ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2005 – December 31, 2005

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

PROJECT LEADER: Thomas H. Tai, Research Geneticist, USDA-ARS
Plant Sciences, UCD

PRINCIPAL UC INVESTIGATORS:

Thomas H. Tai, Research Geneticist, USDA-ARS, Plant Sciences, UCD
Peter M. Colowit, Biological Science Technician, USDA-ARS
Leslie J. Snyder, Graduate Student, Genetics Graduate Group, Plant Sciences, UCD
Virgilio C. Andaya, Research Associate, USDA-ARS

COOPERATORS:

Farman Jodari, Plant Breeder, Rice Experiment Station, Biggs
Carl W. Johnson, Plant Breeder, Rice Experiment Station, Biggs
Junda Jiang, Plant Breeder, Rice Experiment Station, Biggs
Jeffrey J. Oster, Plant Pathologist, Rice Experiment Station, Biggs
Iestyn Roughton, Technician, Rice Experiment Station, Biggs

LEVEL OF 2005 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 to expedite the identification of useful germplasm and streamline the breeding of improved varieties.

1) Disease resistance
    a. Stem Rot: Our objective is to identify DNA markers linked to resistance to stem rot 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 F_2 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 2005 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1) Disease resistance
   a. Stem Rot:
      1. Disease Phenotyping: In order to identify the genes conferring disease resistance to long grain breeding line 87Y550, we repeated field and greenhouse disease phenotyping experiments in 2005. As in 2004, F_3 progeny from F_2 plants derived from the cross R22400 (87Y550/96Y480) were tested for their reaction to inoculation with stem rot. The total number of lines was expanded from 94 to 116 in order to account for the possible loss of lines due to low seed. For stem rot, three greenhouse (using the RES Plant Pathology facility) and six field tests (three reps at the RES and three reps at the UCD rice research facility) were conducted in 2005. Due to the late season and the larger dataset, analysis of the disease rating data is still underway and will be summarized in next year's report.
      2. Population Development: In 2005, emphasis was again placed on the development of a genetic mapping population from the M-206/100912 cross made last year. Last year over 1700 BC_2F_1 seed were collected (at least 2 from each of the 106 BC_1F_1 developed earlier). This year BC_2F_1 seed from each of the 106 BC_1F_1 lines was planted (about 3 to 4 seed per line) and both tissue and BC_2F_2 seed were harvested from about 302 lines (each of the 106 BC_1F_1 lines is represented least once). The tissue will be used for preparing DNA for marker analysis and the seeds will be used for trait assessment (i.e. phenotyping). Single seed descent for the development of recombinant inbred lines was continued for the following crosses: R22400 (87Y550/96Y480; F_4 seed planted), R22115 (94Y561/L-205; F_5 seed harvested and planted).
      3. Molecular Marker Analysis: In order to identify the resistance genes in 87Y550 (i.e. the number and location of these genes) using the R22400 population, DNA marker analysis was performed on the parental lines 87Y550 (resistant) and 96Y480 (susceptible). In 2005, approximately

237 microsatellite markers were assessed (making a total of about 500 markers examined to date) using DNA from the two parents. Of these 500 markers, about 90 appear to be able to distinguish the two parents (i.e. are polymorphic) and may be useful for genetic mapping. About 80 of the 90 polymorphic markers have been used to examine DNA from each of the R22400 lines (a total of 123 lines, 116 of which were used in the disease phenotyping described above). Mapping data for 41 of the 80 markers was obtained this year. Data for another 110 markers is needed to facilitate the genetic mapping of the R22400 population and the analysis to determine the number and location of genes involved in the stem rot resistance exhibited by 87Y550.

b. Blast:
    1. With the release of M-207 which has *Pi-z* resistance, all of our effort this year has been focused on stem rot. We have, however, initiated a project with Jeff Oster who is interested in using DNA markers linked to several blast resistance genes (*Pi-ta/Pi-ta*^{2}, *Pi-b*, *Pi-z*, *Pi-z*^{5}/*Pi-2*, *Pi-k*^{h}, *Pi-k*^{m}, and *Pi-9*) which will be described in the 2006 RB-3 proposal.

2) Cold tolerance
    a. Seedling Stage:
        1. Chromosome 12 (cold-induced wilting): Fine mapping resulted in the narrowing down of the chromosomal region containing the gene(s) to within about 20 kb of DNA. This region contains only 4 genes, two of which encode glutathione S-transferases which are enzymes which have been implicated in stress response in various mammalian and plant systems. We believe that one or both of these glutathione S-transferases is responsible for the tolerance to cold-induced wilting observed in M-202 seedlings. During the process of identifying these candidate genes we have developed a number of DNA markers which may be tested for their usefulness in marker-assisted selection for this trait.
        2. Chromosome 4 (cold-induced yellowing): With the focus on the cold-induced wilting trait on chromosome 12, work on chromosome 4 was not pursued in 2005. It is expected that with the success described above, we will now shift our attention in 2006 to trying to identify markers for this trait and, ultimately, the gene or genes involved.
        3. Population development: In 2005, materials from the M-202/IR50 cross continued to be advanced. Currently, we have focused our efforts on a population of 1,954 recombinant inbred lines. Among these are 496 F_{10}, 518 F_6, and 940 F_5 generation lines.
    b. Booting Stage:
        1. Population development and assessment: Due to the effort expended on fine mapping of the chromosome 12 seedling cold tolerance trait, we were not able identify a set of M-202/IR50 recombinant inbred lines for analysis of booting stage cold tolerance. This work will be pursued in 2006.

3) Grain quality
    a. *Waxy* marker: In 2005, we continued to apply the waxy marker to materials from the RES including advanced lines from the short grain (Jiang) and long grain (Jodari) breeding program. DNA extraction by the RES (Roughton) has become routine and RES personnel (Roughton) have received training in my lab on DNA marker protocols.

PUBLICATIONS OR REPORTS:

Snyder, L.J., Oster, J.J., Colowit, P.M., Caravello, T.I., Jodari, F., and Tai, T.H. 2005. Progress on Molecular Studies of Stem Rot Resistance. Rice Field Day, Rice Experiment Station, Biggs, CA, August 31, 2005. (poster)

Tai, T.H. and V.C. Andaya. Molecular Genetic Analysis of Seedling Cold Tolerance. 5ht International Rice Genetics Symposium, Temperate Rice Workshop, Manila, The Philippines, November 21, 2005. (oral presentation)

V.C. Andaya and T.H. Tai. Fine mapping of *qCTS12* locus, a major QTL for seedling cold tolerance in rice. (manuscript in preparation).

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 2005, 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) 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. Using long grain materials provided by the RES, field and greenhouse disease tests were conducted again this year and the results will be compared to last year. DNA marker work for mapping stem rot resistance genes was continued. With regard to cold tolerance, fine genetic mapping of a region on chromosome 12 conferring tolerance to cold-induced wilting in seedlings resulted in the identification of four candidate genes. Two of these genes are glutathione S-transferases, one of which has been previously reported to have a role in enhancing cold tolerance in rice. Several DNA markers have been developed as a result of this work and will be tested in the coming year. In the area of DNA marker application, assistance and training was provided to the RES in analyzing short and long grain breeding lines with the *Waxy* grain quality marker.