Amylase Activity in Rice Endosperm as Influenced by Gibberellins, Gibberellin-Derivatives, Kaurenoic Acid and Mevalonic Acid with Special Reference to their Growth-Promoting Activity

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Abstract

Relative effectiveness of gibberellins A_1 - A_9 except for A_6 , allogibberic acid, gibberic acid, epiallogibberic acid, kaurenoic acid and mevalonic acid on the leaf sheath elongation of rice seedling and on the release of reducing sugars from rice endosperm and the producing activity of amylases in rice endosperm was examined.

The order of effectiveness in the rice seedling test was $A_3 > A_1 > A_7 > A_2 > A_5 > A_4 > A_9 > \text{allogibberic acid} > A_8 > \text{gibberic acid} = \text{epiallogibberic acid} = \text{kaurenoic acid} = \text{mevalonic acid} = \text{water control}$.

The order of effectiveness in the rice endosperm test was $A_1 \doteq A_3 \doteq A_4 \doteq A_7 > A_2 > A_5 > A_9 > A_8 > \text{allogibberic acid} > \text{gibberic acid} = \text{epiallogibberic acid} = \text{kaurenoic acid} = \text{mevalonic acid} = \text{water control}$.

The discordance in the order for the leaf sheath elongation and for the enzyme producing activity in the endosperm of the same variety shows that the response is different with respect to the different parts of tissues in the same species.

Different effectiveness of the known gibberellins on growth of many kinds of different plant species have been found by Brian *et al.*¹⁾ It is also observed by Paleg *et al.*²⁾, and Griffiths *et al.*³⁾ that various gibberellins behaved differently in stimulating barley endosperm to release reducing sugars and amylases. This paper reports the relative effectiveness of gibberellins A_1 - A_9 except for A_6 , gibberellin A_3 -derivatives, kaurenoic acid and mevalonic acid on the release of reducing sugars from rice endosperm and the producing activity of amylases in rice endosperm in reference to their effectiveness on the growth of rice leaf sheath.

Materials and Methods

Gibberellins and related substances

Samples of the gibberellins A_1 , A_2 , A_3 , A_4 , A_5 , A_7 , A_8 , A_9 , allogibberic acid, gibberic acid, epiallogibberic acid, and mevalonic acid were dissolved in the distilled water. Kaurenoic acid was dissolved in the solution of 1/10 N NaOH overnight at room temperature and then adjusted to pH 6.5 with 1/10 N HCl. The solutions were sterilized by Seitz-filtration and 1 ml of each solution was aseptically placed in a glass tube (3×12 cm) for the seedling test, or in a test tube (1.5×18 cm) for the endosperm test.

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*Seedling test*⁴⁾: Seven seeds of the rice, variety "Kotake-Tamanishiki", sterilized with a 10% sodium hypochlorite solution were placed in the glass tube containing the test solution and grown under a fluorescent light of ca. 1500 lux at 30°. The length of the second leaf sheath was measured on the 7th day.

Endosperm test: Five sterilized embryoless endosperms of the same rice variety were placed in the test tube and incubated at 30°, according to the method reported previously⁵⁾.

Release of reducing sugars from endosperm: The procedures employed were similar to those reported by Nicholls and Paleg with barley endosperm⁶⁾ and Murakami with rice endosperm⁷⁾. After 2-day incubation the ambient solution in each test tube was filled up to 5 ml with distilled water, shaken with the amberite IR-120 (H) resin, and 1 ml of the supernatant fluid was assayed for the reducing sugar content by means of Somogyi-Nelson colorimetric method. The coloured solution was filled up to 15 ml and the optical density was measured with a spectrophotometer at 590 m μ .

α -Amylase and β -amylase activities in endosperm: After 2- or 4-day incubation the endosperms were ground in a glass mortar, extracted with 5 ml of 0.5% calcium chloride solution and filtered. The filtrate was examined for its effect on starch digestion, i. e., 1 ml of the filtrate was added to 4 ml of 0.25% soluble starch of pH 4.8 acetate buffer solution and incubated at 35°. After 15 minutes β -amylase activity was estimated by measuring the amounts of reducing sugars converted from the starch substrate; 0.5 ml of the reaction mixture was assayed by means of Somogyi-Nelson colorimetric methods. The enzyme activity was expressed by the amounts of sugars at 15 minutes minus those of the sugars which were present in the initial solution. After 30 minutes α -amylase activity was estimated by the iodine-starch reaction; 0.5 ml of the reaction mixture was pipetted off to a test tube containing 5 ml of 0.5 M acetic acid and 5 ml of 3×10^{-5} N iodine. The coloured solution was measured at 590 m μ with a spectrophotometer. α -Amylase activity was expressed by the formula, $\frac{C-E}{C} \times 100$ (%), where C and E are the optical densities of the solution at the start and at the end of the incubation, respectively.

