ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2003 – December 31, 2003

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

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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 CRES 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 CRES breeders.

SUMMARY OF 2003 RESEARCH (MAJOR ACCOMLISHMENTS) BY OBJECTIVES:

1) Disease resistance
   a. Stem Rot and Aggregate Sheath Spot: Initially, our efforts focused on reproducing the results of previous work of D. Mackill and colleagues who reported that two stem rot resistance loci were located on rice chromosomes 2 and 3. Unfortunately, after repeated attempts, we were unable to reproduce these results. Efforts are still underway to determine the reason(s) for this. In the meantime, in order to identify markers and develop stem rot and aggregate sheath spot resistant germplasm, new crosses were made between several California cultivars and the resistant *O. rufipogon* accession 100912 (see Table 1). True crosses were confirmed by molecular marker analysis. The F1 hybrids will be used to develop advanced backcross and recombinant inbred line populations for genetic studies including developing molecular markers and isolating resistance genes. It is anticipated that some lines developed as a result of these crosses will represent stem rot and/or aggregate sheath spot resistant germplasm, which may be incorporated into the CRES breeding programs. In addition to the wide crosses, we obtained F_2 seed of crosses developed by Dr. F. Jodari using lines derived from the stem rot resistant breeding lines. F_2 progeny from each of 5 crosses (R22115: 94Y5612/L-205, R22400: 87Y550/96Y480, R24822: 99Y070/99Y017, R26731: 99Y621/98Y511, and R26728: 00Y481/96Y671) were advanced to the F_3 generation and seeds were harvested for field-based stem rot assays to be performed in 2004. Lines will continue to be advanced via single seed descent in order to generate recombinant inbred line populations for further gene mapping studies.

      Since the earlier report by Mackill and colleagues of markers linked to two stem rot resistance loci, a large number of rice molecular markers have become available as a result of various rice genome and gene sequencing projects. A set of 335 markers was used to survey *O. rufipogon* and four California cultivars (see Table 2). Over 200 markers that are informative (i.e. can differentiate between *O.*

rufipogon and the California cultivar) were identified in each case. A subset of 150 markers that are polymorphic (i.e. informative) between *O. rufipogon* and all four cultivars was also identified. These markers will enable genes conferring resistance to stem rot and aggregate sheath spot to be mapped in the populations currently under development. Additional traits such as yield and abiotic stress tolerance may also be examined with the populations and the markers developed in this work.

b. Blast: Although markers for several blast resistance genes (i.e. *Pi* genes) have been reported, the *Pi-z* gene is of primary interest to the CRES breeders. Using three microsatellite markers (RM 6836, RM5936, and RM527) linked to the *Pi-z* gene (see Figure 1), we have examined various lines from Dr. Carl Johnson and initiated a small-scale marker-assisted selection project in cooperation with Dr. Todd Campbell. Analysis of Dr. Johnson's materials indicates that these markers may be used to determine the presence or absence of the *Pi-z* gene to a high degree of probability and should be more robust than phenotypic assessment of resistance or susceptibility to the blast fungus (see Table 3). Analysis of parental lines of various premium quality crosses from Dr. Campbell's program indicated that these three markers should be useful for marker-assisted selection of progeny from two crosses, R27006 and R27007. R27006 F_2 progeny were derived from a cross between 01Y185 (susceptible) and 01Y348 (resistant) and R27007 F_2 progeny were derived from a cross between 01Y350 (resistant) and 01Y337 (susceptible). DNA samples were isolated from seedlings of approximately 260 F_2 progeny of R27007 and 240 F_2 progeny from R27006 and subjected to marker analysis. Results of the analysis were used to select 62 individuals from the R27007 cross and 33 individuals from the R27006. These individuals were allowed to produce seed, which will be provided to Dr. Campbell for phenotypic evaluation by J. Oster. These 95 individuals have been re-evaluated using the three markers and comparison of these results with the initial test results is currently underway.

