J. Japan. Soc. Hort. Sci. 56(4) : 417—425. 1988.

Cytokinin Concentrations in Roots and Root Xylem Exudate of Cucumber and Figleaf Gourd as Affected by Root Temperature[1]

Shoji Tachibana
Faculty of Bioresources, Mie University, Tsu, Mie 514

Summary

Effects of root temperature on cytokinin concentrations in roots and root xylem exudate were compared between cucumber (*Cucumis sativus* L. cvs. 'Suyô' and 'Kurume-ochiai H') and figleaf gourd (*Cucurbita ficifolia* Bouchè). The root-chilling tolerance of the latter plants is known to be considerably higher than that of the former plants.

Cytokinin concentrations in root xylem exudate of cucumber cultivars decreased sharply at lower root temperatures, with 'Suyô' more greatly affected. In contrast, in figleaf gourd the concentrations were relatively unchanged at 14-23°C, and increased to a strikingly high level at 11°C. Cytokinin concentrations in roots were highest at 23°C in 'Suyô' and at 17°C in 'Kurume-ochiai H', and decreased at lower root temperatures in both cultivars. In figleaf gourd, on the contrary, the concentrations were significantly higher at 14°C than at higher temperatures, and increased to a still higher level at 12°C. Major cytokinins in the roots of figleaf gourd and probably of cucumber cultivars were zeatin and zeatin riboside, and the composition apparently changed little with root temperature.

These results strongly suggest that figleaf gourd roots respond to low root temperature by stimulating cytokinin synthesis within the roots. However, cytokinin synthesis in cucumber roots may be greatly inhibited by low root temperature. The implications for the difference in root-chilling tolerance between cucumber and figleaf gourd are discussed.

Introduction

Recently much evidence has accumulated in support of the concept that growth and development of shoots are dependent on hormones derived from roots. Roots and root xylem exudate contain an appreciable amount of several hormones(26). Among them, cytokinins are regarded as being synthesized in roots(29). It is most likely that cytokinins produced in roots move to shoots and there regulate the growth and nature of development of shoots. Thus, cytokinin synthesis may be one of the most fundamental functions of roots, comparable with water and nutrient absorption.

Cytokinin synthesis in roots is influenced by a number of terrestrial environments, such as fertilizer levels(15, 19), moisture(24), aeration(5) and temperature(2, 16). Atkin *et al.* (2) found a decrease in cytokinin concentrations in exudate of corn roots following growth at low root temperature. According to Skene and Kerridge(23), cytokinins in exudate of grape vines differed in composition with root temperature. These changes are regarded as one of the factors which determine growth of shoots at reduced root temperatures.

It seems of great interest to know whether the cytokinin synthesizing capacity of roots at low root temperature has any relation to the sensitivity of plants to chilling root temperatures. To date no information to our knowledge is available which describes the relation between them. In the present paper the effect of root temperature on cytokinin concentrations in roots and root xylem

[1] Received for publication November 19, 1985.

exudate is compared betwen cucumber and figleaf gourd. The root-chilling tolerance of figleaf gourd is considerably higher than that of cucumber (25).

Materials and Methods

Cytokinin concentrations in root xylem exudate as affected by root temperature (Exp. 1)

Seedlings of cucumber (cvs. 'Suyô' and 'Kurume-ochiai H') and figleaf gourd were grown uniformly in 4 culture vessels with 220 *l* of one third strength Hoagland No. 2 solution at 20°C root temperature until about 10 leaves unfolded. Then, root temperatures were changed to 11, 14, 17 and 23°C. After 10 days plants were decapitated at noon, and the exudate from cut ends of stumps was collected for 20 h. Exudate was gathered as frequently as possible during the period and stored immediately at −20°C until cytokinin assay.

