

**ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE**

January 1, 2006 – December 31, 2006

PROJECT TITLE: Application of Molecular Marker-Assisted Selection to Rice Improvement

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LEVEL OF 2006 FUNDING: \$25,000

OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

The overall objective is to integrate molecular genetic approaches and conventional breeding methods to develop improved germplasm for the California rice industry. Primary emphasis will be placed on the development of molecular (DNA) markers that can be used to predict the presence or absence of a trait of interest (e.g. disease resistance, cold tolerance, grain quality) and the application of these markers via molecular marker-assisted selection to expedite the identification of useful germplasm and streamline the breeding of improved varieties.

- 1) Disease resistance
 - a. Stem Rot: Our objective is to determine the genetic basis of resistance/tolerance to the stem rot pathogen *Sclerotium oryzae* and utilize that information to develop and implement tools for improving California rice varieties.

- b. Blast: Our objective is to assist the RES in the development and application of DNA markers for introducing blast resistance genes into California rice varieties.
- 2) Cold tolerance
- a. Seedling Stage: Our objective is to determine the location of genes that confer tolerance to cold-induced yellowing and stunting at the seedling growth stage in M-202.
 - b. Booting Stage: Our objective is to develop populations from the cross M-202/IR50 with similar heading dates in order to assess reproductive stage cold tolerance in a field situation.
- 3) Grain quality
- a. The *Waxy* gene encodes granule bound starch synthase, the enzyme which controls amylose content of rice grains. Our objective is to use the *Waxy* gene marker to assess breeding lines and the progeny of crosses developed by RES breeders.

SUMMARY OF 2006 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

- 1) Disease resistance
- a. Stem Rot:
 - 1. Mapping population development: In 2006, approximately 150 recombinant inbred lines derived from the long grain cross R22400 (87Y550/96Y480) were advanced to the F₆ generation. These lines need to be analyzed with DNA markers to confirm that they are genetically fixed (i.e., homozygous, pure breeding). Typically, the F₆ generation has undergone enough rounds of selfing to provide a mapping population that can be used to assess complex traits such as resistance to stem rot, which appears to be controlled by several genes and which is not a complete resistance. The availability of this mapping population should facilitate more accurate disease scoring for use in conjunction with DNA marker data to identify resistance genes from 87Y550.
 - 2. Characterization of stem rot isolates: In 2006, DNA markers generated by the Amplified Fragment Length Polymorphism (AFLP) technique were used to determine genetic relationships of 45 isolates consisting of 33 isolates from 2005 and 12 isolates collected in 1988 (provided by J. Oster). Based on the AFLP analysis and mating type, 14 isolates were selected for characterization of such traits as growth on complex media, sclerotia production, conidia production, sclerotia viability, and fungicide sensitivity, and other factors that may be involved in determining the prevalence of certain genotypes in nature. We were most interested in determining if there are differences in virulence on rice among these isolates. Towards this end, we used previously described seedling virulence assays and 4 isolates were selected based on virulence and mating type: # 15 (virulence: high, mating type: A), #38 (moderate, a), #9 (low, A), and #11 (low, A). Crosses were made between the isolates (38 x 15, 38 x 9, and 38 x 11) to generate progeny for genetic characterization of virulence. We have selected the

38 x 15 cross (164 progeny) for further characterization and are currently preparing materials for developing a genetic map of *S. oryzae* and for assessing virulence under controlled laboratory conditions.

b. Blast:

1. DNA marker survey: We initiated a project with Jeff Oster who is interested in using DNA markers linked to several blast resistance genes (*Pi-ta/Pi-ta²*, *Pi-b*, *Pi-z*, *Pi-z⁵/Pi-2*, *Pi-k^h*, *Pi-k^m*, and *Pi-9*). DNA was extracted from fourteen rice lines that were provided by J. Oster. DNA marker analysis was conducted using markers linked in previous studies to various blast resistance genes (*Pi-b*, *Pi-z*, *Pi-k*, *Pi-ta*) to determine if any of these markers can be used to assist in a backcrossing strategy with M-206 as the recurrent parent. Some of the results obtained are shown in Table 1 below.

