Fruit Growth Induced by Benzyladenine in *Cucumis sativus* L.: Influence of Benzyladenine on Cell Division, Cell Enlargement and Indole-3-acetic Acid Content

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Summary

The effect of ^6N -benzyladenine (BA) in promoting growth of cucumber (*Cucumis sativus*, cultivars Chojitsu-Ochiai No. 2 and Mogami) fruit was studied. The effect of BA was greatest for unpollinated fruits of 'Mogami', a non-parthenocarpic cultivar. BA did not promote growth of fruits where normal development occurred in response to pollination or natural parthenocarpy (in 'Chojitsu-Ochiai No.2'). The growth promotion induced by BA was mostly due to an increase in cell number. Endogenous indole-3-acetic acid (IAA) level, detected and quantified by an enzyme-linked immunosorbent assay (ELISA), was not enhanced by BA. We conclude that BA induces parthenocarpic fruit development mainly through the promotion of cell division.

Introduction

Fruit growth can be regulated by plant hormones and some plant hormones can induce fruit growth when applied exogenously to unpollinated flowers (9, 16). Auxins can cause such parthenocarpic fruit development in cucumber (8, 21). Also, auxin transport inhibitors can stimulate parthenocarpic fruit set in cucumber (5, 14), possibly by blocking auxin movement down the flower pedicels and thus increasing auxin concentration in the ovaries (1, 21). BA has also been reported to induce parthenocarpic fruit development in cucumber (8, 13) although the mechanism of its growth promoting effect remains unclear (17). Cell division and cell enlargement are important factors influencing the capacity of fruit to import assimilates, and thus the regulation of these factors are critical for fruit growth (2, 3, 4). Despite numerous studies on induction of parthenocarpy by application of growth substances, few efforts have been made to relate the applied growth substances to the endogenous growth substance complement of the young fruits

(9). Accordingly, the present work studied the effects of BA on cell number, cell size and endogenous IAA level in cucumber fruit. IAA was quantified by ELISA which could reportedly give an accurate result as analysis by gas chromatography-mass spectrometry (6, 20).

Materials and Methods

1. Plant materials

Cucumber 'Chojitsu-Ochiai No.2' and 'Mogami' (both are monoecious) were used as experimental materials. The seeds were planted in plastic pots early April and the seedlings were transplanted in a glasshouse late April in each year from 1985 to 1988. Female flowers reached anthesis from June to August were used. Female flowers were covered with paper bags the day prior to anthesis to prevent natural pollination, and artificially pollinated next morning or left unpollinated. Ten μl of a 95:5 ethanol:H₂O solution containing BA or α -naphthaleneacetic acid (NAA) (both purchased from Sigma Chemicals Co., St. Louis, Missouri, U.S.A.) at the desired concentration was applied to the peduncles of individual female flowers on the day of anthesis. Most experiments were carried out using plants on which all fruits were allowed

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to develop. However, the same results were obtained in replicate experiments where only one fruit per plant was allowed to develop and all other female flowers being removed.

2. Measurements of cell number and cell size

The fruits were harvested 3 days after anthesis, and fixed in a mixture of 70:30 ethanol:H₂O, formaldehyde, and acetic acid (90:5:5; by volume). A 5-mm-thick slice was cut out from the middle portion of each fruit and embedded in paraffin. Nine- μ m-thick transverse sections were prepared from the slices by means of a microtome. The sections were mounted, stained with toluidine blue and observed under a microscope. The thickness of fruit wall was measured by means of a micrometer. The number of cells occurring on a line drawn between the fruit surface and the inner epidermal layers (see Fig. 1) was counted as an indicator of cell number. The thickness of the fruit wall was then divided by the number of cells to compute average cell size. The measurements were made on 3 parts of each section, for 4 sections from each of 3 different ovaries in each treatment for each experiment.

