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# Changes in Cytokinin Activities and Mass Spectrometric Analysis of Cytokinins in Root Exudates of Rice Plant (*Oryza sativa* L.)[1]

## Comparison between Cultivars Nipponbare and Akenohoshi

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### ABSTRACT

**Changes in exudation rate and cytokinin activities in the exudates were measured in two varieties of rice (*Oryza sativa* L.), cv Nipponbare (a Japanese normal cultivar) and cv Akenohoshi (a high-yielding cultivar). The exudation rates of Akenohoshi, the leaves of which remained green for a longer time, were higher than those of Nipponbare after the booting stage. Cytokinin activities in the exudates of Akenohoshi were higher than those of Nipponbare during the ripening period. Cytokinins in the exudates collected during the middle of the ripening stage were analyzed with mass spectrometry using deuterium-labeled standards. *trans*-Zeatin, *trans*-ribosylzeatin, and $N^6$-isopentenyladenosine were detected as free cytokinins, and zeatin was detected in the hydrolysates of highly polar fractions ("conjugated zeatin") in the exudates of both cultivars. Conjugated zeatin was the predominant cytokinin in both cultivars. Therefore, we suggest that conjugated zeatin is an important form of cytokinin during the ripening stage. The level of each of the cytokinins in Akenohoshi was higher than that in Nipponbare. Also, we discuss the correlation between the leaf senescence and cytokinin content in root exudates.**

In a previous study to clarify the physiological and ecological characteristics of high-yielding rice varieties (11, 12), it was reported that the yield in Akenohoshi was about 1780 kg/ha higher than that in Nipponbare, a Japanese standard cultivar, and that Akenohoshi had a higher dry matter production capacity compared with Nipponbare. This was attributed to the maintenance of a higher net assimilation rate during the ripening period in the former. The higher net assimilation rate of Akenohoshi resulted from a slower leaf senescence than that of Nipponbare at the ripening stage. For example, the photosynthetic rates in the 14th leaf of Nipponbare and Akenohoshi during the middle ripening stage were 9.2 $\mu$mol of $CO_2$ $m^{-2}$ $s^{-1}$ (44.6% of its maximum rate at the panicle formation stage) and 12.7 $\mu$mol of $CO_2$ $m^{-2}$ $s^{-1}$ (62.0%), respectively. In addition, the cause for these differences between the two cultivars was examined by measuring the active and passive water absorption abilities of the roots. The rate of exudation from the main stem and the resistance to water transport from the root surface to the leaves under intense transpiration were higher and lower in Akenohoshi than in Nipponbare, respectively. These differences between the two cultivars were especially large during the ripening stage. This fact suggests that physiological activity in the roots of Akenohoshi was higher than that in Nipponbare. It is known that a decline in the physiological activity of roots causes leaf senescence and death of the leaves at the lower positions (10), but the mechanism by which this occurs is not understood well.

Cytokinins transported from the roots via the xylem are known to delay leaf senescence (6, 16, 18). In the exudate of rice plants, several cytokinins were identified (15). However, the constitution of cytokinins in the exudate, the level of these cytokinins, and their effects on leaf photosynthesis and senescence have been examined in only a few studies (20).

In this report, we discuss the changes in the exudation rates and cytokinin activities in the exudates of two cultivars, Nipponbare and Akenohoshi, during the growing season. We also report the presence and the levels of endogenous cytokinins in the exudates of the two cultivars during the ripening stage when the leaves senesced.

## MATERIALS AND METHODS

### Plant Material

Two cultivars of rice (*Oryza sativa* L.), Nipponbare and Akenohoshi, were grown at the Honmachi paddy field of the Tokyo University of Agriculture and Technology. In 1987, seeds were sown in an upland nursery bed on May 6 and grown to the seven-leaf stage. The seedlings, three per hill, were transplanted to the paddy field on June 10. Planting density was 22.2 hill/$m^2$ (15 × 30 cm). The paddy field was submerged from the time of transplanting until harvest. Fertilizer, 50 kg of N, 50 kg of P, and 50 kg of K per ha, was

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applied just before transplanting. An additional fertilizer dose of 30 kg of N and 30 kg of K per ha was applied on July 30. Maximum tillering was observed on July 2 in Nipponbare and on July 12 in Akenohoshi. Heading occurred on August 27 in both cultivars. The average number of panicles per plant was 6.1 in Nipponbare and 4.7 in Akenohoshi. The cultivation method used in 1988 was the same as that in 1987, except that seedlings were raised in nursery boxes in 1988.

