

제 3부 - 육종과 생명공학 기술의 산업화

Plant Biotechnology in the 21st Century in Japan

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The first generation of plant biotechnology of late 20th century in Japan mainly focused on micro-propagation as new agricultural practices, protoplast fusion as a new tool for plant breeding and the production of useful compounds using plant cell culture systems. The combination of plant tissue culture technique with molecular biology during the 1980's led to the development of transgenic plants, GMO. During the past a decade rapid progress has been made in the GMO research in Japan. One of major challenges of plant biotechnology in the 21st century is to develop gene engineering crops in relation to productivity, resistance to environmental stresses and nutritional quality of fruits, tubers, roots or grains. Exploitation of genetically modified functional rice grains accumulating coenzyme Q10, human interferon-or allergen-specific T cell epitope peptides for the control of IgE-mediated allergic diseases has a positive impact on human health. Phytoremediation plants which assimilate nitrogen dioxide or degrade dioxin have been developed in Japan. Now plant biotechnology is promising approach which contributes to human welfare by improvement of human health. Horticultural approach to improve characteristics of ornamental flowers is an attractive target of plant biotechnology. Last year many researchers engaged in plant biotechnology enthused about the birth of Blue Rose and celebrated it. In this presentation, the achievement of metabolic engineering of flower color in Japan and its potential for phytomonitoring will be evaluated.

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Analysis of key metabolic enzyme genes regulating rice carbon metabolism

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Cytosolic fructose 1,6-bisphosphatase (cFBPase) plays as a first valve that makes hexose-phosphates from triose-phosphates in the cytosol in higher plants. Rice appeared to contain two cFBPases, OscFBP1 and OscFBP2, and analyses of gene expression, promoter::GUS transgenic plants and OscFBP1 mutants indicated that OscFBP1 plays a major role in the sucrose synthesis in rice leaves. Hexokinase (HXK) is a dual-function enzyme that both phosphorylates hexose to form hexose 6-phosphate and plays an important role in sugar sensing and signaling. We analyzed rice sequence databases and isolated ten rice hexokinase DNAs, OsHXX1 (*Oryza sativa* Hexokinase 1) through OsHXX10. With the exception of the single-exon gene OsHXX1, the OsHXXs all have a

highly conserved genomic structure consisting of nine exons and eight introns. The hexokinase activity of the OsHXKs was confirmed by functional complementation of the hexokinase-deficient yeast strain YSH7.4-3C (*hxx1*, *hxx2*, *glk1*). Cell-wall invertase (CIN) catalyzes the hydrolysis of sucrose into glucose and fructose for supplying carbohydrates to sink organs via an apoplastic pathway. We isolated cDNA clones showing amino acid similarity to the plant cell wall invertase proteins from a search of rice sequence databases. Profile analyses revealed that transcripts of *OsCIN1*, *OsCIN2*, *OsCIN4*, and *OsCIN7* were detected in immature seeds whereas *OsCIN3* gene expression was flower-specific. Further transcript analysis of those genes expressed in developing seeds indicated that *OsCIN1*, *OsCIN2*, and *OsCIN7* might play an important role involving sucrose partitioning to the embryo and endosperm. (Acknowledgements: This work was supported, in part, by grants from SRC for the PMRC, Korea Science and Engineering Foundation (KOSEF) Program, and from the Biogreen 21 Program, Rural Development Administration.)

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- 품질향상을 위한 농업생명공학기술 -

AgroBiotechnology for Quality Improvement:
Coordinate Transgenic Expression of Multiple Carotenoid Genes
Accumulates Provitamin A in Rice Grain

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Plant biotechnology for molecular breeding has been rapidly developed with mining of agricultural useful genes by bioinformatics through genome projects and large mutant screening. Transgene technology into crop has been also advanced recently. Taken all technologies together, it comes a good time to improve crops for better future. As one of examples, carotenoids are essential components of the diet as a source of provitamin A and also play roles in decreasing human age-related-macular degeneration, some cancers and heart disease. As not only the importance of carotenoids to agriculture and human health but also the advance of the coordinate expression technology of multiple metabolic genes, we have targeted the carotenoid pathway to accumulate provitamin A in endosperm of rice grain. Carotenoid biosynthetic genes originated from a Korean red pepper cv. Nockwang and *Erwinia uredovora* were used to make three different vector systems: 1. multi-gene fusion by PCR, 2. viral internal ribosome entry site (IRES) sequence, 3. self-processing virus 2A polyprotein sequence. Recently, we've got transgenic rice plants with yellow seed color like a Golden rice 1 from a single polyprotein construct. Now, we are further developing transgenic rice plants and analyzing their activity and function by molecular biological and biochemical tools. Another quantitative trait loci (QTL) approach will be introduced to identify and clone novel genes or alternate alleles of known biosynthetic genes that positively impact seed carotenoid content on the molecular basis of untapped natural variation between *Arabidopsis* ecotypes.

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