Cytokinins were extracted with 80% methanol from lyophilized exudate. The extracts were reduced to aqueous phase *in vacuo*, and the aqueous phase at pH 2.5 was shaken with ethylacetate to remove lipids. After readjustment to pH 8, the aqueous phase was partitioned against n-butanol to extract cytokinins. Then the butanol extracts, taken to dryness and dissolved in acid water, were streaked on thin layer chromatography (TLC) plates. Chromatography was on silica gel (Whatman LK 6, 250 μm) plates developed with 0.03 M boric acid (pH 8.4). Chromatograms were divided into 10 equal Rf sections, scraped off the plates, eluted with 80% ethanol, and assayed for cytokinin-like activity using soybean callus (*Glycine max* L., cv. Acme) (17).

Cytokinin concentrations in roots as affected by root temperature (Exp. 2)

Young plants of cucumber (cvs. 'Suyô' and 'Kurume-ochiai H') and figleaf gourd with 3 unfolded leaves were grown in one third strength Hoagland No. 2 solution at root temperatures of 12, 14, 17 and 23°C for 12 days, harvested and weighed for fresh weight determination. Roots were frozen on dry ice and stored at −20°C until required.

Cytokinins in roots were extracted with 80% methanol for 24 h at 4°C. Extracts were reduced to aqueous phase *in vacuo*, acidified to pH 3.5, shaken with petroleum ether to remove lipids, and purified by Dowex 50 W × 8 cation exchange resin as described by Van Staden (27). Eluates were evaporated to dryness under reduced pressure. The residue dissolved in acid water was streaked on Toyo No. 526 filter paper and developed with isopropanol-ammonia-water (10 : 1 : 1, v/v). After drying the chromatograms were divided into 10 equal Rf zones, which were assayed for cytokinins as described above.

Preliminary identification of cytokinins in roots was carried out on a Sephadex LH-20 column. Cytokinins were extracted from roots of figleaf gourd grown at 12 and 23°C for 10 days in a separate experiment. Eluates from a Dowex 50 W × 8 column were taken to dryness, dissolved in 35% ethanol and loaded on a Sephadex LH-20 column (2.5 × 70 cm). Elution was performed with 35% ethanol at flow rate of 30 ml/h. Twenty ml fractions were collected, determined for absorbance at 254 nm and assayed for cytokinins as above.

Results

Cytokinin concentrations in root xylem exudate as affected by root temperature (Exp. 1)

Optimum root temperatures for root growth were above 23°C in 'Suyô', 17°C in 'Kurume-ochiai H' and 14°C in figleaf gourd

Table 1. Effect of root temperature on root dry weight and exudation rate (Exp. 1).

Crop	Root temp. (°C)	Dry weight ($g \cdot plant^{-1}$)	Exudation rate ($ml \cdot g^{-1}$ dw $20 h^{-1}$)
'Suyô' cucumber	11	0.99	42.7
	14	1.28	134.2
	17	1.45	260.0
	23	2.00	220.0
'Kurume-ochiai H' cucumber	11	0.71	42.3
	14	1.00	65.0
	17	1.30	93.1
	23	1.05	66.7
Figleaf gourd	11	1.55	30.1
	14	2.23	62.8
	17	1.88	64.9
	23	1.88	28.1

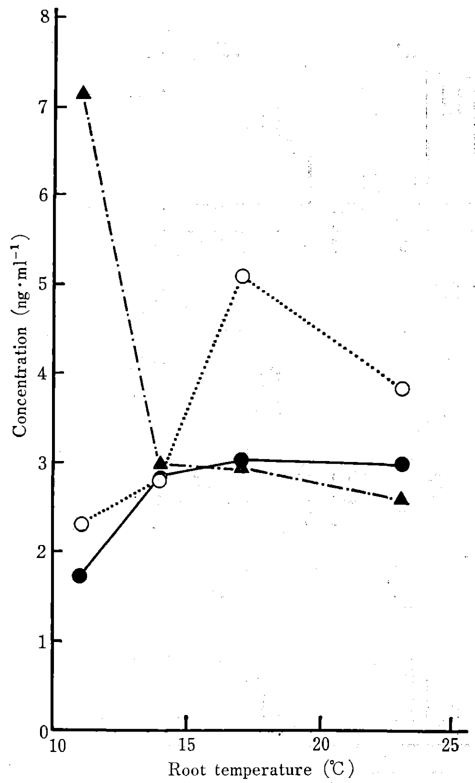

Fig. 1. Cytokinin concentration in root xylem exudate of 'Suyô' cucumber (○), 'Kurume-ochiai H' cucumber (●) and figleaf gourd (▲) as affected by root temperature (Exp. 1).