### Exudate Collection

Each plant was cut at an internode about 15 cm above the soil level at 8 PM. Absorbent cotton was placed on top of each of the decapitated stems and covered with polyethylene sheets. To avoid night dew and direct sunlight, the cotton-set stubble was covered with a paper bag. At 8 AM the next day, the absorbent cotton pieces with exudate were collected. As detailed by Hirasawa et al. (9), the exudation rate (mL/12 h) was estimated from the increase in cotton weight with the assumption that the specific gravity of the exudation sap was 1.0. To the absorbent cotton pieces was added 99% ethanol, and the exudate was extracted three times by squeezing on a suction filter. The extracts thus obtained were combined and stored at −20°C until analysis. For mass spectrometric analysis, the extracts collected from 45 plants were combined, and internal standards (summarized in Table I) were added to them.

### Chl Content

Ten leaf discs, 5.3 mm in diameter, were cut with a leaf puncher from the center of the 14th leaf (third from the flag leaf) on the main stem. The discs were homogenized and extracted with 80% acetone. The *A* of the extract was measured at 663 and 645 nm. Chl content was determined as described by Arnon (1).

### Purification on Sep-pak $C_{18}$ Cartridge

The combined extracts (about 4-fold volume of the absorbed cotton weight) were evaporated under vacuum to an aqueous residue. The residue was dissolved in 2 mL of water and filtered, and the filtrate was placed on a Sep-pak $C_{18}$ cartridge (Waters, Milford, MA). The cartridge was washed with 2 mL of water and then eluted with 20 mL of 40% methanol.

### Bioassay

The samples, purified on Sep-pak $C_{18}$ cartridges, were used for the bioassay. Cytokinin activity was determined by the *Amaranthus* betacyanin bioassay (2) using *Amaranthus tricolor*. The activity was compared with a standard curve made by a serial dilution of a solution of BA, and the results were expressed as "BA equivalent quantities" by multiplying the estimated concentration by the exudation rate.

### Chemicals

$^2$H-labeled standards used for the mass spectrometric analysis were prepared as described previously (7, 19). Z[3] and RZ consisted of the *trans*-isomer (92%) and the *cis*-isomer (8%).

### HPLC

After purification by Sep-pak $C_{18}$ cartridges, the samples for mass spectrometric analysis were further purified by HPLC. A Shimadzu model LC-6A equipped with a UV detector (270 nm) was used, and the flow rate was 1 mL/min. The reverse phase column (150 × 6.0 mm, i.d.) was a Shim-pack CLC-ODS (Shimadzu Co.). The column was eluted with 44% methanol, and the eluate was separated into five fractions (Rt: 0–4, 4–8, 8–12, 12–18, and 18–24 min), which were code-named A, B, C, D, and E, respectively. Fraction E, containing iP and iPA, was further purified using 25% acetonitrile, and each of them was fractionated as a single peak. Fraction B, containing Z and RZ, was further purified by HPLC using 11% acetonitrile to fractionate Z and RZ. Final purification of the Z and RZ fraction was carried out using 33% methanol, and the eluates corresponding to *trans*-Z, *cis*-Z, *trans*-RZ, and *cis*-RZ were collected.

Fraction A, which was supposed to contain conjugated cytokinins, was purified by HPLC using 11% acetonitrile to separate any free form cytokinins. The highly polar fraction (Rt, 1.5–8 min) was evaporated and hydrolyzed as described below. The hydrolysate was repurified on HPLC using 44% methanol and separated into five fractions under the same conditions at the first HPLC purification step; each of them was named a, b, c, d, and e, respectively. $^2$H-labeled Z was added to fraction b (Table I). The final purification was carried out by HPLC using 11% acetonitrile, and the eluates corresponding to *trans*- and *cis*-Z were collected and combined.

