ズッキーニの着果における内生インドール-3-酢酸の役割

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Role of Endogenous Indole-3-acetic Acid in Fruit Set of Zucchini

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Summary

The effects of pollination and 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) treatments at anthesis on endogenous indole-3-acetic acid (IAA) and its influence on fruit set and drop in zucchini (*Cucurbita pepo* L. 'Beruna') fruit were investigated. Pollination and CPPU treatments at anthesis increased the length and fresh weight of zucchini fruit rapidly. CPPU-treated fruit grew faster than did hand-pollinated ones, non-pollinated pistils without CPPU treatment failed to grow during the 7 days after anthesis (DAA). IAA level in the mesocarp of CPPU-treated fruit rapidly increased from 1 to 2 DAA, whereas hand-pollinated fruit increased from 2 to 3 DAA; it subsequently decreased in both treatments. IAA in the placenta set by pollination and/or treated with CPPU reached the highest level 2 DAA. In the non-pollinated control ovary, IAA level fluctuated at low levels. These results indicate that endogenous IAA might be closely related to the fruit set and growth in zucchini.

Key Words: CPPU, fruit set, IAA, zucchini.

Introduction

In a previous study, Yasukawa and Hayata (2002), demonstrated that CPPU, a cytokinin-derivative plant hormone, also possesses auxin-like activity and that 2, 4-dichlorophenoxy-propionic acid also promoted parthenocarpic fruit set in zucchini. This finding implies that CPPU promotes fruit set by altering the level of endogenous IAA. Furthermore, seeds that are the center of IAA biosynthesis (Gustafson, 1939; Hayata et al., 2002; Hocher et al., 1992) contain significantly more IAA than do other organs of fruit, e. g. strawberry (Nitsch, 1950), peach (Miller and Walsh, 1990), tomato (Hocher et al., 1992), and muskmelon (Li et al., 2002). In muskmelon, which belongs to Cucurbitaceae as does zucchini, the IAA levels in the mesocarp and placenta increased after anthesis (Hayata et al., 2002). The non-pollinated ovary of zucchini remaines green and does not drop for one week or more, in striking contrast with muskmelon, whose non-pollinated ovary turned yellow 2 days after anthesis (DAA) and brown by 4 DAA (Hayata et al., 2002). In this study, the levels of endogenous IAA in hand-pollinated and/or CPPU-treated zucchini ovary or fruit were determined before and after anthesis to clarify how the treatments promoted parthenocarpy.

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Materials and Methods

Plant material

Seedlings of zucchini 'Beruna' were transplanted 60 cm apart in two lines (1.2 m wide × 20 m long, 2 beds) in a greenhouse kept between 18 and 28℃ at Hiroshima Prefectural University on Sep. 6, 2001, and cultured through Nov. 3, 2001. The soil was a sandy loam with 2% organic matter and a pH of 6.5. Irrigation was automatically regulated by a tensionmeter (DM-8P Melon, Takemura Electric Works Ltd., Tokyo) between pF 2.0 and 2.2. Fertilizer was broadcast as a preplant application at 33 kg·10a^{-1} of 8N-15P-10K. Female flowers above the 15th node of main shoot were used in this experiment.

Experimentation at anthesis

1) Female flowers were hand-pollinated only (P); 2) flowers were hand-pollinated and sprayed with 500 mg·L^{-1} CPPU (Sigma Chemical Co., MO, USA) (PC); 3) flowers were emasculated and covered with paper bags the day before anthesis (E) and 4) flowers treated as in (E) were sprayed with 500 mg·L^{-1} CPPU at anthesis (EC).

Sampling

The paper bags were removed 3 DAA. Ovaries or fruits were sampled on 5, 3, and 1 days before anthesis, and on 0, 1, 2, 3, 5, and 7 DAA, respectively. The harvested ovaries or fruits were placed immediately in a cold room (4℃) where the placenta (containing ovules) and the mesocarp from the equatorial area of ovary or

fruit were sampled. The samples were stored at -80℃ until analysis.