(Table 1). Root growth was significantly inhibited at 11°C in all crops, with 'Suyô' most greatly influenced. Table 1 also shows exudation rates in terms of exudate volume per unit root dry weight. The rate was highest at 17°C in both cucumber cultivars, and declined linearly with decrease in root temperature. In figleaf gourd it was highest at 14-17°C and declined sharply at 11°C.

Cytokinin concentrations in exudate of 'Suyô' were very high at 17-23°C, as compared with those of the remaining two crops (Fig. 1). The concentration was highest at 17°C in 'Suyô' and at 17-23°C in 'Kurume-ochiai H'. It decreased sharply at 14 and 11 °C in 'Suyô', whereas it only decreased significantly at 11°C in 'Kurume-ochiai H'. In contrast, in figleaf gourd the concentration increased gradually with decrease in root temperature down to 14°C, and increased to a strikingly high level at 11°C.

Table 2. Effect of root temperature on total content of cytokinins in root xylem exudate (ng·exudate^{-1}) (Exp. 1).

Crop	Root temperature (°C)			
	11	14	17	23
'Suyô' cucumber	97	377	1,323	848
'Kurume-ochiai H' cucumber	52	185	371	210
Figleaf gourd	340	417	361	186

Total content of cytokinins in exudate was highest at 17°C in both cucumber cultivars (Table 2). It declined sharply below 14°C, in 'Suyô' in particular. The total content in exudate of figleaf gourd was highest at 14°C and decreased only slightly at 11°C.

Chromatograms of soybean callus assay (Fig. 2) show that, although the separation of cytokinins was not good enough to make a certain conclusion, exudate contained zeatin and zeatin riboside as major cytokinins, irrespective of kinds of crops and root temperatures.

Cytokinin concentrations in roots as affected by root temperature (Exp. 2)

Total fresh weights of plants were greatest at 17-23°C in 'Suyô', at 14-17°C in 'Kurume-ochiai H' and at a broad temperature range from 14 to 23°C in figleaf gourd (data not shown). Maximum root fresh weights were obtained at 17-23°C in 'Suyô' and at 17°C in 'Kurume-ochiai H'. Root growth was greatly inhibited below 14°C and at 12°C in 'Suyô' and 'Kurume-ochiai H', respectively. In figleaf gourd root growth was greatest at 14°C and inhibited only slightly at 12°C.

Cytokinin concentrations in 'Suyo' roots decreased linearly with lower root temperatures (Fig. 3). Concentrations in 'Kurume-ochiai H' were very high at 17°C, and declined sharply below and above it. On the other hand, in figleaf gourd concentrations increased gradually with decrease in root temperature down to 14°C, and increased to a markedly high level at 12°C, as in exudates. Total content of cytokinins in roots was also maximum at 23°C in 'Suyô', at 17°C in 'Kurume-ochiai H' and at 12°C in figleaf gourd (Table 3). Total content in roots of plants