2) Cold tolerance

a. Seedling Stage: F_3 families from the M-202/IR50 cross have been generated to fine map the chromosomal regions surrounding the genetic loci associated with cold tolerance at seedling stage. Several quantitative trait loci (QTL) have been identified previously (see publications), but the fine mapping work is concentrated on two major QTL associated with tolerance to cold-induced leaf yellowing and wilting that are located in chromosomes 4 and 12, respectively. The regions surrounding the two QTL are presently being saturated with new microsatellite markers through analysis of the recombinant inbred line population used in the initial genetic mapping. Markers closest to the loci of interest will be used to find recombinants using a larger population. Assays are also being developed that will enable the testing of large number of families rapidly and more reliably. The screening assays utilize a chiller to bring the temperature of water to about 5-10°C. Two-week old seedlings are exposed to cold water and

normal air temperature in the greenhouse for about one to two weeks or until the susceptible control begins to show advanced low temperature injuries. Preliminary small-scale tests appear promising.

b. Booting Stage: QTL associated with cold tolerance of rice at the booting stage were previously identified. The QTL were detected under controlled environment, but the effects of the QTL have not been tested under field conditions. Recombinant inbred lines (RILs) from the M-202/IR50 cross are being developed and assembled into maturity groups to study cold tolerance at the booting stage under natural field condition. A total of 931 advanced lines derived from M-202/IR50 (F_4: 302 lines with >90% fertility and 244 lines with 80% to 90% fertility, F_6: 249 lines, and F_8: 136 lines) were sent to the winter nursery in Hawaii for generation advance and seed increase. These materials will be evaluated for spikelet fertility under non-cold stress condition and the lines that have a high spikelet fertility rate will be used to assemble the different maturity groups (early, medium, and late). Field screening for spikelet sterility or blanking is effective if low temperature stress occurs approximately 10-14 days before heading. In field screening, variability in heading of test materials complicates phenotypic scoring and QTL detection. Grouping lines with similar heading improves the chances of uniformly exposing the test materials to cold stress at the same reproductive stage, thus resulting in more reliable data.

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 CRES. Staff from the experiment station were involved in harvesting tissue and gained some experience in DNA extraction from plant tissues and with DNA marker technology. Development of a high-throughput, rapid DNA extraction procedure that resulted in DNA samples that could be consistently analyzed with DNA markers was a major accomplishment of the Waxy maker project. Results of the marker analysis were in general agreement with marker analysis performed by CRES' cooperators at the USDA-ARS in Beaumont, TX, indicating that this technology can be performed effectively and more efficiently in cooperation with the USDA-ARS in Davis, CA. Further analyses to clarify observed differences and to examine more lines for the CRES breeders are expected in the coming year. Use of the Waxy DNA marker should assist breeders in making selections well before harvest and may also be used to confirm or clarify results of quality lab testing.

PUBLICATIONS OR REPORTS:

Andaya, V.C. and D.J. Mackill 2003. QTLs conferring cold tolerance at the booting stage in rice using recombinant inbred lines from a *japonica* x *indica* cross. Theor. Appl. Genet. 106:1084-1090.

Andaya, V.C. and D.J. Mackill 2003. Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J. Exp. Bot. 54:2579-2585.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

The application of molecular markers was examined in the context of three major breeding objectives for California: disease resistance, cold tolerance, and grain quality. Genetic crosses between California elite cultivars (i.e. S-102, M-202, and M-206) and a wild species *O. rufipogon* (accession number 100912) were made as a first step in developing populations for genetic analysis of stem rot and aggregate sheath spot and for generating new California germplasm with enhanced disease resistance. Marker analysis of the parental lines has resulted in the identification of a set of DNA markers that will be useful in genetic analysis. Markers tightly linked to the *Pi-z* blast resistance gene were used to assess breeding materials from the medium grain program and in a small scale marker-assisted selection experiment with the premium quality program. Efforts to identify genes conferring cold tolerance at the seedling stage were continued as additional molecular markers became available. Genetic populations for field assessment of booting stage cold tolerance continued to be developed. The Waxy gene marker was used in a small scale experiment to assess grain quality (i.e. amylose content classification) of materials from the long grain breeding program and as an "proof of concept" test for transferring marker technology to the CRES.

