Plant Improvement by Genetic Engineering; Perspectives of transgenic rice plants

Hirofumi Uchimiya
Institute of Molecular and Cellular Biosciences,
Univ. of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan.

Recent development in recombinant DNA technology have made it possible to isolate and characterize plant genes. Generally, such methods rely on the availability of either specific antibodies or appropriate oligonucleotides to target genes. We have initiated a project to identify genes present in randomly selected cDNA clones by virtue of large scale sequencing. We have chosen the graminaceous monocot rice(*Oryza sativa* L.) as the experimental material, because classical genetics has mapped numerous morphological and isozyme markers to their respective chromosomes. To achieve the large scale gene identification, our strategy has been based on the construction of cDNA libraries using a bacterial plasmid vector, and generation of double stranded plasmid DNA for dideoxy sequencing. Partially resolved nucleotide sequences were used to search the GenBank database for nucleotide sequence homology. Here, we show that a number of putative genes can be identified from the cDNA libraries by this method.

We have previously generated transgenic rice plants(*Oryza sativa* L. japonica cv. Yamahoushi) homozygous for a chimeric maize ubiquitin promoter-*bar* gene. Such plants showed a high level of *bar* gene expression, which resulted in herbicide(either bialaphos or phosphinothricin) resistance. We have also obtained R1 transgenic plants of the commercially important rice cultivar(japonica, var. Nipponbare) by introducing the same constructs into protoplasts. Since it has been known for some time that

bialaphos is toxic to fungal pathogens of rice such as *Rhizoctonia solani*, the etiological agent of sheath blight, one of the most severe fungal diseases of rice, and *Pyricularia oryzae*, as well as the host plants, we were prompted to assess the potential application of bialaphos resistant transgenic rice plants for protection against fungal disease. Here, we present evidence that treatment of these transgenic plants with bialaphos can selectively eliminates invasion by the sheath blight fungal pathogen, *R. solani*.

During the last 5 years, dramatic progress has been made in protoplast culture and DNA transformation of rice. Plant regeneration from protoplasts of both japonica and indica rice have been achieved in many laboratories. Various genes have been cloned in the form of cDNA as well as genomic DNA, comprehensive RFLP genomic map has become available and transposition of maize transposable element(Ac) into rice has also recently been demonstrated. However, protoplast regeneration and DNA transformation systems in rice are still not very efficient. Plant regeneration from protoplast is often limited to only specific genotypes and the results are often non-reproducible. The cell suspension often lose regeneration potential. Hence, to incorporate desirable genes into rice plants, it is essential to have efficient plant regeneration systems applicable to wide range of elite germplasm. Also, there is a need to have a large number of cloned genes governing useful agronomic traits such as disease and insect resistance, salinity and drought tolerance, and improved nutritional quality, etc.

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