Recent Progress of the Crop Functional Genomics Program
- 21C Frontier Program of the Ministry of Science and Technology, ROK-

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I. Background

Since its the first introduction of Flavr Savr® tomato in 1994, biotech crops which are developed by introducing useful genes has become a general practice in agriculture these days. In less than 10 years they are cultivated in 67.7 Mha over 18 countries by 2003 (James, 2003). It accounts for about 9% of worldwide arable land. Even though the market value of biotech crop seeds was only $3 billion in 2002, it is expected to account for 80% of seed market by 2010. They might be the fastest adapted crop varieties or agricultural practice in agriculture.

Biotech crops are developed by transformation of conventional crop varieties with useful genes conferring desirable agronomic traits such as herbicide resistance gene and Bt toxin gene. Even though novel and/or more efficient method for crop transformation is still necessary, generation of transgenic plants is now becoming increasingly routine for major crops (Hansen and Wright, 1999; Sinclair et al., 2004). Availability of useful genes might be more crucial especially for the sake of commercial purpose. Industrial countries including US, EU and Japan are putting major efforts to discover novel function, as well as structural determination, of entire genomes.

Once useful genes are available by genomics from any sources, transgenic research can support the breeders by transfer of the genes that are not available in conventional gene pool. It can also support the breeders by transfer of desired genes from one variety to another without bringing undesirable qualities.

Even though 17 species and 75 varieties of biotech crops are commercially available on the market in 2003, even a single commercial variety is developed in Korea. The reason to get behind the trends might be attributed to lack of basic research and negative public perception on biotech crops.

Considering the importance and potential value of novel genes and biotechnology utilizing them, however, the Korean Ministry of Science & Technology (MOST) and the Rural Development Administration (RDA) has started to support systematic and coordinated approach for crop functional genomics since 2001.

II. Crop Functional Genomics Center

1. Introduction

The 21st Century Frontier R&D Program has been developed by the MOST to boost national competitiveness in science and technology, improve the quality of life, and benefit humanity. The Crop Functional Genomics Center (CFGC), which belongs to the program, focuses on the functional genomic study for crop improvement.

For the next 10 years, the CFGC will run target-oriented basic research projects in the fields of plant functional genomics, crop transformation, and plant molecular breeding to improve agronomic performance of major crops. To overcome the limits of novelties in outcome, over 500 novel genes for crop transformation will be identified and characterized using genomic information. To demonstrate the usefulness of genes identified, at least 10 new crop varieties with desirable traits will be developed using transformation or marker-assisted selection technology.

Unraveling the complex relationship between genes and phenotypes and applying this information to developing better crops are dependent on co-operative works in genomics, transformation, and molecular breeding. These efforts are coordinated on the basis of the technology road map made up of 3 major areas over 3 phases interacting each other.

CFGC is a virtual research center made up of 85 principal investigators throughout the country affiliated to 26 universities, 6 research institutes, and 6 of industry. In total 220 PhD scientists are working for the program.

2. Summary of recent progress

During 3 years of the first phase of the program, projects developing methodology, research tools and resources have been emphasized, which can be shared and utilized with other scientists throughout the program duration to identify genes and transform major crops essential for genetic improvement.

1) Construction of insertion mutants pools of rice

Professor Gynheung An of the Pohang University of Science and Technology have generated approximately 100,000 lines of T-DNA inserted population in japonica rice varieties, Dongjin and Whayoung. Goal of this research is to establish reverse genetic methods to maximize utilization of the mutant lines. DNA pools from 60,000 lines of activation tagged insertion mutants have been prepared during last 3 years (Jeong D-H et al., 2002). He is providing the DNA pool screening service when primers are provided. Many scientists including Professors T. Okita of the Washington State University and R. Amasino of the University of Wisconsin have been using the service.

To analyze tag insertion sites, flanking DNA are amplified by iPCR or TAIL-PCR and their nucleotide sequences are determined. Dr An's goal is to establish about 6,000 tag end sequence (TES) database each year. The TES database containing 6,381 flanking sequences is open to the public through CFGC (http://www.cfgc.snu.ac.kr) and the mutant seeds are distributed upon request.

