

**ANNUAL REPORT**  
COMPREHENSIVE RESEARCH ON RICE  
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PROJECT TITLE: Application of Molecular Marker-Assisted Selection to Rice Improvement

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LEVEL OF 2004 FUNDING: \$25,000

OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

The objective of this project is to integrate molecular genetic approaches and conventional breeding methods to develop improved germplasm for the California rice industry. Primary emphasis is on the application of molecular marker-assisted selection (MMAS) to expedite the identification of useful germplasm and streamline the breeding of improved varieties.

- 1) Disease resistance
  - a. Stem Rot and Aggregate Sheath Spot: Our objective is to identify DNA markers linked to resistance to stem rot and aggregate sheath spot in the wild species *Oryza rufipogon* and facilitate transfer of these traits to elite California varieties via marker-assisted selection.
  - b. Blast: Our objective is to use DNA markers linked to the *Pi-z* blast resistance gene to analyze breeding lines and F2 progeny from crosses between resistant and susceptible materials developed by RES breeders.
- 2) Cold tolerance

- a. Seedling Stage: Our objective is to continue to develop a high resolution map of genetic loci in the variety M-202 that confer tolerance to cold-induced yellowing and leaf wilting at the seedling growth stage, leading to the identification of DNA markers for breeding and, ultimately, to the identification of the genes controlling these traits.
  - 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. These populations will be used to identify genes controlling this type of cold tolerance and to develop DNA markers for this trait.
- 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 2004 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

- 1) Disease resistance
- a. Stem Rot and Aggregate Sheath Spot:
    1. Disease Phenotyping: In order to identify the genes conferring disease resistance to long grain breeding line 87Y550, F<sub>3</sub> progeny from 94 F<sub>2</sub> plants derived from the cross R22400 (87Y550/96Y480) were assessed for their reaction to inoculation with stem rot or aggregate sheath spot. For stem rot, two greenhouse (using the RES Plant Pathology facility) and three field tests (two plots at the RES and one at the UCD rice research facility) were conducted in 2004. For aggregate sheath spot, two greenhouse tests (RES) were conducted. The 94 entries exhibited a range of responses to stem rot that was fairly consistent among the various locations and the data are currently be examined more closely and will be used in conjunction with DNA marker data (see #3 below) to determine if specific loci (i.e. chromosomal locations of resistance genes) can be identified. The aggregate sheath spot tests did not produce consistent results and need to be repeated. Due to the labor-intensive nature of disease scoring, additional populations described last year, were not pursued.
    2. Population Development: In 2004, emphasis was placed on the development of a genetic mapping population from the M-206/100912 cross made last year. Four F<sub>1</sub> plants were used to pollinate M-206 plants and 106 BC<sub>1</sub> progeny (i.e. BC<sub>1</sub>F<sub>1</sub>) were generated (crosses confirmed by DNA marker analysis). These BC<sub>1</sub>F<sub>1</sub> were backcrossed to M-206 and over 1700 BC<sub>2</sub> seed have been collected (at least 2 per BC<sub>1</sub>F<sub>1</sub>). In addition to developing an advanced backcross population in a medium grain (M-206) background, F<sub>2</sub> seeds were collected from the following wide crosses: S-102/100912 (about 100 seeds), M-206/100912 (about 630 seeds), 100912/L-205 (about 480 seeds), and M-202/100912 (about

150 seeds). Single seed descent for the development of recombinant inbred lines was continued for the following crosses: R22400 (87Y550/96Y480; F<sub>4</sub> seed harvested), R22115 (94Y561/L-205; F<sub>4</sub> seed harvested), and R22791 (87Y550/L-204; F<sub>3</sub> seed harvested).

3. Molecular Marker Analysis: In order to identify the resistance genes in 87Y550 (i.e. the number and location of these genes), DNA marker analysis was performed on the parental lines 87Y550 (resistant) and 96Y480 (susceptible). To date, 273 microsatellite markers have been used to assess the parents, and 52 of these markers have been polymorphic (i.e. they can distinguish between the two parents).

b. Blast:

1. *Pi-z* marker assisted selection in short grain crosses: In 2003, two short grain crosses R27006 (01Y185, susceptible/01Y348, resistant) and R27007 (01Y350, resistant/01Y337, susceptible) were analyzed with DNA markers tightly linked to the blast resistance gene *Pi-z*. Approximately 260 F<sub>2</sub> progeny from R27007 and 240 F<sub>2</sub> progeny from R27006 were analyzed and used to select 62 individuals from the R27007 cross and 33 individuals from the R27006. These individuals were allowed to produce seed which were provided to J. Oster for blast testing. Those tests were conducted in 2004 and indicated that the DNA markers accurately predicted the resistance or susceptibility in the case of the R27007, but not in the case of R27006. There were no obvious reasons for the discrepancy between the marker scores and the disease scores for R27007 (such as mistakes made in harvesting seed or interpreting data). Seeds of the F<sub>2</sub> plants from R27007 (remnant from the samples tested by J. Oster) are available for re-testing with *Pi-z* markers to confirm/clarify the results; however, this particular cross is not a priority according to Dr. K. McKenzie, who re-assumed responsibility for the short grain breeding program following the departure of Dr. T. Campbell. Other short grain materials of interest to Dr. McKenzie have been analyzed using the *Pi-z* markers.
2. Assessment/confirmation of medium grain and long grain materials: In 2004, DNA markers linked to blast resistance genes were used to assess several materials from the medium grain (192 entries from Dr. C. Johnson, *Pi-z* gene) and long grain (96 entries from Dr. F. Jodari, *Pi-ta*<sup>2</sup> gene) programs.