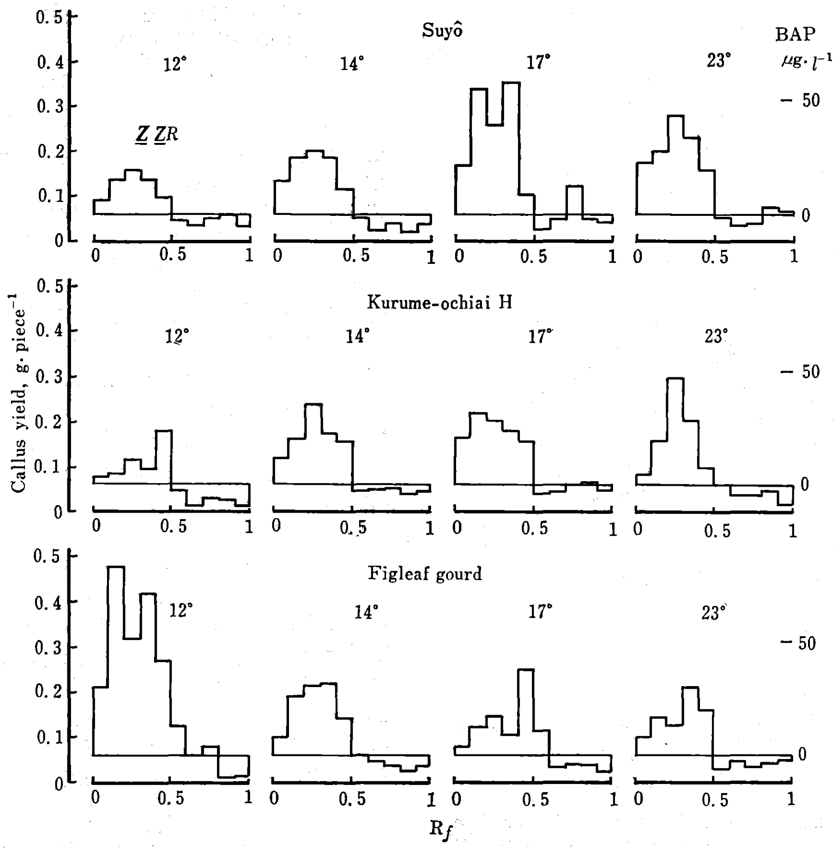

Fig. 2. Chromatorams of cytokinin activities in root xylem exudate. Butanol extract from 200 ml exudate (180 ml for 'Kurume-ochiai H' at 11℃) was chromatographed on a thin layer plate with 0.03 M boric acid (pH 8.4). Z=zeatin, ZR=zeatin riboside (Exp. 1).

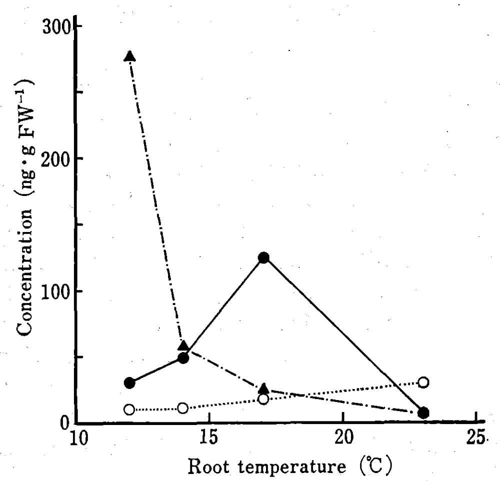

Fig. 3. Effect of root temperature on the cytokinin concentrations in roots of 'Suyô' cucumber (○), 'Kurume-ochiai H' cucumber (●) and figleaf gourd (▲) (Exp. 2).

Table 3. Effect of root temperature on total content of cytokinins in roots ($ng \cdot root^{-1}$) (Exp. 2).

Crop	Root temperature (℃)			
	12	14	17	23
'Suyô' cucumber	124	175	348	523
'Kurume-ochiai H' cucumber	235	538	1,511	158
Figleaf gourd	5,344	1,480	515	200

grown at lower root temperatures (12 and 14 °C) increased in the order of 'Suyô', 'Kurume-ochiai H' and figleaf gourd.

Major cytokinins in roots were apparently zeatin and zeatin riboside in all three crops (Fig. 4). The change in the composition of cytokinins was assumed to be insignificant.

Cytokinins were separated on a Sephadex LH-20 column from roots of figleaf gourd grown at 12 and 23°C root temperatures for

