Preface to the special issue “Technology in tissue culture toward horizon of plant biotechnology”

Yutaka Tabei¹, Toshiya Muranaka²

¹Strategic Planning Headquarters, National Agriculture and Food Research Organization, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8517, Japan; ²Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Transformation and genome editing technology are indispensable technologies used in everything from basic research to practical applications, such as the production of useful materials and plant breeding. In plant research, genetic transformation, genome-editing techniques, individual regeneration, and tissue and cell culture systems are essential.

Tissue culture research began in the early 20th century. Haberlandt (1902) proposed that plant cells have totipotency, which was demonstrated by finding adventitious shoots differentiating from growing calli (White et al. 1939). Subsequently, many researchers attempted to induce adventitious shoot and root differentiation. A breakthrough in tissue and cell culture techniques was the discovery of plant hormones, such as cytokinin and auxin. Controlling the cytokinin to auxin ratio was found to regulate adventitious shoot and root differentiation in tobacco (Skoog and Miller 1957).

Steward et al. (1958) and Reinert (1959) induced somatic embryo from carrot calli to regenerate complete plants. The growth process resembled embryonic development from a fertilized egg morphologically, so the regeneration was called somatic embryogenesis. This knowledge has led to an essential method for studying the mechanisms of differentiation and for applying genetic transformation and genome editing.

Simultaneously, many basal media for culturing tissues and cells were developed, some of which are still in use. Murashige and Skoog (1962) reported a medium (MS medium) in which nutrient concentrations were optimized by culturing tobacco pith cells. Gamborg et al. (1968) reported B5 medium for growing soybean root tip cells. Other established media included White medium (White 1963), LS medium (Linsmaier and Skoog 1965), NN medium (Nitsch and Nitsch 1969), N6 medium (Chu 1978), and AA medium (Müller and Grafe 1978). A suitable medium for each plant material can be developed by adjusting the plant hormone conditions, changing the carbon source, and modifying inorganic salts.

These tissue culture studies demonstrated the potentially unlimited growth of undifferentiated cells and the redifferentiation of roots, shoots, and entire plants. However, these studies did not help to demonstrate whether a single cell has genetic totipotency because of their failure to assure the single cell clonal origin of regenerated plants. Finally, Vasil and Hildebrandt (1965) proved the totipotency of single cells by regenerating a whole plant from protoplasts and provided a way to elucidate the function of single cells. Studies of plant tissue and cell cultures led to other industrial objectives, including the production of specialized metabolites (Ramawat and Merillon 2007), and the micropropagation of trees, crops, and horticultural plants, most notably orchid species (Arditti and Krikorian 1996).

A research group studying tissue and cell culture was organized in Japan at the beginning of these studies and was the predecessor of the Japanese Society for Plant Cell and Molecular Biology. This research group held its first symposium on plant cell culture in 1968. In 1986, 731 of the 2,894 members in the International Society of Plant Tissue Culture were Japanese, implying that Japanese researchers understood the importance of tissue and cell culture.

Production of useful compounds in plant tissue cultures

Most specialized plant metabolites have completely different bioactivities depending on the existence of structural isomers. Chemically, they are often difficult to synthesize or have poor yields. Hence, they are usually extracted from plants. Such specialized plant metabolites can be biosynthesized and accumulated from some tissues and organs in a few plant species. Because important plant species are often endangered, the development of alternative production methods is needed. In the late 1950s and early 1960s, the production of useful compounds in plant tissue cultures

This article can be found at http://www.jspcmb.jp/
Published online June 25, 2020
received attention from Pfizer and other companies and research institutes. In Japan, such attempts peaked in 1984 with the explosive sales of "Lady 80 BIO Lipstick" commercialized by Mitsui Petrochemical Industries (now Mitsui Chemicals) and Kanebo (now Kanebo Cosmetics). The basis of this success was technology for the mass production of shikonin, a specialized plant metabolite produced by cultured cells of *Lithospermum erythrorhizon* (Tabata and Fujita 1985; Yazaki 2017), which had been used as a dye and herbal medicine since ancient times. The technology was established after examining various culture media and conditions. Subsequently, Nitto Denko started producing tissue-cultured ginseng commercially, and many companies and research institutes followed and produced other compounds. However, the huge cost of production and lack of information on the biosynthetic pathways of useful metabolites hindered the commercial application of plant tissue cultures and the technology was thought to be on a downward trend.

Now, plant biotechnology is being reinterpreted and implemented by integrating new technologies, including genetic engineering, genome analysis, omics, and informatics, which have developed remarkably in this century. Some genome editing technology can be considered an extension of conventional breeding, as described below, which has accelerated the movement toward industrialization. The fundamentals of these technologies are old but involve new plant tissue and cell culture techniques. By integrating those technologies, the production of useful metabolites in plants or plant tissue cultures are also being re-evaluated.

