キュウリ果実の発育における外与及び内生ジベレリンの促進作用

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Promotive Effects of Exogenous and Endogenous Gibberellins on the Fruit Development in *Cucumis sativus* L.

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Summary

Promotion of development of cucumber fruit (*Cucumis sativus* L. cv. Chojitsuochiai No. 2) was achieved by the application of gibberellin A_3 (GA_3) and gibberellin A_{4+7} (GA_{4+7}) to its flower. The promotive activity of GA_{4+7} was much greater than that of GA_3. Their activities increased in parallel with higher concentrations. The promotive activities of gibberellin A_4 (GA_4) and gibberellin A_7 (GA_7) were also higher than GA_3.

The level of endogenous gibberellins in the placental tissue and the sarcocarp of parthenocarpic fruits was examined in relation to their development by using thin layer chromatography (TLC) followed by the rice seedling test. Three zones of gibberellin activity were found in the acidic and the nonacidic fraction from each of the fruit tissues. These zones were co-chromatographed on TLC with GA_3, GA_4/GA_7 and gibberellin A_9 (GA_9). Total amounts of three gibberellins in both placental tissue and sarcocarp increased with development of the fruit which fitted with a role for gibberellins in promoting development of cucumber fruit. Existence of larger amounts of the gibberellins in the placental tissue suggests a potential for production of parthenocarpic fruit in this variety of cucumber.

Introduction

Application of gibberellin to the flower of cucumber plants grown in the field or greenhouse from late summer to winter has been proposed by some investigators to increase fruit yield(6, 7, 8). Early experiments carried out by Takayama (8) with the cucumber plants grown in a greenhouse in autumn showed that fruit development was maximally stimulated by 50 ppm GA_3 with a lesser promotion at higher concentrations(8). Ogawa and Aoki (6) reported a prominent promotion of cucumber fruit growth by a mixture of GA_{4+7} or N^6-benzyladenine with slight less promotion by GA_3(6).

On the other hand, there have been some reports dealt with the occurrence of endogenous gibberellins in developing fruit of cucumber (2, 4).

In the present paper the promotive effect of GA_3 and GA_{4+7} on the development of cucumber fruit has been examined over wide range of concentrations. The occurrence of endogenous gibberellins in two parts of parthenocarpic fruit, placental tissue and sarcocarp was also examined in relation to development of the fruit.

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Materials and Methods

Exogenous gibberellins

Plants of cucumber (*Cucumis sativus* L. cv. Chojitsuochiai No. 2) were cultivated in a greenhouse controlled at higher temperature than 13℃ from November to December. During the experiments the plants formed only female flowers produced parthenocarpic fruits. Aqueous solutions of GA_{4+7} and GA_3 were applied with a small sprayer on the flowers formed at 25 to 30 nodes on the main stem. In other experiments, solutions of GA_4 and GA_7 were separately applied to the flowers. Ten flowers of different plants were treated with each test solution. The fruits were harvested after two weeks and fresh weights

of them were measured and represented as mean values. The control flowers were treated with distilled water and some of them aborted shortly after flowering, but these fruits were included for measurement of the averaged fresh weight.

Endogenous gibberellins

Plants of the same variety were cultivated in the greenhouse in May, when conditions were suitable for growth of fruits, as compared with the winter cropping mentioned above. To obtain parthenocarpic fruits, pistil of female flower was removed with a pincette on the day before flowering and the developing fruits were harvested on different days after the flowering, and their length and fresh weight were recorded. Then, they were cut lengthwise with knife and separated into two parts, such as placental and sarcocarpic tissues. Each tissue was ground and extracted with 80% ethanol. After the ethanol was evaporated, the resulting aqueous solution was adjusted to pH 2.5 with 1 N hydrochloric acid and extracted three times with ethylacetate. The combined ethylacetate was washed five times with pH 8 phosphate buffer. The buffer solution was adjusted to pH 2.5 with the hydrochloric acid and extracted five times with ethylacetate. The combined extract then was washed with distilled water and dried with anhydrous sodium sulfate and designated as the acidic fraction. The ethylacetate washed with the phosphate buffer was dried with anhydrous sodium salfate and designated as the nonacidic fraction. Both the acidic and the nonacidic fractions were dried and the resulting residue was dissolved in a small volume of ethylacetate, subjected to silicagel TLC and developed with the solvent system of isopropylether and acetic acid (95 : 5 v/v). The dried plate was divided into 20 sections, each of which was eluted with 0.5 ml of 80% methanol in a small glass tube. The gibberellin activity in the eluate was assayed using the rice seedling test (Tanginbozu) of microdrop method (3) with some modification. Assay responses were not regarded as positive unless they were more than 10% greater than the control. The position of the authentic gibberellins on the TLC plate were detected under UV light after spraying with ethanolic sulphuric acid following heating.

