# Endogenous Gibberellins in the Panicle of Rice Plants Determined by the Rice Seedling Bioassay

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## Introduction

Seasonal changes of GAs in rice plants during development were first reported by Osada et al.[6] on cv. Norin 29. They found that the GA content in the panicle was markedly increased within 4 or 5 days around heading time. Later Kurogochi et al.[1] described the change of $GA_{19}$ content throughout the life cycle of another normal type cv. Nihonbare, determined by GC–SIM. They observed that the amount of $GA_{19}$ reached a maximum 3 to 6 days after heading and rapidly decreased to a very low level. The present author[5] also reported that the peak amount of GAs in the panicle of cv. Ginbozu was observed at the anthesis stage.

The author's previous work[4] revealed that anthers have the largest amount of GAs in floral organs of *Mirabilis jalapa*. The present investigation was undertaken to examine whether or not the sharp change of GA content in the rice panicle is attributable to the anther. Endogenous GAs in the anther of dwarf Tan-ginbozu, which is deficient in GAs in its shoots[7], were also compared with those of normal Ginbozu.

## Materials and methods

*1) Plant materials*

Rice plants (*Oryza sativa*) cv. Ginbozu (normal type) and cv. Tan-ginbozu (dwarf type) were cultivated in the artificial paddy field of the National Institute of Agricultural Sciences at Nishigahara in Tokyo from 1975 to 1978. Forty panicles of each cultivar were harvested about 10 times from Aug. 20 to Sept. 30 for measuring GA levels during their development. To know the distribution of GAs within the panicle, 40 panicles of Ginbozu at full anthesis were separated into anthers and spikelets without anthers. The anthers, 0.5 g in fresh weight, were gathered from the panicles just before the opening of the flower and used for extraction of endogenous GAs.

*2) Extraction and fractionation*

Extraction, fractionation, thin-layer chromatography, and bioassay were similar to those reported in a previous paper[2].

Sample material was homogenized in 70% aqueous acetone with a blender, kept for one day at room temperature, and filtered. After the acetone was evaporated, the aqueous solution was adjusted to pH 2.5 with phosphoric acid and partitioned three times against ethyl acetate. The combined ethyl acetate phase was extracted three times with 1 M phosphate buffer at pH 7. The buffer phase was adjusted to pH 2.5 with phosphoric acid and partitioned three times against ethyl acetate to give an acidic ethyl acetate fraction. After drying over anhydrous sodium sulfate, acidic ethyl acetate fraction was evaporated to dryness. With anthers the initial ethyl acetate phase was dried without partitioning against phosphate buffer.

Abbreviations: GA(s), gibberellins; GAn, gibberellin An GC–MS, combined gas-liquid chromatography-mass spectrometry; GC–SIM, combined gas-liquid chromatography-selected ion monitoring; TLC, thin-layer chromatography

*3) Thin-layer chromatography*

Each evaporated extract was taken in a small volume of acetone and subjected to TLC. Thin-layers of Silica gel H were used with the solvent system, isopropyl ether/acetic acid (95:5). In the case of redevelopment, the solvent used was a 10:1:1 mixture of isopropanol, 28% ammonia, and water. Scraped Silica gel was used for the elution with 50% aqueous acetone.

*4) Bioassay*

Two dwarf rice, Tan-ginbozu and Waito-C, were used to assay GAs. Tan-ginbozu is known to respond to many kinds of GAs, while Waito-C responds to limited kinds such as C-3-hydroxy GAs[3]. Such specificity between the two dwarfs was used to identify the GAs found in the extracts.

## Results and discussion

*1) Changes of GA in rice panicle*

Fig. 1 shows examples of histograms of GA activities in extracts of panicles of Ginbozu at three stages of panicle growth. GA activities were detected in all stages when assayed with Tan-ginbozu seedlings. But no clear GA activity could be found in the histograms when Waito-C seedlings were used, except for the extract at full anthesis.