### Acidic Hydrolysis

Conjugated cytokinin fraction was refluxed at 100°C in 0.2 N HCl for 1 h to form free cytokinins.

### Trimethylsilylation

Each cytokinin fraction was dried overnight under vacuum over phosphorous pentoxide before silylation. Fractions of RZ and iPA were trimethylsilylated with hexamethyldisilazane-trimethylchlorosilane in pyridine (Tokyo Kasei Co.) at 120°C for 1 h, whereas those of Z and iP were silylated with a mixture of acetonitrile/bis(trimethylsilyl)acetamide/trimethylchlorosilane (35:15:3, v/v) at 80°C for 20 min.

### GC-MS and SIM

A Shimadzu LKB 9000 gas chromatograph-mass spectrometer equipped with a multiple ion detector was used under the following conditions: ionization energy, 20 eV; separating

[3] Abbreviations: Z, zeatin; RZ, ribosylzeatin; iP, $N^6$-isopentenyladenine; iPA, $N^6$-isopentenyladenosine; SIM, selected ion monitoring; Rt, retention time.

**Table I.** *Amounts of $^2H$-Labeled Standards Added to the Exudate and the Ions Used for Mass Spectral Quantification*

| Cytokinin | Amount | Monitoring Ions | | |
|---|---|---|---|---|
| | | Identity | m/z | |
| | | | Labeled | Unlabeled |
| | μg | | | |
| Z-$d_5$ | 3.0 | Z-3TMS ($M^+$) | 440 | 435 |
| RZ-$d_5$ | 3.3 | RZ-4TMS ([M-15]$^+$) | 629 | 624 |
| iPA-$d_6$ | 2.3 | iPA-3TMS ($M^+$) | 557 | 551 |
| iP-$d_6$ | 3.2 | iP-2TMS ($M^+$) | 353 | 347 |
| Z-$d_5$[a] | 2.7 | Z-3TMS ($M^+$) | 440 | 435 |

[a] Added to the conjugated zeatin fraction.

port temperature, 280°C; and ion source temperature, 290°C. A glass column (1 m × 3 mm, i.d.) was packed with 1% OV-1 for the analysis of free cytokinins but with 1.5% OV-1 in the case when Z occurred as the hydrolysate of conjugated Z. The column oven temperatures for the respective fractions were 170°C (iP), 185°C (Z), 210°C (iPA), 225°C (RZ), and 205°C (Z as hydrolysate of conjugated Z).

## RESULTS

### Changes in Total Chl Content

The changes in total Chl content of the 14th leaves of Nipponbare and Akenohoshi are shown in Figure 1. Chl content of Akenohoshi was higher than that of Nipponbare at every measurement. Onset of degradation in Nipponbare was earlier than in Akenohoshi, and there was a slight tendency for the differences to get larger with age.

### Changes in Exudation Rate

Changes in the rate of exudation from the panicle initiation stage to the late ripening stage are shown in Figure 2. After August 21 (booting stage), the exudation rate of Akenohoshi was higher than that of Nipponbare. There were significant differences ($P < 0.01$) after the booting stage except for August 29, when there was rain.

### Total Cytokinin Activity of the Exudates

Total cytokinin activity measured by the *Amaranthus* betacyanin test (2) after purification is shown in Figure 3. The cytokinin activities of the two cultivars were low before heading but rapidly increased on August 29, when the plants headed. Thereafter, the activities in Nipponbare declined rapidly, whereas those in Akenohoshi, which maintained a higher level of Chl content in the leaves, remained remarkably high.

### Qualitative and Quantitative Determination of Cytokinins

Endogenous cytokinins in the exudates collected on September 13 (17 d after heading) during the middle ripening stage were analyzed by MS. The internal standards, summarized in Table I, were added to the ethanol extracts of the exudates collected from 45 plants. The aqueous solutions obtained after evaporation of the ethanol were purified on Sep-pak $C_{18}$ cartridges, and the eluates were purified by HPLC. The column was eluted with 44% methanol and fractionated as shown in Figure 4. Fraction B, containing Z and RZ, was further purified by HPLC using 11% acetonitrile and 33% methanol, and fraction E, containing iPA and iP, was purified using 25% acetonitrile.

