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Current Status of Plant Transformation Research in Korea

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Why transformation?

There are two extreme opinions on the consequence of genetically engineered plants. The pros says that in order to be able to feed ever-increasing population with the existing area of agricultural land without degrading the natural resources base, application of modern biotechnology is the only solution. On the other hand the cons insist that transgenic plants will eventually bring about a disastrous effect on environment and human safety.

In reality however trials and application of transgenic plants have been sharply increasing on the global basis. According to James(1997), during the twelve year period 1986 to 1997, approximately 25,000 transgenic crop field trials were conducted globally on more than 60 crops with 10 traits in 45 countries. Of this total of 25,000, 15,000 field trials (60 percent) were conducted during the first ten year period, 1986 to 1995, and 10,000 (40 percent) in the last two year period, 1996-1997. The Peoples Republic of China was the first country to commercialize transgenics in the early 1990s with the introduction of virus resistant tobacco. In 1994, Calgene obtained the first approval in the USA to commercialize a genetically modified food product, when the company marketed its Flavr Savr TM tomato. In 1997, the global area of transgenics increased 4.5 fold from 2.8 million hectares in 1996 to 12.8 million hectares with 7 crops grown in 6 countries.

The countries in descending order of transgenic crop area were: USA, with 8.1 million hectares representing 64 percent of the global acreage, China with 1.8 million hectares equivalent to 14 percent, Argentina with 1.4 million hectares representing 11 percent of global acreage, Canada with 1.3 million hectares representing 10 percent of global area and Australia with almost 0.05 million hectares and Mexico, 0.03 million hectares, both representing less than 1 percent.

Transgenic soybean ranked first in 1997, accounting for 40 percent of global acreage, and replaced tobacco (13 percent in1997) which was the highest ranking crop in 1996 with 35 percent of the global area. Corn, which ranked fourth in 1996 (10 percent of global area) moved up to second position in 1997 with 3.2 million hectares, equivalent to 25 percent of the global transgenic area. The proportion of global acreage occupied by transgenic canola increased from 5 percent in 1996 to 10 percent in 1997, whereas the area of cotton decreased from 27 percent to 11 percent and similarly tomato from 4 percent to 1 percent.

Herbicide tolerance occupied the top ranking position in 1997 with 54 percent of the global area. Insect resistance was 31 percent, out of which 14 percent with virus resistance; quality

traits occupied less than 1 percent.

The trend summarized above is clear enough to convince that transgenic plants are not insignificant any more, and its importance in agriculture has been and will continuously grow in a rapid rate. Development of the methods to recover intact plants from dedifferentiated or organized tissues and to introduce exogenous DNA into plants are known to be the essential step and furthermore such gene transfer technology is the practical barrier between model species and important crops in exploitation of plant genetic engineering(Christou 1996).

Advancements in plant transformation in Korea

This paper is to examine the current status of plant transformation in Korea, to identify the bottleneck limiting progress and, if possible, to make suggestions for national strategy to best use the research fund available in this field. Leading laboratories arbitrarily selected by the authors were surveyed by a questionnaire on the crop(s), transgene(s), research accomplishments in terms of progress from protocol development for plant regeneration to variety development, and factor(s), they feel, limiting the advancement of their research.

Rice is the most important crop in both cultivated area and economic value of the product in this country. Dept. of Biology, Kyongsang National Univ. reported successful regeneration of fertile transgenic rice plants from a Korean variety, Nakdongbyeo, in 1995(Lee et al 1995). Protoplast from embryogenic suspension cultures were co-transformed with hygromycine phosphotransferase(HPT) and GUS genes in separate plasmids in the presence of PEG. GUS transgene expression and Mendelian segregation pattern of HPT transgene were confirmed in T1 plants. Kim and Choi(1996) of Kangwon National Univ. obtained GUS transgenic plants from suspension culture by electroporation. An and his colleagues have established a successful transformation system in rice and are producing T-DNA inserted transformants at a rate of 2,000 transformants/year/person for the purpose of identifying function of genes(An 1998). They were able to produce transformants where OsMADS1 gene controlling flower organ development is inserted. Progenies of the crosses involving the transgenic lines are presently undergoing performance test in the field. National Institute of Agricultural Science and Technology succeeded or is making progress in obtaining rice transformants of genes of herbicide resistant bar, ribosome inactivation protein, glucose oxidase and chitinase(Chung; personal communication). They aim at developing breeding lines resistant to rice blast, bacterial leaf spot and herbicide by pyramiding the genes by crosses among transformants, which will be later combined with high yield, insect resistance and good quality.

Solanaceous crops attracted much attention of transformation workers in Korea. Tobacco, tomato, pepper and white potato are among these. Plant Molecular Biology Lab., Genetic Engineering Research Institute of the Korea Institute of Science and Technology(KIST) recovered transgenic tobacco plants and confirmed stable expression of the inserted cucumber mosaic virus satellite RNA in T1 generation(Kim et al 1992). Park and Lee(1996) of the Korea Ginseng and Tobacco Research Institute reported transformation of *N. tabacum* L. Nc82 with TMV coat protein cDNA and 80 to 90% inhibition of virus replication in R4 generation. In the Dept. of Horticulture, Chungnam National University, similar results were obtained(Lee et al 1996) independently and the expression of the inserted gene was confirmed in T0 generation.