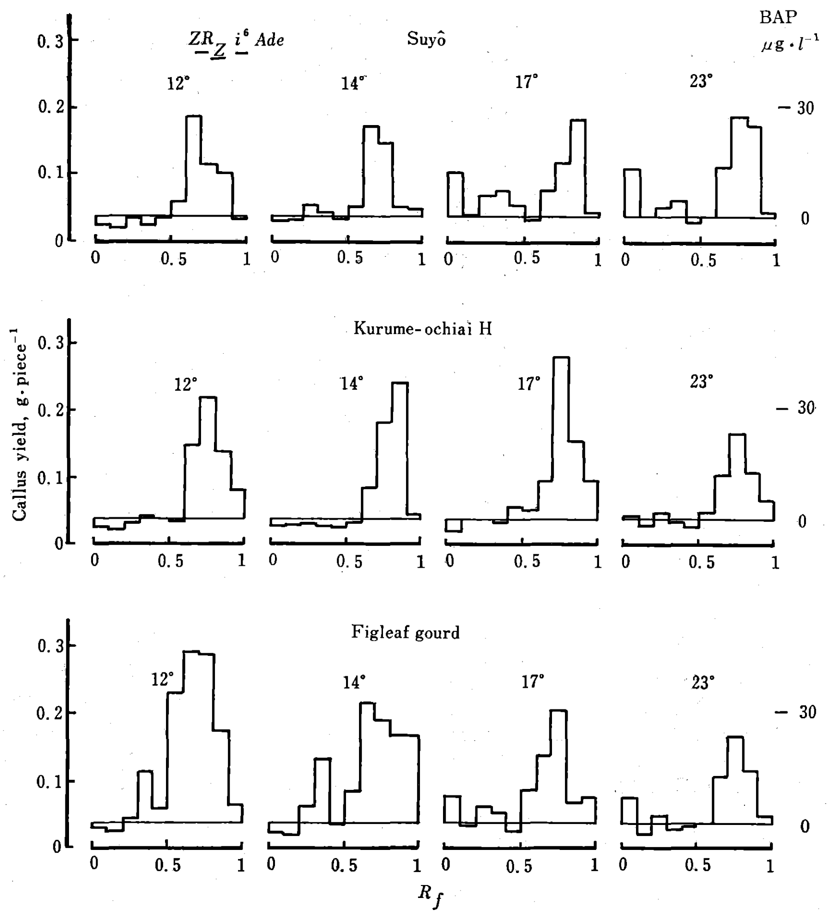

Fig. 4. Chromatograms of cytokinin activities in roots. Dowex purified extract was chromatographed on paper with isopropanol-ammonia-water (10 : 1 : 1). Z=zeatin, ZR=zeatin riboside, i^6Ade=N^6-isopentenyladenine (Exp. 2).

10 days (Fig. 5). This results indicated that figleaf gourd roots contained mostly zeatin and zeatin riboside, and that both increased in concentration at 12°C. Dotted lines in the figure show the absorbance at 254 nm. The retention volume of the first large peak agreed with that of adenosine-5′-monophosphate(1).

Discussion

The most characteristic feature of evidence from the present study is that cytokinin concentrations in both roots and root xylem exudate of figleaf gourd increased significantly upon chilling the roots. This was unexpected, since there is no evidence which shows an increase in cytokinin synthesis within roots at reduced root temperatures.

Recently, Yamada *et al.* (30) found higher cytokinin activity in roots of *Citrus unshiu* grown at 5°C root temperature than in those grown at higher temperatures. They attributed this to reduced transport of cytokinins out of roots, since the activities in both stem and leaves declined simultaneously at 5°C. However, in figleaf gourd not only concentrations in roots but the total content in exudate increased at low root temperatures. This indicates that the increase in roots is not due to reduced transport out of roots, but rather to increased synthesis within the roots.

