

**ANNUAL REPORT  
COMPREHENSIVE RESEARCH ON RICE**

January 1, 2007 – December 31, 2007

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

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**LEVEL OF 2007 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 2007 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:**

- 1) Disease resistance
  1. Stem Rot:
    1. Mapping population development: In 2007, approximately 150 recombinant inbred lines derived from the long grain cross R22400 (87Y550/96Y480) were advanced to the F<sub>7</sub> generation. Sufficient seeds were collected from these lines, which will be used in disease tests in 2008. Plants from each line should essentially be identical at this point and should produce consistent disease scores when inoculated with a pure isolate of the stem rot fungus. In addition to the R22400 long grain mapping population, a second population derived from a cross between 87Y550 and S-102 was advanced in 2007. F<sub>3</sub> seeds were harvested from approximately 330 individual F<sub>2</sub> plants from this cross. These lines will continue to be advanced using the single seed descent method in order to produce recombinant inbred lines. In our experience, S-102 has consistently scored as very susceptible compared to 87Y550.
    2. Characterization of stem rot isolates: In 2007, tests of stem rot fungal isolates identified in 2006 were not consistent with previous results. Differences in virulence were observed and may be due either to changes in virulence of the isolates over time or to yet undefined differences in assay conditions. In addition, sclerotia preparations (i.e., standard fungal inocula) of parental isolates did not produce consistent disease scores; therefore, assessment of the virulence of the resulting progeny of crosses between parental isolates was not performed. In 2007, several useful DNA markers were identified using the Randomly Amplified Fragments of DNA (RAPD) marker approach. The data from these RAPD markers and the AFLP markers identified in 2006 were used in cluster analysis to examine the genetic relationship of our collection of stem rot isolates (Figure 1).