3. Analysis of IAA

Fruits were harvested 3 days after anthesis and immediately homogenized with sodium N,N-diethyldithiocarbamate in cold 80:20 methanol:H₂O (45 mg and 10 ml per g fresh weight of tissue, respectively) with a blender. Four fruits were homogenized for each treatment. The homogenate was filtered and the methanol then removed in vacuo. The aqueous phase was centrifuged at 4,280 $\times$ g for 10 min. The supernatant was partitioned 3 times at pH 2.5 with equal volumes of ethyl acetate. The ethyl acetate-soluble fraction was dehydrated by adding anhydrous sodium sulfate and taken to dryness in vacuo. The dry residue was then dissolved into 1 ml of 30:70 methanol:H₂O, including 1% acetic acid, and loaded on a C₁₈ Sep-Pak cartridge (Waters Associates, Milford, Mass., U.S.A.) and eluted with 80:20 methanol:H₂O, including 1% acetic acid. The 80% methanol eluate was taken to dryness in vacuo. The dried residue was then dissolved in a small amount of 40:60 methanol:H₂O, including 1% acetic acid, and injected onto a glass column (6 mm in diameter and 20 mm long) packed with

Fig. 1. Diagram of a transverse section of fruit in *Cucumis sativus*. The distance between fruit surface and epidermal layers (indicated with a two-headed arrow) was considered as thickness of fruit wall. Number of cells which stand on a line along the two-headed arrow was counted for use as an indicator of cell number.

ODS material (Nomura Chemicals, Seto, Japan) equipped to a low pressure liquid chromatograph, UVILOG ALPC-100 (Oyo Bunko, Tokyo, Japan) and chromatographed with 40:60 methanol:H₂O, including 1% acetic acid, at a flow rate of 2.5 ml·min⁻¹. The eluate of retention volume of 5 to 20 ml was collected as the IAA fraction. This fraction was taken to dryness in vacuo at ca. 35°C and methylated with diazomethane. Three aliquots, 50 mg fresh weight of tissue equivalent each, were analyzed by ELISA (22) using Phytodetek-IAA kit (Idetek, Inc., San Bruno, Calif., U.S.A.). The dose-response curve for authentic IAA plus 50 mg fresh weight equivalent of the cucumber extract (purified as above) was linear and parallel to that for IAA alone ($y = 28.19 + 1.00x$, $r = 0.999$), thus indicating no interference by extract contaminants on the ELISA response (15).

Results

1. Growth promoting effect of BA

Fruits that had developed from ovaries of pollinated and unpollinated flowers in both cultivars which were treated with BA or NAA were harvested 7 days after anthesis and fruit length was then measured (Table 1). The length of ovaries at anthesis was 4.5 ± 0.19 and 2.3 ± 0.13 cm in 'Chojitsu-Ochiai No.2' and 'Mogami', respectively. The unpollinated 'Chojitsu-Ochiai No.2' ovary developed to a "natural" parthenocarpic fruit. However, unpollinated 'Mogami' ovaries grew very little, indicating that parthenocarpic fruit development did not occur in this cultivar. The greatest

Table 1. Effect of BA and NAA on growth of pollinated and unpollinated fruits in *Cucumis sativus* 'Chojitsu-Ochiai No.2' and 'Mogami'.

Plant growth regulator	Dose ($\mu\text{g}/\text{fruit}$)	Length of fruit (cm)			
		Chojitsu-Ochiai No.2		Mogami	
		Unpollinated	Pollinated	Unpollinated	Pollinated
	0	11.1 $\pm$ 1.4	12.8 $\pm$ 1.5	2.7 $\pm$ 0.12	5.3 $\pm$ 0.39
BA	0.1	10.0 $\pm$ 1.1	12.7 $\pm$ 1.2	2.7 $\pm$ 0.11	6.6 $\pm$ 1.9
	1	10.5 $\pm$ 1.1	11.3 $\pm$ 0.9	3.2 $\pm$ 0.07*	5.6 $\pm$ 0.56
	10	10.1 $\pm$ 1.5	13.1 $\pm$ 1.3	4.8 $\pm$ 0.30*	6.3 $\pm$ 0.31
NAA	0.1	11.8 $\pm$ 0.8	13.5 $\pm$ 1.4	2.9 $\pm$ 0.28	4.9 $\pm$ 0.51
	1	11.9 $\pm$ 1.5	12.6 $\pm$ 1.2	2.7 $\pm$ 0.17	5.4 $\pm$ 0.73
	10	11.4 $\pm$ 1.5	14.2 $\pm$ 1.6	3.3 $\pm$ 0.16*	5.1 $\pm$ 0.77

The flowers were artificially pollinated or left unpollinated, and 10 μl of 95 : 5 ethanol : H₂O containing BA or NAA at several concentrations was applied to the peduncle on the day of anthesis. The length of fruits was scored 7 days after anthesis. Each value is mean of 10 to 20 fruits $\pm$ SE. The values with asterisk (*) are significantly bigger than control at 5% level in a t-test.

effect of BA was observed for unpollinated ovaries of 'Mogami'. BA had no or only a slight promotive effect on the growth of naturally parthenocarpic 'Chojitsu-Ochiai No.2' fruit, or on pollinated ovaries of 'Mogami'. The visible effect of BA on fruit growth was essentially the same as that of NAA, which was examined simultaneously.