Fig. 2 shows changes in the quantity of GAs per panicle of Ginbozu in relation to panicle growth. The amount at different stages, expressed as $GA_3$ equivalents, was calculated by comparing the responses of Tan-ginbozu rice seedlings to the eluates with those to standard solutions of $GA_3$. The amount of GA in one panicle 3 days before heading was 0.65 ng. It reached 1.4 ng at full anthesis, then fell off to 0.45 ng 7 days after heading, and remained low during the seed ripening. The peak for GAs was observed when the panicle was at full anthesis.

Fig. 3 shows changes of GA content in one panicle of dwarf Tan-ginbozu. GA was detected only in the flowering panicle. The maximum amount 0.8 ng in one panicle of Tan-ginbozu was a little more than half that of Ginbozu.

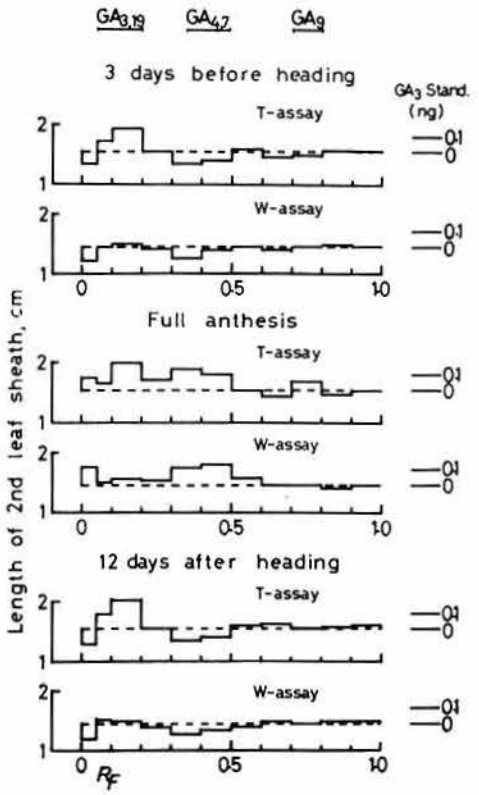


Fig. 1. Histograms showing GA activities of extracts taken from panicles of cv. Ginbozu and separated with TLC using isopropyl ether/acetic acid (95:5) as the developing solvent
Eleven eluates were tested on Tan-ginbozu rice seedlings (T-assay) or Waito-C rice seedlings (W-assay). This legend also applies to Fig. 4 to Fig. 7.

Fig. 4 shows the distribution of GA within the panicle of Ginbozu at full anthesis. The amount of GAs in the anthers of one panicle was about 1.2 ng. The spikelets without anthers showed 0.15 ng. The amount of GAs in the anthers mainly accounts for the quantity of GAs in the panicle at full anthesis.

*2) GA in rice anther*

Histograms of GA activities in extracts from anthers of Ginbozu and Tan-ginbozu are

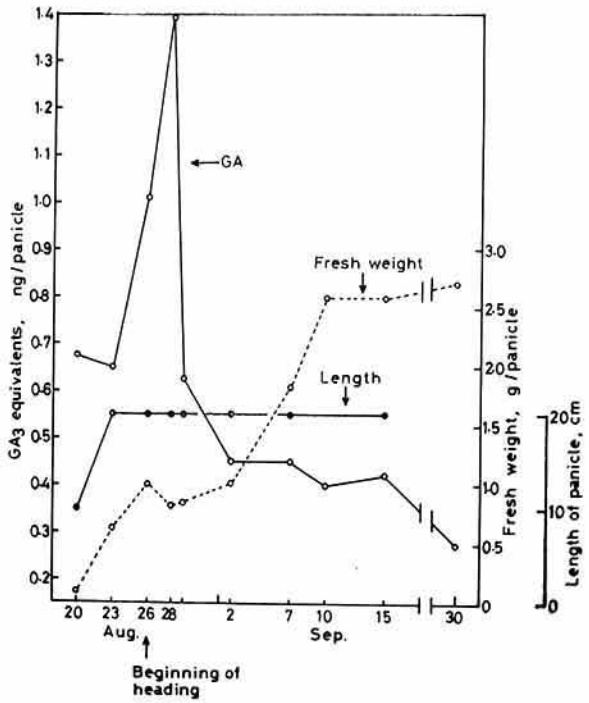


Fig. 2. Changes in the amount of endogenous GAs per panicle of Ginbozu at different stages of development

The amount is expressed as $GA_3$ equivalents by comparing the responses of Tan-ginbozu rice seedlings to the TLC eluates with responses to standard solutions of $GA_3$. This legend also applies to Fig. 3.