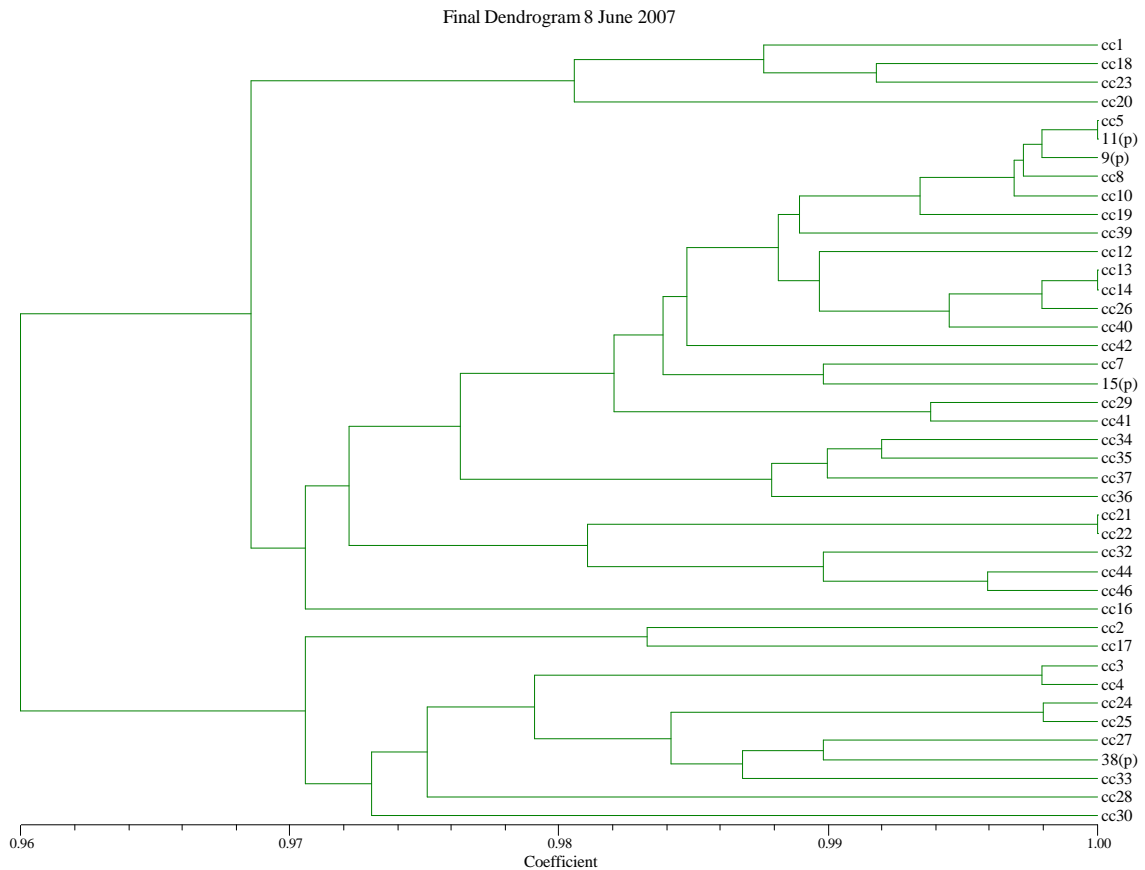


Figure 1. Genetic diversity of stem rot isolates. AFLP and RAPD marker data were analyzed using NYSTS software to produce the dendrogram (phylogenetic tree). Isolates are noted at the far right (cc = core collection, isolates with “p” designations were used as parents in crosses in 2006).

In addition to DNA characterization, the ability of some of these isolates to cause disease on young rice plants (3 to 4 weeks old) was examined using a new inoculation method in which fungal cultures were grown over paper discs which were then used to inoculate stems/sheaths of plants which had been placed between two acrylic boards. In this arrangement, the site of infection and progression of the fungus was more defined than in standard field inoculations with sclerotia. Preliminary tests suggest that this method produces consistent results and clear differences in virulence were observed among isolates tested (Figure 2). Crosses between more virulent and less virulent isolates of stem rot were not successful in 2007. Previous crosses from 2006 were successful in producing progeny; however, subsequent testing of parental isolates of those crosses suggests that the difference in virulence between those isolates may not be sufficient for analysis. Furthermore, there were inconsistencies in

the test tube/sclerotia inoculations used in those experiments. Testing with this new method may address the inconsistencies observed.

