生育時期および生育条件による水稲の内生ジベレリンの変化

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Changes of Endogenous Gibberellins in Rice Plants as Affected by Growth Stage and Different Growth Conditions*

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Growth of rice plants has generally been studied from the aspect of photosynthesis or plant nutrition and the role of endogenous growth regulators in the growth has so far received only minor attention. Recently, however, role of gibberellins in plant growth and development has well been recognized. It has been known that gibberellins (GA) may be most abundant in cotyledon or leaf except for developing mmature fruit and seed[3,4,5]. In rice plant, although ichanges of endogenous GAs were reported in relation to dwarfism[7] and photoperiodic floral induction[6], no information was available in relation to growth stages and different growth conditions. Thus, the experiment, whose results are reported in this paper, was undertaken to light on the changes of endogenous GAs as affected by growth stages and different growth conditions.

MATERIALS AND METHODS

Norin No. 29, a rice cultivar, was used throughout the experiments. Extraction, fractionation, chromatography and bioassay of GAs were carried out as follows. Immediately after harvesting, the plants were ground in a mixer with 70% acetone. The resulting brei was shaken for about 8 hr at room temperature and then filtered. The residue was re-extracted once more in a similar manner. The combined filtrate were evaporated under reduced pressure. The resulting aqueous solution, after being adjusted to pH 2.0 with phosphoric acid, was extracted three times with ethyl acetate and the ethyl acetate fraction was then extracted with 1 M phosphate buffer at pH 7.0. This phosphate buffer solution was acidified to pH 2.0 with phosphoric acid and extracted five times with ethyl acetate. The ethyl acetate fraction was dehydrated with anhydrous sodium sulfate overnight and then the solvent was distilled off under reduced pressure. The resulting residue was taken up in a small volume of acetone and was subjected to paper chromatography.

Ascending paper chromatography was carried out on Toyo No. 50 filter paper with the mixture of isopropanol/water/ammonia (10:1:1), the solvent front being allowed to travel 30 cm. The developed chromatogram was dried and divided transversely into 15 equal strips. Each strip was eluted with 50% acetone and the eluate was put into a test tube (2.3 × 6 cm). Then acetone was dried off and 0.5 ml of water was added.

In this test tube, five rice seedlings (semi dwarf variety "Tan-ginbozu"), whose coleoptile attained about 1 mm, were planted and allowed to grow under artificial light (4000 lux) conditions at 30°C. They were supplied with 0.5 ml water after 3 days. The length of the second leaf sheath was measured after 5 days.

RESULTS

Experiment I: Changes of endogenous GAs as affected by growth stages

Rice plants used in this experiment were rised according to ordinary cultural practices. The seeds were sown in seed bed in May 12 and transplanted into paddy field in June 24. Plants were harvested 11 times in different growing period and subjected to the extraction of GAs. Several examples of histograms showing GA activities, are presented in

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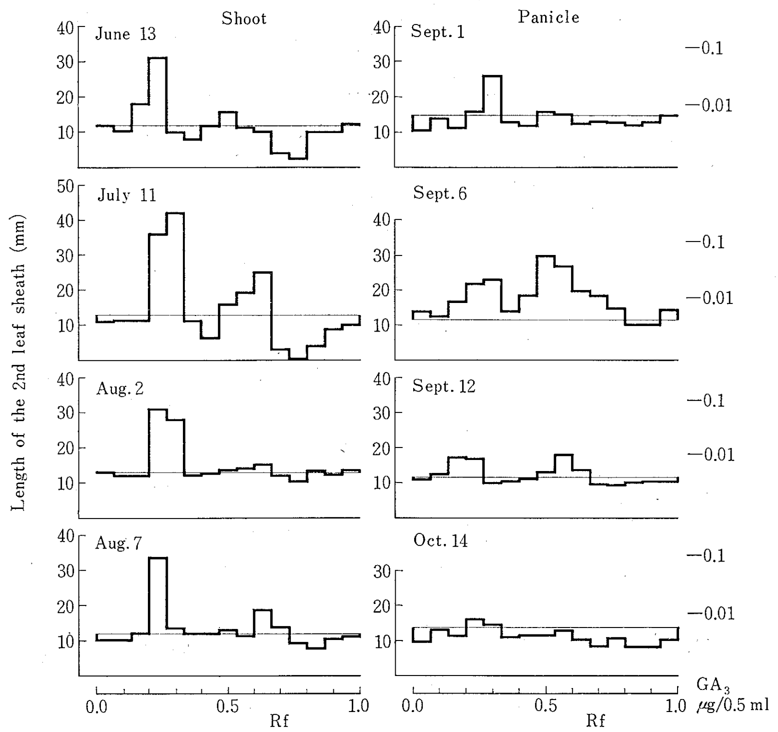

Fig. 1 Histograms showing examples of gibberllin activity of extracts of shoots (100 gram fresh weight) and panicles (50 gram fresh weight) harvested in different growth stages after paper-chromatographic development with ammonical isopropanol. Broken line denotes water control.