The fractions containing the four internal standards together with endogenous cytokinins were trimethylsilylated

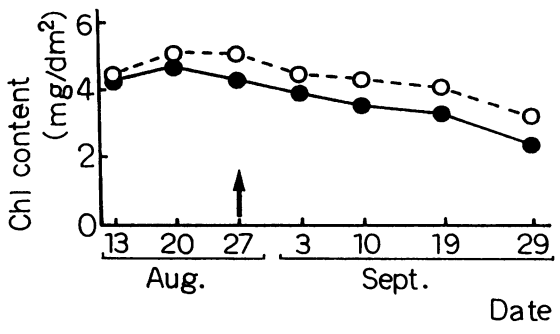


**Figure 1.** Changes in total Chl contents of 14th leaves of Nipponbare (●) and Akenohoshi (O). Arrow indicates heading date.

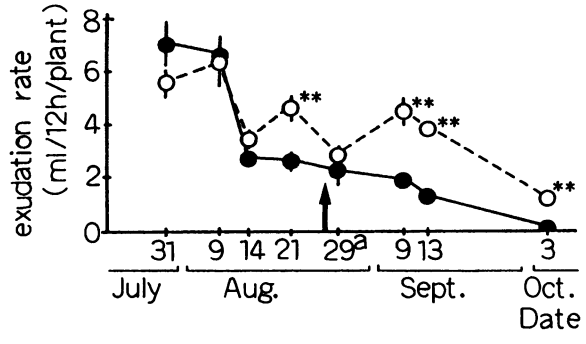


**Figure 2.** Changes in exudation rate per plant in Nipponbare (●) and Akenohoshi (O). Bars indicate ±SE. [a] Rainy day. ** Significant at $P = 0.01$.

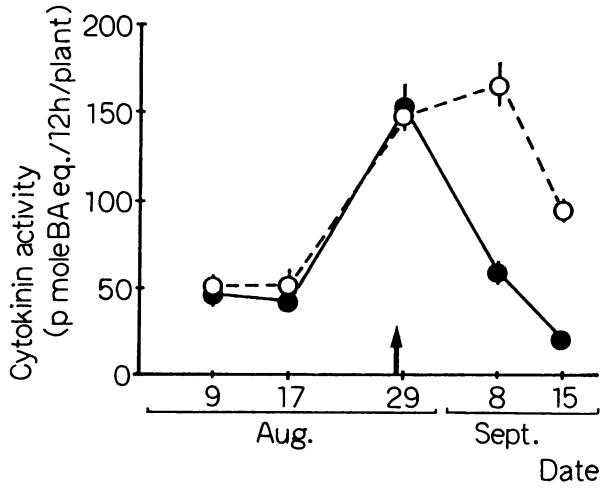


**Figure 3.** Changes in cytokinin activity in exudates of Nipponbare (●) and Akenohoshi (○). Bars indicate ±SE of three replicates.

and subjected to GC/SIM analysis. Peak height ratios of endogenous cytokinins to the internal standards were determined, and corrections for the unlabeled materials were made from the standard calibration curve obtained in previous studies (7). The identities of the peaks were confirmed by complete mass spectra. Figure 5 shows the SIM chromatograms of the iPA fractions. The $[M]^+$ ions of trimethylsilyl derivatives of endogenous iPA (m/z 551) and the $^2H$-labeled iPA (m/z 557) were monitored and detected as peaks with an Rt of 6.0 min. The levels of endogenous iPA, calculated as described above, were 0.8 pmol/plant in Nipponbare and

