

Quantitative Changes of Six Cytokinins in Tuberous Roots During The Development of Sweet-Potato Plants

メタデータ	言語: English 出版者: 明治大学農学部 公開日: 2012-05-24 キーワード (Ja): キーワード (En): 作成者: 杉山, 民二 メールアドレス: 所属:
URL	http://hdl.handle.net/10291/12434

Quantitative Changes of Six Cytokinins in Tuberos Roots During The Development of Sweet-Potato Plants*

Tamizi SUGIYAMA

(Accept: June 7, 1996)

Summary

Presence and levels of cytokinins in both the tuberos roots and the fibrous as well as pencil-like roots of sweet potato (*Ipomoea batatas* Lam, cv. Kohkei No. 14) were determined at five different stages during the tuberization process by means of mass spectrometry using deuterium-labeled standards. *Trans*-ribosylzeatin was maintained at a relatively high level throughout the development, except for the 92nd day. The compound reached the maximum level at an earlier stage, which shares approximately 78% of the amount of cytokinin found. The level of 9-glucosyl-N⁶-isopentenyladenine was high in the fibrous and pencil-like roots. It was observed that the level of *trans*-ribosylzeatin markedly decreased with increase of *cis*-ribosylzeatin level, and the relative proportion of *cis*- and *trans*-isomer consequently varied on the 92nd day, but the physiological significance of the phenomenon is not clear. It seems therefore that *trans*-ribosylzeatin plays a causative role in the thickening of tuberos roots of sweet potato.

INTRODUCTION

The tuberization of sweet-potato and potato is known to be regulated by the combined action of several phytohormones^{1),10),14),15),17)}. In particularly, cytokinins have often been considered to be major principle in the tuberization, because which are known to stimulate cell division and involve a sink-source relationship in crop^{5),7),8),16-20)}. This is supported by findings that the endogenous cytokinin activity is high in developing tuberos roots^{6),12)}. Several workers previously reported on the base of determination by EIA method and GC-MS that *trans*-RZ content in the roots of sweet potato increased rapidly when the thicken roots began to appear^{12),17)}. In potato, *cis*-RZ has been identified as the major cytokinin species, so *cis*-RZ appear to be a functional cytokinin which plays a causative role in the tuberization process^{4),8),14),19)}. In this paper, attempts have been made to determine the endogenous six cytokinins, which were *trans*- and *cis*-RZ, *trans*- and *cis*-Z, iPA and iPG, in both the tuberos roots and fibrous as well as pencil-like roots, and to compare the quantitative changes in their levels in correlation with the tuberization process of sweet potato.

* Part of this study was made at Tokyo University of Agriculture and Technology.

Abbreviations: Z, Zeatin; RZ, Ribosylzeatin; iP, N⁶-isopentenyladenine; iPA, N⁶-isopentenyladenosine; iPG, 9-Glucosyl-N⁶-isopentenyladenine; HPLC, High performance liquid chromatography; TLC, Thin layer chromatography; GC-MS, Gas chromatography-mass spectrometry; SIM, Selected ion monitoring; TMS, Trimethylsilyl; EIA, Enzyme immunoassay.

MATERIALS AND METHODS

Plants. Vine cuttings of sweet potato (*Ipomoea batatas* Lam cv. Kohkei No. 14) were planted at the university farm of Tokyo University of Agriculture and Technology in Fuchu-shi on April 30, 1983. The plants grown under the field conditions were harvested five times from June 5 to September 27, and then divided into three parts; tuberous roots, fibrous and pencil-like roots, and stem and leaves. The growth curve shown in Fig. 1 was presented as the average 50 plants (36th), 10 plants (61st), 7 plants (92nd) and 5 plants (120th and 150th).

Extraction and purification of cytokinins. For cytokinin determinations were used maximal tuberous-roots per a plant on each stages. They were 50 g (36th), 280 g (61st), 400 g (92nd), 400 g (120th) and 470 g (150th) by the fresh weight. Fibrous and pencil-like roots were also used 160 g (36th), 100 g (61st), 200 g (92nd), 120 g (120th) and 100 g (150th) by the fresh weight. Their samples were sliced and soaked in 99% ethanol for one month at 4°C. Further,