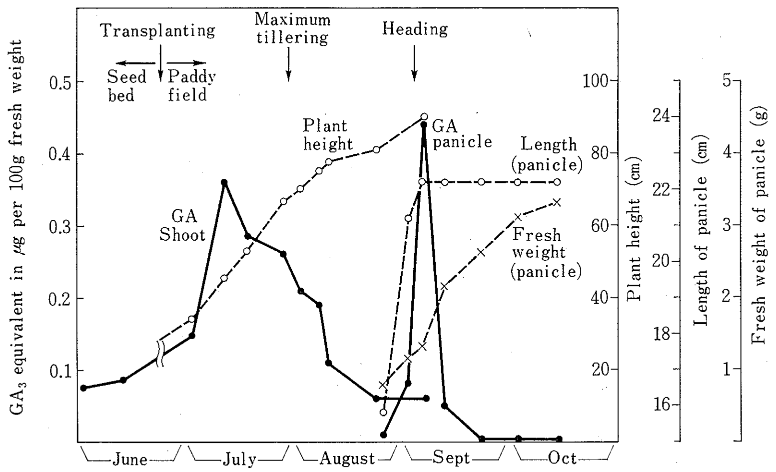

Fig. 2 Changes in gibberellin concentration, plant height, panicle length and fresh weight of panicle in rice plants from seedling stage to maturity

Table 1 Effect of shading on the content of endogenous gibberellins

Treatment	Plant height at the time of extraction (cm)	Plant organ	GA_3 equivalent in μg per 100 g fresh weight
Control	70.6	Leaf blade	0.394
		Leaf sheath	0.200
Shading	77.0	Leaf blade	0.517
		Leaf sheath	0.181

fig. 1. Changes of GA content in fig. 2 are values expressed by GA_3 equivalent in μg per 100 gram fresh weight. The GA activity in shoots reached to maximum in early July and that in panicle was found to reach maximum at a few days after the heading time.

Experiment II: Effect of shading on the GA activity in shoots

For testing the effect of shading on the GA activity in shoots, shading treatment was carried out to rice plants that had been grown in paddy field using gray-colored cheese cloth. Rate of shading was 90%. Shading was started July 19 and terminated July 28. GA extraction was made just after the end of shading treatment. As shown in table 1, shading plants resulted in increase of GA activity as well as that of plant height.

Experiment III: Effect of temperature on the GA activity in shoots

In order to study the effect of temperature on the GA activity in shoots, experiment was conducted using pot-grown plants. Seeds were sown in August 5 in 1/5000 *are* pots. On September 12, pots were separated into 3 groups and transferred to three different temperature conditions; 17, 25 and 33°C. Ten days after the transfer, plants were harvested for the extraction of GAs. Results are presented in table 2. GA activity was found to be lower under low temperature condition.

Experiment IV: Effect of nitrogen deficiency on the GA activity in shoots

Table 2 Effect of temperature on the content of endogenous gibberellins

Temperature (°C)	Plant height at the time of extraction (cm)	GA_3 equivalent in μg per 100 g fresh weight
17	55.6	0.047
25	61.5	0.097
33	62.5	0.081
Start of temperature treatment	54.4	0.087

Changes in the GA activity in shoots under nitrogen deficient conditions were determined using solution culture method. Germinating seeds were transplanted to cultural solution in 1/5000 *are* pots on August 8. For providing uniform seedlings, plants were thinned several times. All materials were grown under the condition of 20 ppm nitrogen until September 22 from which half of them was transferred to the solution of 0 ppm nitrogen concentration. As the control, 20 ppm nitrogen was continuously supplied until the time of GA extraction. On October 6, plants were harvested and subjected to GA extraction. As shown in table 3, GA activity was greatly reduced by ceasing to supply nitrogen.