In both cases , *Agrobacterium tumefaciens* was employed as a host of vectors.

For tomato transformation, binary vectors of *Agrobacterium tumefaciens* were constructed and protocol was developed using kanamycin resistance as a selective marker in Dept. of Horticulture, Chungnam National University(Park et al 1991). Choi and Kim(1993) of Kangwon National Univ. reported that they obtained tomato transformants inserted with BTT toxin gene and that kanamycin resistance segregated in a ratio of 3:1 in R1 generation. Southern blot analysis and DNA analysis on these transgenic plants showed that one copy of the vector plasmid fraction was integrated into the plant genome and the inserted BTT-toxin gene had been stably transmitted to the R1 generation.

Lee et al(1993) of Genetic Engineering Research Institute, KIST reported an efficient system for in vitro regeneration of *Capsicum* pepper plant and they succeeded in transformation of pepper with *Agrobacterium tumefaciens* LBA4404 carrying a binary vector pRok1/105 harbored with cDNA of CMV I17N-satellite RNA. Integration and stable expression of the transgene were confirmed by PCR and Northern blot analyses, respectively. Yu et al of the Catholic Univ. of Taegu Hyosong obtained transformant with coat protein gene of TMV-OM strain by Agrobactrium method and confirmed expression of the transgene in two transformed plants. Yang et al (1997) also proposed a regeneration and transformation protocols from their experiments with hypocotyl/cotyledon as explants cocultured with *Agrobacterium tumefaciens* pDY183 for two days after two days of preculture and ADA(mouse adenosine deaminase) and NPTII as selectable markers. Breeding Research Institute of HungNong Seed Co., Ltd. has been involved in transformation of OsMADS1, RIP and CMV resistance genes into pepper. Expression of the latter transgene was confirmed by artificial screening with CMV and the transformants are now in T2 generation, while transformants of the former two genes are in T1 generation. Authors were also successful in regeneration of plantlets from cotyledon explants with and without cocultivation with *Agrobacterium* in a series of experiments in the National Horticultural Research Institute. Expression of RIP gene was confirmed in biological test with *Xanthomonas campestris* pv. *vesicatoria* in T2 generation of the obtained transformants. We however later found that a great majority of the regenerants survived the selection media of Kanamycin were non-transformants.

Tissue Culture Research Unit, KRIBB, KIST succeeded in transformation of potato leaf roll virus coat protein gene(Joung et al 1996), herbicide resistant pGA-bar gene(Choi et al 1996) and cold regulated gene(BN115)(Choi et al 1996b). They applied *Agrobacterium* method and used kanamycin resistance as the selectable marker. They also found that mouse adenosine deaminase gene was a useful marker in selection of transformants from regenerants(Choi et al 1996b).

Among *Curciferous* crops, regeneration of A genome species and radish had been known very difficulty, but it has recently become possible mostly from cotyledon/hypocotyl explants largely due to ethylene inhibitor effect of AgNO3 added in the medium. The Bioresouces Research Group of Genetic Engineering Research Institute, KIST reported that Chinese cabbage transformant of TMV coat protein gene was obtained, where cotyledon was used as explant and kanamycin resistance as selectable marker(Jun et al 1995). Son and his colleagues of Kyungbuk National Univ. established transformation method in *Brassica napus* using GUS gene and later succeeded in obtaining transformants of rol C

gene which is known to confer root initiation and dwarfness(Sohn and Cho 1991; Sohn et al 1994). Efficiency of transformation is still believed way below the level of practical application in Chinese cabbage and radish and no data are available on advanced generations of transformants of rapeseed.

Choi et al (1994) succeeded in transformation of GUS gene into watermelon by *Agrobacterium* method using cotyledonary explants. But no other success has been reported in spite of a number of attempts made elsewhere. Lee et al(1996) reported acquisition of TMV coat protein gene transformed cucumber plants and the stable expression of the gene in To generation. But again this still remains the only report on the transformant obtained in this crop.

Reports are available on the success of transformation of Korean ginseng(Lee et al 1993; Lee et al 1995; Yang et al 1996), lettuce(Choi et al 1994), water dropwart(Bin and Kim 1995) and *Codonopsis lanceolata*(Choi et 1994). All of these cases employed Agrobacterium method with kanamycin resistance as the selectable marker. Transgenes included chitinase and adenosine deaminase genes in addition to GUS gene. Transformants were also obtained in ornamental species such as petunia(Ahn et al 1993; Kim and Paek 1995) and carnation(Yu and Bae 1996). Breeding Research Institute of HungNong Seed Co., Ltd. transformed OsMADS1 gene into chrysanthemum and is trying to insert the same gene into lily species(Unpublished data).