### Development and application of transformation technology

Cohen et al. (1973) successfully cut out DNA from two plasmids using restriction enzymes and spliced them in vitro. This study is considered the start of modern recombinant DNA technology. Genetically modified tobacco was reported in 1984 (De Block et al. 1984; Horsch et al. 1984), and two years later there was a field trial of transgenic plants in the United States. In 1994, a genetically modified tomato cultivar (*Flavr Savr*) with prolonged shelf life was cultivated commercially and sold in the United States. Since 1996, transgenic crops have been released for commercial cultivation, including herbicide-tolerant soybean, pest-resistant corn, and potatoes. The total area cultivated with genetically modified crops was 191.7 million hectares in 2018, and the area is increasing worldwide (ISAAA 2018). The recalcitrance of plant species, especially monocotyledons, to *Agrobacterium* transformation led to modification of the *Agrobacterium* transformation conditions (Hiei et al. 1995) and other methods for delivering foreign DNA into plant cells. These included electroporation, microinjection, floral dipping, and particle bombardment (Peña 2005).

In Japan, the importance of recombinant DNA technology was described in White Papers on Science and Technology in 1981 (Science and Technology Agency 1981). Subsequently, intensive biotechnology research on genetically modified crops was supported by several ministries. Much progress has also been made in tissue and cell culture, although the practical application of genetically modified crops and research on genetic modification for social implementation stalled around 2004 due to the worsening economic situation, public concerns about genetically modified foods, and the enactment of regulations by local governments restricting the cultivation of genetically modified crops.

### Toward the spread of genome-editing technology and its application

Genome editing technology is a new biotechnology that alters genes by cutting a target DNA sequence in an extremely specific manner. Kim et al. (1996) developed zinc finger nuclease (ZFN) and Christian et al. (2010) developed transcription activator-like effector nuclease (TALEN). In 2012, a new genome editing tool called CRISPR/Cas9 was reported (Gasiunas et al. 2012; Jinek et al. 2012). Because CRISPR/Cas9 was a simpler tool for genome editing and had greater cleavage efficiency, this genome editing technology rapidly became popular.

In 2014, Japan started the Cross-ministerial Strategic Innovation Promotion Program, which accelerated basic research and application of the social implementation of genome-edited crops and fish. Genome editing is now being used in many fields of research. The demand to apply genome editing to various crops has also increased, and it is necessary to develop novel tissue cell culture systems for plant species that are difficult to culture to accelerate efficiency of genome editing.

### Handling rules for the use of genome-edited organisms

To use genome-edited organisms properly, it is necessary to establish handling rules. Internationally, genetically modified organisms are regulated by the Cartagena Protocol on Biosafety and by the Cartagena Domestic Act in Japan (Ministry of the Environment, MoE 2004). Genetically modified foods and feeds are regulated by the Food Sanitation Act (Ministry of Health, Labour and Welfare, MHLW 2000) and Feed Safety Act (Ministry of Agriculture, Forestry and Fisheries, MAFF 2002), respectively. Handling policy has become necessary for the social implementation of genome-edited organisms. In late 2019, the MoE formulated a policy for handling...
genome-edited organisms (Ministry of Environment 2019) and the MHLW established a policy for handling genome-edited foods and food additives (Ministry of Health Labour and Welfare 2019). Subsequently, the Ministry of Education, Culture, Sports, Science and Technology (Ministry of Education, Culture, Sports, Science and Technology 2019), Ministry of Economy, Trade and Industry (Ministry of Economy, Trade and Industry 2019), and MAFF (Ministry of Agriculture, Forestry and Fisheries 2019) set out their respective policies. According to the MoE handling policy, when a genome-edited organism is categorized in SDN-1 and it does not contain a foreign DNA sequence (null segregant), the organism may not need to be regulated as a genetic recombinant if notified to the regulatory authorities. The MHLW has indicated that genome-edited foods and food additives may not need to be regulated as genetically modified foods if they are derived from null segregants and the modified DNA sequence is within the range of natural mutation.

This policy has markedly influenced research and application. This special issue contains a study on genome editing in potato and tomato that does not incorporate foreign genes by transient Agrobacterium infection. Using the iPB method (Hamada et al. 2017), the possibility of genome editing by introducing ribonucleoprotein or protein instead of DNA has been examined. Southern blot analysis and PCR are now used to prove null segregants, although a new method using a next-generation sequencer has been reported (Ito et al. 2020).

Currently, crops with established tissue and cell culture systems have advanced, while crops that are difficult to culture have been left behind. Under these circumstances, this special issue has two main purposes: to organize expertise in established tissue culture and transformation techniques, and to introduce new culture systems and new transformation and genome-editing technologies.

This special issue introduces culture systems and transformation conditions for ten crops species (corn, wheat, liliaceous species, grape, apple, tall fescue, cucumber, Cucurbita species, cedar, and tea) and plastid transformation of tobacco. Novel genome editing methods that do not integrate foreign genes and industrialization using tissue culture are also described, including the low-cost, highly efficient mass-propagation of potato using microtubers. The issue also introduces cryopreservation of the growth points of cedar and mutation detection using wheat.

We hope that the information described in this issue will contribute to research using transformation and genome-editing technology.