Van Staden and his colaborators(4, 28) found that chilling the dormant seeds of

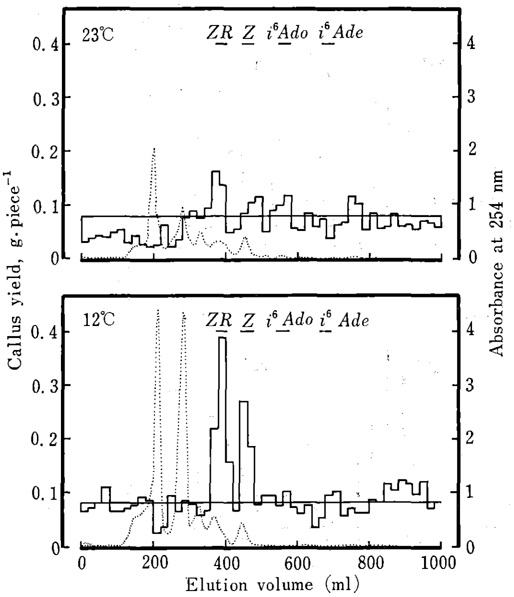

Fig. 5. Soybean bioassay of roots of figleaf gourd grown at 12 and 23°C root temperatures. Dowex purified extract (equivalent to 70g fresh weight) was fractionated on Sephadex LH-20. Absorbance at 254 nm is shown by the dotted line. Z=zeatin, ZR=zeatin riboside, i^6Ado=N^6-isopentenyladenosine, i^6Ade=N^6-isopentenyladenine (Exp. 2).

Acer, *Leucandendron* and *Protea* resulted in increase in cytokinins in the seeds. An interconversion from water soluble to butanol-soluble cytokinins was stimulated in the first two genera, and possibly increased *de novo* synthesis took place in the third. The mechanisms by which cytokinin synthesis was stimulated at low root temperature in figleaf gourd are not known. Probably, synthesis at the root apex *per se* was stimulated, since apical meristems are regarded as sites of cytokinin synthesis(21), and since the increased root growth at 12–14°C was not due to increase in numbers of lateral roots (=increase in apices) but to increased thickening.

In cucumber, on the other hand, low root temperature caused cytokinin concentrations to decrease in both roots and exudate, certainly due to inhibition of cytokinin synthesis within the roots. Decrease in cytokinin concentrations in plants grown at low root temperature has been demonstrated by several investigators(2, 16). According to Shôno and Furuya(20), growth of a cytokinin-non-requiring strain of tobacco calli was suppressed by low temperature without cytokinin, possibly due to reduced cytokinin biosynthesis.

It has been well documented that growth of shoots and fruits is stimulated by certain concentrations of exogenously-applied cytokinins (3, 14, 22). According to Menhenett and Wareing(16), leaf discs of plants grown at low root temperature exhibited a greater expansion growth on kinetin solution than those grown at high root temperature. This indicated that cytokinin concentrations in leaves of low-temperature grown plants were lower than those in leaves of high-temperature grown plants. Although cytokinin concentrations in leaves were not determined in this study, the data on total cytokinin content in exudate suggest that figleaf gourd leaves may have contained cytokinins at much higher levels than cucumber leaves at low root temperature. This is probably one of the physiological factors that cause a difference between cucumber and figleaf gourd in shoot growth at low root temperature.

Cytokinins are also involved in chloroplast build-up and chlorophyll formation(10, 11). Upper leaves exhibited chlorosis at 11–12°C in cucumber cultivars but not in figleaf gourd, as in the previous study(25). Involvement of cytokinin concentrations in leaves in low-root temperature induced chlorosis is under study.

Cytokinin concentration in cucumber roots was highest at temperatures where root growth was maximum in both cultivars. It declined at lower root temperatures concomitantly with decrease in root growth. In figleaf gourd roots, the concentration increased significantly at 14°C where root growth was most stimulated. But it increased still more at the lowest temperature where root growth was restricted.

The literature shows that exogenously-applied cytokinins stimulate or inhibit growth of roots and their functions such as water and nutrient absorption(7, 13). Probably, cytokinins elicit these responses dependent upon the applied concentrations and their interaction with other hormones. Butcher and Street(6) found that kinetin stimulated growth of excised tomato roots in high-sucrose media but inhibited it in low media. According to Radin and Loomis(18), zeatin-based cytokinins extracted from radish roots stimulated secondary thickening of roots of the plant.

Figleaf gourd roots thicken characteristically upon chilling at 12–14°C, with increased cell enlargement(25). Furthermore, it is known that low root temperature increases sugar concentration in roots(8), and that cytokinins stimulate photosynthate translocation(9). Unpublished data by the present author showed a greater increase in sugar concentrations in roots of figleaf gourd than in cucumber at low temperature. It seems therefore that in figleaf gourd, at least at 14 °C, increased cytokinin concentration could have stimulated root meristem activities and photosynthate translocation to roots, and thereby could have resulted in greater cell enlargement and eventually greater root growth. Whether the surprisingly high level of cytokinins at 11–12°C has some causal relation to retardation of root growth, particularly in Exp. 1, needs further investigation.