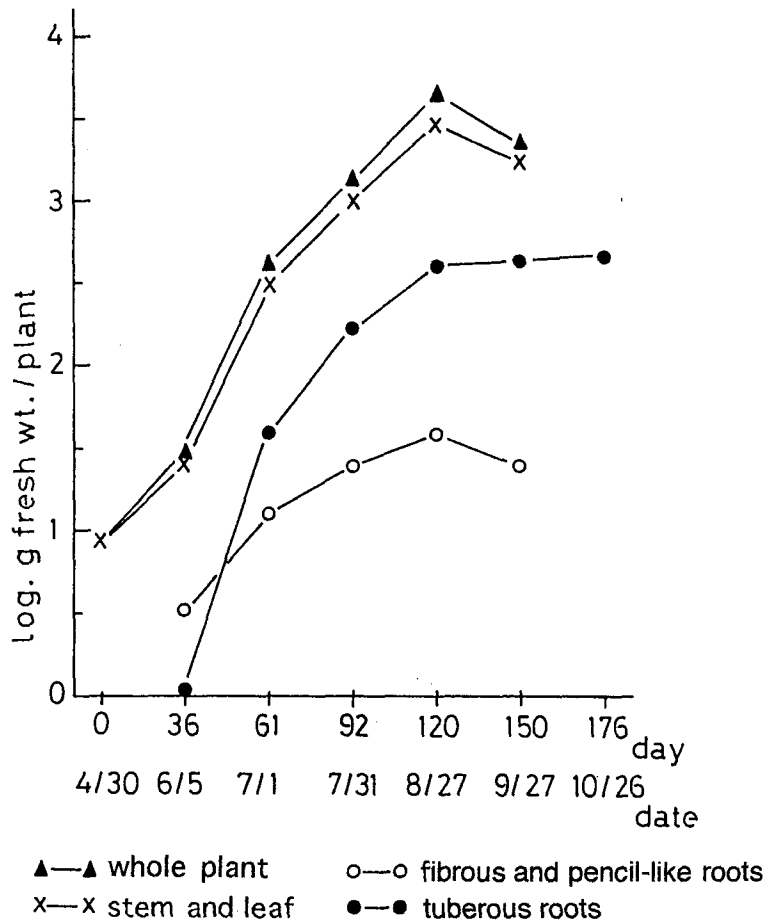


Fig. 1 Growth curve of sweet-potato plant.

Table I AMOUNTS OF THE ROOTS USED FOR CYTOKININ ANALYSIS.

Stage	Tuberous roots(g, fw.)	Fibrous and pencil-like roots(g, fw.)
36th day	50	80
61st day	70	100
92nd day	80	100
120th day	50	60
150th day	47	50

ethanol extraction was repeated three times every a week. Each of the ethanol extracts was combined, respectively. To part of their extracts shown in Table I was added Z-d₅ (2.6 μg), RZ-d₅ (4.7 μg), iPA-d₆ (2.8 μg) and iPG-d₆ (4.95 μg) as internal standards.

Cytokinin purification. The purification procedure of cytokinins is summarized Fig. 2. Procedural details were described in previous papers^{(21), (22)}.

