Plant Biotechnology 39 (2022)
DOI: 10.5511/plantbiotechnology.22.editorial

Editorial

From the Editors

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Here, we take this opportunity to introduce several notable recent articles published in *Plant Biotechnology*.

The first of the two invited review articles that appeared in *Plant Biotechnology* last year was an overview of cytoplasmic male sterility (CMS) in rice by Toriyama (2021), who won the JSPB Award for Distinguished Research in 2020. His review also describes the detailed mechanisms involved in the cause and restoration of CMS through genetic interactions between the mitochondrial and nuclear genomes. The second review article examines efforts to establish a cost-effective, scalable, safe and sustainable expression system for useful recombinant proteins in plants by Miura and colleagues (Nosaki et al. 2021), who won the JSPB Award for Technical Advance in 2020.

Applied biotechnology, tissue culture, and plant cell engineering form the strong backbone of *Plant Biotechnology*. In the area of prevailing CRISPR/Cas9 technology, Basso et al. (2021) reported its application to C4 grass, *Setaria viridis*. The CRISPR/Cas9 binary vector that exploits the non-homologous end joining system was optimized to efficiently knock out a GFP transgene. In another report focused on this technology, CRISPR/Cas9 attached to the translation enhancer dMac3 was employed to modify the composition ratio of amylose and amylopectin of storage starch in potato (Takeuchi et al. 2021). dMac3, a translation enhancer derived from 5′UTR of *OsMac3* mRNA (Aoki et al. 2014), was put in front of Cas9 ORF and used to edit the potato granule-bound starch synthase gene. This resulted in the creation of a potato plant lacking starch branching enzyme StSBE3, which is expected to contribute to elucidation of the function of SBE in starch biosynthesis.

In the areas of transgenic technologies, several examples of improvement of *Agrobacterium*-mediated plant transformation were reported. Optimization of the *in planta* transformation protocol of rice (*Oryza sativa*, indica cultivar; Sarkar et al. 2021) was reported to include piercing imbibed embryos and then vacuum-infiltrating *Agrobacterium* suspension into the embryos. Other notable reports include establishment of *Agrobacterium*-mediated transformation protocol for radish (*Raphanus sativus* L.) using hypocotyl-derived explants (Muto et al. 2021), enhancement of the transformation efficiency of *Arabidopsis* T87 cell line by preculturing the cells in an excess amount of Murashige and Skoog macronutrients before cultivation in B5 medium (Hata et al. 2021), and optimization of a floral dip protocol with smearing sticky *Agrobacterium* suspension mixed with a spreading agent onto flower buds of tomato (*Solanum lycopersicum*; Honda et al. 2021).

In the areas of cell and tissue culture, methods for stress-induced somatic embryogenesis and artificial seed production in Japanese honewort (*Cryptotaenia japonica*) were reported (Kato and Shiota 2021). Shoot apex explants of Japanese honewort were subjected to hyperosmotic stress (i.e., 0.7 M sucrose) for 3–6 weeks and then transferred to stress-free culture conditions to obtain somatic embryos. Hyperosmotic stress-induced somatic embryos were then encapsulated with alginate beads to obtain bead- and sponge-type artificial seeds. In vitro propagation of axillary buds of *Dalbergia congestiflora*, a woody Fabaceae plant in Mexico, by optimizing the hormone concentrations (Hernández-García et al. 2021) will assure sustainable usage of this endangered species.

Studies on biostimulant and biofertilizer are gaining interest as they can contribute to achieving sustainable development goals. In the fermentation process of an organic fertilizer made of plant residue, called "Bokashi" fertilizer in Japan, 3-phenyllactic acid (PLA) produced by lactic acid bacteria was shown to be a root-promoting compound (Maki et al. 2021). PLA showed a synergistic effect with tryptophan on the promotion of adventitious root. PLA did not increase endogenous indole-3-acetic acid, whereas tryptophan did. The structure of PLA is similar to that of L-2-aminooxy-3-phenylpropionic acid, which inhibits IAA biosynthesis. These results suggest that the root-promoting activity of PLA is not simply due to the increase in endogenous auxin, and further study is needed to clarify the mechanism underlying the root-promoting activity of PLA.