The present study suggests that cytokinin concentrations of roots may be responsible in part for the difference in growth rate of roots and shoots at low root temperature. High root-chilling tolerance of figleaf gourd may have some bearing on the stimulation of cytokinin synthesis within roots under low root temperature.

Acknowledgement

The author wishes to express his sincere appreciation to Dr. S. Kuraishi, Professor of Hiroshima University, for the gift of soybean seeds cv. Acme.

Literature Cited

1. ARMSTRONG, D. J., W. J. BURROWS, P. K. EVANS and F. SKOOG. 1969. Isolation of cytokinins from tRNA. Biochem. Biophys. Res. Commun. 37 : 451—456.
2. ATKIN, R. K., G. E. BARTON and D. K. ROBINSON. 1973. Effect of root-growing temperature on growth substances in xylem exudate of *Zea mays*. J. Exp. Bot. 24 : 475—487.
3. BISWAS, A. K. and S. K. MONDAL. 1986. Regulation by kinetin and abscisic acid of correlative senescence in relation to grain maturation, source-sink relationship and yield of rice (*Oryza sativa* L.). Plant Growth Reg. 4 : 239—245.
4. BROWN N. A. C. and J. VAN STADEN. 1973. The effect of stratification on the endogenous cytokinin levels of *Protea compacta* and *Leucandendron daphnoides*. Plant Physiol. 28 : 388—392.
5. BURROW, W. J. and D. J. CARR. 1969. Effect of flooding the root system of sunflower plants on the cytokinin content in the xylem sap. Physiol. Plant. 22 : 1105—1112.
6. BUTCHER, D. N. and H. E. STREET. 1960. Effects of kinetin on the growth of excised tomato roots. Physiol. Plant. 13 : 46—55.
7. COLLINS, J. C. and A. P. KERRIGAN. 1974. The effects of kinetin and abscisic acid on water and ion transport in isolated maize roots. New Phytol. 73 : 309—314.
8. CROWFORD, M. M. and T. J. HEXTER. 1977. Root growth and carbohydrate metabolism at low temperature. J. Exp. Bot. 28 : 917—925.
9. DÜRING, H. and G. ALLEWELDT. 1980. Effect of plant hormones on phloem transport in grapevines. Ber. Deutsch. Bot. Ges. 93 : 339—347.
10. FLETCHER, R. A., C. TEO and A. ALI. 1973. Stimulation of chlorophyll synthesis in cucumber cotyledons by benzyladenine. Can. J. Bot. 51 : 937—939.
11. HARVEY, B. M. R., B. C. LU and R. A. FLETCHER. 1974. Benzyladenine accelerates chloroplast differentiation and stimulates photosynthetic enzyme activity in cucumber cotyledons. Can. J. Bot. 52 : 2581—2586.
12. ITAI, C. and Y. VAADIA. 1965. Kinetin-like activity in root exudate of water-stressed sunflower plants. Physiol. Plant. 18 : 941—944.
13. KATSUMI, M. 1972. Cytokinins. p. 193—250. In : Y. MASUDA, M. KATSUMI and H. IMASEKI (eds) Plant hormones. Asakura Shoten, Tokyo (In Japanese)
14. KURAISHI, S. and F. S. OKUMURA. 1956. The effect of kinetin on leaf growth. Bot. Mag. (Tokyo). 69 : 300—306.
15. MENARY, R. C. and J. VAN STADEN. 1976. Effect of phosphorus nutrition and cytokinins on flowering in the tomato, *Lycopersicon esculentum* Mill. Aust. J. Plant Physiol. 3 :

201—205.

16. MENHENETT, R. and R. F. WAREING. 1975. Possible involvement of growth substances in the response of tomato plants (*Lycopersion esculentum* Mill.) to different soil temperatures. J. Hort. Sci. 50 : 381—397.