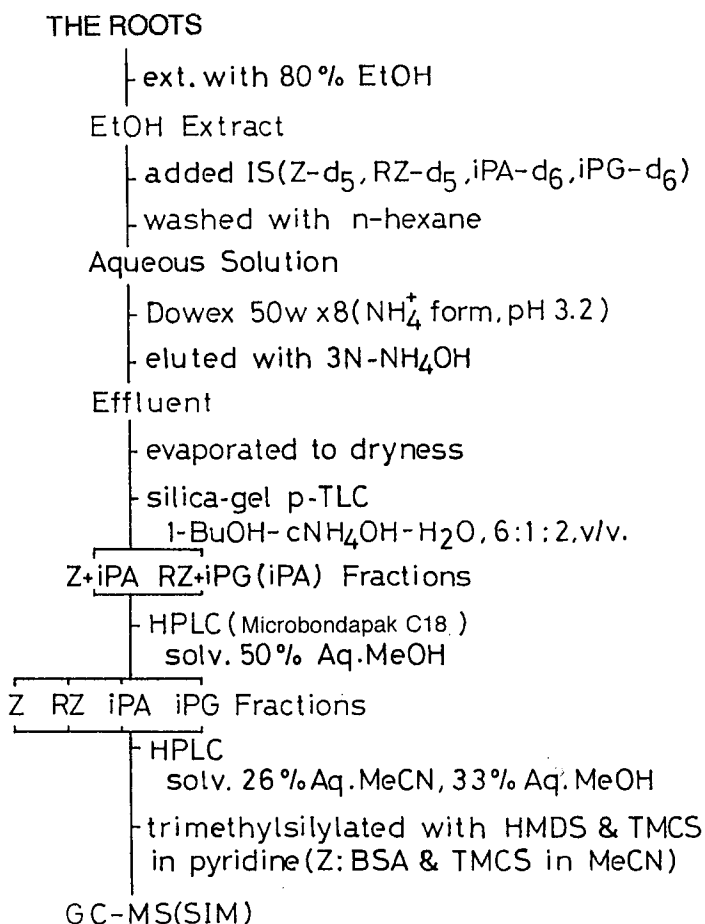


Fig. 2 Procedures for the separation of cytokinins from the roots extracts of sweet potato.

Chemicals. Deuterium-labeled standards used for the experiments were prepared as described previously^{2),21)}. Z and RZ consisted of the *trans*-isomer (92%) and *cis*-isomer (8%).

High-performance liquid chromatography. A Shimadzu model LC 3A equipped with a UV detector (270 nm) was used. The column (30 cm × 7.8 mm i.d.) was prepacked with Microbondapak C18 (Waters Associates Inc., USA). Elution was done with three kinds of solvent (40% aq. methanol, 15% aq. acetonitrile and 30% aq. methanol) at a flow rate of 2 ml/min.

Trimethylsilylation. Each fractions of RZ, iPA and iPG in a minivial was dried in vacuo over phosphorous pentoxide at 90°C for 30 min. To the dry residues were added pyridine (35 μl), hexamethyldisilazane (10 μl) and trimethylchlorosilane (5 μl). The mixtures were heated at 120°C for 1 hr. Fractions of each of *cis*- and *trans*-Z in a mini-vial were also dried over phosphorous pentoxide, and then silylated with a mixture of acetonitrile, bis(trimethylsilyl)acetamide and trimethylchlorosilane (20 : 10 : 1 3 v/v) at 70°C for 10 min.

Selected ion monitoring. A Shimadzu LKB9000 gas chromatograph mass spectrometer equipped with a multiple ion detector was used under the following conditions: ionizing energy, 20 eV; separating port temperature, 280°C; and ion source temperature, 290°C. OV-1 (1.5%) on silylated Chromosorb in a glass column (1 m × 3 mm i.d.) was used at 30 ml of helium/min. The column oven temperatures were at 210°C (Z), 250°C (iPA), 260°C (RZ) and 270°C (iPG).

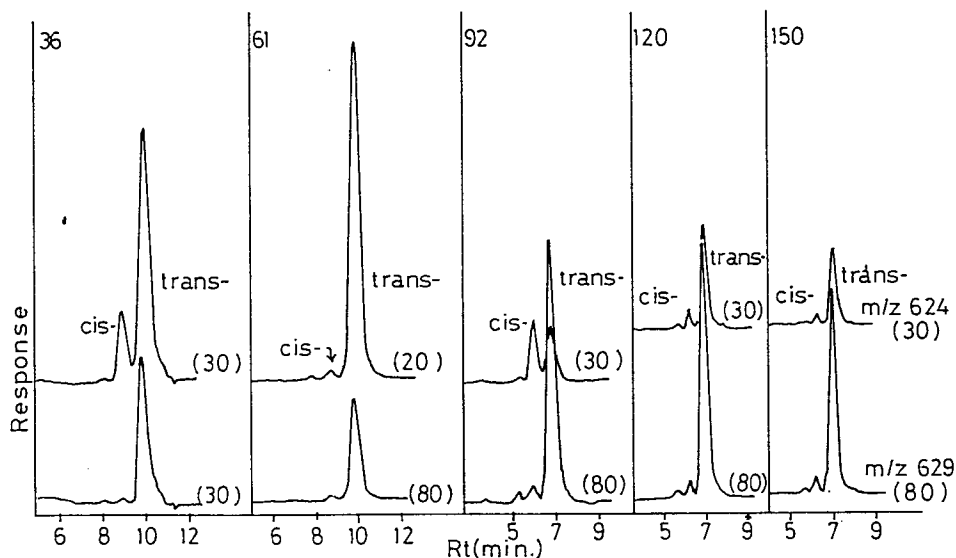
RESULTS

Determination of cytokinins

To the ethanol extracts presented in Table I was added deuterium-labeled cytokinins as internal standards. The aqueous solution shown Fig. 2 was purified by a cation exchange resin. The ammoniacal eluate was subjected to preparative TLC to fractionate two fractions. Each of the two fractions was further purified by HPLC to recover the four internal standards together with endogenous cytokinins. Furthermore, the stereoisomers of Z and RZ were separated by HPLC. The trimethylsilylated products of the fractions recovered are analyzed by GC-MS and SIM. The identification of the peaks was made by the retention time with those of the authentic compounds and complete mass spectra. Peak height ratios of the endogenous cytokinins to the internal standards using the ions summarized in Table II were determined on SIM chromatograms. Fig. 3