Results

The lengths of the second leaf sheaths of rice grown in the solutions containing different concentrations of gibberellins and the related substances are shown in Fig. 1.

All the gibberellins stimulated the leaf sheath elongation significantly, except for A₈ which was ineffective. A₃ was most effective, whereas A₁, A₇, A₂, A₄, A₅, A₉, allogibberic acid and A₈ were less effective in the given order. Epiallogibberic acid, gibberic acid, kaurenoic acid and mevalonic acid were ineffective.

Figure 2 shows the amounts of reducing sugars released from the endosperm to the surrounding fluid and α -amylase activity in the endosperms cultured in the test solutions for 2 days.

The substances tested can be divided into four groups, i. e., (1) A₄, A₁, A₇ and A₃ which were effective at the lowest concentrations; (2) A₂ and A₅ which were less effective; (3) A₉, A₈ and allogibberic acid which showed weak activities; and (4) gibberic acid, epiallogibberic acid, kaurenoic acid and mevalonic acid which were ineffective. Figure 3 presents the activities of α -amylase and β -amylase in the endosperm cultured in the test solutions for 4 days.

A₁, A₄, A₃ and A₇ were effective at 10^{-5} and 10^{-4} μ g/ml, and A₈ and allogibberic

Fig 1. Effects of gibberellins (A_1 - A_9 except for A_6), allogibberic acid (AL.), gibberic acid (G.), epiallogibberic acid (E.), kaurenoic acid (K.) and mevalonic acid (M.) on the rice leaf sheath elongation.

acid at 10^{-1} $\mu\text{g/ml}$. Gibberic acid, epiallogibberic acid, kaurenoic acid and mevalonic acid had still no activity.

Discussion

Minimum concentrations required for the elongation of rice leaf sheath and for the amylase producing activity in the rice endosperm of the same variety differ with the kinds of gibberellins. The release of reducing sugars from the endosperm as well as the activity of α - and β -amylases in the endosperm occurred in response to gibberellins and allogibberic acid at lower concentrations than those required for the leaf sheath elongation. The sensitivity of the endosperm test, which was estimated after 4-day incubation, is 100 or 1000 times as high as that for the seedling test. A_8 , which showed very low activity for many kinds of growth test¹⁾ was effective in the endosperm test at even 10^{-1} $\mu\text{g/ml}$. The order of effectiveness of the gibberellins and related substances at 1 $\mu\text{g/ml}$ for the seedling test was $A_3 > A_1 > A_7 > A_2 > A_5 > A_4 > A_9 > \text{allogibberic acid} > A_8 = \text{gibberic acid} = \text{epiallogibberic acid} = \text{kaurenoic acid} = \text{mevalonic acid} = \text{water control}$. A similar order of these substances was observed in the seedling test of many other dwarf varieties of rice¹¹⁾. Hashimoto and Yamaki investigated promoting effects of gibberellins A_1 , A_2 , A_3 and A_4 on the elongation of the seedlings of the rice variety Norine 29⁸⁾. According to their result, the order of effectiveness was $A_1 = A_3 \gg A_4 > A_2$. Discrepancy in the order of gibberellins A_1 , A_2 , A_3 and A_4 between their result and the present one might be due to the difference in the rice varieties used or to that in the conditions of bioassay. A stimulating activity of allogibberic acid on the rice seedling was reported by Yabuta

*et al.*⁹⁾ It has been found by Katsumi *et al.*, that kaurenoic acid had no activity on the growth of the the rice seedling, although it showed some promoting activity on the growth of dwarf maize¹⁰⁾.

