(W- I -1) :

GENE TRAMSFPRMATION TECHNOLOGY IN DICOTS

Hak-Tae LIM

Division of Applied Plant Sciences, Kangwon National University,

Chunchon, Kangwon-Do, 200-701, Korea

Genetically altered crops, into which new genes have been introduced, are moving out of the lab and into the field at the rapid pace in recent years. Many of them are already appeared in supermarkets and kitchens, at least in the U. S. A. Clearly, gene transformation technology is in fashion. Methods of transfer DNA from one organism to another were already known in the 1940s. In plants, however, the major breakthroughs for introducing foreign DNA were the development of shuttle vectors for harnessing the natural gene transfer system of *Agrobacterium tumefaciens* and use of these vectors in direct transformation system. Most of the genetically engineered crops are dicots such as tomato, squash, cotton, soybean, canola, potato, cantaloupe, and sunflower, which are susceptible to *Agrobacterium tumefaciens*. Use of direct gene transfer techniques to introduce DNA into plant protoplasts, like PEG-uptake and electroporation, also have been used to generate stably transgenic plants, though this technique has been more widely used to study transient expression of DNA-construct and gene promoters. But, the usefulness of these gene transfer methods in dicots is heavily dependent upon the efficiency of gene transfer to cells and tissues which are highly regenerable. There are several components involved in the frequency of plant regeneration and gene transformation. In this workshop, I would like to discuss about factors influencing selection efficiency, plant regeneration, and genetic transformation, hardening-off, and field application in dicots based on the results obtained from ginseng, potato, Chinese cabbage, broccoli, hot pepper, and chicory in last few years in my lab.

(W- I -2) :

GENE TRANSFORMATION TECHNOLOGY IN MONOCOT

Hyo-Yeon LEE

Department of Resource Plant, Sunchon National University

The bialaphos is a potent inhibitor of glutamine synthease in higher plants and is used as a non-selective herbiside. We have used the bialaphos resistant (Bar) gene encoding for an acetyltransferase isolated from Streptomyces hygroscopicus SF1293. Callus derived from mature seeds of rice (*Oryza sativa* L. cv. Dong Jin) were co-cultivated with *Agrobacterium tumefaciens* EHA101 carring

a plasmid pGPTV-HB containing genes for hygromycin resistance (HygR) and Bar. Transgenic plants showing in vitro resistance to 50 mg/l hygromycin and 10 mg/l bialaphos were obtained by using a two-step selection/regeneration procedure. Transformation efficiency of rice about 30% which was as high as reported in dicotyledons. Progenies(T_1 generation) derived from primary transformant of 17 lines segregated with a 3 resistant : 1 sensitive ratio in medium containing hygromycin and bialaphos. Stable integration of bar gene into chromosomal DNA was proven by Southern blot analysis of genomic DNA isolated from T_2 progenies. Transgenic plants(T3) grown in the field were resistant to bialaphos (Basta) at a dosage lathal to wild type plants. These results show that the Bar and HygR gene have proven to be a useful selectable marker for the transformation of rice plants and for the production of herbicide-resistant plants.

(W-Ⅰ-3) :

BAC CLONING AND LARGE BAC DNA TRANSFORMATION TECHNOLOGIES

Sang dun CHOI

Division of Biology, 139-74, California Institute of Technology, Pasadena, CA 91125, USA, e-mail : schoi@cco.caltech.edu

BAC (bacterial artificial chromosome) cloning has served an important role in agricultural and mammalian genomics since its introduction in 1992. BAC libraries are currently in use or under development for virtually every important genome. The primary reasons BACs are so useful is that they can maintain large DNA inserts (up to 350 kb) in *E. coli.* and are amenable to virtually all of the sophisticated molecular biology techniques developed over the last 20 years for small 2-10 kb plasmids. I have been constructing BAC libraries of a variety of organisms including rice, *Arabidopsis*, cotton, citrus, *Ashbya*, and human in the last several years. Recently, I have begun constructing a series of BAC libraries with a much larger insert size (182 - 202 kb) from human (http://www.tree.caltech.edu/lib_status.html). The larger insert BAC library will provide significant improvement to applications in physical mapping, positional cloning, and DNA sequencing.

DNA transfer into plants has been accomplished by several methods including *Agrobacterium*-mediated transformation, biolistic bombardment, and microinjection. BAC vectors have been engineered for transformations of large DNA inserts into plant genomes. A pBACwich system has been developed to achieve site-directed integration of DNA into the genome (Choi et al., unpublished work). A 150 kb cotton BAC DNA was transferred into a specific *lox* site in tobacco by biolistic bombardment and Cre-*lox* site specific recombination. These results open up a number of new possibilities for plant molecular biology and genetic engineering of plants. These systems will streamline positional cloning and the transfer of desirable traits into plants.