IAA analysis

The extraction of IAA was performed according to Chen et al. (1988) with some modifications. Experiments were carried out at 4℃. Five grams of ovary or fruit samples were homogenized in 20 mL of 65% isopropanol containing 0.2 M imidazole buffer (pH 7.0). ^{13}C-IAA (Hayata et al., 2002) was then added as the internal standard and the mixture centrifuged at 13000 g_n for 20 min. The supernatant was concentrated by a decompression concentrator, adjusted to pH 2.5 with 1N HCl, and then passed through SPE C18 column (Mallinckrodit Baker, Inc., NJ, USA). After the column was rinsed with distilled water, the trapped IAA was eluted with 2 mL acetonitrile. Eighteen ml of 20 mM imidazole buffer (pH 7.0) was added to the eluate and the mixture was passed through SPE NH_2 column (Mallinckrodit Baker, Inc., NJ, USA). The NH_2 column was then rinsed successively with 2 mL each of hexane, methyl acetate, acetonitrile, and methanol; the IAA was eluted with 6 mL methanol containing 2% acetic acid. After the eluate was evaporated to dryness by a decompression concentrator and re-dissolved with 100 μL methanol (pH 7.0), the solution was injected into a TRIROTAR-VI high performance liquid chromatography (HPLC) system (JASCO CO., Ltd., Tokyo) equipped with an UVIDEC-100-VI UV spectrophotometer at 254 nm for further purification. The Mightsil RP-18 GP column (150 × 6.0 mm i.d., KANTO Chemical Co., Inc., Tokyo) kept at 35℃, was eluted with the carrier solvent; 40% methanol solution was adjusted to pH 3.5 with acetic acid at a flow rate of 1 mL·min^{-1}. The IAA fraction was collected, concentrated to dryness by a decompression concentrator, and then methylated by adding excess diazomethane in diethyl ether. The methylated IAA was injected into a QP5050 GC-MS system (Shimadzu Co., Ltd, Kyoto) equipped with a DB-1 (J & W Scintific, CA, USA) capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness). The injector and interface of GC-MS system were maintained at 250℃; the helium carrier gas pressure at the inlet was 50 kPa. The column oven was held at 50℃ for 2 min, then raised to 250℃ at a rate of 20℃·min^{-1}, and then maintained at 250℃ for 10 min. The selected ion monitor was used for determination of IAA by monitoring ion *m/z* 189/130 and 195/136.

Results and Discussion

The length of the ovary increased rapidly from 5 days before anthesis, but the fresh weight did not. The fruits in CPPU treatment with and without pollination (PC and EC) elongated faster after anthesis than did those in the pollination-only treatment (P). The fresh weight in PC and EC treatments increased immediately after anthesis, while that in P treatment increased from 1 DAA, a day later; neither length nor weight of the emasculated ovary (E) increased (Fig. 1). These results indicate that CPPU induces parthenocarpy and promotes growth of zucchini fruit as it did in muskmelon (Hayata et al., 2002). That is, in the previous study, muskmelon, treated with CPPU at anthesis, increased the fresh weight of during the early growth stages and induced parthenocarpy regardless of whether it was pollinated or not. The length or weight of the ovary in E failed to increase from anthesis to 7 DAA (Fig. 1).

The levels of endogenous IAA in the mesocarp and placenta of zucchini ovary decreased from 5 days before anthesis to anthesis, while those in the mesocarps of fruits in PC and EC treatment increased at 1 DAA and reached the highest level at 2 DAA. The auxin level in P treatment started increasing from 2 DAA to 3 DAA, while that in the placenta in PC and EC treatment increased after 1 DAA; that in the P treatment did immediately at anthesis. IAA reached the highest level at 2 DAA; that in the placenta in PC treatment was the highest, followed by P and EC treatment. In E treatment, IAA in the mesocarp of the ovary decreased immediately from anthesis to 1 DAA and remained low, while that in the placenta stayed constantly low after anthesis (Fig. 2). That suggests that pollination and CPPU treatment increased IAA levels in fruit after anthesis, which is