Table 1. DNA marker analysis of various rice lines.

Entry	Marker Name	Pibdom	RM 208	RM 527	RM 5963	RM 6836	RM 1233	RM 144	RM 224	OSM 89	RM 155	RM 7102	OSM 91	pita 440	pita 403
	Gene	Pib	Pib	Piz	Piz	Piz	Pik	Pik	Pik	Pita	Pita	Pita	Pita	Pita	Pita
	Chrom. #	II	II	VI	VI	VI	XI	XI	XI	XII	XII	XII	XII	XII	XII
M206	none	-	2	1	1	1	1	2	1	1	1	1	1	-	-
Tsuyuake	Pik	-	2	2	2	6	3	5	2	1	[2/3]	1	1	-	-
IRBLPi-km	Pik	-	2	3	3	4	3	5	2	1	4	1	-	-	-
Cocodrie	Pik	1	3	3	3	2/4	3	1	1	1	1	2	1	1	1
IRBLPi-z5	Pi-z	-	1	4	2	5	3	4	1	2	1	3	[1]	1	1
IRBLPi-z5	Pi-z	-	1	1	2	6	3	5	2	1	1/4	4	2	-	-
Lafitte	Pi-z	1	2/3	2	2	4	2	5	3	1	4	1	[1/2]	-	-
M207	Pi-z	-	2	2	1	1	1	2	1	1	3	1	1	-	-
97Y315vE	Pi-b	-	2	2	1	1	1	2	1	1	3	1	1	-	-
Daegwanbyeo	Pi-b	1	3	2	1	1	[3]	1	3	2	1	2	1	1	1
Drew	Pi-ta	-	1	3	2	6	3	5	1	1	1	4	1	-	-
IR65482-4	Pi-ta	-	1	1	2	7	1	3	3	1	4	1	2	-	-
IRBLPi-9-V	Pi-9	-	1	2	3	1/4	3	5	1	1	5	4	-	-	-
Unknown	Unknown	-	2	2	2	-	[1]	5	1	[1]	-	1	-	-	-

Notes: Top row indicates the names of the DNA markers used. Second row indicates the blast resistance genes associated with each marker. Third row indicates on which rice chromosome (in roman numerals) the genes are located. Rice accessions examined are listed in first column under entry. Second column indicates which blast resistance gene is present in the accession.

Remaining columns indicate the marker class (i.e., allele) identified for each accession. Those accessions with the same number under a given marker would not be distinguishable using that marker. For example, the *Pi-b* marker RM208 cannot be used to distinguish M-206 from 97Y315vE although that accession contains the *Pi-b* gene. However, RM208 can be used when comparing M-206 and another possible *Pi-b* donor, Daegwanbyeo. Occasionally two different marker classes were identified (e.g., Lafitte RM208 = 2/3). This may indicate that the sample is not pure. The brackets [] indicate a weak “signal” relative to the other results.

2) Cold tolerance

a. Seedling Stage:

1. Tolerance to yellowing/stunting (Chromosome 4, *qCTS4*): In 2006, the *qCTS4* locus of M-202, which is associated with tolerance to cold-induced yellowing and

stunting, was fine mapped to a region of about 560 kb on chromosome 4. This region contains many genes (>50) based on analysis of the DNA sequence. One of the predicted genes encodes a putative cationic peroxidase, which co-segregates with the cold tolerance trait. Peroxidases are enzymes that reduce hydrogen peroxide (toxic compound) to water and oxygen. This role as a detoxifying enzyme and its co-segregation with the tolerance phenotype make this gene a possible candidate for *qCTS4*. During the process of fine mapping *qCTS4*, we have developed a number of DNA markers which may be useful for marker-assisted selection for this trait.