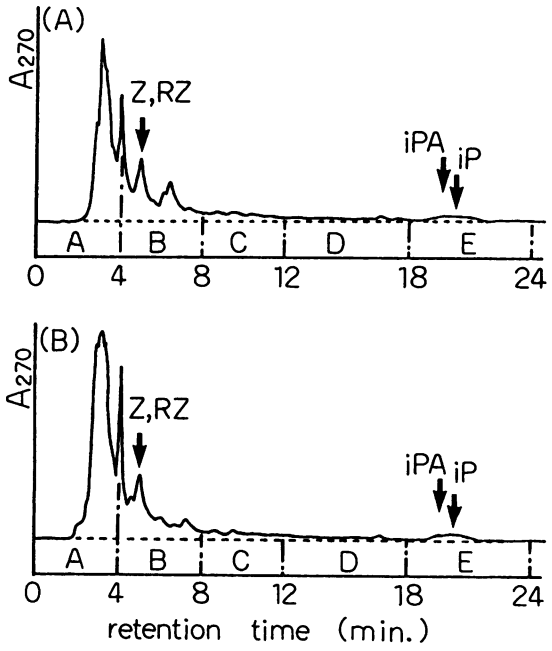


**Figure 4.** HPLC elution profiles of root exudates collected from Nipponbare (A) and Akenohoshi (B). "A," "B," "C," "D," and "E" were fractionated under HPLC conditions as described in "Materials and Methods."

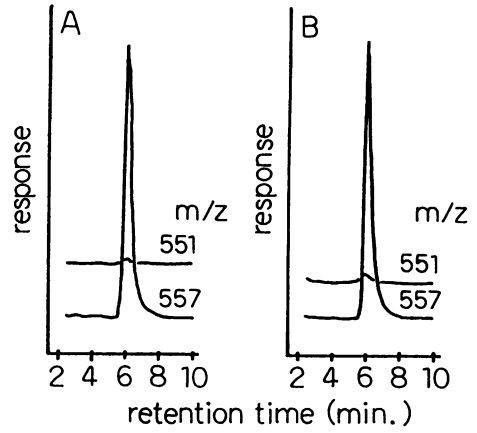


**Figure 5.** SIM chromatograms of the iPA fraction recovered from the exudate of Nipponbare (A) and Akenohoshi (B).

2.4 pmol/plant in Akenohoshi (Fig. 6). Figure 7 shows the SIM chromatograms of the RZ fractions. *cis*-RZ was not detected in either cultivar. The levels of endogenous *trans*-RZ were 2.9 pmol/plant in Nipponbare and 5.7 pmol/plant in Akenohoshi (Fig. 6). Similarly, the levels of *trans*-Z were lower (0.9 pmol/plant) in Nipponbare than in Akenohoshi (7.0 pmol/plant) (Fig. 6). iP was, however, not detected in either cultivar. *cis*-Z could not be identified because of the presence of impurities.

### Detection of Conjugated Z

These results show that the level of each of the free cytokinins (*trans*-Z, *trans*-RZ, and iPA) detected in Akenohoshi was higher than in Nipponbare. However, the total levels of these free cytokinins were much lower than the values estimated by bioassay (see Fig. 3). Therefore the cytokinin activity in the fractions that were not subjected to GC/SIM analysis (fractions A, C, and D) was measured by bioassay. Activity was high in fraction A, especially in that of Akenohoshi, whereas that in fractions C and D was much