Table II IONS MONITORED USED FOR QUANTITATIVE MEASUREMENTS.

compound	Ion identity	m/z	
		Unlabeled	Internal standard
Z-3TMS	M ⁺	435	440
RZ-4TMS	[M-15] ⁺	624	629
iPA-3TMS	M ⁺	551	557
iPG-4TMS	M ⁺	653	659



GC:1.5% OV-1,1m,30ml/min He,oven temp. 245 C, SIM was obtained by monitoring RZ-d₅(m/z629) and endogenous RZ(m/z624). (): gain

Fig. 3 SIM of RZ fractions recovered from the ethanol extracts of the tuberous roots.

shows the SIM chromatograms of the RZ fraction recovered. *Trans*- and *cis*-isomer was equal to their peak height at the 92nd day throughout the development of sweet potato. The levels of endogenous cytokinins determined were summarized in Fig. 4 and 5. The values given in the both Fig. are the means from three measurements. The errors inherent in the measurement were a few % in the iPG, iPA and Z fractions as a percentage standard deviations, while those of RZ fraction were the range of 5% to 13%. Therefore, we are also able to demonstrate the presence and levels of six cytokinins, namely *trans*- and *cis*-Z, *trans*- and *cis*-RZ, iPA and iPG in both the tuberous roots, and the fibrous as well as pencil-like roots.

Changes of endogenous cytokinin levels in tuberous roots during development

Fig. 4 shows the quantitative changes in endogenous cytokinin levels during the development of tuberous roots. The content of iPG was a maximum in the tubers harvested at the 36th day, and then the level gradually decreased during the development. The level of iPA also gradually decreased during development. *Trans*-RZ reached the highest content at the 61st day, which shares approximately 78% of the amount of cytokinins found. After one month (92nd day), the level suddenly decreased, and conversely *cis*-RZ increased. The level of *cis*-RZ became approximately equal to that of *trans*-RZ. The content of *trans*-RZ hereinafter increased during development, whereas *cis*-RZ decreased. The levels of iPA and *trans*-Z were relatively high level at the 36th day to the 61st day, while *cis*-Z was very low level during the development.

