sucrose at the peduncle in melon (Hughes et al., 1983; Hughes and Yamaguchi, 1983). These studies also showed that a large amount of ^{14}C-stachyose was present in melon leaves exposed to $^{14}CO_2$, but that no ^{14}C-stachyose was detected in the fruit after assimilates had been transported. It is possible that the effect of *p*-CPA described in the previous paragraph promotes the importation of assimilates into fruits, since it has been found that auxins act as plant growth regulators and are closely related to assimilate translocation in pea (Patrick and Wareing, 1973, 1978), grape (Weaver et al., 1969), and tomato (Shishido and Hori, 1989). Ofosu-Anim et al. (1998) reported that the application of ABA to excised melon discs enhanced the simple diffusional uptake of glucose, fructose and sucrose, whereas the application of IAA stimulated the carrier-mediated system of these three sugars but did not stimulate the simple diffusional uptake system for sucrose. However, there is still insufficient information available to fully understand the overall effects of seeds and plant growth regulators on the import of photoassimilates into fruit.

In the present study we investigated the influence of CPPU and *p*-CPA on the growth and sugar accumulation of seeded and parthenocarpic muskmelon fruit in terms of photoassimilate translocation. We studied the effects of CPPU and *p*-CPA on the translocation and distribution of ^{14}C-sugars in the pathway between leaves exposed to $^{14}CO_2$ and the fruit of muskmelon plants with and without pollination.

Materials and methods

Plant culture

Muskmelon plants (*Cucumis melo* L. cv. Crest Earl's) were cultured in pots (ca 13 L) in a greenhouse at Hiroshima Prefectural University. Plants were trained vertically to a single main stem and topped at the 24th node. Lateral shoots between the 13th and 15th stem nodes were cut above the 2nd node, while other lateral shoots were removed. Fruits were thinned at 6 DAA to leave only one per plant.

Plant growth regulator treatments

There were three plant growth regulator treatments: (1) female flowers were pollinated by hand at anthesis, without any plant growth regulator application; (2) female flowers were emasculated and covered with a paper bag the day before anthesis, the ovaries were sprayed with 20 mg L^{-1} CPPU solution (0.1 mL fruit^{-1}) the next day, and the bag was removed at 3 DAA; (3) fruits derived from emasculated ovaries that had been treated with CPPU at 0 DAA were sprayed with 100 mg L^{-1} *p*-CPA solution at 10 DAA (2 mL fruit^{-1}) and 25 DAA (5 mL fruit^{-1}).

$^{14}CO_2$ pulse treatments

At 7 DAA and 32 DAA, three days before the respective $^{14}CO_2$ pulse treatments, plants were placed in a growth chamber that was installed in radioisotopic facilities. The growth chamber had a 14 h photoperiod (temperature; 30 ± 2 °C day/20 ± 2 °C night), with a photosynthetic photon flux density of 0.333 mmol m^{-2} s^{-1} (PAR, photosynthetically active radiation) at the level of the pulsed leaf, provided by fluorescent and metal haloid lamps. Four plants were used in each pulse treatment. At 10 DAA and 35 DAA, respectively, a reaction vial containing 1.9 mg $Ba^{14}CO_3$ (ca 20 MBq, Amersham Life Science, Buckinghamshire, England) and 79 mg cold $BaCO_3$ was placed on the petiole of the leaf (fully expanded mature) at the 2nd node acropetal from the node-branched lateral shoot setting a fruit at the 1st node. The leaf and vial were enclosed in a 32 cm × 52 cm cellophane film bag (MST No. 300, photo transmissivity 96%, PAR), which was sealed with clay and cellophane tape and checked for leaks (Sawamura et al., 1973). The air in the bag was discharged by a suction pump. The bag was then immediately refilled with 500 mL of air, and 1.5 mL of 30% perchloric acid was added to the reaction vial using a syringe. The bag was removed after a 30 min $^{14}CO_2$ pulse period, and the plant was left undisturbed until sampling (Hughes et al., 1983).

Sampling

Five hours after the $^{14}CO_2$ pulse treatment at 10 DAA each plant was divided into the following fractions: (a) leaf blade of the leaf exposed to $^{14}CO_2$, (b) petiole of the leaf exposed to $^{14}CO_2$, (c) the stem from the node of the leaf exposed to $^{14}CO_2$ to the node-branched lateral shoot on which the fruit set, (d) the lateral shoot from the bisipetal end of the stem to the peduncle of the fruit, (e) peduncle, (f) fruit, and (h) the rest of the plant. Five hours after the $^{14}CO_2$ pulse treatment at 35 DAA each plant was divided into a, b, c, d, e and h fractions, as described for the divisions at 10 DAA. In addition, the fruits were divided into seed or empty seed, placenta, and mesocarp tissues. After this division, each fraction (a–f) was immediately soaked in 95% (v/v) hot ethanol (70 °C) in a polypropylene bottle for 30 min these bottles were then stored at −80 °C. The remaining samples (h) were enclosed in paper bags and dried at 70 °C for 70 h, followed by another drying period of 2 h at 106 °C using a convection drying oven. Dried samples were powdered using a mill and stored in a desiccator.

Sugar extraction and determinations

Samples soaked in ethanol were homogenized for 2 min using a GLH homogenizer (Yamato Scientific Co., Ltd., Tokyo, Japan) running at 30,000 rpm. The homogenates were then passed through filter paper (4 μm) and the residues were washed with 80% ethanol. Filtrates were evaporated to dryness at 40 °C and re-dissolved in 10 mL of distilled water. Four grams of each powdered sample were soaked in 100 mL of 80% ethanol, boiled for 30 min and then filtered. The filtrates were evaporated to dryness at 40 °C and re-dissolved in 10 mL of distilled water. Aliquots of the soluble sugars were passed through a membrane filter (0.45 μm) and injected into a high performance liquid chromatography (HPLC) system. Stachyose, raffinose, and sucrose were separated using a JASCO HPLC system (Japan Spectroscopic Co., Ltd, Tokyo, Japan) equipped with a Shodex Asahipak NH2P-50 4E column (Showadenko Co., Ltd, Tokyo, Japan) at 35 °C with 65% acetonitrile solution (0.5 mL min^{-1}) and determined using a JASCO RI-930 refractive index detector. Glucose and fructose were separated by a Gilson HPLC system (Gilson Medical Electronics, Inc., Middleton, USA) equipped with a Shodex Sugar SC 1011 column at 80 °C using redistilled water (0.6 mL min^{-1}) and determined in a Gilson 133 refractive index detector. Each sugar was identified by comparing its retention times with authentic standards.

^{14}C-radioactivity determination

The HPLC systems described above were both equipped with a JASCO SF-212 N super fraction collector to collect each sugar