Duke of Edinburgh Prize to:

Makoto Matsuoka
Professor, Bioscience and Biotechnology Center,
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for “A Study on the Phytohormone, Gibberellin,
Contributing to Species Conservation,
Plant Diversity, and Crop Productivity”

Outline of the work:

Gibberellin (GA) is a phytohormone that affects a wide range of plant growth, development, and environmental responses, including seed germination, stem elongation, leaf expansion, and flower induction. GA was first identified in 1926 by a Japanese scientist in a study of the “foolish seedling” disease in rice and was isolated in 1935 by Drs. Yabuta and Sumiki from a fungus, *Gibberella fujikuroi*, hence, its name.

Using rice as an experimental material, Prof. Makoto Matsuoka started to study the molecular mechanism of GA synthesis and signaling. By studying the molecular and biochemical pathways, he found major components for GA synthesis and perception. Among the GA synthesis-related genes, he found a “green revolution” gene, which contributed to the rapid increase in rice crop yield from the 1960s to 1970s. Based on the results obtained from rice, he expanded his GA research to lower plants, such as moss and fern, and found that GA and its perception system function for gametophyte sex determination in fern. This suggests that both function plastically for various biological events (e.g., longitudinal growth and sex determination) across species in the process of plant evolution, and such plastic roles have contributed to the maintenance of genetic diversity and conservation of plant species.

1. Studies on the molecular mechanism of GA biosynthesis and perception and their application in molecular breeding

Prof. Matsuoka’s major successes using rice can be categorized in the following two areas: First, he investigated rice genes involved in all the six steps of GA biosynthesis and identified all mutants that are defective in each of the steps (Plant Physiology, 2004). Through the study of rice GA-deficient mutants, he identified a gene that contributed to the “green revolution” of rice. The gene encodes GA 20-oxidase 2 (GA 20ox2), the fifth enzyme in the GA biosynthetic pathway (Nature, 2002). The mutant defective in GA 20ox2, called *semi-dwarf 1* (*sd1*), shows semi-dwarfism and higher tolerance to lodging. Thus, farmers can fertilize it more to boost crop yield without unnecessarily enhancing lodging. After finding the *SD1* gene, he proposed various approaches on how to improve the rice architecture for increased crop yield by modifying the expression or function of other GA-related genes. For example, GA 2-oxidase, a GA catabolic enzyme, is efficacious in decreasing GA content in rice to suitable levels enough to bring about lodging resistance by directing its expression at the elongating culm and leaf using the promoter of a GA synthesis gene, GA 3-oxidase (Nature Biotechnology, 2003).

The second part of his GA study is GA signaling. Prof. Matsuoka greatly contributed to the elucidation of the GA perception mechanism using rice GA insensitive mutants. He identified the two important components of the GA perception machinery, namely, the GID1 GA receptor (Nature, 2005) and GID2 GA-specific F-box protein gene (Science, 2003). Based on these findings, he proposed the molecular mechanism of GA perception as follows: a) There are three important components for GA perception, namely, the GID1 nuclear receptor, SLR1 repressor protein (Plant Cell, 2002), and GA-specific F box protein or GID2; b) GA binding to the GID1 receptor induces the formation of a GID1-GA-SLR1 protein complex; and c) This is followed by the GID2-dependent degradation of SLR1, resulting in various GA actions (Annual Review of Plant Biol., 2007).

Later, Prof. Matsuoka also succeeded in analyzing the 3D structure of the GID1 receptor bound to a molecule of GA and deciphered the important amino acid residues for their binding, as reported in *Nature* (2008). This study also revealed that the global structure of GID1 is similar to that of an enzyme, carboxyl esterase, referred to as α/β hydrolase structure; its interaction site with GA also corresponds to the catalytic site of carboxyl esterase. Based on these observations, he proposed the molecular mechanism of GID1-GA interaction as follows: when GA binds to GID1, the N-terminal potion of GID1 receptor (called a lid) covers the GA binding site to stabilize the GA-GID1 interaction.

2. GA contributes to plant diversity and species conservation

Furthermore, Prof. Matsuoka studied the molecular mechanism of establishment and improvement of the GID1 and GA perception system in the process of plant evolution. First, he retrieved the homologs of the three important components of GA perception, GID1, GID2, and SLR1 from lower plants such as moss and fern, and found that *Selaginella moellendorffii*, a club moss, contains all components; however, *Physcomitrella patens*, a moss, does not have any components. A comparative analysis of the important residues of GID1 for GA binding in rice and club moss revealed that some amino acids recognizing GA in GID1 in club moss had been replaced with more suitable ones in the process of plant evolution. Based on these observations, he proposed the hypothesis that GID1 had probably evolved to recognize GA molecules with higher affinity and more stringent specificity (Plant Cell, 2007).