17. MILLER, C. O. 1968. Naturally occurring cytokinins. p. 33—45. In : F. WIGHTMAN and G. SETTEFIELD (eds) Biochemistry and physiology of plant growth substances. Runge Press, Ottawa.

18. RADIN, J. W. and R. S. LOOMIS. 1971. Changes in the cytokinins of radish root during maturation. Physiol. Plant. 25 : 240—244.

19. SATTELMACHER, B. and H. MARSHNER. 1978. Nitrogen nutrition and cytokinin activity in *Solanum tuberosum*. Physiol. Plant. 42 : 185—189.

20. SHÔNO, K. and T. FURUYA. 1971. Effects of temperature on the cytokinin requirement of tobacco calluses. Plant Cell Physiol. 12 : 61—71.

21. SHORT, K. C. and J. G. TORREY. 1972. Cytokinins in seedling roots of pea. Plant Physiol. 49 : 155—160.

22. SKENE, K. G. M. 1975. Cytokinin production by roots as a factor in the control of plant growth. p. 365—396. In : J. G. TORREY and D. T. CLARKSON(eds) The development and function of roots. Acad. Press, London.

23. SKENE, K. G. M. and G. H. KERRIDGE. 1967. Effect of root temperature on cytokinin activity in root exudate of *Vitis vinifera* L. Plant Physiol. 42 : 1131—1139.

24. SUZUKI, S., M. OKAWA and T. USHIJIMA. 1975. The effect of cytokinin on the growth and senescence of radish plants under water stress. Environ. Control in Biol. 13 : 55—64.

25. TACHIBANA, S. 1982. Comparison of effects of root temperature on the growth and mineral nutrition of cucumber cultivars and figleaf gourd. J. Japan. Soc. Hort. Sci. 51 : 299—308.

26. TORREY, J. G. 1976. Root hormones and plant growth. Ann. Rev. Plant Physiol. 27 : 435—459.

27. VAN STADEN, J. 1976. Occurrence of a cytokinin glucoside in the leaves and honeydew of *Salix babylonica*. Physiol. Plant. 36 : 225—228.

28. VAN STADEN, J., D. P. WEBB and P. F. WAREING. 1972. The effect of stratification on endogenous cytokinin levels in seed of *Acer saccharum*. Planta 104 : 110—114.

29. VAN STANDEN, J. and J. E. DAVEY. 1979. The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ. 2 : 93—106.

30. YAMADA, H., H. MUKAI, N. UTSUNOMIYA, A. SUGIURA and T. TOMANA. 1985. The effect of low root temperature on the cold hardiness of citrus species and avocado. J. Japan. Soc. Hort. Sci. 53 : 419—426. (In Japanese)

キュウリとクロダネカボチャの根及び木部出液水のサイトカイニン含有率に及ぼす根温の影響

橘　昌司
三重大生物資源学部　514　津市上浜町1515

摘　要

キュウリ（品種；四葉及び久留米落合H型）とクロダネカボチャの根及び根の木部出液水のサイトカイニン含有率に及ぼす根温の影響について調べた.

キュウリ2品種の木部出液水のサイトカイニン含有率は低根温によって低下し，それは特に‘四葉’において顕著であった．これに対して，クロダネカボチャのそれは14～23℃では大きな変化がなかったが，11℃では著しく高い値になった．一方，根組織のサイトカイニン含有率は，‘四葉’では23℃で，‘久留米落合H型’では17℃で最大になり，ともにそれ以下の根温では低下したのに対して，クロダネカボチャでは14℃まで根温の低下につれて次第に増大し，12℃では更に顕著に増大した．根組織，出液水ともに，ゼアチンとゼアチンリボシドが主要なサイトカイニンであると推定されたが，根温による組成の変化は顕著ではなかった.

以上の結果から，キュウリでは低根温によって根のサイトカイニン生成が抑制されるのに対して，クロダネカボチャでは低根温に感応して根端でのサイトカイニン生成が高まることが示唆される．このような根のサイトカイニン生成能の違いがキュウリとクロダネカボチャの根の低根温下での生長やひいては低根温耐性に違いをもたらす生理的要因のひとつであると推察される.