Secondary metabolism, or specialized metabolism, is another backbone of *Plant Biotechnology*. Last year, three articles reported analysis of the production of

This article can be found at http://www.jspcmb.jp/
Published online March 25, 2022

glycyrrhizin, a triterpenoid saponin produced by *Glycyrrhiza* species (licorice). First, in an invited article by Inui et al. (2021), of which the corresponding author is a winner of the JSPB Award for Technical Advance in 2017, the authors established a hydroponic cultivation system of *G. uralensis* and selected superior *G. uralensis* clones with high glycyrrhizin content. They identified DNA sequence polymorphisms in intron 7 of *CYP88D6*, encoding a key enzyme of glycyrrhizin biosynthesis, which is associated with glycyrrhizin content. Although more genetic data are required, this finding suggests that the intronic region of species-specific biosynthetic genes is effective in the selection of superior clones. Second, four active isoforms of UDP-glucose dehydrogenase (UGD) in *G. uralensis*, which supply UDP-glucuronic acid for triterpenoid saponin biosynthesis, were isolated (Kawasaki et al. 2021). Among the four UGDs, GuUGD1–4, GuUGD2 had the highest catalytic constant and gene expression level, suggesting that it is the major isoform contributing to the transition from UDP-glucose to UDP-glucuronic acid *in planta*. Third, glycyrrhizin content in *G. uralensis* was reported to be enhanced by rhizobial symbiosis (Kusaba et al. 2021). The authors conducted genome analysis of rhizobia of *G. glabra* and isolated *Mesorhizobium* sp. J8, which was then inoculated to *G. uralensis* roots to form nodules. Interestingly, the relative expression level of *CYP88D6* was increased in the inoculated roots, which coincided with an increase in glycyrrhizin content. It might be of interest to test the effect of other symbiotic organisms, such as mycorrhizal fungi, on glycyrrhizin content.

Flower color has been one of major targets of plant metabolic engineering. Co-introduction of *CYP76AD1*, *DOPA 4,5-dioxygenase* (*DOD*), and cyclo-DOPA 5-*O*-glucosyltransferase (5GT), which were derived from *Beta vulgaris*, *Mirabilis jalapa*, and *M. jalapa*, respectively, into lisianthus (*Eustoma grandiflorum*) increased betalain, comprising violet betacyanins and yellow betaxanthins (Tomizawa et al. 2021). The color of the flower petals of T_1 progenies of the transformants varied, depending on the betalain and anthocyanin accumulation levels. Although anthocyanins and betalains generally exhibit mutually exclusive distribution in plants (Timoneda et al. 2019), the results of this study showed that anthocyanins and betalains can co-exist within one plant through transgenic technology. Lisianthus plants produced in this study might be useful for revealing how the biosynthesis of anthocyanin and betalain interact with each other on the levels of gene expression, enzymatic activity, transport, and accumulation. 5-*O*-Glucosyltransferase involved in anthocyanin biosynthesis in cyclamen (*Cyclamen purpurascens*) flower was also characterized (Kang et al. 2021). Gene expression analysis demonstrated that *Cpur5GT* shows strong expression in fully opened petals, suggesting that *Cpur5GT* will be useful in analysis of the mechanism of A5GT-mediated flower coloration in cyclamen.

Studies on pollen are regarded as a basis for plant proliferation and breeding. Mori et al. (2021) focused on the presence of fluorescent and non-fluorescent pollen in Japanese apricot (*Prunus mume*). Fluorescent pollens are sterile, while non-fluorescent ones are fertile. Combining HPLC analysis and MALDI-MS/MS imaging analysis, the authors identified compounds responsible for fluorescence, including chlorogenic acid, 1-*O*-(*E*)-feruloyl-*β*-D-glucose, and 1-*O*-(*Z*)-feruloyl-*β*-D-glucose, and they demonstrated that chlorogenic acid present on the surface of sterile pollen grains is probably deposited on the pollen surface through tapetal degradation. They hypothesize that the fluorescent sterile pollen is induced by low-temperature stress and that it enhances visual signaling to its pollinator, the honeybee. Kobayashi et al. (2021) demonstrated that the exine-defective mutant of *Arabidopsis thaliana*, *cyp704b1*, did not have normal exine but had localized high-electron-density granules that probably contain polymerized and aggregated sporopollenin precursors. The high-electron-density granules may contribute to retaining the pollen coat of the *cyp704b1* mutant and to its fertility.

An article concerning the environmental risk assessment of transgenic plants was published in the last year. Hiwasa-Tanase et al. (2021) attempted to evaluate the environmental risk of growing genetically modified (GM) tomato that produces a taste-altering protein, miraculin, from two points of view: competitiveness (the ability to compete with wild plants for nutrients, sunlight, and space in ways that prevents their growth) and the production of toxic substances (the ability to produce substances that interfere with the habitat and growth of wild plants, animals, and microorganisms) by testing whether GM and non-GM tomato showed biologically meaningful differences or not. Assessment endpoints related to competitiveness, including plant morphology and growth characteristics, as well as tolerance to low temperature during early growth and overwintering, showed no significant differences. Tests for toxic substances were performed by quantitatively measuring crop growth parameters in the same soil in which GM tomato was grown, and by surveying the soil in which GM tomato was grown for fungal or bacterial counts. There were no statistical differences in these parameters, indicating that GM tomato does not secrete toxic substances. This article describes the first environmental risk assessment of confined field-grown GM tomato in Japan and is the first to provide assessment information to the public.

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