Agrobacterium tumefaciens had been found to be a very effective vehicle for introducing foreign genes into plants but its host specificity limited widespread use in many important agricultural crops including rice until quite recently. Alternative methods were proposed such as chemical treatments, electroporation, particle bombardment, infiltration of DNA into seeds under vacuum, use of microscopic needles or fibers to facilitate penetration of DNA into specific tissues, pollen tube pathway, and incubating seeds or intact plants with *Agrobactrium*. As seen above, *Agrobacterium* method is becoming more and more widespread over a wide range of crop species, as its host specificity is overcome. Considerable portion of published papers are dealing with genes, such as GUS, appropriate for protocol development rather than those for agronomic improvement and yet the efficiency of transformation is not high enough to be applied for practical crop improvement. As has been foreseen by Liu(1994) , transformation still seems the most serious bottleneck in many crop species important in this country.

Regulatory aspects

Related to genetically modified organism, two types of regulations generally apply: 1) those that are desired to protect the health and safety of the personnel conducting the experiments in laboratories or greenhouses and 2) those that are designed to initially contain transgenic crops and safeguard the environment by governing field experiments with and field release of transgenic crops. Other than these, regulations governing the commercialization of transgenic crops are often subject to independent approvals from more than one regulatory agency in the same country. For example, in the United States, USDA/APHIS issues permits for field trials, and later for general environmental release, any crop containing a gene for a pesticide also requires approval from the Environmental Protection Agency. If the product from a transgenic crop is for food or feed use, the Food and Drug Administration is also involved in the approval of the product.

In April 1997, Ministry of Health and Welfare(1997)

officially released the Guidelines for Gene Recombination Experiments. The guideline covers isolation, treatment of recombinants, training and health care, safety measures and evaluation provisions. The guidelines inquire heads of central government organizations to provide and implement more specific guidelines in the relevant areas. In this regard, Rural Development Administration has drafted the guidelines to be applied for genetically modified plants, animals and microorganisms for agricultural use. Before finalizing the guidelines, there will be opinion polls within the community of agriculture and discussions with other government organizations.

Intellectual property right aspects

Intellectual property right is primarily to encourage inventions made elsewhere by allowing inventors to enjoy exclusive right for a given period of time. Patent system was available only for vegetatively propagated plants in the past, but plant variety right system has become available in 27 crops by the Seed Industry Law which was enacted on 31 December 1997. The number of crops will be gradually expanded. Plant variety right protection is also to encourage investment on plant breeding and, by doing so, provide farmers with better varieties. IPR systems have costs royalty payments being the most obvious- but the cost of absence of protection in terms of no or delayed access of technologies shall be considered(Lesser 1997).

Components of gene technology contributing to the development of transgenic variety are protected in general by the patent system. Not only the techniques but also the DNA are the subject of patents in many countries. Chandler(1996) listed examples of technology which are protected: recombinant DNA methods; antisense, sense and ribozyme processes; specific gene sequence(with known function); promoter sequences; selectable marker genes; regeneration and/or transformation protocols; and binary vector systems. If you want commercialize any transformant, you will have to approach the owners of the involved technology components. But it also has to be remembered that both protection of variety and involved technology components are operated by each government independently. International organizations such as UPOV and WIPO are primarily for harmonization of the systems among member countries. Protection of transgenic varieties of seed-propagated plants are available only by the plant variety right system in Korea, but it is available by patent systems too in the United States. European Union recently decided to provide protection of genetically modified plant varieties by patent system.

Under the Seed Industry Law which in principle follows the 1991 Convention of UPOV, breeder of transgenic variety may have to pay royalty to the breeder of the original variety, although further deliberation and discussions will be needed before practical enforcement. Recently, particularly since the Leipzig declaration in 1996, IPR protection for farmers right on indigenous genetic resources has become a hot issue among the international society. Farmers right defenders argue that the present plant variety right of UPOV system will let transnational companies take over national breeding systems of the developing countries, exacerbate erosion of biodiversity, and is not in harmony with TRIPs. But it seems too early to predict how these different views will reach an agreement.

Conclusion and strategic approach

Rapid increase of cultivation of transgenic varieties particularly in developed counties tells us about the need for more vigorous research for the development of genetically modified plant

varieties. Quite a number of laboratories have been rigorously involved in transformation research in recent years and considerable progress is observed. However, except for a few crops such as rice and tobacco, efficient transformation protocols are yet to be developed. Reported transformation studies in Korea are mostly not extended to field trials or to practical variety development. Transformation seems a serious, if not only, bottleneck in the advancement of the exploitation of outcomes of molecular biology. To cope with international environment changes led by transnational companies and the public research systems of the developed world, wise national strategies are greatly needed, taking into account the limited resources particularly during the adverse period of IMF-aided economy. In consideration of the economical importance, likelihood of success in regeneration/transformation and potential gains from transgenic varieties, priority crops and priority subject areas may have to be selected to concentrate the available resources and efforts nationally. Proper regulatory systems related to experiments with and utilization of genetically modified plants have to be established shortly. IPR systems also shall continue to improve to effectively adapt to the academic advancement and international systems development.

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