Gibberellins and Inhibitors in the Rice Plant

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The occurrence of gibberellin(GA)-like substances in the rice plant (*Oryza sativa* L.) was shown by Murakami¹⁾ for the first time, and later, the presence of endogenous GAs in many cultivars of rice plant has been proved with exceptions in the case of dwarf varieties, "Tan-ginbozu" and "Sankei-10."²⁾ Murakami suggested that the major GA in the rice plant was identical with GA in bamboo shoots by thin-layer chromatography(TLC) using various solvent systems and bioassay.³⁾ However, the identification of GAs in rice plants has not been reported yet, because of small content of GAs in vegetative tissues. This paper deals with the identification of GAs in *Oryza sativa* L. cv. "Nihonbare" (Japonica) and cv. "T-136" (Indica) as well as identification of two growth inhibitors in Japonica rice.

Shoots (9 kg, fresh weight) and roots (3 kg, fresh weight) of *Oryza sativa* L. cv. "Nihonbare" were collected from paddy field two months after sowing, and ears (1 kg, fresh weight) were harvested at full anthesis. Seedlings (10 kg, fresh weight) were harvested two weeks after sowing into the trays filled with soil. After harvesting plants were homogenized and extracted with methanol twice using approximately 5 liters of solvent per kilogram of tissue. Methanol was removed *in vacuo* and the aqueous residue was worked up to obtain ethyl acetate-soluble acidic fraction as follows; the aqueous solution was adjusted to pH 7 and was partitioned three times against the half volume of ethyl acetate, the residual aqueous

solution being partitioned three times against ethyl acetate after adjustment to pH 3 to give an acidic fraction. The acidic fraction was purified by the following sequence of column chromatographies, (1) charcoal adsorption chromatography, eluted with acetone/water, (2) silicic acid adsorption chromatography, ethyl acetate/hexane, (3) silicic acid partition chromatography, ethyl acetate/hexane, 0.5 M formic acid as stationary phase, (4) Sephadex LH-20 chromatography, hexane-ethyl acetate-acetic acid-methanol-water (100: 80: 5: 40: 7, v/v), lower layer being used as stationary phase and upper layer eluting solvent. The active fraction thus obtained was subjected to TLC using Silica gel GF₂₅₄ (Merck) and ethyl acetate-chloroform-acetic acid (20: 8: 1, v/v). GAs in the above purification process were monitored by water culture⁴⁾ and micro drop bioassay⁵⁾ using dwarf rice seedlings, "Tan-ginbozu" and "Waito-C."

The *R_f* value of TLC and the feature of biological activities on dwarf rice seedlings, "Tan-ginbozu" and "Waito-C," of the most purified fraction from the shoots of Japonica variety suggested that the major GA might be GA₁₉. The active fraction was subjected to GC-MS analysis after derivatization to methyl ester trimethylsilyl ether (MeTMS) using diazo-methane and pyridine-*bis*-trimethylsilyl acetamide-trimethylchlorosilane (1: 2: 1, v/v). The fragment ions at *m/e* 462 (M⁺), 434, 402 and 374, which are characteristic of GA₁₉MeTMS, were observed in the mass spectrum obtained by scanning at the GC peak with the same retention time as that of the authentic specimen. Thus, it was unequivocally confirmed that the major GA in the rice plant was GA₁₉.

In the same way as in the case of the shoots, the roots and ears of Japonica variety cv. "Nihonbare" were also examined and these tissues were found to contain GA₁₉ as the major GA. The content of GA₁₉ in various tissues of the rice plant was estimated to be ranged 0.5~1.5 μ g GA₃ equivalent per kg of tissue. Minor GAs in the rice were detected by bioassay but identification by GC-MS was unsuccessful due to their minute amount. However, pre-

FIG. 1. The Purification Procedure for Acidic Ethyl Acetate-soluble Fraction (AE) from the Seedlings of *Oryza sativa* L. cv. "Nihonbare."

CAC: charcoal adsorption chromatography, SAC: silicic acid adsorption chromatography, SPC: silicic acid partition chromatography, TLC: silica gel thin-layer chromatography, EtOAc: ethyl acetate.

liminary mass fragmentography analysis suggested that they might be GA₁, GA₃ and GA₄. Further experiments are under way to identify the minor GAs more firmly.

Two acidic growth inhibitors in the seedlings of Japonica variety were detected by GA bioassay. One was isolated in crystalline state and identified as salicylic acid by the direct comparison of IR spectrum with that of authentic specimen. The content of salicylic acid was 1~1.5 mg per kg of fresh weight. The other was identified as abscisic acid by GC-MS. The isolation and identification process of plant regulators from seedlings of the rice plant was summarized in Fig. 1.

GA₁₀ was also found to be the major GA in seedlings of Indica cv. "T-136." This suggests that both Japonica and Indica variety have the same GA, though they exhibit the different feature of growth pattern.

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