Atsushi Komamine (1929–2011): a tribute

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Abstract Atsushi Komamine was one of the pioneers of plant tissue culture in Japan and a researcher with many friends throughout the world. His objective was to determine the potential capacities of plant cells, and he contributed considerably to plant tissue cultivation research by establishing several unique culture systems that controlled cell division and differentiation. He emphasized that the uniformity of cultured cells and the synchrony of the plant cell response at a high frequency were necessary to study the functions of plant cells using biochemical and molecular biological methods. He trained over 300 people, and many outstanding individuals in academia and industry emerged under his tutelage. He was an outstanding mentor: with his accommodating attitude, he was loved dearly by students and foreign researchers alike. He set up the Asia Pacific Association of Plant Tissue Culture and Agribiotechnology in 2000, and he established the international journal *Plant Biotechnology Reports* with Jang R. Liu in 2006, thereby contributing to Asian plant sciences. In addition to describing his achievements and activities, this article aims to paint a portrait of the man himself.

Introduction

Most researchers who knew Atsushi Komamine claim say that there has never been a kinder advisor than he was. He was active in his research right up to his death. His research career from his graduation until his death spanned 60 years. In his later life, foreign researchers who met with him were surprised by his age. When he retired as the Director of the Kihara Institute for Biological Research, Yokohama City University (2006–2010), he published his memoirs (Komamine 2010). In this paper, I would like to give an overview of his research and describe his contributions to academic societies by referring to his memoirs. His academic career was recounted in his obituary, which was published in *Plant Biotechnology Reports* (Kodama 2011).

He first became interested in plant physiology after reading “Jack and the Beanstalk” in the early grades at elementary school. He placed soaked absorbent cotton wool into a cup, put a bean on top, and covered it with

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drawing paper. When he looked in the cup the next day, he was disappointed that the bean had not sprouted, but several days later he was elated when he noticed that the shoots had lifted the cover. He said that this was what inspired him to study plant physiology. Of interest, a plant material used in his first studies was a velvet bean cultured in Japan (*Stizolobium hassjoo*, cv. Hassjoomame; now renamed as *Mucuna puriens* cv. Yokohama velvet bean). Velvet bean contains dihydroxyphenyl alanine (DOPA) in abundance. The damaged part soon turns red, because DOPA is oxidized by polyphenol oxidase. Komamine started by identifying amino acids (stizolobic acid and stizolobinic acid) in velvet beans (Hattori and Komamine 1959). Thereafter, he studied abroad under the Finnish Nobel prize winner, Artturi Virtanen. At that time, there were few Asians, including Japanese, studying in northern Europe. In fact, besides the diplomatic staff, there were very few Japanese in Finland at the time. After his return, he started plant physiology research using experimental systems based on tissue culture techniques. He conducted plant physiology research at the University of Tokyo (1961–1984), Tohoku University (1984–1993), Japan Women's University (1993–1998), and the Research Institute of Evolutionary Biology, Tokyo University of Agriculture (1998–2011). He emphasized the logic that is required when students and researchers establish physiological experimental systems. He motivated students to think about how they could develop superior experimental systems (Fig. 1). He explained an outline of experimental systems for the determination of plant cellular function as follows: the function of a plant cell was recognized to be a “black box.” The experimental systems for plant physiology comprise the stimulus applied to plant cells (input), the black box (plant cell), and cellular responses (output). Plant cells are surrounded by various factors, including physiological, chemical, and biological. These extracellular input factors can activate signal transduction, which results in cellular responses that include cell proliferation, differentiation, and changes in metabolism. After the development

of plant genetic transformation techniques, he added genetic transformation as a potential output of plant cells. He emphasized the importance of elucidating the relationships between the input stimuli and output responses. To obtain reproducible cellular responses, the following requirements must be fulfilled: (1) control of the physical and chemical conditions in the environment and (2) a homogeneous plant cell population. To analyze the output by using biochemical and molecular biological methods, the ideal experimental systems must exhibit cellular responses at a high frequency and in synchrony. In addition, he considered that experimental systems based on single cells were desirable. His teachings were based on the analytical techniques available between the 1970s and the 1990s. Techniques for single cell analysis using microscopy and highly sensitive mass spectrometry, etc., were not available in those days; therefore, large amounts of plant materials were necessary for biochemical and molecular biological analyses. However, the concept proposed by Komamine is still important to us, and it would be useful if every researcher could develop a definite philosophy like him.