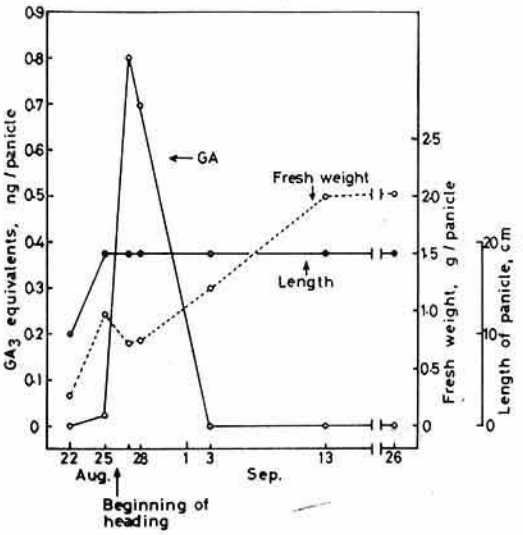


Fig. 3. Changes in the amount of endogenous $GA_3$ per panicle of Tan-ginbozu at different stages of development

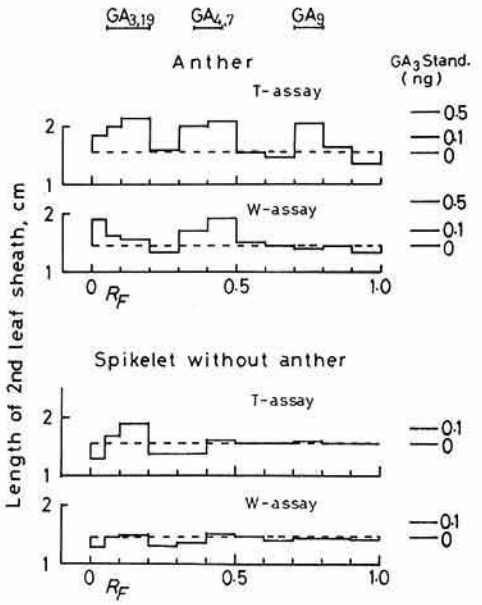


Fig. 4. Histograms showing GA activities of extracts taken from anthers and from spikelets without anthers of Ginbozu at full anthesis

The TLC solvent was a 95:5 mixture of isopropyl ether and acetic acid.

summarized in Fig. 5. A general glance shows that GA activities were detected in three zones, Rf 0–0.2, Rf 0.3–0.5, and Rf 0.7–0.8, of the histograms of both cultivars, when the eluate was assayed with Tan-ginbozu seedlings. The difference between Ginbozu and Tan-ginbozu was observed in the GA activity at Rf 0–0.2. Other GA activities at Rf 0.3–0.5 and Rf 0.7–0.8 were similar between the normal and the dwarf. The zone of Rf 0.3–0.5 detected with Tan-ginbozu and Waito-C seedlings was at the position of $GA_4$ and $GA_7$. $GA_4$ and $GA_7$ are C-3-hydroxy GAs and are equally active to both rice seedlings[2]. The activity of Rf 0.3–0.5 may be explained by the presence of either $GA_4$ or $GA_7$ or a combination of both. The activity at Rf 0.7–0.8 was detected with Tan-ginbozu seedlings but not with Waito-C seedlings. Its Rf value was very close to that of $GA_9$. $GA_9$ was almost as active as $GA_3$ to the Tan-ginbozu seedlings but was only slightly active to the Waito-C