He further studied the broad and diverse range of GA function in *Lygodium japonicum*, a Japanese climbing fern. In some types of ferns, it has been known that a GA-like compound called antheridiogen functions as a pheromone that induces male gametophyte formation. In a fern population, the early-maturing gametophytes (from spores that germinated first) produce and secrete antheridiogens into the aqueous environment, which promote differentiation of the neighboring younger gametophytes into males. These early-maturing antheridiogen-producing gametophytes will mainly develop the egg-forming female sex organ. This phenomenon was first discovered in 1950; however, little has been known about this unique mechanism of antheridiogens. Prof. Matsuoka showed that antheridiogen is produced via the conserved GA biosynthetic pathway in higher plants, but it needs to be modified to become an active GA for male organ induction (Science, 2014). Several genes for the production of GA intermediate are highly expressed in the early-maturing gametophyte, whereas the gene encoding the last enzyme of the GA synthesis pathway is preferentially expressed in the late-maturing young gametophytes to produce bioactive GA, GA_4. Such partitioning of gene expression of GA synthesis explains why the early-maturing gametophytes mainly produce the antheridiogen and only late-maturing gametophytes respond to the pheromone. GA_4 produced by such a split biosynthesis pathway interacts with its receptor, GID1, which is preferentially expressed in the late-maturing young gametophyte, and finally activates the GA signaling pathway (Science, 2014). The remaining critical question is how the fern is able to respond to the low levels of antheridiogen being produced and secreted by mature gametophytes into the environment. Antheridiogen is more efficiently captured than GA_4 by young gametophytes, probably because of the higher hydrophobicity of antheridiogen, which causes it to easily diffuse through the cell membrane. Gathering from these observations, Prof. Matsuoka proposed the model that although GA functions as a growth substance to enhance the longitudinal growth and development in higher plants, it also functions as a sex pheromone in fern, inducing male gametophyte formation. This mechanism results in a few female gametophytes being surrounded by male gametophytes, promoting outcrossing and the maintenance of genetic diversity and species conservation.

As previously described, moss has neither GA nor its perception system, but even in moss, there is a GA-mediated mechanism similar to that in higher plants, which is under the control of a transacting factor, GAMYB (Nature Communication, 2011). GAMYB functions as a master regulator in the GA signaling pathway of higher plants, which induces the expression of starch-degrading enzymes and development of anthers and pollen (Plant Cell, 2009). Prof. Matsuoka revealed that moss GAMYB can function in higher plants in a similar way, e.g., promoting anther and pollen development in rice. Furthermore, the disruption of GAMYB in moss causes a defect in male organ development, which corresponds to the above phenomenon of a GA derivative, antheridiogen, found in fern. Taking together the observations in moss and fern, Prof. Matsuoka proposed the following evolutionary model of GA and its perception system: in the earliest land plant, moss, there is no GA or its perception system; however, even moss has a male organ-developing system under the control of GAMYB that is essentially the same as that used in GA signaling in higher plants. Later, when fern plants emerged, GA and its perception system were established with the development of the GID1 receptor and suppressor protein by recruiting the pre-existing GAMYB control system for male organ development (Nature Communication, 2011).

The concept of "species conservation" has usually been understood in the context of protecting and sustaining a particular species, particularly those endangered by human activities. In contrast, the study on the evolution of GA and its perception by Prof. Matsuoka suggested that for the survival of existing species and emergence of novel species, plants have themselves aggressively used pre-existing mechanisms from which to produce new mechanisms that are adaptable to novel biological events for the sake of their survival, thus conservation. Indeed, the development and improvement of GA and its perception mechanism that he so meticulously studied, such as the emergence of GID1 from carboxyl esterase, improvement of the affinity and specificity of the GA-GID1 interaction, and co-evolution of the GA catalytic and GID1 perception systems, clearly involved the modification of their structure or components to become adaptable to novel environmental conditions. A specific example is the antheridiogen system in fern because they succeeded in using the GA system to communicate between neighboring individuals by splitting the GA synthesis pathway, which was originally thought of as an all-in-one system in one cell. This is a good example of an intrinsic system of "species conservation," which helped attain the subsistence and success of land plants.

Knowledge on how to modify the molecular mechanism of GA perception without disturbing its intrinsic function or the environment should be very useful for further improvement of crop productivity. Prof. Matsuoka is actually doing crop improvement based on such knowledge obtained from basic science. For example, the transplanting system of rice seedlings is an essential way for sustainable crop productivity in Japan, although this system entails high cost and labor. He found a new mechanism with rapid growth at the young seedling stage by enhancing GA synthesis (Plant Cell Physiol., 2012), which might be useful for an alternative rice cultivation system using direct seeding that does not require transplanting.

The above findings were reported in more than 50 scientific papers published in highly reputable journals such as *Nature*, *Science*, *Nature Sisters*, and *PNAS*, to name a few, and in more than 250 papers published in other international journals. Prof. Matsuoka has also served as a plenary speaker at many international meetings and has successively held editorship of different international journals of plant science. For his achievements in GA study and rice molecular breeding, Prof. Matsuoka has received the following awards and recognitions: Tokyo Techno Forum 21 Gold Medal (1998), Kihara Foundation For The Advancement Of Life Science Prize (2006), Japanese Society of Breeding Award (2010), American Association for Advancement of Science Fellow (2009), International Plant Growth Substance Association's Silver Medal (2012), and Chunichi Cultural Award (2014).

Major Publication List

1. Original Papers

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