The T-DNA insertional mutant pools are supplemented by maize transposon Activator/Dissociations-tagged mutants generated by Drs Moo Young Eun of the Agricultural Biotechnology Research Institute and Chang Deuk Han of the Kyungsang National University (Kim et al., 2002). Over 34,000 lines of Ds transposants of Japonica type rice (Dongjin) were selected and insertion sites in 6,381 Ds transposants has been analyzed by PCR amplification and flanking sequences each year. These flanking sequence database is also open to the public through the web (http://www.niab.go.kr) and seeds are also available upon request.

2) Microarray preparation

A rice 60K genome-wide oligomeric DNA microarray was developed by the GreenGene Biotech (GGB), a bio-venture company founded by Professor Nahm. The new rice microarray was made in collaborative efforts with Professor Minkyun Kim at the Seoul National University and Professor David W. Galbraith of the University of Arizona. The Rice 60K microarray is currently available from GGB for worldwide collaborative rice research.

For microarray-based gene expression studies of hot pepper, cDNA microarray containing 10,797 unigene clones was also prepared. This microarray was used in analysis of gene expression profiles of hot pepper infected with pathogen Xanthomonas axonpodis pv. glycines to screen defense-related useful genes. In addition, effects of treatments with salicylic acid, jasmonic acid, ethylene and abiotic stressors are also being analyzed. They also support other researchers interested in pepper microarray-based expression analysis system. To date about 98 sets of experimental results are collected on the CFGC database and is open to the public.

3) Large scale identification of functional genes through proteome analysis

A new paradigm, 'the combinatorial functional genomics', is attempted employing proteomics to identify functional and novel genes at a large scale in Arabidopsis (Rossignol, 2001). Professor Hong Gil Nam of Pohang University of Science and Technology is analyzing proteomic patterns of numbers of distinct GUS expression lines including visible phenotype mutants as well as reference lines. They have generated massive 2D gel images and analyzed the proteomic patterns with sophisticated classification techniques. Protein expression profile libraries from Arabidopsis mutants containing hundreds of useful traits are generated by this approach.

4) Development of high frequency transformation systems

The current plastid transformation method is applicable to only a few plant species including tobacco, potato, and Arabidopsis, with an extremely low frequency of transformation. Dr Jang Ryol Liu of the Korea Research Institute of Bioscience and Biotechnology targeted the prokaryotic *RecA* gene to the plastid to enhance the frequency of homologous recombination. Plastids in the cell were reduced in number but their size was enlarged by over-expressing the *FtsZ* gene for controlling plastid devision (Jeong WJ et al., 2002). The possible hazard resulting from

pollen-mediated foreign gene flow from transformed crops to neighbor species could be overcome.

Pepper is notorious for its recalcitrance to genetic transformation. Drs Pill-Soon Song and Young Soon Kim of Kumho Life and Environmental Science Laboratory and Chee Hark Harn of the Nong Woo Bio Co. has been trying to establish an efficient protocol. Han and his colleagues established callus-induced transformation protocol in inbred lines by extensive trial with TMV-CP and PPI1 genes at the frequency of 0.84% by using kanamycin as a selective agent. Song and Kim used highly morphogenetic tissues derived from the hypocotyls and cotyledons of aseptic plants. The pre-cultured pepper explants were inoculated with *Agrobacterium*. The integration and expression of gusA gene were confirmed by Southern and Northern hybridizations, respectively. All the progenies showed an expected segregation pattern of hygromycin resistance, indicating that the T-DNA was stably maintained in the progeny through the generation. The amount of transgenic PepEST, a member of the esterase family, accounted for 0.01% of the soluble protein in the transgenic plants by immunological analysis. Transgenic pepper showed strong resistance against inoculated anthracnose fungus.