Komamine particularly valued the teachings of Hans Molish (later to become the president of Vienna University, 1856–1937), who was the first professor in the plant physiology laboratory at Tohoku University. According to Molish's teaching, Komamine always strived to perform his plant physiology research from a perspective that was as broad as possible with respect to the potential of plant cells.

Physiological studies of plant cells using tissue culture systems

Komamine and colleagues investigated the basic metabolism of cultured plant cells, e.g., the metabolism of carbohydrates (Morohashi et al. 1967), nucleotides (Kanamori et al. 1979), and cell wall polysaccharides (Takeuchi and Komamine 1978; Suzuki et al. 1990). In addition to these biochemical analyses, he established several unique experimental systems for the analysis of cell proliferation and differentiation, which are described in brief in the following passages.

Study of the cell cycle using synchronous cell cultures

It is important to regulate the proliferation of in vitro-cultured plant cells. Suspension-cultured Madagascar periwinkle (*Catharanthus roseus*) cells are homogeneous in their size and shape. Clusters of cultured *C. roseus* cells can be macerated easily to yield protoplasts with a very high efficiency, which facilitates the estimation of cell

Fig. 1 Experimental design of plant physiology experiments

numbers in cultures. Synchronous cell division can be induced by manipulating the availability of phosphate in the medium (Amino et al. 1983), which was an innovative experimental system for cell cycle studies in plants. Using synchronous cell cultures, various aspects of molecular events in the cell cycle were investigated by Komamine's students, including Masaki Ito and myself (Ito et al. 1991; Kodama et al. 1991). Masaki Ito reviews the progress in cell cycle studies in the present issue (Ito 2013).

Tracheary element differentiation

Tracheary elements, cells that constitute the vessels and tracheids, are characterized by visible patterns in the secondary cell wall thickenings such as annular, spiral, and reticulate patterns. Using this feature, tracheary element formation has been investigated for many years as a model of plant cell differentiation, although the techniques available were quite limited in the early studies because of a lack of efficient experimental systems. Hiroo Fukuda and Komamine established a cell culture system in which a large proportion of single cells isolated from the mesophyll of *Zinnia elegans* directly differentiated into tracheary elements with good synchrony (Fukuda and Komamine 1980). This experimental system opened the door to biochemical and molecular biological analyses of tracheary element differentiation. Taku Demura summarizes the findings obtained during subsequent studies in the present issue (Demura 2013).

Somatic embryogenesis

Plant cells have the potential to regenerate an entire organism, which can often be observed in the differentiated cells of mature tissues. This ability is known as “totipotency”, a representative phenomenon of which is somatic embryogenesis (Reinert 1958; Steward et al. 1958). Fujimura and Komamine (1979) obtained carrot cell clusters that comprised 3–10 cells by sieving and density gradient centrifugation, and synchronous embryogenesis was induced by transferring these cell clusters to auxin-free conditions. Subsequently, Nomura and Komamine (1985) established a culture system in which single cells differentiated into embryos at a high frequency. This study showed that single plant cells could express their totipotency in defined culture conditions. Tatsuhito Fujimura reviews these studies of somatic embryogenesis in the present issue (Fujimura 2013).

Study of organogenesis in *Arabidopsis*

Since the late 1980s, *Arabidopsis thaliana* has been used as a model plant species. Munetaka Sugiyama and colleagues

established an experimental system suitable for studying callus, root, and/or shoot induction from *Arabidopsis* explants (Yasutani et al. 1994; Ozawa et al. 1998). Using this system, they isolated and characterized many mutants, which led to the identification of genes involved with dedifferentiation and also in organogenesis. Munetaka Sugiyama reviews molecular genetic studies of in vitro organogenesis in the present issue (Sugiyama 2013).