Results

1. Effects of GA_3 GA_{4+7}, GA_4 and GA_7

Aqueous solutions of GA_3 and GA_{4+7} over wide range of concentrations were applied to the flowers. The results are shown in Fig. 1. GA_{4+7} was much more active than GA_3 at all the concentrations examined, and the activity of GA_3 and GA_{4+7} each increased in parallel up to concentrations as high as 1,000 ppm. Aqueous solutions of GA_4 or GA_7 at lower than 20 ppm were applied separately to the

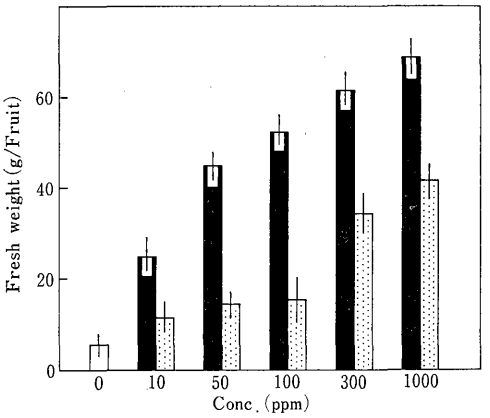

Fig. 1. Effects of GA_{4+7} and GA_3 at different concentrations on fruit fresh weight of cucumber. Blacked bar, GA_{4+7}; Dotted bar, GA_3. Standard error is given by vertical line.

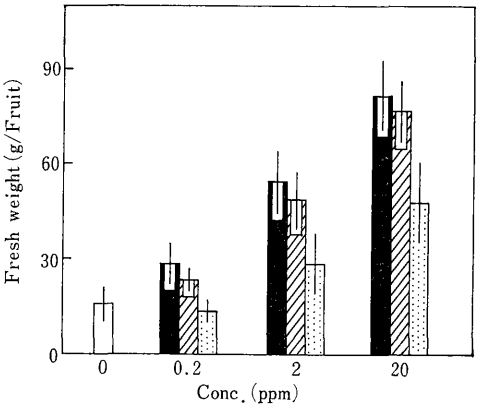

Fig. 2. Effects of GA_4, GA_7 and GA_3 at different concentrations on fruit fresh weight of cucumber.
Blacked bar, GA_7; Oblique bar, GA_4; Dotted bar, GA_3. Standard error is given by vertical line.

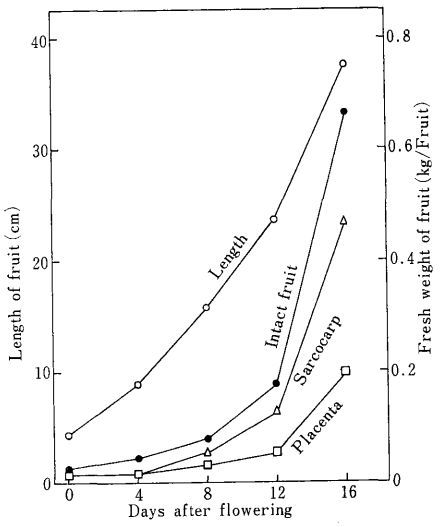

Fig. 3. Changes in length and fresh weight of intact fruit, sarcocarp and placental tissue in cucumber fruit on different days after the flowering.
—○—, Fruit length; —●—, Fresh weight of intact fruit; —△—, Fresh weight of sarcocarp; —□—, Fresh weight of placental tissue.

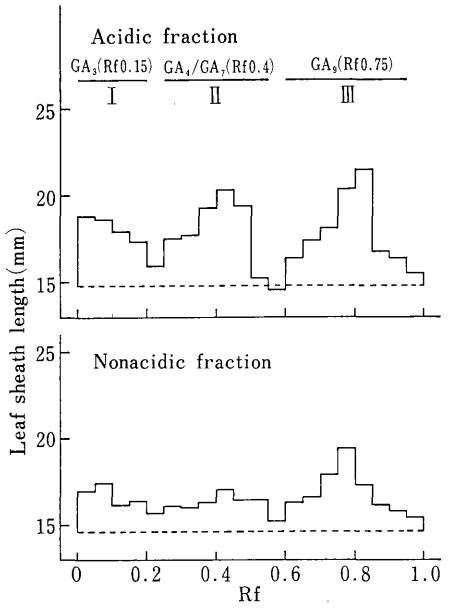

Fig. 4. Gibberellin activity on TLC of acidic and nonacidic fraction from placental tissue.