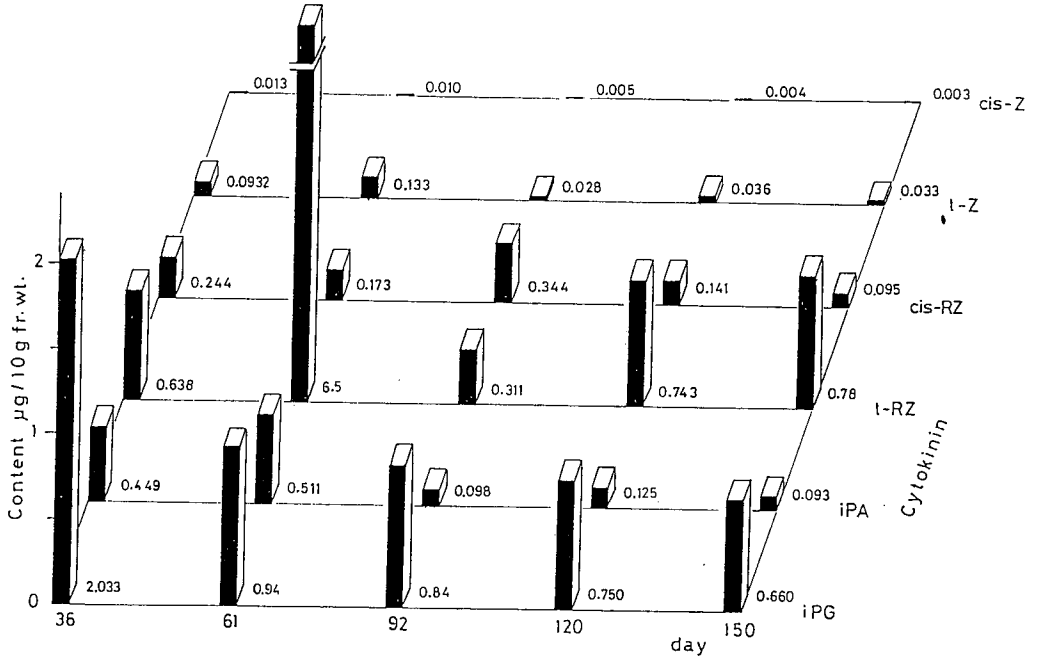


Fig. 4 Cytokinins found in the tuberous roots of sweet potato.

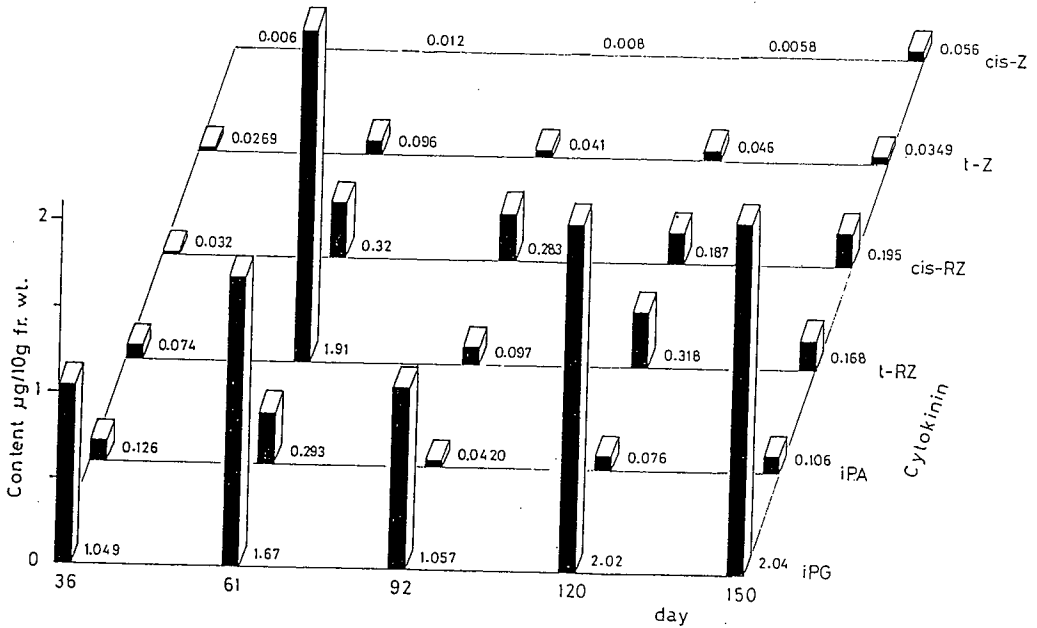


Fig. 5 Cytokinins found in the fibrous and pencil-like roots of sweet potato.

Changes of endogenous cytokinin levels in fibrous and pencil-like roots during development

As shown Fig. 5, the quantitative changes of the levels of endogenous cytokinins, except for iPG, was likely to those of tuberous roots. It was also confirmed to decrease the level of *trans*-RZ with increase of *cis*-RZ at the 92nd day. Furthermore, the ratio of both isomer of RZ levels varied to increase of *cis*-isomer. The fluctuation of iPG level was different from that of another cytokinins. The level was much higher throughout the development, with a share of over 70% of total cytokinin contents.

DISCUSSION

The present data indicated that six cytokinins were identified in both tuberous roots and fibrous as well as pencil-like roots, and that iPG levels was relatively high in the fibrous and pencil-like roots. However, the cytokinin activity of iPG is demonstrated to be one thousandth of that of 6-benzylaminopurine on *Amaranthus betacyanin* bioassay³⁾. It seems, therefore, that iPG do not play important physiological functions in the tuberization process. The rest of endogenous cytokinins, in particularly *trans*- and *cis*-RZ, *trans*-Z, and iPA which have a high cytokinin activity in Tobacco callus bioassay¹¹⁾, was relatively high levels in the tuberous roots. These compounds appear to be functional cytokinins which play a causative role in the tuberization process during the development of sweet potato.