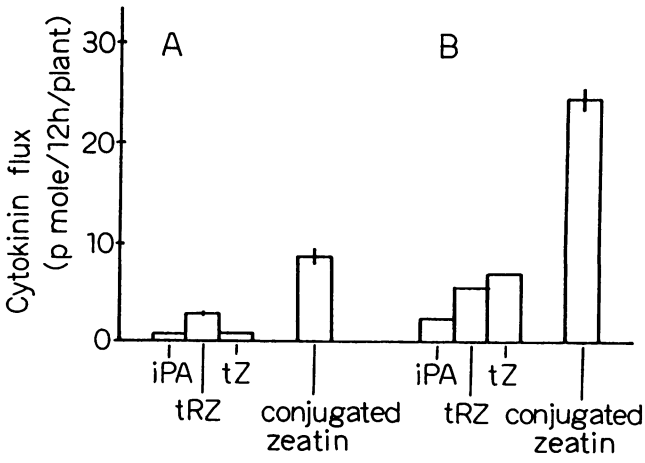


**Figure 6.** Cytokinins found in the exudates of Nipponbare (A) and Akenohoshi (B). Bars indicate ±SE. t, *trans*.

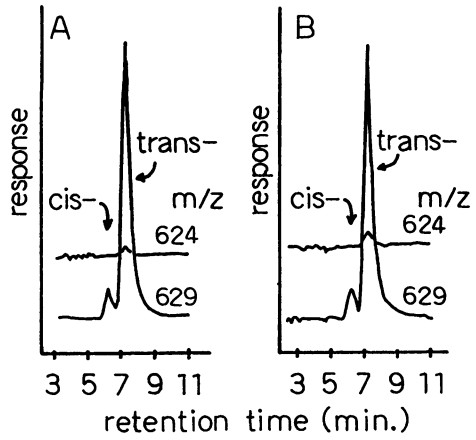


**Figure 7.** SIM chromatograms of the RZ fraction recovered from the exudate of Nipponbare (A) and Akenohoshi (B).

lower. It is not known which factors induced weak activities in fractions C and D in this study.

Fraction A, which was supposed to contain conjugated cytokinins such as glucosides and/or nucleotides, was further purified by HPLC and then hydrolyzed. The hydrolysates were fractionated by HPLC under the same condition shown in Figure 4, and cytokinin activities in each fraction were measured by bioassay. Because fraction b in each cultivar showed bioactivity, suggesting the presence of Z (Fig. 8), $^2$H-labeled Z described in Table I was added to the fraction b, which was then purified by HPLC. The Z fraction, referred to as the conjugated Z fraction, was trimethylsilylated and subjected to GC/SIM analysis. Figure 9 shows the SIM chromatograms. The ratio of the peak height of endogenous conjugated Z to $^2$H-labeled Z in Akenohoshi was much higher than in Nipponbare. The levels of endogenous conjugated Z were 8.8 pmol/plant in Nipponbare and 24.4 pmol/plant in Akenohoshi (Fig. 6), making the level in the latter 2.8 times higher than that in the former.

The level of conjugated Z was the highest among all cytokinins found in the exudates of both cultivars, and the level of each cytokinin in Akenohoshi was significantly higher than the same substance in Nipponbare (Fig. 6). The total level of cytokinins was 13.4 pmol/plant in Nipponbare

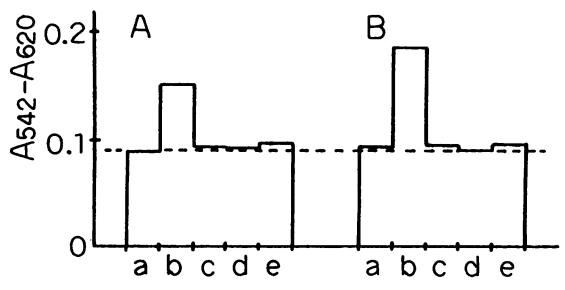


**Figure 8.** HPLC bioassay profiles of hydrolysates of fractions A from Nipponbare (A) and Akenohoshi (B). Broken line indicates value of the control. "a," "b," "c," "d," and "e" were fractionated under the HPLC conditions described in "Materials and Methods."

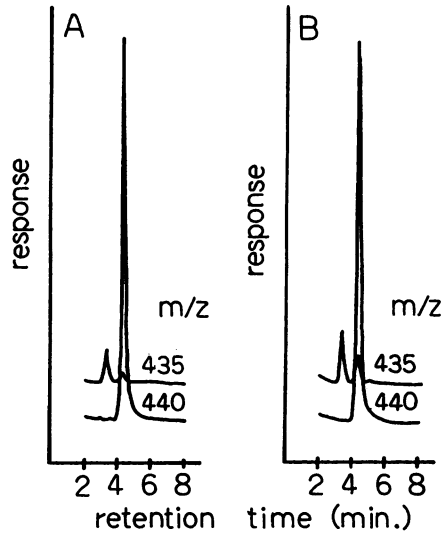


**Figure 9.** SIM chromatograms of the Z fraction recovered from the hydrolysates of fractions A of Nipponbare (A) and Akenohoshi (B).

and 39.5 pmol/plant in Akenohoshi. The level in Akenohoshi was therefore 2.9 times higher than that in Nipponbare.

## DISCUSSION

The Chl content of the 14th leaves of Akenohoshi was higher than that of Nipponbare, especially after heading. As shown by Jiang et al. (12, our unpublished data), leaf senescence was slower in Akenohoshi than that in Nipponbare during the ripening period.