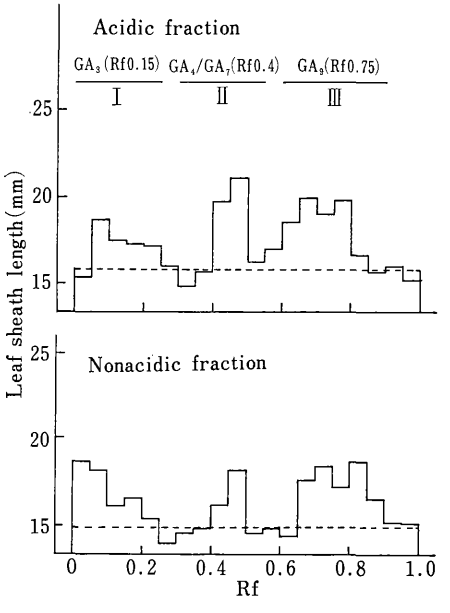

Fig. 5. Gibberellin activity on TLC of acidic and nonacidic fraction from sarcocarp.

flowers. As shown in Fig. 2, they were found to be active even at 0.2 ppm. Their activities were almost the same as each other and also higher than GA_3.

2. Endogenous gibberellins

Changes in the fruit length and the fresh weight are shown in Fig. 3. The fruit began to elongate almost immediately after flowering and continued to increase for 16 days. The fresh weight of intact fruit and the respective fruit tissues increased slowly until 8 days and, thereafter, very rapidly. The fruit harvested on the 16 th day consisted of 70% sarcocarp and 30% placental tissue on a fresh weight basis.

The histograms of gibberellin activity on TLC of both acidic and nonacidic fractions prepared from the placental tissue and the sarcocarp in the fruit harvested on the 12 th day are shown in Fig. 4 and 5, respectively. Gibberellin activity was found in three zones at similar Rf values on all the plates, although there were some variations in their Rf values. These were referred to as zones I (Rf 0–0.3), II (Rf 0.3–0.5) and III (Rf 0.6–0.9) and they co-chromatographed with GA_3 (Rf 0.15), GA_4/GA_7 (Rf 0.4) and GA_9 (Rf 0.75), respectively, as reported by Murakami(4). The amounts of gibberellins from the histograms of TLC plate were estimated as GA_3 eqivalents based on the activity of GA_3 in the bioassay.

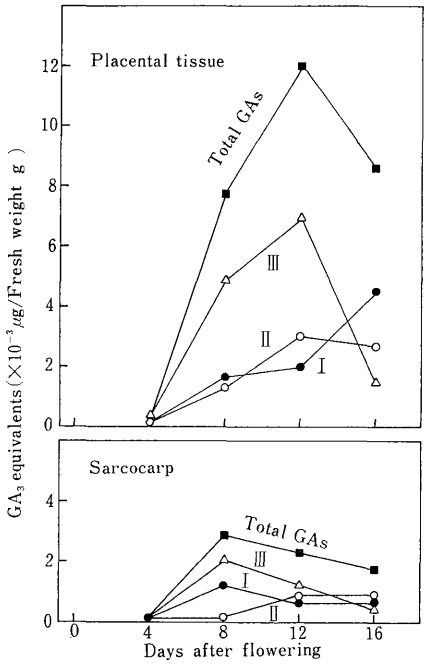

Fig. 6. Changes in amounts of three zones and total amounts of gibberellins in placental tissue and sarcocarp in cucumber fruit on different days after the flowering.
—●—, Zone I ; —○—, Zone II ; —△—, Zone III ; —■—, Total amounts of gibberellins (Tatal GAs).