The levels of iPA, RZ and Z in the tuberous roots are higher than those in the fibrous and pencil-like roots at the 36th day. Among of these compounds, *trans*-RZ was relatively higher level than other compounds. Therefore, *trans*-RZ may be presumed to be a functional cytokinin species, but *trans*-Z was not negligible because the cytokinin activity of *trans*-Z is the highest, whose activity is about ten fold higher than that of *trans*-RZ¹¹⁾. Furthermore, *cis*-RZ and iPA were less active than that of *trans*-RZ¹¹⁾. It seems that the thickening in tuberous roots at the 36th day may be stimulated by *trans*-isomers of both Z and RZ on the base of their levels.

Thereafter, *trans*-RZ increased markedly, and reached a maximum level which share about 78% of total cytokinin contents of the tuberous roots throughout the development. In the fibrous and pencil-like roots, *trans*-RZ achieved a maximum level at this stage. It should be noted that *trans*-RZ associated with the thickening of tuberous roots as well as growth of fibrous and pencil-like roots of sweet potato.

Matsuo et al^{12),13)}, and Nakatani and Komeichi¹⁸⁾ pointed out that the cytokinin activity and *trans*-RZ level in the tuberous roots significantly decreased at late in July that correspond to the 92nd day in this study. The present data noted that *trans*-RZ markedly decreased with increase of *cis*-RZ level and consequently the relative proportion of *cis*- and *trans*-RZ varied at the 92nd day. There is no report deal with the variation of ratio in stereoisomers during the development of plants. The physiological significance is not clear now. In potato, *cis*-RZ appears to be a functional cytokinin species which play an important role in tuberization process⁸⁾, but the compound is not primary stimulator for tuber initiation, and specific primary stimulator has been characterized⁹⁾. It is presumed that *cis*-RZ functions to stimulate the sink potential in tuberous roots like in the tuber of potato.

Trans-RZ was only cytokinin species to increase its level at both the 120th and the 150th day

during the development. The levels in tuberous roots were 2–4 times higher than those of in the fibrous and pencil-like roots. It is known that the thickening activity in tuberous roots mostly increase at two stages, namely, on June and at late in August⁶⁾. It is indicated that *trans*-RZ levels increased with increase of thickening activity of tuberous roots in this study. It can be, therefore, concluded that *trans*-RZ stimulates the activation of the primary and secondary cambium¹⁸⁾, and consequently functions the thickening of tuberous roots in sweet potato.

Acknowledgement: I am grateful to Professor N. Watanabe of the University farm of Tokyo University of Agriculture and Technology for supplying the samples.

References

- 1) Goodwin, P. B. (1978). Phytohormones and growth and development in organs of the vegetative plant. *Phytohormones and Related Compounds—A—Comprehensive Treaties*, Vol. II, ed. by D. S. Letham, P. B. Goodwin and T. J. V. Higgins, Elsevier/North-Holl and Biomedical Press 1978, Amsterdam, pp. 31–173.
- 2) Hashizume, T., T. Sugiyama, M. Imura, H. T. Cory, M. F. Scott and J. A. McCloskey (1979). Determination of cytokinins by mass spectrometry based on stable isotope dilution. *Anal. Biochem.*, **92**: 111–122.
- 3) Hashizume, T., S. Suye, T. Soeda and T. Sugiyama (1982). Isolation and characterization of a new glucopyranosyl derivative of 6-(3-methyl-2-butenylamino)purine from sweet potato tubers. *FEBS Lett.*, **144**: 25–28.
- 4) Hashizume, T., S. Suye and T. Sugiyama (1985). Mass spectrometric determination of endogenous cytokinins in potato tubers. *Agric. Biol. Chem.*, **49**: 387–390.
- 5) Hojyo, Y. (1973). The callus formation of tissue explant derived from tuberous roots of sweet potato plants, *Ipomoea batatas* Poiret. *Nogyo Gijutsu Kenkyusho Hokoku D*, **24**: 1–33.
- 6) For example, Hojyo, Y. (1975). *nogyoujijyututaikei*, vol. 5, pp 25, nosanngyosonn-bunnkakyoukai.
- 7) Jameson, p.E., J. A. McWha and R. M. Haslemore. (1985). Changes in cytokinins during initiation and development of potato tubers. *Physiol. Plant.*, **63**: 53–57.
- 8) Koda, Y. and Y. Okazawa. (1983). Characteristic changes in the levels of endogenous plant hormones in relation to the onset of potato tuberization. *Japan. J. Crop Sci.*, **52**: 592–597.
- 9) Koda, Y., E. A. Omer, T. Yoshihara, H. Shibata, S. Sakamura and Y. Okazawa, (1988). Isolation of a specific potato tuber-inducing substance from potato leaves. *Plant Cell Physiol.*, **29**: 1047–1051.
- 10) Latham, D. S. (1978). Cytokinins. In *Phytohormones and Related Compounds—A—Comprehensive Treaties*, Vol. I, ed. by D. S. Letham, P. B. Goodwin and T. J. H. Higgins, Elsevier/North-Holland Biochemical Press, Amsterdam, pp. 205–263.
- 11) Matsubara, S. (1980). Structure-activity relationships of cytokinins. *Phytochemistry*, **19**: 2239–2253.
- 12) Matuo, T., T. Yoneda and S. Ito (1983). Identification of free cytokinins and the changes in endogenous levels during tuber development of sweet potato (*Ipomoea batatas* Lam.), *Plant Cell Physiol.*, **24**: 1305–1312.
- 13) Matsuo, T., H. Mitsuzono, R. Okada and S. Ito. (1988). Variations in the levels of major free cytokinins and free abscisic acid during tuber development of sweet potato. *Plant Growth Regul.*, **7**: 249–258.
- 14) Mauk, C. S. and A. R. Langille (1978). Physiology of tuberization *Solanum tuberosum*. *cis*-zeatin riboside in the potato plant its identification and changes in the endogenous levels as influenced by temperature and photoperiod. *Plant Physiol.*, **62**: 438–442.
- 15) Meils, R. J. M., and J. von Staden (1984). Tuberization and Hormones. *Z. Pflanzenphysiol.*, **113**: 271–283.
- 16) Mothes, K. and L. Ngelbrecht. (1961). Kinetin-induced directed transport of substances in excised leaves in the dark. *Phytochemistry*, **1**: 58–62.

