

2.1.2 Laboratory : Plant Breedign

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A. Research Activities (2010.4-2011.3)

A-1. Main Subjects

a) First discovery of an active transposon in rice

A mutant slender glume gene *slg* was induced with gamma-ray irradiation to seeds of the rice variety Gimbozu. But this slender glume gene occasionally reverts to its wild type allele *slg+*. We considered that such mutability of *slg* results from precise excision by a transposon inserted in it. As a result of analyzing the molecular structure of *slg*, we could demonstrate that a transposon mPing (belonging to one of the non-autonomous transposon family called MITEs) was inserted in the fourth exon of *slg*, the same gene as *Rurm1m* (Rice ubiquitin related modifier-1), and that reversion from *Rurm1m* to its wild type allele resulted from precise excision of the mPing. Thus we successfully identified an active rice transposon by analyzing the mutability of a slender mutation of glume. No active transposons have so far been reported in intact rice plants, and there have been no reports on active MITEs mobile both in intact higher plants and in animals. Mobile transposons used as gene tags in intact plants are powerful tools for function analysis because transposon insertions often inactivate genes. Therefore, this discovery is a milestone for function analysis of rice genes.

b) Genetic analysis of major agronomic characters in rice

A number of japonica- and indica-rice varieties and a large number of mutant lines induced and preserved in our laboratory were analyzed for genetic factors controlling important agronomic characters, such as heading time, plant height and resistance to blast. These genes were subjected to the RFLP or SSR analysis to determine their locations on chromosomes. Some of these genes were also examined for the effects on phenotypic expression including pleiotropy and gene-gene interaction, and evaluated for their agronomic values.

c) Molecular-genetic analysis of chitinase gene family in rice.

We had identified that there are twelve independent loci of chitinase gene (Cht1 to Cht12) in rice genome. These genes were analyzed for the primary structure and function of enzyme protein in relation to disease - resistance after isolation and purification using *E. coli* expression system.

d) Analysis of genetic variation in wheat seed storage protein, glutenin

Asian hexaploid wheat (*Triticum aestivum* L.) cultivars were investigated for the genotype of high molecular weight glutenin subunits (HMGS). HMGS is the most important factor determining the bread-baking quality and noodle quality. We are investigating the genetic diversity of HMGS among East Asian wheat cultivar to identify the valuable genotypes for the improvement of wheat quality in Japanese varieties. Then, we found the new HMGS in one variety which showed a specific dough quality comparing to other varieties. It is important to disclose effects of this new HMGS on the characteristics of dough and bread-baking quality.

e) Genetic analysis of major agronomic characters in soybean

A number of varieties and 96 recombinant inbred lines (RILs) derived from the F₂ population of 'Peking' and 'Tama-homare' which was developed in our laboratory were analyzed for genetic factors controlling major agronomic characters such as filling duration, stress resistance, seed quality. The genetic map consisting 342 SSR markers loci, three phenotypic gene loci (I, T and W1 locus) was constructed using above RILs. Based on this map, we found several QTLs (Quantitative Trait Loci) for pre-germination flood tolerance and isoflavone accumulation in seeds (cotyledon).

A-2.Publications and presentations

a) Publications

Original Papers(including book-reviews)

- Tsukiyama, T., J. Lee, Y. Okumoto, M. Teraishi, T. Tanisaka and K. Inoue:
Gene cloning, bacterial expression, and purification of a novel rice (*Oryza sativa* L.) ubiquitin-related protein, RURM1. *Biosci. Biotechnol. Biochem.* 74; 430-432, 2010

- Yoshikawa, T., Y. Okumoto, D. Odata, T. Sayama, M. Teraishi, M. Terai, T. Toda, K. Yamada, K. Yagasaki, N. Yamada, T. Tsukiyama, T. Yamada and T. Tanisaka:
Transgressive segregation of isoflavone contents under the control of four QTLs in a cross between distantly related soybean varieties. *Breed. Sci.* 60; 243-254, 2010

- Nishizawa, K., K. Takagi, M. Teraishi, A. Kita and M. Ishimoto:
Application of somatic embryos to rapid and reliable analysis of soybean seed components by RNA interference-mediated gene silencing. *Plant Biotech.* 27;409-420, 2010

- Motoyama, T., Y. Okumoto, T. Tanisaka, S. Utsumi and N. Maruyama:
Co-expression of α' and β subunits of β -conglycinin in rice seeds and its effect on the accumulation behavior of the expressed proteins. *Transgenic Res.* 19; 819-827, 2010

- Saito H., Y. Okumoto, Y. Yoshitake, Q. Yuan, M. Teraishi, T. Tsukiyama, H. Nishida and T. Tanisaka:
Complete loss of photoperiodic response in the rice mutant line X61 is caused by deficiency of phytochrome chromophore biosynthesis gene. *Theor. Appl. Genet.* 122; 109-118, 2011

Reviews

- Y. Okumoto: Strategies for breeding a seed-flood tolerant soybean variety. *J. of Crop Res.* 55; 65-67, 2010

b) Conference and seminar papers presented

- Congress of Japanese Society of Breeding: 7 presentations
- Congress of the Society of Crop Science and Breeding in Kinki: 2 presentations
- Congress of Japanese Society of Genetics: 1 presentation

A-3.Off-campus activities 1

Membership in academic societies

- Yutaka Okumoto : Japanese Society of Breeding (Treasurer), The Society of Crop Science and Breeding in Kinki (Vice-president)
- Takuji Tsukiyama : Japanese Society of Breeding (Council member), The Society of Crop Science and Breeding in Kinki (General affairs)

A-3.Off-campus activities 2

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Monbusho Research Grant: Scientific Research (B) : Tsukiyama, Takuji : Transposition mechanism of mPing and development of high-efficiency transposon-tagging system
- Monbusho Research Grant: Scientific Research (B) : Okumoto, Yutaka : Dynamic modification of gene expression network by mPing in rice

2.Other Research Grants

- Independent Administrative Institute (National Institute of Agrobiological Sciences) : Okumoto, Yutaka : Genetic and molecular dissection of quantitative traits in rice
- Research and development projects for application in promoting new policy of Agriculture Forestry and Fisheries : Okumoto, Yutaka : Advancement of productivity and functionality of large-seed black soybean for novel demand expansion
- Fujikko Co. Ltd. : Okumoto, Yutaka : Collaboration “Breeding of soybean varieties with high quality”

B.Educational Activities(2010.4-2011.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level : Plant Breeding I, II (Okumoto), Biometrics (Okumoto), Seminar in Crop Science (Okumoto), Laboratory in Bioresource Science I (Okumoto, Teraishi, Tsukiyama), Introduction to Research (Okumoto, Teraishi, Tsukiyama)
- Graduate level : Progress in Mutation Breeding (Okumoto), Plant Breeding Seminar (Okumoto), Special Laboratory Work in Plant Breeding (Okumoto, Teraishi, Tsukiyama)

B-3.Overseas teaching 1

International students

- International students : Master 1 (Brazil) Doctoral 4 (Egypt 1, Bangladesh 2, Laos 1)