Amounts of each of the three zones were obtained by adding together those of the same zones in both acidic and nonacidic fractions. Changes in gibberellin amounts in the placental tissue and the sarcocarp per their fresh weight on different days are shown in Fig. 6. There were larger amounts of gibberellins in the placental tissue and their increasing pattern differed between the gibberellins zones. Amounts of zone III gibberellins increased rapidly after 4 days until 12 days, then decreased. Amounts of zones I and II gibberellins increased gradually also after 4 days until 12 and 16 days. A similar pattern of their changes was observed also in the sarcocarp, though smaller amounts than in the placental tissue. Total amonts of the gibberellins were estimated by summing up those of three zones in each of the fruit tissues. The amounts of the gibberellins in the placental tissue reached a high level on the 12 th day. On the contrary, those in the sarcocarp began to decrease already after 8 days falling to a low level. On the 12 th and 16 th day, the amounts of gibberellins in the placental tissue were about nine and five times as much as those in the sarcocarp.

Discussion

Application of GA_3 and GA_{4+7} enhanced development of cucumber fruits, and the later mixture was much superior to the former in activity. The early experiment by Takayama reported that 50 ppm GA_3 was optimum for fruit growth. However, the promotive activity of both of the gibberellin, GA_3 and GA_{4+7} increased more with higher concentrations. (Fig. 1) and there was no optimum concentration over wide range of concentrations applied in the present experiment. Such dose-response curves for gibberellins are also found for elongation of the shoot and are typical of vegetative growth in various plant species(10). GA_4 and GA_7 were active in almost the same degree as each other and they were also more active than GA_3 (Fig. 3). The promotive effect of GA_{4+7} could be obtained by summating of each gibberellin individually. Incidentally, greater elongation of the shoot in cucumber seedling treated with GA_4 and GA_7 than that with GA_3 has also been reported by some other investigators(1, 11). Response of cell enlargement to different gibberellins would no doubt occur whether the cells are present at the shoot apex or at the flower ovary of the same individual.

The placental tissue and the sarcocarp in cucumber fruit contained three zones of gibberellin activity on TLC of both acidic and nonacidic fractions (Figs 4 and 5). The gibberellins found in the nonacidic fraction might be glucosyl-esters of the respective acidic gibberellins(9). In *Sechium edule* developing seeds also contained gibberellins in the nonacidic fraction as well as the acidic fraction(5). From these facts, it is assumed that enzymic conversion of gibberellins to their glucosyl-esters occurs in fruit tissue of *Cucurbitaceae* as do other steps in gibberellin metabolism.

The endogenous gibberellins of a cucumber fruit might be GA_3, GA_4/GA_7 and GA_9 and their related gibberellins, particularly since applied GA_3, GA_4 and GA_7 promoted develop-

ment of cucumber fruit. Moreover, their amounts in both placental tissue and sarcocarp increased with rapid growth of the fruit tissues (Figs 3 and 6). Therefore, these endogenous gibberellins could operate as promoters of fruit development. Especially, existence of larger amounts of bioactive gibberellins in the placental tissue suggests enhanced ability of this variety to produce a parthenocarpic fruit in cucumber.

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キュウリ果実の発育における外与及び内生ジベレリンの促進作用

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摘　要

抑制栽培で11～12月に大型ガラス室内でM式水耕法により育成しているキュウリ(長日落合2号)の開花中の花に，各種濃度(10～1000 ppm)のジベレリン A_3(GA_3)とジベレリン A_{4+7} (GA_{4+7}) の溶液を処理すると果実の発育が促進される．この両ジベレリンの促進作用は，濃度の増加に伴って強くなるが，GA_{4+7} の促進作用は GA_3 のそれよりも強い．また，GA_4 と GA_7 (0.2～20 ppm) の促進作用は殆んど同じ程度の強さであり，GA_3 よりも強い．

半促成栽培で5月に同じ方法で育成した同品種の単為結果の果実の組織，胎座組織と果肉における内生ジベレリンの消長を調査した．各組織のアルコール抽出物を酢酸エチルで抽出し，酸性と非酸性の層に分画した．それを薄層クロマトグラフィー (TLC) で展開して，イネテスト（短銀坊主）でジベレリン活性を調べた．いずれのTLCで3つの区分にジベレリン活性が認められるが，それは，GA_3, GA_4, GA_7, GA_9 或はそれらの関連物質であると思われる．両分画のジベレリンの総和量は，開花4日後から急速に増加し，12日目の胎座組織で，1.2×10^{-2} μg GA_3 当量/新鮮重（g）に達し，この量は果肉の約9倍になる．この生理的活性のある内生ジベレリンの存在がキュウリの単為結果性を示す要因の一つであると推察される．

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