Recent topics of rice studies in Graduate School of Agricultural Science, Tohoku University

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Produced in more than 110 countries, rice is a staple food grain for more than half the world's population. It accounts for about 30% of total cereal production, almost equal to the level of wheat production. Unlike wheat, 92% of world's rice production is in Asia, home to 60% of world's population. It is estimated that the world population will be about 50% larger by 2025 (IRRI 1995). This projected increase will be mostly in Asia. It is therefore crucial to increase rice production within a relatively short period, and that the increases in rice production are achieved by increases in yield from the land presently used. Thus, a better understanding of the growth and functional performance of rice plants from molecular to whole plant levels is required for further development of rice varieties and improvement of cultivation practices (Mae 1997). Here, we will briefly review the recent topics of rice research held in the Graduate School of Agricultural Science, Tohoku University.

1. Improvement of leaf photosynthesis : Decrease in Rubisco by antisense rbcS leads to a higher N-use efficiency of photosynthesis under conditions of high CO_2

Rice (*Oryza sativa L.*) plants with decreased Rubisco were obtained by transformation with the rice *rbc*S antisense gene under the control of the rice *rbc*S promoter. The transformants were screened for their Rubisco to leaf N ratio. Plants transformed with 65% wild-type Rubisco were selected as having the optimal Rubisco content at saturating CO_2 partial pressures for photosynthesis under conditions of high irradiance. Although the plants with decreased Rubisco content showed 20% lower rates of light-saturated photosynthesis in normal air (36 Pa CO_2), they had 5-15% higher rates of photosynthesis in elevated partial pressures of CO_2 (100-115 Pa CO_2) than the wild-type plants for a given leaf N content. It is concluded that rice plants with 65% wild-type Rubisco show higher N-use efficiency of photosynthesis under conditions of saturating CO_2 and high irradiance (Makino et al., 1997, 2000).

2. Improvement of sink capacity : Increase in grain size of japonica-type rice leads to a higher grain yield

A new large-grain cultivar of japonica-type rice (*Oryza sativa* L), Akita 63, recorded a high yield (brown rice) of 936-1057 g m^{-2} (Av. = 982 g m^{-2}, n = 3), that was comparable to the past highest yield record of japonica-type rice in Japan. Although total biomass and total plant nitrogen of Akita 63 at harvest did not differ from those of a local high-yielding cultivar, Yukigesyou, grown under the same conditions, the harvest index of Akita 63 was higher than that of Yukigesyou (0.59 versus 0.47). The high yield of Akita 63 could be attributed to its large yield capacity, mainly due to its large grain size (31 mg versus 23 mg for Yukigesyou) with an efficient translocation of dry matter into spikelets throughout the ripening period (Inaba et al., unpublished data).

3. Molecular basis for nitrogen recycling : Analysis of mutants inserted retrotransposon Tos-17 and QTL mapping

When 20 seeds, in which *Tos17* is possibly inserted into *GS1* gene, were germinated and grown in a green house, 4 lines of mutants were successively isolated. In these lines, *Tos17* was homozygously inserted into the exon-8 of *GS1* gene. GS1 protein and its activity in leaves were hardly detectable in these lines. The GS1 mutants also exhibited an extreme delay of plant growth and a dwarf phenotype. They were needed for a long period from heading to flowering and most spikelets were not filled. A few seeds were obtained,

although these were sterile. Amongst the remaining 16 seeds, 11 lines were heterozygously inserted and 5 lines had no insertion. These lines showed no difference in phenotype or GS1 protein contents when compared to the wild type. These phenotypes suggest that GS1 is essential for normal growth and development of rice.

A QTL on chromosome 2 determines the GS1 content (positive allele : Nipponbare), panicle weight (positive allele : Kasalath) and spikelet number (positive allele : Kasalath) of rice. To isolate near isogenic lines (NILs), backcrossed isogenic lines (BILs) originated from Koshihikari's genetic background (C lines) were used. Linkage analysis showed that the QTL was mapped to be close to a CAPS marker, R1843 on chromosome 2. One line (C-22) was selected from self-pollinated progeny derived from C lines. This line was nearly isogenic with the target QTL consisting of the chromosomal segment from Kasalath. This line (C-22) showed less content of GS1 protein in leaf blades than wild type Koshihikari, indicating that the replacement of this region with the Kasalath chromosome has a negative effect on GS1 content. C-22 also had a higher tiller number at early stages of growth, higher panicle number, and greater total panicle weight than Koshihikari in both green-house and field conditions, particularly grown with low-nitrogen supply. These results suggest that the target QTL is important in the development of tiller and panicle in rice (Obara et al., 2001, Yamaya et al., 2002).

4. Improvement of chilling resistance : An increase in unsaturation of fatty acids by introduction of genes for glycerol-3-phosphate acyltransferase enhanced low-temperature tolerance in rice seedlings

Chilling-sensitive plants, such as rice, contain a high proportion of saturated fatty acids in phosphatidylglycerol (PG) of chloroplast membranes, while chilling-tolerant plants, such as spinach and *Arabidopsis thaliana* tend to contain lower levels. Glycerol-3-phosphate acyltransferase (GPAT; EC 2. 3. 1. 15) in chloroplasts plays an important role in determining the saturation levels of fatty acids in PG. A cDNA for GPAT of spinach and *Arabidopsis* under the control of a maize ubiquitin promoter was introduced into rice. The percentage of the sum of the minimum proportions of high-melting point molecular species of PG (Sat (%)) in leaves of transgenic rice were found to be 58% that of wild-type plants when they were transformed with spinach GPAT and 67% when transformed with *Arabidopsis* GPAT. The rates of photosynthesis of leaves at 17°C and 14°C and the fresh weight of seedlings after exposure to 17/14°C (day/night) for 6 weeks were examined in transgenic lines with spinach GPAT, *Arabidospsis* GPAT, a segregated non-transgenic line and wild-type plants. There was a significant correlation between the Sat (%) value and the chilling tolerance with respect to the rate of photosynthesis and the fresh weight of seedlings. These results indicate that the introduction of cDNA for spinach GPAT causes greater unsaturation of fatty acids in PG and confers more chilling tolerance upon rice seedlings (Ariizumi et al., 2002).

5. Improvements of field practices : Single basal application of total fertilizer-N into nursery box saves labor's cost and improves the nitrogen-use efficiency

A unique technology of single basal application of the total amount of fertilizer nitrogen into rice nursery box was first developed in the Experimental Farm of Tohoku University. It saved labor costs of fertilization and improved the nitrogen-use efficiency of fertilizer by rice plants. All fertilizer nitrogen needed by rice plants for entire growing season was applied in nursery box by co-situs placement using a sigmoid, 100 day-type of polyolefin coated fertilizer (POCUs-100), which is a class of slow-release fertilizer. Rice seedlings grown by these methods showed no significant difference in morphological properties compared to those grown by conventional methods, rather, the dry weight and nitrogen content of the plants were often somewhat greater. Innovative no-tillage transplanting cultivation of rice with a combination of single basal application of total fertilizer nitrogen in nursery box greatly improved the nitrogen use efficiency of fertilizer. This cultivation method was first demonstrated in very poorly drained soils, later applied to almost all soil types, and proved to be effective. The nitrogen recoveries from the single basal application of POCUs-100-N by rice plants (73-83%) were

much higher than those from basal application of ammonium sulfate-N in the conventional cultivation (20-39%). Brown rice yields of this cultivation were the same to those of conventional ones or even greater. The method was also proved to be effective in decreasing methane emissions, water pollution, and lodging of rice plants (Sato and Shibuya 1991, Saigusa et al., 1996).

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