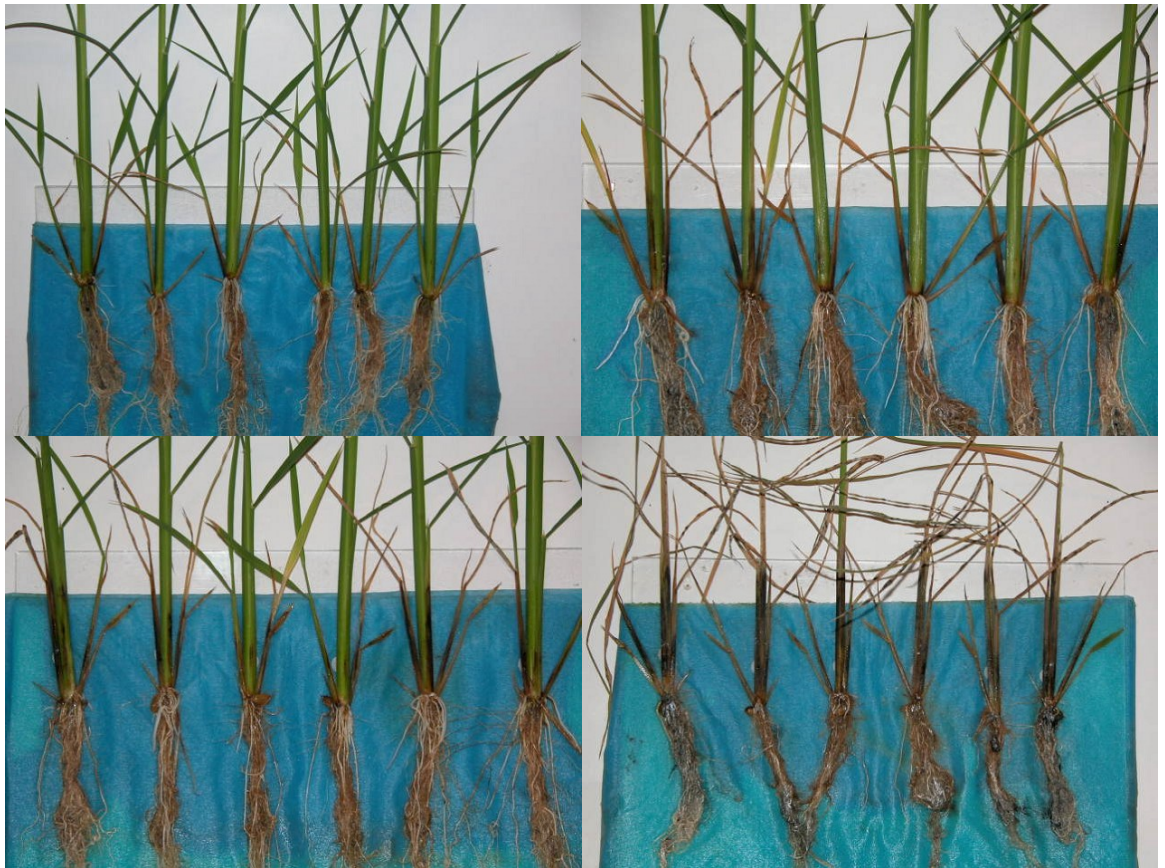


Figure 2. Paper disc inoculation of rice seedlings (S-102) with stem rot isolates (discs removed prior to photographing). Discs covered with stem rot mycelia are placed on stem about 2-4 cm above roots. Un-inoculated control (top left), cc23 isolate (upper right), cc3 (lower left), cc21 (lower right). Virulence cc21>>>cc3>cc23.

The information obtained concerning the genetic relationships and virulence of the stem rot isolates provides a foundation for identifying genes needed by the pathogen to cause disease on rice. Once identified, these genes will be targets for strategies to control stem rot.

## 2. Blast:

In 2007, the RES hired Dr. Virgilio C. Andaya as the short grain/premium quality program breeder. Dr. Andaya has been a primary contributor to the marker-assisted selection and development projects in my group. With his move to the RES, this work is now essentially being performed at the RES. My program remains open to support or cooperate with Dr. Andaya and the other staff members at the RES.

## 2) Cold tolerance

### 1. Seedling Stage:

1. In 2007, two sets of rice varieties from the USDA core collection (each consisting of 96 entries) were identified for further testing of seedling cold tolerance (one set for testing under *qCTS4* conditions which result in leaf yellowing and seedling stunting, and one set for testing under *qCTS12* conditions which result in leaf necrosis and seedling wilting). Some of these varieties appear to be more tolerant than M-202 and may provide a novel source of seedling cold tolerance. These lines will be re-tested in 2008 and DNA markers developed from analysis of M-202 cold tolerance will be tested on these lines to examine any potential relationship between the tolerance exhibited by M-202 and these varieties.
2. Advanced backcross lines (IR50 background with introgression of *qCTS4* and *qCTS12* from M-202) were advanced in 2007. These lines will continue to be advanced and seed will be collected in 2008 for field and controlled environment testing in subsequent years.

### 2. Booting Stage:

1. Population development and assessment: A set of 483 recombinant inbred lines (F<sub>10</sub> generation) derived from the cross of M-202/IR50 were planted in the UC Davis rice nursery and in the RES nursery during the 2007 season for assessment of heading date (Biggs and Davis) and fertility (Davis). 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. In 2008, data from the 2006 and 2007 plantings will be analyzed to identify lines for further study.

## 3) Grain quality

- a. *Waxy* marker: As with the blast marker, this work has moved to the RES. It is expected that the RES programs will exploit the expertise of Dr. Andaya to advance the application of this marker work with the USDA-ARS program providing support and cooperation as needed.

4) Additional 2007 research activities

Semidwarf marker: Analysis of the semidwarf (*sd-1*) gene in U.S. rice varieties was completed in 2007 and a manuscript is being prepared.

**PUBLICATIONS OR REPORTS:**

None at this time.

**CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:**

In 2007, the use of molecular markers for rice improvement in California was examined in the context of basic studies aimed at identifying traits of interest and developing markers that may be used in selection for these traits. Primary 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 consistent stem rot disease scoring and a new inoculation method also aimed at improving the consistency of disease ratings was examined. These resources will be used in 2008 to determine if stem rot resistance genes can be identified and if useful DNA markers can be developed for this trait. Molecular genetic characterization of stem rot fungal isolates continued with the ultimate objective of understanding the basis for virulence of this pathogen on rice. With regard to cold tolerance, new genetic materials for seedling cold tolerance were identified for more detailed analysis in 2008 and germplasm development continued with the advancement of backcross lines that will be tested in the field in the coming years. In the area of DNA marker application, the recent hire of Dr. Virgilio Andaya by the RES has shifted this effort to the RES with the USDA-ARS program playing a supporting role in the future.