Production of secondary metabolites in cultured cells

Secondary metabolites are produced in specific tissues and cells at specific stages of growth in higher plants, which implies a close correlation between secondary metabolism and growth and morphological differentiation of cells. Komamine realized that plant red pigments were good indicator metabolites for use in metabolic studies of plant cell growth and differentiation (Komamine et al. 1989). Komamine and his colleagues found that anthocyanin accumulation in *Vitis* cells had a negative correlation with cell division, whereas betacyanin biosynthesis in *Phytolacca* cells had a clear positive correlation with cell division (Sakuta and Komamine 1987). Furthermore, they showed that anthocyanin synthesis is closely correlated with morphological differentiation, i.e., embryogenesis and negatively correlated with cell division or proliferation in suspension cultures of carrot (Ozeki and Komamine 1981, 1986). Recent advances in pigment biosynthesis are summarized in the present issue by Masaaki Sakuta (2013).

Promotion of Asian plant biotechnology

Komamine made great efforts to ensure that several international conferences were held in Japan. At the 5th International Congress of Plant Tissue and Cell Culture held in Tokyo and Lake Yamanaka (1982; Chairman, Akio Fujiwara), he dedicated all of his efforts to the management of 31 symposia sections and the presentation of 365 papers. In addition, he liked entertaining guests and paid careful attention in organizing a welcome party. He set up the welcome dinner party in a Japanese-style room and he also planned entertainment in the form of a traditional Japanese summer festival, Bon Odori. A stage was assembled in the corner of the hotel and a genuine Bon Odori was held. Many foreign researchers enjoyed participating in the Bon Odori dance. The congress was an international networking forum and an opportunity for many young Japanese researchers to spread their activities abroad. This congress was an important turning point in the progress of Japanese plant biotechnology, and it laid the foundations for plant biotechnology in Japan.

Komamine proactively traveled abroad to meet foreign researchers, which is why he became to be known as the “Flying Professor” among staff members and other professors at Tohoku University. He contributed to the industrial applications of plant biotechnology in Asia from the 1980s and nurtured young scientists in the areas of plant science and biotechnology in Southeast Asian nations. He set up the International Agri-Biotechnology Association of Japan (IABA-J, a nongovernmental organization) in 1992 to manage staff training in the Southeast Asian area, particularly Cambodia, Myanmar, Laos, and Vietnam. Initially, IABA-J supported technology transfer to Vietnam, but it shifted to agricultural biotechnology support for Myanmar around 2000. He donated his library to Yezin Agricultural University in Myanmar with the support of IABA-J. Furthermore, in 2000, he set up the Asia Pacific Association of Plant Tissue Culture and Agribiotechnology to promote activities and cooperation among Asian plant science researchers. In addition, Komamine and Jang R. Liu made their best efforts as the co-founders of the international journal *Plant Biotechnology Reports* in 2006, the first issue of which was published in 2007. There is no doubt that plant tissue culture in Japan and Asia made great progress thanks to his contact with researchers and his generous support of young scientists in developing Asian countries.

“Mother” of research

Figure 2 shows a photograph of Komamine’s signboard, which hung on his room door. This signboard shows a flask, in which many shoots have been produced using tissue cultivation techniques. This was designed by Tatsuhito Fujimura, and it was very much treasured by Komamine. The flask symbolizes Komamine’s laboratory and the shoots represent the many young peoples who studied there. His hospitality was greatly appreciated by

his students and foreign researchers. He always encouraged young researchers who were experiencing difficulties by using the phrase “Do the best you can at this time.” Many of the students he mentored now have their own laboratories. The “flask” provided an ideal environment for students and his students received many “nutrients” from the medium prepared by Komamine. Remembering Komamine, Hiroo Fukuda had the following to say: “For me, Dr Komamine was like the mother of research.” These words reflect the deep appreciation we all feel for him.

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Fig. 2 Signboard on the Komamine Laboratory door

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