- 17) Nakatani, M. (1990). Role of plant hormones in formation and thickening of tuberous roots in sweet potato. *Chemical Regul. Plants.*, **25**: 183-191.
- 18) Nakatani, M., and M. Komeichi. (1991). Changes in the endogenous level of zeatin riboside, abscisic acid and indole acetic acid during dormation and thickening of tuberous roots in sweet potao. *Japan Jour. Crop Sci.*, **60**: 91-100.
- 19) Obata-Sasamoto, H. and H. Suzuki (1979). Activity of enzyme relating to starch synthesis and endogenous levels of growth regulators in potato stolon tips during tuberization. *Physiol. Plant.*, **45**: 320-324.
- 20) Sattelmacher, B. and H. Marshiner (1978). Cytokinin activity in stolons and tubers of *Solanum tuberosum* during the period of tuberization. *Plant Physiol.*, **44**: 69-72.
- 21) Sugiyama, T., S. Suye and T. Hashizume (1983), Mass spectrometric determination of cytokinins in young sweet-potato plants using deuterium-labeled standards. *Agric. Biol. Chem.*, **47**: 315-318.
- 22) Sugiyama, T. and T. Hashizume (1989), Cytokinins in developing tuberous roots of sweet potato. *Agric. Biol. Chem.*, **53**: 49-53.

サツマイモ塊根形成時期における6種サイトカイン含量の定量的解析

杉山 民二

要 旨

サツマイモ塊根形成時期における内生サイトカイン6種の含量を質量分析法を用いて定量することにより、塊根形成に果すサイトカイン分子の役割を明らかにすることを目的とした。trans-リボシルゼアチンは塊根形成期を通して相対的に高い含量を維持していた。特に、61日目ではその含量が最大となり、全サイトカイン量の78%を占めるほどであった。一方、細根・梗根では9-グルコシル-N⁶-イソペンテニルアデニンが高い含量を一様に維持していた。塊根形成中期にtrans-リボシルゼアチンは急激に低下した。逆にcis-リボシルゼアチン含量は増加した。その結果、リボシルゼアチンのcis-体とtrans-体の相対比が逆転することを観察した。これまでの研究報告では、サツマイモ塊根のサイトカイン活性はこの中期頃に低下することは指摘されていた。従って、この相対比の変動は塊根の肥大生長において何らかの生理・生化学的な変化を反映した結果と考えられるが、文献的にも推察できなかった。本研究により、サツマイモ塊根の形成にはtrans-リボシルゼアチンが深く関与していることが判明した。