2. Tolerance to necrosis (tissue death)/wilting (Chromosome 12, *qCTS12*): Our previous work suggests that the gene underlying the M-202 tolerance encodes an enzyme called glutathione S-transferase (GST). There are two GST genes located in the region. Based on work by other researchers, one of these genes (designated *OsGSTZ1*) appears most likely to be *qCTS12*. During this year, the *OsGSTZ1* gene was sequenced from both M-202 (tolerant) and IR50 (susceptible). Although some differences were observed, none appear to be responsible for the difference in cold tolerance observed between the two parents. It is possible that expression of the *OsGSTZ1* gene is responsible for the difference in tolerance. This will be examined in 2007.
- b. Booting Stage:
1. Population development and assessment: A set of 483 recombinant inbred lines (F₁₀ generation) derived from the cross of M-202/IR50 were planted in the UC Davis rice nursery and in the RES nursery during the 2006 season for assessment of heading date and fertility. These lines have previously been used in our seedling cold tolerance research and have been extensively characterized with DNA markers. Data on heading dates were collected and representative panicles were harvested for assessing fertility from both locations. These data will be used to identify a set of lines that may be used for genetic analysis of tolerance to low temperature-induced blanking.
- 3) Grain quality
- a. *Waxy* marker: We continued to apply this DNA marker to materials from the RES from the long grain (Jodari) breeding program. Approximately 1,000 lines were assessed for the RES.
- 4) Additional 2006 research activities
- a. Semidwarf marker: Since the late 1970s, most California varieties have been semidwarf or short stature. This is the result of breeding efforts that have incorporated different forms of the *Sd-1* gene, which encodes an enzyme involved in the synthesis of the hormone gibberellin. These recessive forms of *Sd-1* (referred to as *sd-1* alleles) confer the semidwarf trait that enables high yields in response to fertilizers without lodging. In California, the two main sources of *sd-1* are Calrose76 and Dee-Geo-Woo-Gen (via the variety IR8). DNA markers that can differentiate the sources of semidwarf are available. Analysis of all the varieties released by the RES indicates that the IR8 allele of *sd-1* is present in 15 of them (M9, M-201, L-202, M-202, A-

301, L-203, M-204, A-201, L-204, Calmati-201, L-205, M-205, M-208, L-206, and Calmati-202). In addition, DNA sequence analysis of the *sd-1* gene from M-401 (an induced semidwarf mutant of the variety Terso) revealed that M-401 has a unique version of the *sd-1* gene that is distinguishable from all previously known sources of *sd-1*. Confirmation of the identity of the *sd-1* alleles in the other semidwarf California varieties is pending although all of these varieties most likely contain the Calrose 76 source. Whether any of the different *sd-1* alleles has an advantage over the others in terms of agronomic performance in the California environment is unknown.