The exudation rates of Akenohoshi were higher than those of Nipponbare after the booting stage, except on the rainy day. Therefore, it was confirmed that the physiological activity was higher in the roots of Akenohoshi than in those of Nipponbare.

Short and Torrey (17) reported that cytokinin content was greatest in the 0- to 1-mm root tip of pea seedlings. Water absorption ability in this part is very low in rice plants (13). As such, the exudation rate and cytokinin biosynthesis in the root may be controlled by different systems. In fact, lack of similarities between exudation rate and cytokinin flux has been shown in tomato (3), lupine (4, 5), sunflower (18), and soybean (8). Hence, we presented our results as amount of cytokinin per plant per 12 h and not as concentration.

The cytokinin activities of the two cultivars studied here were low before heading and increased at the heading stage. Such a phenomenon, i.e. a low cytokinin content in exudate at the early reproductive stage, has been observed in rice (23) and soybean (8). After heading, the cytokinin activities declined as leaves senesced and grains filled. The activity of Nipponbare declined more rapidly than that of Akenohoshi, the leaves of which remained green for a longer time.

To measure the precise level of each cytokinin species, we used mass spectrometric analysis. In exudates of rice plants, several species of cytokinins have been identified by chromatographic behavior (21) and physicochemical analysis (15). In the present study, *trans*-RZ, *trans*-Z, and iPA were identi-

fied as free cytokinins, but *cis*-RZ and iP were not detected in the exudates during the middle of the ripening stage by mass spectrometric analysis. Murofushi et al. (15) identified *cis*-RZ but did not detect iPA and iP in the exudate of rice plants during the panicle initiation stage. Therefore, *cis*-RZ may occur in the exudate at panicle initiation stage and not at the ripening stage, whereas the opposite may be the case with iPA.

Z was identified in hydrolysates of highly polar fractions on reverse phase HPLC (conjugated Z fraction). *trans*-Z ribotide and Z glucoside have been identified in the exudate (15) and roots (22), respectively, of rice plants. In the present study, however, the substance conjugating to the conjugated Z was not identified.

Conjugated Z was the predominant cytokinin in both cultivars, making up 66% of the total cytokinins found in Nipponbare and 62% in Akenohoshi. Noodén et al. (16) reported that RZ and dihydrozeatin riboside were the predominant cytokinins in the xylem sap of soybean, whereas the contents of *O*-glucosides and ribotides were low. Davey and van Staden (4, 5) reported that Z and RZ were predominant in the exudates of white lupine plants. Letham and Palni (14) concluded that ribosides are an important translocation form of cytokinins in the xylem. These reports differed from our finding that the levels of conjugated Z were highest in the exudates of the two cultivars. Our finding is supported by the study of Murofushi et al. (15). These authors proposed that *trans*-Z ribotide was the translocation form in the rice plant because of its comparatively high content in the root exudate.

The level of each cytokinin species in the exudate of Akenohoshi was much higher than that of Nipponbare. Furthermore, the total level of cytokinins in Akenohoshi was found to be 2.9 times higher than that in Nipponbare during the middle of the ripening stage, when the leaves at every position senesced. This difference was found to be greater if cytokinin flux was expressed as amount per shoot per 12 h, because Nipponbare and Akenohoshi had 6.1 and 4.7 shoots per plant, respectively. These results suggested that the slower leaf senescence in Akenohoshi, compared with that of Nipponbare, correlated with the larger amount of cytokinins in the exudate. In the intact plant, however, xylem sap flow may be controlled much more strongly by evaporative pull from the leaf surfaces than by root pressure. We have no evidence that root exudation and xylem flux under transpiration pull are similar or parallel. However, even though the link between these two parameters may not be fully established, the differences in export of cytokinins from the roots of plants are of interest.

Further research is needed to clarify the precise relationship between leaf senescence and cytokinin content, especially of conjugated Z, in rice exudates. Therefore, the goal of our next work will be to measure the quantity of each cytokinin throughout the ripening process.

