Japan Academy Prize to:

Yuji Kamiya
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for “Study on the Biosynthesis and Regulation of Plant Growth Hormones”

Outline of the work:

Two plant growth hormones, auxin and gibberellin, act throughout the life cycle of plants to regulate seed germination, stem elongation, flower development, and in response to diverse environmental stimuli. Charles Darwin was the first to observe some “influence” for phototropism in 1880, leading to the discovery of auxin. Its simple chemical structure was identified as indole-3-acetic acid (IAA) in 1931; however, its biosynthetic pathway remained unknown for a long time. In Japan in 1926, another plant growth hormone, gibberellin (GA), was originally found as a toxin produced by a pathogenic fungus, *Gibberella fujikuroi,* causing the abnormal elongation of rice plants (Bakanae). In 1958, the homologous GA was isolated from immature seeds of a bean, *Phaseolus multiflorus,* and recognized as endogenous hormones. The biosynthesis of GA was also unconfirmed due to its complex chemical structure.

Dr. Yuji Kamiya cloned the key enzyme genes involved in GA biosynthesis for the first time from both higher plants and the plant pathogenic fungi and revealed the diversity and regulatory mechanisms of GA biosynthesis. During the course of the experiment, he developed a highly sensitive and comprehensive analyzing system for endogenous plant hormones. The system was successfully used to elucidate the long-standing mystery of IAA biosynthesis.

1. Biosynthesis of GA and its regulation

GA biosynthetic pathways are divided into two parts. The first part is the formation of a straight-chain terpene, geranylgeranyl-diphosphate, following its cyclization to a tetracyclic diterpene, GA_{12}. The second part includes a cascade of oxidation and hydroxylation of several GA_{12} sites to produce various physiologically active and inactive GAs.

The first part’s key reaction is cyclization, successively catalyzed by *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS). For KS, Dr. Kamiya purified the enzyme from pumpkin and obtained its partial amino acid sequences, based on which he cloned the full-length KS cDNA. For CPS, he reconstructed the cDNA, according to the exon-intron analyses of *Arabidopsis* dwarf CPS mutants. These cDNAs were expressed in *Escherichia coli*, and their enzyme activities were characterized. He further cloned a full-length cDNA of *G. fujikuroi* homologous gene with plant CPS. The fungal CPS was found to possess CPS and KS activities, indicating that the fungal enzyme is bifunctional.

The second part's key reaction involves sequential reactions producing many GA homologs. Dr. Kamiya also contributed to this part by revealing the regulatory mechanisms of GA3β- and GA2β-hydroxylases in producing physiologically active and inactive GAs, respectively. The GA3β-hydroxylase plays a critical role in the light-induced germination of various plant seeds. The precise elucidation of these enzymes involved in GA biosynthesis contributes to the breeding of dwarf plants and developing new synthetic plant growth regulators.

2. Development of a highly sensitive and comprehensive plant hormone analyzing system

During the course of the above-mentioned work on GA biosynthesis, accurate analysis of microamounts of GA homologs and related substances had frequently been a bottleneck. To overcome this, Dr. Kamiya constructed an improved analytical system based on the three elemental technologies, including (i) high-performance liquid chromatography that allows the separation of target substances without forming derivatives, (ii) tandem mass spectrometry for the identification of substances, and (iii) isotope-labeled internal standards for quantifying recovery yield. The constructed system allowed the simultaneous analysis of multiple hormones and related substances with a sensitivity of approximately 100–1000 times higher than that of the previous method with a reliable accuracy. This system was used to analyze GAs and other plant hormones, such as abscissic acid, brassinosteroids, and strigolactones, and continues to contribute to the field of plant science.

3. Elucidation of the main IAA biosynthetic pathway with a solution of the long mystery

As for the biosynthesis of IAA in plants, four pathways starting from tryptophan had been proposed, but none of them proved to be correct, and the real pathway remained unknown for more than 70 years. Dr. Kamiya assumed that the major cause of this failure is due to the intermediates' chemical instability, and he applied the above-mentioned system for accurate quantification of possible candidates in plants.

Definitive results were obtained with the IAA-dependent dwarf mutants of *Arabidopsis* of the two genes, *TAA* and *YUC*, which had been assigned in the different pathways proposed. The deficient mutants of *TAA* contained decreased amounts of idole-3-piruvic acid (IPA) compared to a wild-type strain, in contrast, the deficient mutants of *YUC* showed a marked increase in IPA. Alternatively, overexpression mutants of *TAA* showed an increase in IPA, but not IAA. Overexpression mutants of *YUC* showed an increase in the endogenous amounts of IAA and its derivatives. These results indicated that TAA and YUC worked in a single pathway from tryptophan to IAA, and *YUC* must code the direct synthetic enzyme of IAA. A final confirmation was made by expressing the cloned *YUC* cDNA in *E. coli*, which showed activity to catalyze the oxidative decarboxylation of IPA to IAA by a novel flavin-containing monooxygenase. These and associated evidences distinctly proved this two-step reaction as the main pathway of IAA biosynthesis, which will become a new target for plant breeding.

The creative work of Dr. Yuji Kamiya contributed to a better understanding of the biosynthesis and the regulation of plant growth hormones, especially GA and IAA. He is globally recognized in the field of plant hormone biochemistry and has received the following awards: The Japan Society for Bioscience, Biotechnology and Agrochemistry, Young Scientists Award (1980), The Japanese Society of Chemical Regulation of Plants Award (1988), Foreign Corresponding Membership Award of the American Society of Plant Biologist (2003), International Plant Growth Substance Association Distinguished Research Award (2004), and Japan Prize of Agricultural Science (2010).

List of Main Publications

1. Kamiya, Y. and Graebe, J.E., The biosynthesis of all major pea gibberellins in a cell-free system from *Pisum sativum*, *Phytochemistry* **22**: 681–689 (1983).
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4. Koshiba, T., Kamiya, Y., and Iino, M., Biosynthesis of Indole-3-acetic acid from L-tryptophan in coleoptile tips of maize (*Zea mays* L.), *Plant Cell Physiol.* **36**: 1503–1510 (1995).
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13. Kawaide, H., Sassa, T., and Kamiya, Y., Functional analysis of the two interacting cyclase domains in *ent*-kaurene synthase from the fungus, *Phaeosphaeria* sp. L487, and a comparison

with cyclases from higher plants, *J. Biol. Chem.* **275**: 2276–2280 (2000).

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15. Kasahara, H., Hanada, A., Kuzuyama, T., Takagi, M., Kamiya, Y., and Yamaguchi, S., Contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthesis of gibberellins in *Arabidopsis*, *J. Biol. Chem.* **277**: 45188–45194 (2002).
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