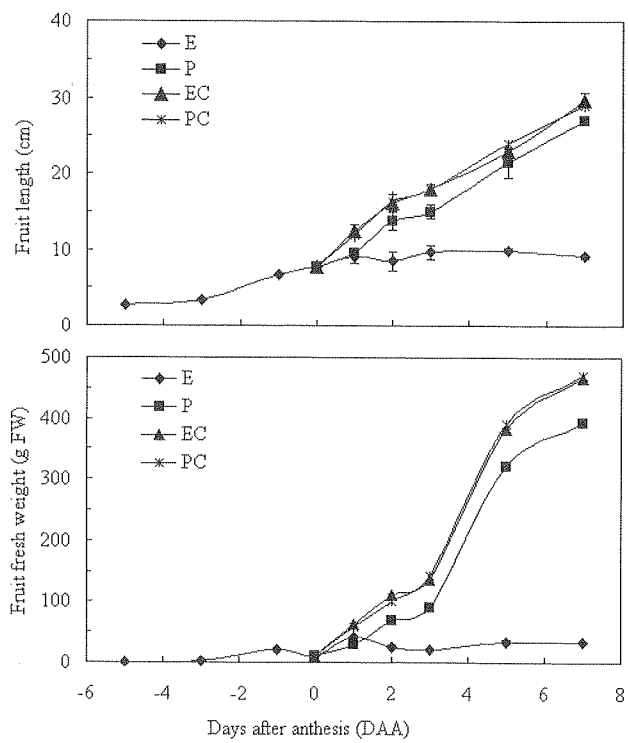

Fig. 1. Changes of the length and fresh weight of zucchini fruits by CPPU treatment with or without pollination. E, emasculation the day before anthesis; P, pollination at 0 DAA; EC, emasculation the day before anthesis and CPPU treatment at 0 DAA; PC, pollination and CPPU treatment at 0 DAA. Each value is the mean of three replicates ± SE.

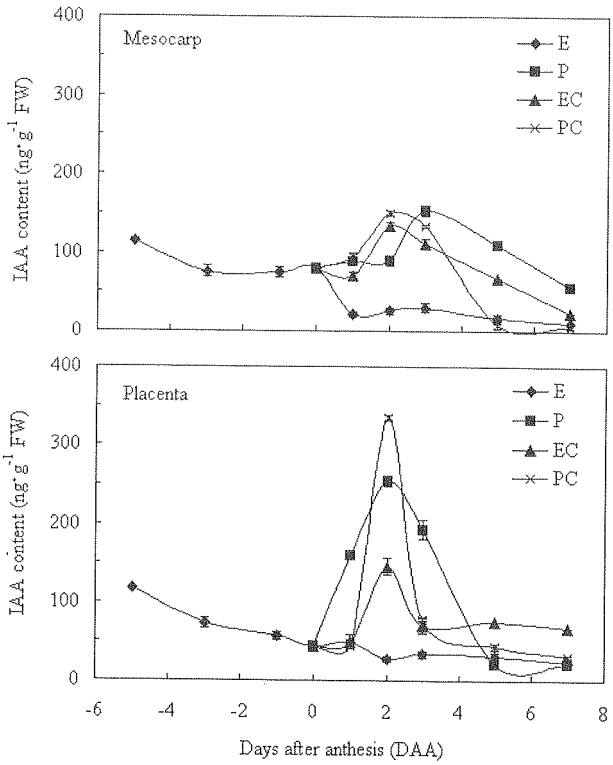

Fig. 2. Changes of the endogenous IAA contents of the mesocarp and placenta of zucchini fruits by CPPU with or without pollination. E, emasculation the day before anthesis; P, pollination at 0 DAA; EC, emasculation the day before anthesis and CPPU treatment at 0 DAA; PC, pollination and CPPU treatment at 0 DAA. Each value is the mean of three replicates ± SE.

closely related to the fruit set. These results are consistent with those of muskmelon (Hayata et al., 2002). The response indicates that IAA moves from the placenta to the mesocarp as demonstrated in muskmelon fruit (Li et al., 2002). Hence, we conclude that endogenous IAA might be closely related to the fruit set and growth in zucchini.

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ズッキーニの着果における内生インドール-3-酢酸の役割

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摘　要

開花時の人工受粉または1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU)処理がズッキーニ幼果の内生インドール-3-酢酸 (IAA)含量に及ぼす影響を調査し，着果におけるIAAの役割について調査した．果実縦径および果実生体重は受粉またはCPPU処理によって開花後急速に増加し，特にCPPUで処理した幼果の成長速度は受粉のみ処理区に比べて速まった．一方，未受粉区の幼果の縦径および生体重は開花7日後までほとんど増加しなかった．CPPUで処理した幼果の果肉部IAA含量は開花1日後から2日後にかけて急速に増加したが，受粉のみ処理区では1日遅く，開花2日後から3日後にかけて増加した．CPPUおよび受粉によって着果した幼果の胎座部のIAA含量はともに開花2日後に最も高いレベルに達した．また，CPPU無処理で未受粉区の幼果のIAA含量は開花7日後までに低レベルで推移した，これらのことから，内生IAAはズッキーニの着果および幼果の成長に強く関連していることが示唆された．