Biotechnology of Crop Production and International Consortium

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Abstract

Session 2 of this Forum, which I chaired, tackled the topic on Biotechnology for Sustainable Bioproduction. All speakers shared with us their research findings and technical knowledge on carbon metabolism and nitrogen metabolism in relation to biotechnology for crop productivity; enzyme engineering of lipids and dietary antioxidants as related to biotechnology for food production; and biotechnology for biotic stress tolerance. While we discussed various concerns about agriculture in Asia, we did not discuss about abiotic stress tolerance in plants. So, I would like to share with you some problems and recent developments in research in this field, which result from our works.

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Recently, global warming has accelerated desertification all over the world. To prevent the desertification and keep agriculture sustainable, we have been studying on the mechanism of salt, drought and heat tolerance in barley (11,12,13) and highly drought- tolerant sheep grass, *Aneurolepidium chinense* (6). We obtained hundreds of salt, drought and heat inducible genes and are now looking for strong genes to confer salt, drought and heat tolerance to rice (Japonica). We have been discussing about possible collaboration with Kasetsart University because Thailand has salty desert area and it is a serious problem not only in Thailand, but also the whole of Asia and other countries including developed countries. Our group has established transformation systems of Japonica rice and Indica rice (poplar trees also recently). We now recognize that collaboration between universities in Asia and our University is of great advantage to resolve this serious environmental problem for sustainable agriculture.

I would like to introduce the following strong transgenic model and rice plants towards abiotic stress analyzed in my laboratory:

WT Dnak-rice

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1) Transgenic tobacco and rice with DnaK from highly salt-tolerant cyanobacterium Aphanothece halophytica

I have been using *Aphanothece halophytica* for quite some time to study on subunit structure and function of CO_2 fixation enzyme, RuBP carboxylase/oxygenase (Rubisco). *Aphanothece halophytica* accumulates glycinebetaine ca. 2 M in the cell. I noticed that this cyanobacterium has quite unique enzymes depending on glycinebetaine or highly salt-tolerant enzymes. We then started cloning a kind of heat shock protein (HSP70) gene, *DnaK*. Usually, DnaKs have a molecular weight of 70kD. But our DnaK has an extra C-tail (9 kD) at the end of the protein compared to other HSP 70 groups. This C-tail is important to make the DnaK strong under both salt and heat stress (1). We introduced this gene into tobacco plants first (8). The transgenic tobacco acquired resistance to salt stress at the vegetative stage. We also confirmed that the transgenic tobacco has highly enhanced heat tolerance at the germination stage and young vegetative stage (5). We were astonished that these transgenic plants have a high productivity (almost twice compared to that of WT) of seeds under heat stress at the reproductive stage. We also made transgenic rice plants using *DnaK*. DnaK-rice acquired high heat tolerance at both young vegetative and reproductive stages. The DnaK-rice has grown better than WT at the normal temperature (28 ℃ /23 ℃) and at high temperature (35 ℃ /23℃). The yield of seeds also increased twice in the transgenic rice like in transgenic tobacco as described above. We are now field-testing the phenotype of DnaK-rice in a strictly regulated area for the safety of genetically modified crops under the collaboration with Dr. Takiko Shimada (Ishikawa Prefecture Univ.). This transgenic rice could be applicable to agriculture under salt, drought and heat stress conditions. We also introduced this gene into poplar and are now analyzing the phenotype. We are expecting that DnaK-poplar trees grow better under normal conditions and stress conditions.

2) Transgenic rice transformed with GSII gene

GSII is glutamine synthetase, which is localized in chloroplasts and involved in photorespiration. GSII is the limiting step in photorespiration as well as Calvin-Benson cycle. Photorespiration is essential in plants that grow under stressful conditions, such as high light. We tested this transgenic rice with salt tolerance. We confirmed that the transgenic rice plants have highly increased tolerance to salt stress (2).

3) Transgenic rice plants transformed with KatE and yeast mitocondrial Mn-SOD genes

We also made transgenic rice plants using catalase gene (*KatE*) from *E. coli* . This transgenic rice also showed a strong phenotype about salt tolerance.

We also examined transgenic rice overexpressing yeast mitochondrial Mn-SOD in chloroplasts. Actually this transgenic rice came from Plantec Research Institute in Japan. Seeds were T7, which showed a weak phenotype of salt tolerance (9). We believe that gene silencing occurred in this transgenic rice (T7). About 50% of the seeds lost the yeast Mn-SOD gene. This is a problem of genetic engineering of plants sometime. We also made transgenic *Arabidopsis* and rice using heat-inducible peroxisomal APX. Those transgenic plants showed enhanced heat tolerance at both vegetative and reproductive stage (7).

We found that glycinebetaine synthesis is induced by H_2O_2 (3). This is an interesting finding. A low level of H_2O_2 enhanced growth of rice under salt and heat stress (10), although it is toxic with high concentrations.

4) Other transgenic plants and rules for collaboration

We have been making transgenic rice producing glycinebetaine (4). Rice is a very salt-sensitive plant and does not accumulate glycinebetaine. We used modified gene (*betA*) of choline oxidizing enzyme from *E. coli* to transform rice, because codon usage is different between *E. coli* and rice. We collaborated with a Japanese company. The company obtained very good data about salt tolerance, although we were competing with another big group. Finally they did not publish the paper at all and obtained the patent for the genetically engineered rice without me. Although I do not mind, I think there are some difficulties to collaborate with companies. However we have to be patient in breeding new crops for our future. Both the university and company people must mutually understand more. Although almost five years have passed after collaboration with the company, I am looking for a possibility of studying the transgenic rice from other points of view, such as acid rain or heavy metal stresses. This experience became a case story in my laboratory, after we made many stronger transgenic plants towards abiotic stresses. I also had a similar experience in collaboration with an Asian university. I realized that any genes from developed countries are kind gifts for Asian people. I think it is a great idea and I am very pleased if they can use the genes we developed for agriculture. However I started the research as a collaborative work. But the Asian scientists wanted to study by themselves. We must establish rules on how to collaborate with not only companies, but also Asian universities, before implementing any activity. Rules on patents and authorships should be well defined to avoid problems.

5) Academic International Consortium

Our laboratory is now open to anyone in the world, if collaboration is honestly carried out between two or more collaborating institutions. We have many candidate genes to develop strong transgenic rice or other plants including poplar trees. Our genes can be used for sustainable agriculture in Asia and an international consortium for this purpose is welcome anytime. The Japanese professors must have at least one postdoctoral fellow and one technician, just like in research institutes or universities in the USA. Our situation in Japan is too stiff to consider any collaborative work with Asian scientists now. I am training two graduate students from Asian countries. It is not easy to train them by myself, although it gives me great pleasure and I enjoy guiding them. The School of Bioagricultural Sciences in Nagoya University needs not only high impact papers on basic sciences, but also high levels of application studies to produce crops and develop and improve tree species for forestry in Asia, including Japan. It is my worry that the time will come when shortage of food will be experienced in Japan. It is my hope that grants are given to the more applied fields in agriculture and forestry.

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