Promotive Effects of Different Cytokinins on the Fruit Growth in *Cucumis sativus* L.

Yukiyoshi OGAWA, Shiro NISHIKAWA, Noboru INOUE* and Shohei AOKI

Faculty of Bioresources, Mie University, Tsu, Mie 514-22

Summary

Promotive effects of endogenous and exogenous cytokinins on the growth of cucumber fruits (*Cucumis sativus* L. cv. Chojitsuochiai No. 2) were examined.

Cytokinin activity in decreasing order was: N⁶-benzyladenine (BA), N⁶-isoamyl adenine (iso Am), Δ^2 -isopentenyladenine (2iP), *trans*-zeatin (t-Z), *cis*-zeatin (c-Z) and kinetin (K).

The endogenous cytokinin levels in the cucumber fruits were examined in relation to fruit growth using the *Amaranthus* assay. The increase in the amounts of the cytokinins corresponding to zeatin in extracts as bioassayed on paper chromatogram coincided with the increase in fresh weight, suggesting promotive effect of the endogenous cytokinins for fruit growth.

Introduction

There have been many reports concerning promotive activity of cytokinins externally applied on fruit development in many plant species (2, 3). The promotion of fruit growth by cytokinins was also shown in Cucurbita plants such as melon (4, 11) and cucumber (9) which were grown in a greenhouse as well as in a field. Ogawa and Aoki (9) have reported that prominent promotion in fruit growth by N⁶-benzyladenine or gibberellin A₄₊₇ was observed for cucumber plants grown in a greenhouse during winter season.

On the other hand, there have been many reports on the occurrence of endogenous cytokinins in growing fruits in many plant species (2, 3). This paper deals with promotive effect of cytokinins, endogenous and exogenous, on the growth of cucumber fruits and the occurrence of endogenous cytokinins of the fruits during their growth.

Materials and Methods

Exogenous cytokinins

Cucumber plants (*Cucumis sativus* L. cv. Chojitsuochiai No. 2) were grown in a greenhouse main-

tained at 13°C or higher from November to December. During the experimental period, the plants formed only female flowers and parthenocarpic fruits were produced. About 25 μ l aqueous solution of various cytokinins was applied with a small brush to each ovary of the opening flowers formed at the nodes 25 to 30 of the stem. Ten or twelve flowers on different plants were treated with each test solution (Fig. 1). Control fruits at comparable positions were treated with distilled water. About two weeks later, fruits were harvested and their fresh weights measured. Some ovaries grew very slowly or aborted after the flowering. The fruit set was represented as percentage of the number of the fruits more than 10 g in fresh weight to the number of those tested. The averaged fresh weight of aborted fruits was also measured.

Endogenous cytokinins

The cucumber plants were grown in the glasshouse in October, the plant growing conditions being more favourable for fruit growth than in the winter season mentioned above. Developing parthenocarpic fruits were harvested on different days after flowering and their length and fresh weights were measured. After immersing in 80% methanol, fruits were ground by a homogenizer and the homogenate filtered. The filtrate was evaporated at reduced pressure at 40°C; the residual aqueous so-

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*Present address: Handa Agricultural High School, Handa, Aichi.

lution was adjusted to pH 3 and washed with ethylacetate to remove fatty materials. The aqueous layer was passed through a Dowex $\times$ 50W (H^+ form) column. After washing the column with water, basic components were eluted with 2 N ammonia. The eluate was concentrated at 40°C and partitioned with n-butanol. The n-butanol fraction was evaporated to near dryness and the residue streaked on a Toyo No 51 filter paper. The chromatogram was developed at about 15°C using water-saturated butanol as solvent. The chromatogram was equally cut into 10 pieces and each piece extracted with 1.2 ml of distilled water in a test tube (1.4 $\times$ 10 cm). Cytokinin activity of each Rf zone was assayed using the *Amaranthus* betacyanin method of Bindington and Thomas (1). The amounts of the endogenous cytokinins were calculated as zeatin equivalents from the histogram.

Results and Discussion

Exogenous cytokinins

Various N^6 -substituted adenines with different normal and isoalkyl substituents together with N^6 -benzyladenine (BA) were compared for their effects on fruit growth of the cucumber. When aqueous solutions of these compounds ($10^{-3}M$)

were applied to the flowers, BA induced all of the flower ovaries to develop normally. Contrarily, most derivatives tested did not promote growth as strongly as BA and yielded in some abortive fruits (Figs. 1, 2). In the isoalkyl series, promotive activity was evidently observed for the derivatives having a C_3 to C_7 chain length and reached to the maximum at C_5 , i.e., N^6 -isoamyladenine (iso-Am). On the other hand, in the normal series, derivatives with a C_4 to C_7 chain length showed almost similar activity in strength. The activity of both the normal- and isoalkyl derivatives did not exceed that of BA. Similar effectiveness of the alkyl derivatives with respect to chain length has been reported in other assays such as leaf growth of *Raphanus* where N^6 -substituted adenines with a normal alkyl substituent of C_3 to C_6 exhibit strong activity (6). Additionally, those with isoalkyl substituent of C_5 and C_6 have been found to promote markedly lettuce seed germination and *Amaranthus* betacyanin biosynthesis (8).