References

Arditti J, Krikorian AD (1996) Orchid micropropagation: The path from laboratory to commercialization and an account of several unappreciated investigators. Bot J Linn Soc 122: 183–241 (London)

Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF (2010) Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186: 757–761

Chu CC (1978) The N6 medium and its application to another culture of cereal crops. In: Proceedings of Symposium on Plant Tissue Culture. Science Press, Beijing, pp 43–50

Cohen S, Chang A, Boyer H, Helling R (1973) Construction of biologically functional bacterial plasmids in vitro. Proc Natl Acad Sci USA 70: 3240–3244

De Block M, Herrera-Estrella L, Van Montagu M, Schell J, Zambryski P (1984) Expression of foreign genes in regenerated plants and their progeny. EMBO J 3: 1681–1689

Gamborg OL, Miller RA, Ojima L (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50: 151–158

Gasianus G, Barrangou R, Horvath P, Siksnys V (2012) Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci USA 109(39): E2579–E2586

Haberlandt G (1902) Culturversuche mit isolierten Pflanzenzellen. Sitz-Ber Mat Nat Kl Akaad Wiss Wien 111: 69–92

Hamada H, Linghu Q, Nagira Y, Miki R, Taoka N, Imai R (2017) An in planta bioilistic method for stable wheat transformation. Sci Rep 7: 11443

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (Oryza Sativa L.) mediated by agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J 6: 271–282

Horsch RB, Fraley RT, Rogers SG, Sanders PR, Lloyd A, Hoffmann N (1984) Inheritance of functional foreign genes in plants. Science 223: 496–498

ISAAA (2018) Global Status of Commercialized Biotech/GM Crops in 2018: Biotech Crops Continue to Help Meet the Challenges of Increased Population and Climate Change. ISAAA Brief No. 54. ISAAA, Ithaca, NY

Ito T, Onuki R, Tsuda M, Oshima M, Endo M, Sakai H, Tanaka T, Ohsawa R, Tabei Y (2020) Foreign DNA detection by high-throughput sequencing to regulate genome-edited agricultural products. Sci Rep 10: 4914

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816–821

Kim YG, Cha J, Chandrasegaran S (1996) Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci USA 93: 1156–1160

Linsmaier EM, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 18: 100–127

Ministry of Agriculture, Forestry and Fisheries (2002) http://www.famic.go.jp/fsis/feed/kokujiki/k14n1780.html (in Japanese)

Ministry of Agriculture, Forestry and Fisheries (2019) https://www.maff.go.jp/j/syoun/nouan/carta/tetuduki/attach/pdf/nbt_tetuzuki-2.pdf (in Japanese)

Minister of Economy, Trade and Industry (2019) https://www.meti.go.jp/policy/mono_info_service/mono/bio/cartagena/genome_yoroyo.pdf (in Japanese)

Ministry of Education, Culture, Sports, Science and Technology
Preface to the special issue “Technology in tissue culture toward horizon of plant biotechnology”

Ministry of Environment (2004) Domestic law and regulation. http://www.biodic.go.jp/bch/english/law.html
Ministry of Environment (2019) To Genome Editing Technologies Users. http://www.biodic.go.jp/bch/download/genome/genome_chirashi_english.pdf
Ministry of Health, Labour and Welfare (2000) Report on Mandatory Requirement for Safety Assessment of Foods and Food Additives Produced by Recombinant DNA Techniques. https://www.mhlw.go.jp/english/topics/food/3-8.html
Ministry of Health, Labour and Welfare (2019) Food Hygiene Handling Procedures for Food and Additives Derived from Genome Editing Technology. https://www.mhlw.go.jp/content/000550824.pdf
Müller AJ, Grafe R (1978) Isolation and characterization of cell lines of Nicotiana tabacum lacking nitrate reductase. Mol Gen Genet 161: 67–76
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497
Nitsch P, Nitsch C (1969) Haploid Plants from Pollen Grains. Science 163: 85–87
Peña L (2005) Transgenic Plants: Methods and Protocols. Humana Press Totowa, NJ
Ramawat KG, Merillon JM (2007) Biotechnology: Secondary Metabolites, Plants and Microbes, 2nd ed. Science Publishers Enfield, NH
Reinert J (1959) Über die kontrolle der morphogenese und die induktion von adventivembryonen an gew-ebekulturen aus karotten. Planta 53: 318–333
Science and Technology Agency (1981) White Paper on Science and Technology (in Japanese)
Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 11: 118–130
Steward FC, Mapes MO, Mears K; Organization in Cultures Grown from Freely Suspended Cell (1958) Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. Am J Bot 45: 705–708
Tabata M, Fujita Y (1985) Production of shikonin by plant cell cultures. In: Zaitlin M, Day P, Hollaender A (eds) Biotechnology in Plant Science. Orlando, pp 207–218
Vasil V, Hildebrandt AC (1965) Differentiation of tobacco plants from single, isolated cells in microcultures. Science 150: 889–892
White PR (1939) Controlled differentiation in a plant tissue culture. Bull Torrey Bot Club 66: 507–513
White PR (1963) A Handbook of Plant Tissue Culture. Jacques Cottell, Pennsylvania, USA
Yazaki K (2017) Lithospermum erythrorhizon cell cultures: Present and future aspects. Plant Biotechnol 34: 131–142