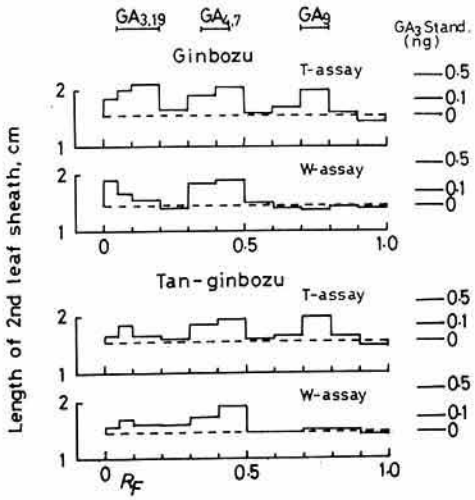


Fig. 5. Histograms showing GA activities of extracts from anthers of Ginbozu and Tan-ginbozu
The TLC solvent was the same as in Fig. 4.

seedlings (Table 1). On the basis of both Rf value and biological activity, the GA at Rf 0.7–0.8 was identified as $GA_9$.

GA activities located in the zone of Rf 0–0.2 of both cultivars were further examined by another chromatographic solvent system, isopropanol/28% ammonia/water (10:1:1). Fig. 6 shows the result of Rf 0–0.05 and Fig. 7 that of Rf 0.05–0.2. The eluate from Rf 0–0.05 of Ginbozu was divided into two active zones, one in the broad zone of Rf 0.05–0.5 and the other at Rf 0.6–0.7. The activity at Rf 0.6–0.7 was at the position of $GA_3$. Recently Suzuki et al.[8] confirmed the occurrence of $GA_1$ in ears at the anthesis stage by GC–SIM. Since

**Table 1. The activity of $GA_3$ and $GA_9$ on Tan-ginbozu and Waito-C rice seedlings**

| Dwarf rice | $GA_3$ dosage (ng/plant) | | | $GA_9$ dosage (ng/plant) | | |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 1 | 0 | 0.1 | 1 |
| Tan-ginbozu | 15.6 | 18.4 | 25.0 | 16.2 | 18.6 | 21.8 |
| Waito-C | 14.8 | 17.6 | 25.8 | 14.6 | 14.6 | 15.2 |

Data expressed as length of the second leaf sheath in mm by the microdrop method[2].

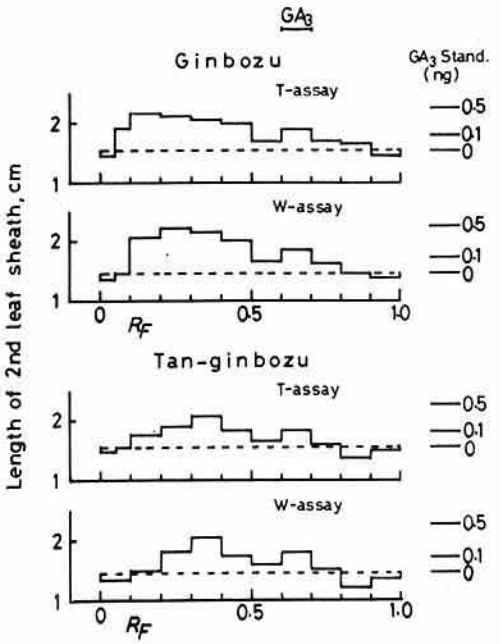


Fig. 6. Histograms showing GA activities of the eluate from Rf 0–0.05 in Fig. 5 after TLC redevelopment using isopropanol/28% ammonia/water (10:1:1) as the solvent.

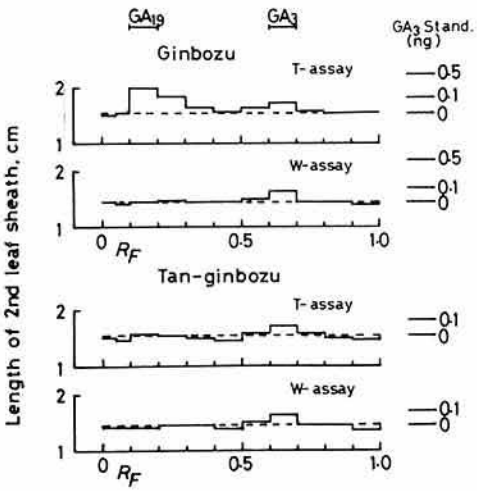


Fig. 7. Histograms showing GA activities of the eluate from Rf 0.05–0.2 in Fig. 5 after TLC redevelopment using isopropanol / 28% ammonia / water (10:1:1) as the solvent.