Experiment V: Changes of endogenous GAs during seed germination

Twenty gram of dry seeds was placed in a large petri dish and 25 m*l* of water was added. Zero, 4, 8 and 12 days after imbibition, sample was subjected to extraction of GAs. Shoot lengths were 0.5, 43 and 120 mm at 4, 8 and 12 days after the

Table 3 Effect of nitrogen deficiency on the content of endogenous gibberellins

Nitrogen concentration (ppm)	Plant height at the time of extraction (cm)	Number of stems at the time of extraction	GA_3 equivalent in μg per 100 g fresh weight
0	55.9	2.1	0.027
20	56.9	3.5	0.103

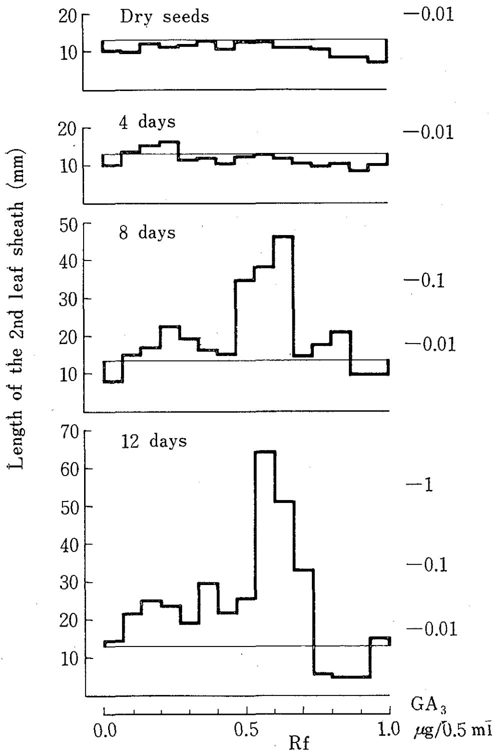

Fig. 3 Histgrams showing changes of gibberellin activity during seed germination. Extract was obtained from two samples originated from twenty gram of dry seeds. Broken line denotes water control.

imbibition, respectively. Histograms of GA activity are presented in fig. 3. No GA activity was detected in dry seeds and slight activity appeared in 4 days after the imbibition. However, activity increased dramatically within next 4 days.

Discussion

The occurrence of GA-like substances in rice shoots, roots and grains was first reported by Murakami[4]. He found that on the extracts from rice shoots, roots and grains (the milk ripe stage), the GA-like activity was spread over the lower half of each chromatogram with the maximum peak near Rf 0.3 when the mixture of isopropanol/water/ammonia (10:1:1) was used as the developing solvent. He also found that the total active substances in 100 gram fresh weight of rice grains in the milk ripe stage were approximately 0.7 μg in GA_3 equivalent. In shoots, however, there was a much lower concentration of GA-like substances. The concentration was roughly estimated to be 0.1–0.5 μg per 100 gram fresh weight in cereal grasses. In the present experiment, GA content in shoots was found to reach maximum in early July, about 15–20 days before the maximum tillering stage. After that GA in shoots was found to decrease gradually toward late August and early September, the heading stage.

On the other hand, GA content in panicle was dramatically increased within 4 or 5 days around heading time and again decreased rapidly. Maximum GA activity in panicle was detected at few days after heading. Although our sample contains panicle axis, changes of GA activity presented in fig. 2 may be mainly due to the GAs in grains. No activity was detected in full ripening stage.

Hashimoto and Rappaport[1,2] studied the variation of endogenous GAs in developing bean seeds and found that as the seed matures, neutral substances increase in activity. On the other hand, the acidic ethyl acetate substances initially increase and then almost disappear. They considered that the neutral fraction may serve as a reserve form of GAs in the dry seed and the acidic ethyl acetate substances may be required for normal development of the bean seed. In this experiment, the neutral fraction was not examined. However, striking increase of GA activity in acidic ethyl acetate fraction during seed germination may suggest the existence of conversion mechanism in rice seeds also.

Treatments that resulted in promotion of elongation such as shading, nitrogen supply or high temperature were found to increase GA content, whereas treatments that resulted in retardation of growth were found to decrease GA content. Those results are suggestive that endogenous GAs play important regulatory roles in the growth and development in rice plants.

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〔和 文 摘 要〕

生育時期および生育条件による水稲の内生ジベレリンの変化

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生育時期および生育条件による水稲農林29号の酸性酢酸エチル分画における内生ジベレリン活性の変化をしらべた．茎葉中のジベレリン含量はおおむね生体 100 g 当り 0.1～0.4 μg の間にあり，最高分けつ期の約 15～20 日前頃に最大に達し，以後 9 月初めの出穂期にむかつて漸減した．穂のジベレリン含量は出穂の数日後に著しい急増を示し生体 100 g 当り約 0.5 μg に達したのち急激に減少した．遮光や高温処理のように生長量を増加させる処理を加えるとジベレリン含量は増加し，窒素欠乏処理のような生長を減退させるような処理によりジベレリン含量は減少した．また，乾燥種子にはジベレリン活性は検出されないが，吸水 4 日目頃より活性があらわれ，次の 4 日間に活性は著しく増大した．これらのことから，水稲の生育に内生ジベレリンが重要な役割をはたしているものと推定される．

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