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Production of rice transformant with useful gene

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Rice is the staple food for more than one third of world's population. The vast majority of cultivated rice is *Oryza sativa*, which consists of three subspecies, indica, japonica, and javanica genotypes. Rice improvement through conventional breeding methods has met with considerable success. Because of the consistent efforts by plant breeders, rice production has doubled between 1966 and 1990, but it must increase further by 60% up to 2025 in order to feed the additional rice consumers (Khush, 1997). In fact, the problem is compounded by the loss of land to urban development, degradation of productive land, and the decreasing yield of several conventinal varities. Besides, every year all over the world, crop worth several million dollars are lost due to damage caused by various biotic and abiotic stresses.

Recently, international attention has been focused on developing new techninques for rice genetic engineering, as among the cereals rice is considered efficiently regeneration systems for several varieties already exist. These biotechnologies are intended to supplement the conventional breeding program aimed at rice improvement by way of providing a breeding line having the desired traits which include resistance against pests, pathogens, salinity, and drought and also improvement of the nutritional quality of rice.

To protect plant pathogen, we have made rice transformants which contain a disease resistance gene recently. Crop protection is one of the most challenging problem in agriculture worldwide. In effort to screen useful disease-resistance gene, antimicrobial protein gene (Asa-AMP1) was cloned by PCR from genomic DNA of garlic (*Allium sativum*). Specific primer were selected from N- and C- terminus containing region of *Ace*-AMP1. Determination of the DNA sequence revealed one intron and indicated a 396-nucleotide open reading frame, which encodes 132 amino acids including 27 a.a signal peptide on N-terminus and 12 a.a pro-peptide on

C-terminus. The intron was removed and the two exons were linked by inserting oligonucleotide linker. The Asa-AMP1 has high homology, 88.6% in amino acid sequence, with Ace-AMP1. Both AMPs belong to nonspecific lipld transfer protein (nsLTP) family based on the sequence homology. Unlike other known LTPs, both AMPs have additional pro-peptide extension in C-terminus. All known LTPs are secretory proteins. The fact that Asa-AMP1 and Ace-AMP1 contain signal and pro-peptide suggests that they are secretory proteins and their final destination is vacuole. Ace-AMP1 was also known to have broad resistant spectrum to phytopathogenic fungi *in vitro*, which implicates similar activity for Asa-AMP1. To develop disease resistant transgenic rice plant using Asa-AMP1 gene, two plasmid vectors were constructed, pSBM-RAB and pSBM-RTA. pSBM-RAB contains whole region of Asa-AMP1; whereas pSBM-RTA contains the mature form of Asa-AMP1 that was translationally fused with transit peptide (TP) of rbcS protein for chloroplast targeting. Rice transformation was performed by *Agrobacterium*-mediated method and transgenic plants were selected against phospinotricin(bar) selection. The efficiency of transformation reached to about 15% as calculated by the number of the callus that gave rise to regenerated plants. Genomic Southern blot and Northern blot analyses demonstrated that the Asa-AMP1 was integrated into rice genome and expressed. The transgenic plants growing in greenhouse showed herbicide resistance. MAR sequence appeared to affect the integration and expression pattern by lowering its copy number and conferring copy number dependency on expression. The gene product was detected by Western blot in transgenic plants of pSBM-RAB, but not of pSBM-RTA, presumably because of incompatibility of Asa-AMP1 with the chloroplast targeting system. The transgene was stably transmitted to next (T1) generation being normally expressed in T1 plants as examined by Northern- and Western-blot analysis with segregation. The T1 progenies of pSBM-RAB transgenics showed elevated disease resistance to sheath blight caused by *Rhizoctonia solani.*

To obtain new rice varieties with high yield productivity, we made rice transformants which contain ADP-glucose pyrophosphorylase(AGPase) gene. AGPase is a key enzyme in starch biosynthetic pathway by regulating the production ADP-glucose from glucose-1-phosphate and ATP. Since the activity of AGPase is regulated by cellular energy state represented by the ratio of 3-PGA and Pi, simple over-expression of AGPase does not increase the yield productivity of transgenic crops. One strategy to achieve this

goal is to modify the regulatory property of AGPase in plants. Although bacterial AGPase gene, *glgC-16* and the plant gene are available currently, using the plant gene has an advantage over the bacterial gene. Plant AGPase is a heterotetramer consisting of two large and two small subunits, of which the large one functions as the regulatory subunit. Therefore, it would be possible to modify the regulatory property of the enzyme if a mutated large subunit gene is used. A mutated AGPase large subunit gene from potato, *upreg1,* is a candidate to be used for such objective. In order to achieve this, it is necessary to demonstrate that the mutant phenotype of *upreg1* is maintained regardless the type of small subunit to form AGPase holoenzyme. For this purpose, *upreg1* was coexpressed in *E. coli* with AGPase small subunit genes. AGPases with different subunit composition were then purified and their regulatory properties were examined. Results showed that the S0.5 of 3-PGA and I0.5 of Pi to activate and inactivate the enzyme containing Upreg1 are lower and higher, respectively, as compared with wild type enzyme. In order to examine whether the modified regulatory property of Upreg1 could change starch synthesis in plants, rice was transformed with the gene. As results, 38 transformants were obtained and the stable insertion of the gene into rice genome was confirmed by Southern blot analysis at T0 and T1 stage. The transgene was also shown to be expressed in rice immature seeds as confirmed by Northern and Western blot analyses. Kinetic properties of AGPase in transgenic seeds were changed in that the enzyme from the transgenic was activated by 3-PGA at lower concentration and inhibited by Pi at higher concentration than that from nontransgenic seeds. This result implicated that the transgenic expressed mutant phenotype of *upreg1.*

As mentioned above which are doing in our lab, significant progress has been made in developing appropriate techniques for rice biotechnology. Rice transformation/Biotechnology has been progressed very rapidly during the past few years, so that number of transformants are currently under field trials. We hope that this new field of science will lead the agriculture for food security as well as for agricultural sustainability.