Professor Ju-Kon Kim of the Myongji University transformed rice with trehalose synthesis gene construct in which trehalose 6-phosphate synthase gene and trehalose 6-phosphate phosphatase gene of *E. coli* was fused in frame (Jang et al. 2003). The transgenic plant was resistant to drought stress as well as to salinity and cold. The project was carried out by collaboration with Professor Ray Wu of the Cornell University (Garg et al., 2002). Wu transformed indica type rice and Kim did japonica type, respectively, but the performace of transgenic rice were pretty much the same. These results also drew attention from world-wide public media such as BBC, CNN, NY Times etc. Currently it is under the process to evaluate food and environmental safety of the transgenic rice.

5) Database construction

To coordinate collaborative research, a database, Kropbase, has been constructed for 41,746 DNAs (ESTs, DNA markers, etc.), 4,560 proteome spots, and 11,342 flanking sequences of rice and *Arabidopsis* insertion mutant lines. In parallel, we have established a seed and genetic resource bank that contains 36,520 seeds, 756 bacterial strains, and 2 libraries obtained from the research projects supported by the CFGC program. For this purpose, 101,259 rice and soybean ESTs have been analyzed for contig construction and sequence homology search using the bioinformatic tools installed in PC clustering system. The DB will be further expanded based on data collection from the PIs in the program. In the same context, the seed and genetic resource bank will be further expanded.

III. Discussion

Most of transgenic crop researches have concentrated on the improvement of agronomic traits such as resistance to biotic, abiotic stresses and herbicides. The research focuses are now moving onto the improvement of grain quality traits including micronutrients and vitamins. With the increasing number of genes discovered in various organisms and the increasing knowledge of their functions, transgenic research can contribute more and more to the improvement of rice yield and grain quality. As such, making the important crop rice a perfect food and/or crop by the advancing agricultural biotechnology is not very far from reality.

Functional genomics and bioinformatics will greatly accelerate the acquisition of knowledge about the function of all plant genes (Somerville and Somerville 1999). The genomes of *Arabidopsis* (The Arabidopsis Genome Initiative, 2000) and rice (Yu et al., 2002) has been completely sequenced. The genomes of wheat, maize, sorghum, millet, and other cereals can be deciphered on the basis of extensive synteny among the cereal genomes. cDNA sequence information will become available for a majority of the genes of many plant species. As genes areassociated with functions or traits in one plant, it will usually be possible to use a database search to identify orthologs responsible for the trait in other plant species. Thus, unraveling the complex relationship between genes and phenotypes and applying this information to developing better crops will be dependent on the cooperative work of genomics, transformation and plant breeding, which will eventually lead to significant contribution to global food security. The initial phase of a revolution in agriculture appears to be taking place. On large areas in the world, genetically modified crops of soybeans, corn, cotton, canola, and others have been grown.

Rice is the first cereal crop from which fertile transgenic plants were obtained. Since then, many fertile transgenic rice lines have been produced by protoplast-mediated DNA transformation, by microprojectile bombardment, or by *Agrobacterium*- *mediated*

DNA transfer. Thus, generation of transgenic plants is now becoming increasingly routine for many rice varieties. Despite many efforts have been made using transgenesis to improve important agronomic traits of rice, however, there are only few cases where transgenic plants were showing acceptable agronomic performance.

Since we believe that promoting basic science is the best way to improve scientific competitiveness in the world market, this program placed a strong emphasis on original and creative ideas. The idea was supported by the development of research tools and resources which can be shared and utilized with other scientists throughout the program duration to identify genes and transform major crops essential for genetic improvement. Tagnology, microarray analysis and proteomics made high-throughput screening of agronomically useful genes possible and more efficient. Development of efficient transformation protocol will accelerate functional annotation of useful genes and commercialization of transgenic varieties.

Science and technology have made extraordinary progresses in the last century and have contributed tremendously to improving human life. We are among those responsible for leading 21st century science, and we are convinced that all our goals can be achieved by establishing a new paradigm for global collaboration. Crop functional genomics programs to identify useful genes of agronomic importance are also running in Japan, China and other industrial countries. Coordinated efforts to utilize genetic resources and to share research tools make functional genomics study more effective and molecular breeding more productive.

IV. Literature cited

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