2) Cold tolerance

a. Seedling Stage:

1. Chromosome 12 (cold-induced wilting): Emphasis was placed on the fine mapping of the chromosome 12 quantitative trait locis (QTL) conferring tolerance to cold-induced wilting due to the availability of more DNA markers on this chromosome. Fine mapping resulted in the narrowing down of the chromosomal region containing the gene(s) to

within 1.7 Mb (i.e. million base pairs of DNA, chromosome 12 is about 27 Mb) flanked by the microsatellite markers RM 5746 and RM 7003. Four additional microsatellite markers were examined in the region between RM 5746 and RM 7003, but all of these markers were unable to distinguish M-202 and IR50 and were therefore not useful.

2. Chromosome 4 (cold-induced yellowing): Due to the availability of relatively few markers on this chromosome, fine mapping of the QTL for tolerance to cold-induced wilting was not pursued in 2004 in favor of cold-induced wilting and development of additional plant materials for use in genetic analysis.
3. Population development: In 2004, materials from the M-202/IR50 cross were advanced including 3500 F<sub>3</sub> families, 1900 F<sub>5</sub> lines, 528 F<sub>6</sub> lines and 318 F<sub>10</sub> lines. These materials are in addition to the 186 recombinant inbred lines (RILs) initially used to identify the QTL on chromosome 4 and 12. Backcrossing of selected lines to the IR50 parent was also initiated to develop near isogenic lines through marker-assisted selection (i.e. germplasm development).

b. Booting Stage:

1. Population development: Locating QTL associated with tolerance to cold-induced spikelet sterility under field conditions is difficult due to variability in stress level or duration and the differences in maturity of test materials. Additional recombinant inbred lines from the M-202/IR50 cross were developed in order to assemble group of lines with similar maturity dates. Of 546 F<sub>4</sub> lines sent to the winter nursery in Hawaii, 528 were selected for spikelet fertility (>80%) and F<sub>5</sub> seeds were planted in Davis in 2004. F<sub>6</sub> seeds have been harvested and will be grown in 2005. Flowering of these lines will be assessed to identify those falling in the same maturity group for booting stage cold tolerance evaluation. More advanced lines (i.e. the 186 RILs used in the original study and the 318 F<sub>10</sub> lines) show a great deal of variability in fertility, which could be the result of the intersubspecific nature of the cross (i.e. japonica/indica), and are not being pursued for booting stage evaluation.

3) Grain quality

- a. *Waxy* marker: The *Waxy* marker has been applied to a set of long grain breeding materials from F. Jodari. Analysis of these samples was performed in cooperation with the RES. In 2004, the RES hired Mr. Iestyn Roughton who was tasked with establishing an on-site lab effort to extract DNA from plant materials. This effort included the procurement of necessary equipment to process tissue samples and extract DNA. A reproducible protocol was developed and applied to the preparation of DNA samples for assessment using the *Waxy* and blast resistance gene markers mentioned earlier. The *Waxy* marker proved somewhat problematic in terms of getting consistent results; however, modification of our protocols for marker analysis led to results comparable to those obtained by the USDA-ARS Rice Research Unit in Beaumont, TX which uses the *Waxy* marker extensively.

PUBLICATIONS OR REPORTS:

Andaya, V., Aragones, D., and Tai, T.H. 2004. Progress in mapping genes associated with cold tolerance of M-202. Rice Field Day, Rice Experiment Station, Biggs, CA, August 25, 2004. (poster)

Snyder, L.J., Oster, J.J., Jodari, F., and Tai, T.H. 2004. Identification of QTL conferring resistance to stem rot and aggregate sheath spot. Rice Field Day, Rice Experiment Station, Biggs, CA, August 25, 2004. (poster)

Tai, T., Colowit, P., Roughton, I., Jodari, F., Johnson, C. and McKenzie, K. 2004. Marker-assisted selection for California rice improvement. Rice Field Day, Rice Experiment Station, Biggs, CA, August 25, 2004. (poster)

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 2004, the use of molecular markers for rice improvement in California was examined in the context of development (disease resistance and cold tolerance) and application (disease resistance and grain quality). Work continued in the development of genetic populations for use in identifying markers and genes for stem rot resistance and cold tolerance. Using long grain materials provided by the RES, preliminary disease screening for stem rot and aggregate sheath spot were carried out and DNA marker work was initiated. Fine genetic mapping was conducted to define the region on chromosome 12 conferring tolerance to cold-induced wilting. With regard to application of DNA markers, assistance was provided to the RES in establishing on-site DNA extraction. DNA samples from the RES were analyzed using markers for the blast resistance genes *Pi-z* and *Pi-ta<sup>2</sup>* as well as the *Waxy* grain quality marker. The focus of these efforts was primarily to assess the reproducibility/robustness of the system.