Table 1. Genetic crosses between California cultivars and *Oryza rufipogon* (accession number 100912). True crosses were confirmed using DNA markers that distinguish between the parents. F1 individuals will be backcrossed to the elite parental cultivar with the goal of establishing advanced backcross populations for genetic analysis. In addition, F1 plants will be allowed to self and recombinant inbred line populations will be developed via single seed descent.

Crosses Attempted	Number of confirmed F1s
S-102 / *O.rufipogon* 100912	4
O. rufipogon 100912 / S-102*	not tested
M-206 / *O. rufipogon* 100912	4
O. rufipogon 100912 / M-206*	not tested
L-205 / *O. rufipogon* 100912	0
O. rufipogon 100912 / L-205*	not tested
O. rufipogon / M-202	4

* Emphasis is being placed on crosses with the elite cultivar as the maternal parent (with the exception of the M-202 / *O. rufipogon* cross which did not yield any seeds) and those involving short and medium grain parents due to the availability of stem rot resistant long grain germplasm (e.g. 87Y550).

Table 2. Number of DNA markers per chromosome identified for genetic analysis of stem rot and aggregate sheath spot resistance derived from *O. rufipogon* 100912. These markers are able to distinguish the cultivars listed at the top of each column from *O. rufipogon* 100912 and may be used to map genes for disease resistance and other traits from *O. rufipogon*. Universal markers refer to those that detect differences between *O. rufipogon* and all four of the cultivars listed. Although M-206 has not been analyzed, it is likely that markers useful for M-202 and the other cultivars will also be useful for analyzing progeny from M-206/*O. rufipogon*.

Chromosome	**L201***	**S102**	**M202**	**L205**	**Universal Markers**
1	25	28	26	25	14
2	26	28	28	27	18
3	23	22	23	21	16
4	8	18	14	12	7
5	24	26	26	24	18
6	26	22	21	21	12
7	25	24	21	25	16
8	23	27	24	23	17
9	15	15	15	14	9
10	11	14	14	12	8
11	10	14	14	11	8
12	9	8	8	7	7
Total Markers	**225**	**246**	**234**	**222**	**150**

*L-201 is in the pedigree of the most stem rot resistant long grain germplasm currently available (i.e. 87Y550).

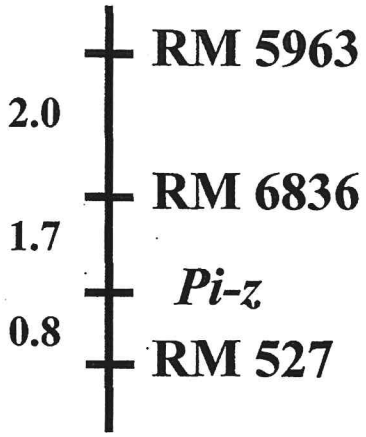

Figure 1. Genetic map of the region of chromosome 6 containing the *Pi-z* resistance gene. Numbers to the left reflect centiMorgans based on analysis of 323 F3 families derived from the cross Bengal/Maybelle (this information is courtesy of Dr. Robert Fjellstrom, USDA-ARS Rice Research Unit, Beaumont, TX).

Table 3. *Pi-z* marker analysis of various breeding lines and parents from C. Johnson indicates that three markers (RM6836, RM5963, and RM527) are in strong agreement with disease scoring (R = resistant, S = susceptible, designations of R and S provided by CRES). Marker analysis was also detected heterozygous (H) individuals (i.e. those having DNA from both the resistant and the susceptible parent).