The order of the effectiveness of gibberellins and related substances at $0.1 \mu\text{g}/\text{ml}$ in the endosperm test was as follows: $A_1 \div A_3 \div A_4 \div A_7 > A_2 > A_5 > A_9 > A_8 > \text{allogibberic acid} > \text{gibberic acid} = \text{epiallogibberic acid} = \text{kaurenoic acid} = \text{mevalonic acid} = \text{water}$

Fig. 2. Effects of gibberellins (A_1 - A_9 except for A_6), allogibberic acid (AL.), gibberic acid (G.), epiallogibberic acid (E.) and kaurenoic acid (K.) on the release of reducing sugars from rice endosperm (above) and on the activity of α -amylase in the rice endosperm (below) cultured for 2 days.

control. A similar order of these substances was observed by the present author with the oat endosperm¹¹⁾, and by Paleg *et al.*²⁾, and Griffiths *et al.*³⁾ with the barley endosperm. Recently, Katsumi found that kaurenoic acid promoted amylase activity in barley endosperm¹²⁾. Yomo and Iinuma found that mevalonic acid was converted in barley embryo to gibberellin-like substances as estimated by the barley endosperm test¹³⁾.

Fig. 3. Effects of gibberellins (A₁-A₉ except for A₆), allogibberic acid (AL.), gibberic acid (G.), epiallogibberic acid (E.), kaurenoic acid (K.) and mevalonic acid (M.) on the activities of β-amylase (above) and α-amylase (below) in the rice endosperm cultured for 4 days.

The order of the effectiveness of gibberellins and the related substances regarding the enzyme producing activity differs from that observed for the leaf sheath elongation. For instance, A_4 was very effective in the former test, but less effective in the latter one. The discordance in the order of the effectiveness of various gibberellins for the leaf sheath elongation of rice and for the enzyme producing activity in the endosperm of the same variety shows that the response is different with respect to the different part of tissues in the same species.

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References

- 1) Brian, P.W., Hemming, H.G., and Lowe, D., *Nature* **193**: 946 (1962).
- 2) Paleg, L.G., Aspinall, D., Combe, B., and Nicholls, P., *Plant Physiol.* **16**: 291 (1965).
- 3) Griffiths, C.M., MacWilliam, I.C., and Reynolds, T., *Nature* **202**: 1026 (1964).
- 4) Ogawa, Y., *Plant and Cell Physiol.* **4**: 227 (1963).
- 5) ———, *ibid.*, in press (1966).
- 6) Nicholls, P.B., and Paleg, L.G., *Nature* **199**: 823 (1963).
- 7) Murakami, Y., *Bot. Mag. Tokyo* **79**: 315 (1966).
- 8) Hashimoto, T., and Yamaki, T., *ibid.* **73**: 64 (1960).
- 9) Yabuta, T., Sumiki, Y., Aso, K., Tamura, T., Igarashi, H., and Tamari, K., *J. Agr. Chem. Soc. Japan* **117**: 721 (1941).
- 10) Katsumi, M., Phinney, B.O., Jefferies, P.R., and Henrick, C.A., *Science* **144**: 889 (1964).
- 11) Ogawa, Y., unpublished data.
- 12) Katsumi, M., *Proc. 1st Meeting Society of Chemical Regulation of Plant, Japan* (1966).
- 13) Yomo, H., and Iinuma, H., *Abstracts of the Papers Presented at the U.S.-Japan Seminar on Plant Growth Regulation* (1966).

摘 要

小川幸持：イネ胚乳のアミラーゼ活性およびイネ芽生えの伸長におよぼす種々のジベレリン類、ジベレリン A_3 誘導体類、カウレン酸およびメバロン酸の作用について

種々ジベレリン類 (A_1 , A_2 , A_3 , A_4 , A_5 , A_7 , A_8 , A_9), A_3 -誘導体類 (gibberic acid, allogibberic acid, epiallogibberic acid), カウレン酸およびメバロン酸のイネ種子の胚乳のアミラーゼ活性およびその芽生えの伸長におよぼす有効性の大小を調べた。

芽生えの伸長に対しては $A_3 > A_1 > A_7 > A_2 > A_5 > A_4 > A_9 > \text{allogibberic acid} > A_8 > \text{gibberic acid} = \text{epiallogibberic acid} = \text{kaurenoic acid} = \text{mevalonic acid} = \text{water-control}$ の有効性の大小がみられた。

また胚乳アミラーゼに対しては $A_1 \doteq A_3 \doteq A_4 \doteq A_7 > A_2 > A_5 > A_9 > A_8 > \text{allogibberic acid} > \text{gibberic acid} = \text{epiallogibberic acid} = \text{kaurenoic acid} = \text{mevalonic acid} = \text{water-control}$ の有効性の大小がみられた。(京都大学農学部応用植物学研究室)