## LITERATURE CITED


1. **Arnon DJ** (1949) Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15
2. **Biddington NL, Thomas TH** (1973) A modified *Amaranthus* betacyanin bioassay for the rapid determination of cytokinins in plant extracts. Planta **111:** 183–186
3. **Davey JE, van Staden J** (1976) Cytokinin translocation: changes in zeatin and zeatin-riboside levels in root exudate of tomato plants during their development. Planta **130:** 69–72
4. **Davey JE, van Staden J** (1978) Cytokinin activity in *Lupinus albus*. I. Distribution in vegetative and flowering plants. Physiol Plant **43:** 77–81
5. **Davey JE, van Staden J** (1978) Cytokinin activity in *Lupinus albus*. II. Distribution in fruiting plants. Physiol Plant **43:** 82–86
6. **Garrison FR, Brinker AM, Noodén LD** (1984) Relative activities of xylem-supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. Plant Cell Physiol **25:** 213–224
7. **Hashizume T, Sugiyama T, Imura M, Cory HT, Scott HF, McCloskey JA** (1979) Determination of cytokinins by mass spectrometry based on stable isotope dilution. Anal Biochem **92:** 111–122
8. **Heindl JC, Carlson DR, Brun WA, Brenner ML** (1982) Ontogenic variation of four cytokinins in soybean root pressure exudate. Plant Physiol **70:** 1619–1625
9. **Hirasawa T, Araki T, Matsuda E, Ishihara K** (1983) On exudation rate from the base of leaf blade in rice plants. Jpn J Crop Sci **52:** 574–581
10. **Honya K** (1966) Fundamental Conditions for High-Yielding Rice Cultivation. Nosangyosonbunkakyokai, Tokyo, Japan, pp 15–36 (in Japanese)
11. **Jiang C-Z, Hirasawa T, Ishihara K** (1988) Physiological and ecological characteristics of high yielding varieties in rice plants. I. Yield and dry matter production. Jpn J Crop Sci **57:** 132–138
12. **Jiang C-Z, Hirasawa T, Ishihara K** (1988) Physiological and ecological characteristics of high yielding varieties in rice plants. II. Leaf photosynthetic rates. Jpn J Crop Sci **57:** 139–145
13. **Kawata S, Lai K-L** (1968) On the correlation between the endodermis differentiation and the water absorption in the crown roots of rice plants. Proc Crop Sci Soc Jpn **37:** 631–639
14. **Letham DS, Palni LMS** (1983) The biosynthesis and metabolism of cytokinins. Annu Rev Plant Physiol **34:** 163–193
15. **Murofushi N, Inoue A, Watanabe N, Ota Y, Takahashi N** (1983) Identification of cytokinins in root exudate of the rice plant. Plant Cell Physiol **24:** 87–92
16. **Noodén LD, Singh S, Letham DS** (1990) Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. Plant Physiol **93:** 33–39
17. **Short KC, Torrey JG** (1972) Cytokinins in seedling roots of pea. Plant Physiol **49:** 155–160
18. **Sitton D, Itai C, Kende H** (1967) Decreased cytokinin production in the roots as a factor in shoot senescence. Planta **73:** 296–300
19. **Sugiyama T, Hashizume T** (1982) Mass spectrometric determination of cytokinins in the top of tobacco plant using deuterium-labeled standards. Nucleic Acids Res **11:** 61–64
20. **Takagi M, Yokota T, Murofushi N, Ota Y, Takahashi N** (1985) Fluctuation of endogenous cytokinin contents in rice during its life cycle: quantification of cytokinins by selected ion monitoring using deuterium-labeled internal standards. Agric Biol Chem **49:** 3271–3277
21. **Yoshida R, Oritani T** (1971) Studies on nitrogen Metabolism in crop plants. X. Gas chromatographic isolation of cytokinins from rice plants. Proc Crop Sci Soc Jpn **40:** 318–324
22. **Yoshida R, Oritani T** (1972) Cytokinin glucoside in roots of the rice plant. Plant Cell Physiol **13:** 337–343
23. **Yoshida R, Oritani T** (1974) Studies on nitrogen metabolism in crop plants. XIII. Effects of nitrogen top-dressing on cytokinin content in the root exudate of rice plant. Proc Crop Sci Soc Jpn **43:** 47–51