Effectiveness of N^6 -substituted adenines with different C_5 side chain as well as BA on the cucumber fruit growth was examined at two concentrations. Among the cytokinins, only BA and iso-Am were active at $10^{-4}M$ (data not shown) but at $10^{-3}M$, these and Δ^2 -isopentenyladenine (2iP) and *trans*-zeatin (t-Z) significantly increased

Fig. 1. Effects of various substituted N^6 -alkyladenines on fruit set of cucumber. Methyl (Me), ethyl (Et), n-propyl (n-Pr), n-butyl (n-Bu), n-amyl (n-Am), n-hexyl (n-Hex), n-heptyl (n-Hep), isopropyl (iso Pr), isobutyl (iso Bu), isoamyl (iso Am), isohexyl (iso Hex), isoheptyl (iso Hep) and benzyl-adenine (BA) at $10^{-3}M$. Aqueous control (C).

Fig. 2. Effects of various substituted N⁶-alkyladenines on fresh weight of cucumber fruits. Vertical bars indicate standard errors. Abbreviations of each compound are the same as those described in Fig. 1. The concentration at 10^{-3} M. Aqueous control (C).

Fig. 3. Effects of various cytokinins on fruit set of cucumber fruits. Kinetin (K), *cis*-zeatin (c-Z), *trans*-zeatin (t-Z), Δ^2 -isopentenyladenine (2iP), isoamyladenine (iso Am) and N⁶-benzyladenine (BA) at 10^{-3} M. Aqueous control (C).

Fig. 4. Effects of various cytokinins on fresh weight of cucumber fruits. Kinetin (K), *cis*-zeatin (c-Z), *trans*-zeatin (t-Z), Δ^2 -isopentenyladenine (2iP), isoamyladenine (iso Am) and N⁶-benzyladenine (BA) at 10^{-3} M. Vertical bars indicate standard errors. Aqueous control (C).

fruit growth (Figs. 3, 4). *Cis*-zeatin (c-Z) was much less active than t-Z but it was equally as active as kinetin (K). That t-Z was more active than c-Z has been reported earlier (5, 8).

Our results show that BA and other cytokinins can stimulate the development of cucumber fruit to varying degrees. At present, we cannot explain the results as to why BA was always highly active and c-Z and K were much less so, particularly when K exhibited weaker activity as compared to results of other assays where K is found to be highly active (7). There is a possibility that the difference in permeability of the ovary tissue and the metabolism of cytokinins *in vivo* might alter cytokinin activities.

Endogenous cytokinins

Extract of fruits which were harvested 10 days

after flowering was fractionated by solvents and constituents separated by paper chromatography. Cytokinin activity of the fractions were bioassayed using the *Amaranthus* betacyanin method (Fig. 5). Significant activity was found at Rf 0.6~0.8, which corresponded to the Rf of t-Z. Additionally, weak activity was found at lower Rf 0.1~0.3, suggesting the presence of polar cytokinins as based on their chromatographic mobility. The amounts of endogenous cytokinins were calculated from the histograms as zeatin equivalents. Changes in the amounts of the endogenous cytokinins and fruit size with age are shown in Fig. 6. The amounts of the cytokinins increased rapidly 4 to 10 days after anthesis but slowly thereafter. The increase in the cytokinin amounts fairly coincided with the increase in fruit length and fresh weight during the growing period.

Fig. 5. Histogram of cytokinin activity derived from a paper chromatogram of an extract obtained from cucumber fruits harvested 10 days after flowering using the *Amaranthus* test. Aqueous control (- -).

Fig. 6. Growth curve of cucumber fruits and changes in amounts of endogenous cytokinins expressed as zeatin equivalents.

The present results provide an evidence for promotive role of endogenous cytokinins such as zeatin and its related compounds on the early growing period of cucumber fruit. Recently, Take-no *et al.* (12) reported that when treated with BA, an increase in cell number and size of pericarp tissue of enlarging cucumber fruits were accompanied with an increase in the levels of endogenous auxins. Thus, we suggest the idea that exogenous or endogenous cytokinins in conjunction with other endogenous plant hormones including gibberellins (10) regulate the fruit growth of cucumber.

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キュウリ果実の生長における各種サイトカイニンの促進作用

小川幸持・西川司朗・井上 昇*・青木勝平

三重大学 生物資源学部 514-22 津市高野尾町

摘 要

大型ガラス室の、キュウリ(長日落合 2号)の抑制栽培(9月上旬定植)では11~12月、開花後の子房すなわち果実の生長が著しく遅れるか、あるいは止り、“流れ果”になるものが多い。この時期に、開花中の子房に各種のサイトカイニンの溶液を処理するとその生長が促進される。この促進作用の順位はN⁶-ベンジルアデニン(BA)が最も強く、次いでN⁶-イソアミルアデニン(isoAm), Δ²-イソペンテニルアデニン(2 iP), トランス-ゼアチン(t-Z)であり、シス-ゼアチン(c-Z)およびカイネチン(K)は最も弱い。

抑制栽培の10月、正常に生長している単為結果の果実における内生サイトカイニンの消長を調査した。果実のアルコール抽出物から精製して得た塩基性の分画をペーパークラマトグラフィで展開してヒモゲイトウ幼苗のベタシアニン形成による、検定法を用いてサイトカイニン活性を調べた。ゼアチンとその関連物質のサイトカイニンの全量は、開花して4日後から著しく増加し、果長および果重の生長と並行して増加する。キュウリ果実の初期生長に内生サイトカイニンは促進的な作用をしていると推察される。

*現在：半田農業高等学校，愛知県 半田市