The time course of early fruit growth induced by BA was studied at a dosage of 10 $\mu\text{g}/\text{fruit}$ (Fig. 2). The effect of BA treatment was clearest in unpollinated 'Mogami' fruits. The difference in fruit length between BA-treated fruits and control, untreated fruits became significant 2 days after anthesis. The BA-treated fruits were longer than fruits which developed by pollination during the first 3 days although the latter became longer than BA-treated fruits thereafter (Table 1). The difference in fruit length between the BA treatment and control was evident 1 day after anthesis in the pollinated fruits. For 'Chojitsu-Ochiai No.2', the growth patterns were almost the same in fruits for all treatments and BA did not influence fruit growth.

2. Cell number and cell size

The influence of BA treatment on fruit growth measured as thickness of the fruit wall (Table 2) was essentially the same as that scored as fruit length (Table 1), and a growth-promoting effect of

Fig. 2. Time course in growth of fruits with and without BA treatment in *Cucumis sativus*, 'Chojitsu-Ochiai No.2' (left) and 'Mogami' (right). The pollinated (circle) and unpollinated (triangle) flowers were treated with 10 μg BA (closed symbol) or not (open symbol) as in Table 1.

BA on wall thickness could be detected in unpollinated 'Chojitsu-Ochiai No.2' (Table 2). The increased fruit growth brought about by BA was due to an increase in both cell number and cell size. The increase in cell number was more prominent than that in cell size when fruit growth was in-

Table 2. Effect of BA on growth of fruit wall, cell number and cell size in pollinated and unpollinated fruits in *Cucumis sativus* 'Chojitsu-Ochiai No.2' and 'Mogami'.

Cultivar	Pollination	BA treatment	Thickness of fruit wall (mm)	Cell number	Cell size (μm)
Chojitsu-Ochiai No.2	—	—	1.51 ± 0.046 a	80.7 ± 1.98 a	18.7
	—	+	1.92 ± 0.025 b	96.4 ± 1.05 b	19.9
	+	—	2.38 ± 0.039 c	102 ± 1.96 c	23.3
	+	+	2.32 ± 0.051 c	103 ± 1.80 c	22.5
Mogami	—	—	0.82 ± 0.027 a	50.0 ± 1.69 a	16.4
	—	+	1.00 ± 0.041 b	56.9 ± 1.44 b	17.6
	+	—	1.24 ± 0.008 c	64.1 ± 0.78 c	19.3
	+	+	1.15 ± 0.061 c	63.4 ± 1.56 c	18.1

The flowers were treated as in Table 1, but only with $10 \mu\text{g}$ BA, and were harvested 3 days after anthesis. The transverse sections of the fruit were prepared and thickness of fruit wall and cell number (see Fig. 1) were scored. Cell size was computed as fruit wall thickness/cell number. Each value is mean of 36 measurements $\pm$ SE. Values followed by the same symbol do not differ significantly at 5% level in a t-test in each cultivar.

duced by BA treatment, whereas the increase in these 2 parameters was the same in fruits that developed after pollination. Thus, BA treatment increased cell number and cell size by 19 and 6%, respectively, over control in 'Chojitsu-Ochiai No.2'. However, the pollination increased these same parameters by 26 and 25% over control. Similar results were obtained for 'Mogami' but the difference between the fruits developed by BA treatment, or after pollination was smaller than found for 'Chojitsu-Ochiai No.2'. There was no significant effect of BA on either cell number or cell size in pollinated fruits of either cultivar.

3. Endogenous IAA level

IAA contents in fruits detected 3 days after anthesis are shown in Table 3. Parthenocarpically developed fruits had the same IAA level as pollinated fruits in 'Chojitsu-Ochiai No.2'. The BA treatment did not influence the IAA level in the unpollinated fruits and significantly decreased it in the pollinated fruits. In 'Mogami', the unpollinated fruits had higher levels of IAA than pollinated fruits. The BA treatment did not influence the IAA level in either unpollinated or pollinated fruits.