	RM 6836	**RM 5963**	**RM 527**
total R plants analyzed	73	73	73
R plants missing data	3	2	1
R plants miscalled	4	3	10
% R called correctly	94.5	95.9	86.3
total S plants analyzed	23	23	23
S plants missing data	3	2	1
S plants miscalled	0	2	2
% S called correctly	100.0	91.3	91.3
total H called	25	20	32

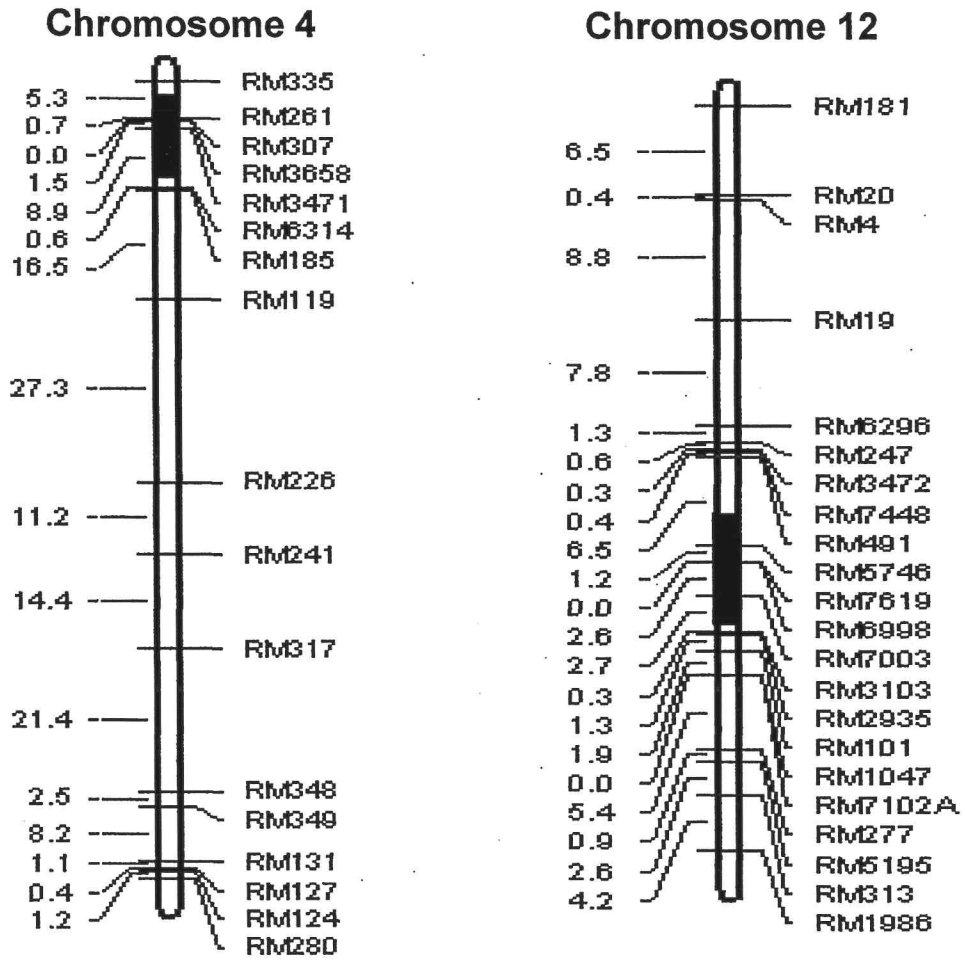

Figure 2. Location of the two major quantitative trait loci (QTL) associated with tolerance to cold-induced leaf yellowing (chromosome 4) and wilting (chromosome 12). The gene(s) at these locations are responsible for at least 40% of the phenotypic variance observed for these traits. The loci are indicated by the shaded in area of each chromosome. Rice DNA markers are noted to the right of each chromosome and genetic distances (in centimorgans) are noted to the left.