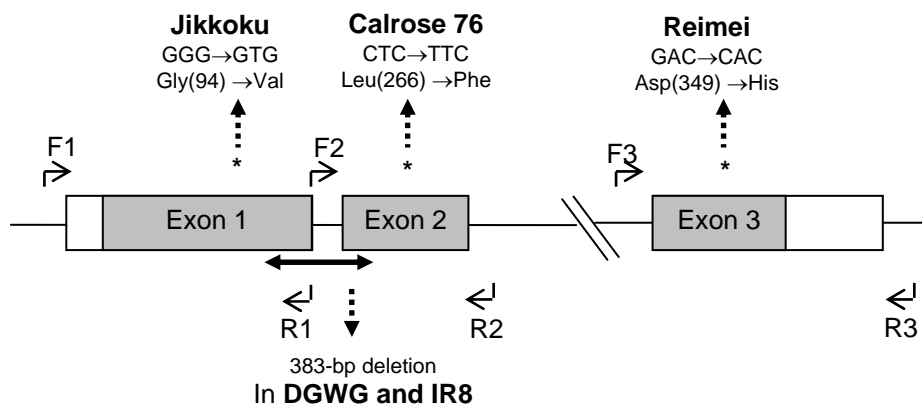


Figure 1. Diagram of the structure of the *Sd-1* gene encoding the enzyme GA20-oxidase-2 and the changes in the DNA found in various *sd-1* alleles of this gene. Some mutations in this gene result in the semidwarf phenotype which is characterized by shorter stature and increased productivity. Several *sd-1* (recessive) alleles of this gene have been used in rice breeding. The Calrose76 and DGWG (IR8) alleles are the most prevalent sources of the semidwarf trait in California varieties. M-401 has a unique allele resulting from changes in Exon 3 (not shown).

- b. RES DNA marker lab: In addition to conducting the various research activities described above, assistance in establishing the RES DNA marker lab was provided. Training and other support were given to Mr. A. I. Roughton and Mrs. B. Deol enabling on-site DNA marker analysis to be accomplished for the first time. In addition to training of technicians, discussions with the RES director and breeders were held regarding the establishment and operation of the marker lab and DNA marker technology in general. These efforts will be extended in 2007.

PUBLICATIONS OR REPORTS:

Snyder, L.J., Oster, J., Colowit, P.M., Jodari, F., and Tai, T.H. Molecular genetic characterization of the interaction of stem rot (*S. oryzae*) and rice (*Oryza sativa*). Rice Field Day, Rice Experiment Station, Biggs, CA, August 30, 2006. (poster)

Andaya, V.C. and Tai, T.H. Genetic mapping of the *qCTS12* and *qCTS4* loci controlling M202 rice seedling response to low temperatures. Rice Field Day, Rice Experiment Station, Biggs, CA, August 30, 2006. (poster)

Andaya, V., Colowit, P., Jodari, F., Jiang, J., Johnson, C., Oster, J., Kim, S., Roughton, A.I., McKenzie, K.S., and Tai, T.H. Application of DNA markers to the Rice Experiment Station Breeding Programs. Rice Field Day, Rice Experiment Station, Biggs, CA, August 30, 2006. (poster)

Andaya, V.C. and Tai, T.H. Fine mapping of the *qCTS12* locus, a major QTL for seedling cold tolerance in rice. 2006. *Theor. Appl. Genet.* 113:467-475.

Andaya, V.C. and Tai, T.H. Fine mapping of the *qCTS4* locus associated with cold tolerance at the seedling stage in rice. (manuscript in preparation)

Kim, S.I., McKenzie, K.S. and Tai, T.H. A survey of the *Sd-1* gene in California rice varieties. (manuscript in preparation)

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 2006, the use of molecular markers for rice improvement in California was examined in the context of development and application of DNA markers. Work continued in the area of genetic population development for use in identifying markers and genes for stem rot resistance and cold tolerance. Long grain materials, originally provided by the RES, have been advanced by selfing to a generation that is amenable to more accurate stem rot disease scoring and will be used to identify resistance genes and develop markers for this trait. In addition, molecular genetic characterization of stem rot fungal isolates was advanced with the ultimate objective of understanding the basis for virulence of this pathogen on rice. With regard to cold tolerance, fine genetic mapping of a region on chromosome 4 conferring tolerance to cold-induced yellowing and stunting of seedlings resulted in the identification of several candidate genes. The fine genetic mapping results from this year and the previous year will now be applied to develop materials for field testing to determine if these mapped regions confer seedling cold tolerance under typical production conditions. Rice germplasm with greater seedling cold tolerance than M-202 were identified by screening a worldwide core collection using our low temperature assays. In the area of DNA marker application, long grain breeding lines were analyzed with the *Waxy* marker, a blast resistance gene marker survey was conducted on rice materials for use in a backcrossing program, and all the varieties released by the RES were examined with the *sd-1* (semidwarf) marker. Assistance was also provided to the RES in establishing an on-site DNA marker lab.