Discussion

The growth promoting effect of BA was most apparent for unpollinated ovaries of non-parthenocarpic 'Mogami'. The same result on fruit

Table 3. Endogenous IAA content in pollinated and unpollinated fruits with and without BA treatment in *Cucumis sativus*, 'Chojitsu-Ochiai No.2' (naturally parthenocarpic) and 'Mogami' (requires pollination for fruit development).

Cultivar	BA treatment	IAA (ng/g FW of tissue eq.)	
		Unpollinated	Pollinated
Chojitsu-Ochiai No.2	—	119 ± 9.78 a	111 ± 17.9 a
	+	105 ± 16.4 ab	65.9 ± 16.2 b
Mogami	—	110 ± 13.4 a	73.6 ± 6.11 ab
	+	124 ± 19.6 ab	68.8 ± 4.76 b

The flowers were treated as in Table 1 but only with $10 \mu\text{g}$ BA, and fruits were harvested 3 days after anthesis. Endogenous IAA in the fruits was extracted and quantified by ELISA. Each value is mean of 3 assays $\pm$ SE. Values followed by the same symbol do not differ significantly at 5% level in a t-test in each cultivar.

growth was obtained with NAA, which is known to induce parthenocarpy in cucumber (21). Therefore, it was concluded that BA induced parthenocarpic fruit development in cucumber as did auxins. The length of fruits that developed parthenocarpically or after pollination was not further increased by BA. This is in striking contrast to the effect of the auxin transport inhibitor, chlorflurenol, which could promote the growth of fruits which developed parthenocarpically and after pollination (7). The chlorflurenol treatment may prevent the movement of auxin from fruits as shown

for some other inhibitors of auxin transport (1, 10). If so, it would cause an increase in auxin level, even in the auxin-rich parthenocarpic cultivar resulting in additional growth promotion. Such a difference in the mode of growth promotion between BA and the auxin transport inhibitor suggests that the growth promotion by BA is not a result of increase in auxin level. In fact, BA did not increase IAA level (Table 3). Not have obvious increases in IAA level been reported in parthenocarpic fruit growth that was induced by 4-chlorophenoxyacetic acid, morphactin and gibberellin A₃ in tomato (18, 19). Because zeatin-like cytokinin activity in cucumber fruit increased during the fruit growth proceeded (13), cytokinin may promote the fruit growth per se, not through increasing auxin level. The growth promoting effect of BA was apparent in the early phase of fruit development (Fig. 2), suggesting that BA stimulated cell division, and in fact it did (Table 2). This is consistent with the cytokinin-stimulated cell division in tomato fruits (11) and the high cytokinin level that was detected during the early cell division period of tomato fruits (3).

Thus, the induction by BA of parthenocarpic fruit development in cucumber is probably due to increased cell numbers, with cell enlargement being stimulated by endogenous IAA, which exists at an "adequate" level in either pollinated or unpollinated fruits.

Ovaries capable of setting parthenocarpic fruit have been assumed to contain higher auxin at anthesis than ovaries of cultivars that require fertilization for fruit growth (12). But, the present study showed that a non-parthenocarpic cultivar, 'Mogami' ovaries contained the same IAA level as a parthenocarpic one, 'Chojitsu-Ochiai No.2', at 3 days after anthesis. Thus, the non-parthenocarpic nature of 'Mogami' is probably not due to low auxin level, but rather may be due to low level of endogenous cytokinins, the latter being required to stimulate cell division in early phase of fruit development (3).

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ベンジルアデニンによるキュウリ果実の生長促進:

細胞分裂, 細胞伸長および内生インドール-3-酢酸含量に対する影響

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摘 要

キュウリ, '長日落合2号'と'もがみ'の果実の生長に対するベンジルアデニン(BA)の作用を調べた。BAの生長促進作用は非単為結果性品種, 'もがみ'の無受粉果において最も顕著であり, 受粉または遺伝的な単為結果性('長日落合2号')によって結果した果実の生長がBAによってさらに促進されることはなかった。BA

による果実の生長促進は主に細胞数の増加によるものであった。果実の内生インドール-3-酢酸(IAA)の含量を酵素イムノアッセイによって測定したところ, BAによってIAA含量が高まることはなかった。これらの結果から, BAは, 主に, それ自身の細胞分裂促進作用によって単為結果を誘導するものと考えられた。