$GA_1$ and $GA_3$ can not be distinguished from each other on the basis of Rf value and biological activity, the GA at Rf 0.6–0.7 appears to be $GA_1$. The active zone of Rf 0.05–0.5 may be explained by the presence of much more polar GAs than $GA_1$ and $GA_3$ because of its lower Rf values. Further investigation using chemical techniques is needed for the identification of these GAs. The eluate from Rf 0.05–0.5 of Tan-ginbozu showed lower GA activity than that from Ginbozu, while the activity at Rf 0.6–0.7 of Tan-ginbozu was similar to that of Ginbozu.

The histogram of Ginbozu in Fig. 7 shows that the GA activity appeared in two zones, one at Rf 0.1–0.3 and the other at Rf 0.6–0.7. The eluate from the zone of Rf 0.1–0.3 had no effect on Waito-C seedlings and the Rf value was the same as $GA_{19}$. Recently Kurogochi et al.[1] identified the major GA in the rice plant as $GA_{19}$ by GC–MS and GC–SIM. The activity of Rf 0.1–0.3 is attributable to $GA_{19}$. The active zone of Rf 0.6–0.7 was already observed and discussed in the redevelopment of the zone of Rf 0–0.05. The activity of Rf 0.1–0.3 corresponding to $GA_{19}$ was not present in the histogram of Tan-ginbozu in Fig. 7. The Tan-ginbozu dwarf has been known to contain no $GA_{19}$ in shoots[3,7]. It is a big difference between Ginbozu and Tan-ginbozu that $GA_{19}$ is not detected in extracts from anthers and shoots of Tan-ginbozu.

## Conclusion

The peak of GA levels during the panicle development was observed at the anthesis stage in rice plants. The anther had the largest amount of GAs; that accounts for the change of GAs during the development of panicles. GAs were present even in anthers of dwarf Tan-ginbozu, which has little GA activity in extracts from leaves and seeds. A big difference of dwarf Tan-ginbozu from normal Ginbozu was lack of $GA_{19}$ in the anther as well as in the shoot and seed.

## References


1) Kurogochi, S. et al.: Identification of gibberellins in the rice plant and quantitative changes of gibberellin $A_{19}$ throughout its life cycle. *Planta*, **146**, 185–191 (1979).
2) Murakami, Y.: New rice seedlings test for gibberellins—Microdrop method. *JARQ*, **5**, 5–9 (1970).
3) Murakami, Y.: Dwarfing genes in rice and their relation to gibberellin biosynthesis. *In* Plant Growth Substances. Carr, D. J. ed., Berlin, Heidelberg, New York, Springer, 166–174 (1972).
4) Murakami, Y.: The role of gibberellins in the growth of floral organs of *Mirabilis jalapa*. *Plant & Cell Physiol.*, **16**, 337–345 (1975).
5) Murakami, Y.: Comparison of gibberellins in developing ears of rice and rye. *In* Soc. Chem. Regulation Plants, Ann. Meet., Oct. 1979, Tokyo, Abst., 13–14 (1979) [In Japanese].
6) Osada, A. et al.: Changes of endogenous gibberellins in rice plants as affected by growth stage and different growth conditions. *Proc. Crop Sci. Soc. Jpn.*, **42**, 41–45 (1973).
7) Suge, H. & Murakami, Y.: Occurrence of a rice mutant deficient in gibberellin-like substances. *Plant & Cell Physiol.*, **9**, 411–414 (1968).
8) Suzuki, Y. et al.: Seasonal changes of $GA_1$, $GA_{19}$ and abscisic acid in three rice cultivars. *Plant & Cell Physiol.*, **22**, 1085–1093 (1981).


(Received for publication, March 7, 1983)