ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 1999 - December 31, 1999

PROJECT TITLE: RB-3: Rice Genetics and Germplasm Development

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LEVEL OF 1999 FUNDING: $ 15,000

OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1. **Microsatellite markers**. The objective is to explore the use of microsatellite markers using the ABI377 DNA sequencer for nonradioactive detection of microsatellite alleles. This project aims to facilitate breeding of japonica rice through use of these markers. (Level of funding: $5,000)

2. **Molecular markers for disease resistance genes**. The objective is to identify molecular markers linked to blast and stem rot resistance genes. A microsatellite or PCR-based marker is being sought for the putative stem rot resistance gene in 87-Y-550 inherited from *Oryza rufipogon*. The project aims to increase the efficiency of breeding for resistance to these diseases. (Level of funding: $ 0)

3. **Cold tolerance**. The objective is to conduct the initial mapping of cold tolerance in the cross M-202 X IR50R. The cold tolerance of this population is being reassessed in the growth chamber. (Level of funding: $5,000)

4. **Male sterile mutants and interspecific gene introgression**. The objective is to evaluate the usefulness of putative PGMS mutants for hybrid rice seed production. Populations developed from crosses with *Oryza nivara* and *O.*

glaberrima are being evaluated for agronomic traits and assayed with molecular markers in an attempt to identify useful genes. (Level of funding: $5,000)

SUMMARY OF 1999 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE

1. **Microsatellite markers for California rice cultivars**

We studied the variability found in 34 rice varieties including California japonica cultivars and indica cultivars (Table 1). All the cultivars could be distinguished with a relatively small number of these DNA markers. The Japanese cultivars Akitakomachi and Koshihikari were distinctively different from the California medium grains. Interestingly, in the entire sample, markers on chromsomes 1 and 2 were less diverse (polymorphic) than markers on chromosomes 10 and 11 among japonica cultivars. This was not observed for the indica cultivars. The dataset is being supplemented with additional varieties. This dataset will form the basis for genetic studies in japonica rice, specifically in the California cultivars.

2. **Molecular markers for disease resistance genes**

We previously identified an AFLP marker linked to stem rot resistance in the germplasm 87-Y-550. Unfortunately, this marker could not be applied in medium grain populations. The marker was mapped on rice chromosome 2 in a separate mapping population. We attempted to identify additional markers linked to this marker that could be used in medium grain populations. However, none of the markers we tested were variable in the medium grain populations. This variability is required for genetic studies. We therefore attempted to identify microsatellite markers linked to stem rot resistance. We found two markers closely associated with resistance in the long grain populations (Fig. 1). These markers were mapped on rice chromosome 3. It is not clear if this represents a second gene in this population. Unfortunately, these markers were not segregating in the medium grain populations, so we could not use them to map resistance in these populations.

We conducted surveys of California varieties and blast resistance donors with molecular markers to determine if they could be used to select for resistance. The data are still being analyzed in this experiment. Five crosses were made at Biggs between Egyptian japonica cultivars with blast resistance and California cultivars. F_3 seeds were harvested in 1999. One population was screened for resistance. This will be used to find a molecular marker that can be used to select for resistance.

3. **Cold tolerance**

Low temperature effects in plants take on many forms such as poor germination, necrosis, wilting, leaf yellowing or pollen and seed sterility. We studied the genetics of tolerance to cold-induced injury as measured by leaf necrosis and recovery from stress at the seedling stage. One of our main objectives was to identify quantitative trait loci (QTL), using molecular markers, that are responsible for cold tolerance of rice at the seedling stage.

A cross between a tolerant japonica variety M-202 and a susceptible indica variety IR50 was made to constitute a mapping population of 198 F_2 plants. The F_3s from each F_2 plant were used for cold tolerance screening at two stress regimes. Twelve seeds from each F_3

family were planted in plastic pots and placed in a growth chamber with the following settings: 20 °C /25 °C night/day temperature and 12 h light period. At the 3-leaf stage, the chamber was adjusted to 9°C /11°C night/day temperature to start the cold treatment. The chamber used for 6°C treatment were set as follows: 6°C /8°C night/day temperature and 10 h light period. At 7 and 12 days of treatment at 6°C and 9°C, respectively, we counted the number of necrotic plants in each family. The plants were then placed in the greenhouse for 10 days after which we counted the number of plants that were able to recover per F_3 family. The distribution of F_3 families classified according to percentage normal plants and plants that were able to recover after the stress treatments is found in Fig. 2. In both treatments, IR50 was severely necrotic and unable to recover after the stress while M-202 was slightly affected and recovered well.

A linkage map comprised of 62 microsatellite and 3 RFLP markers was constructed using the DNA extracted from the F_2s. In Fig. 3, we identified a major QTL controlling tolerance to cold-induced necrosis which is close to an RFLP marker RZ397 in the short arm of rice chromosome 12. The interval between the two closest markers (RM247-RG341 ~ 23 cM apart) flanking RZ397 on average explains 60% of the phenotypic variance when the data from 6 °C screening temperature was used. Another major QTL for recovery from stress was identified in the area closest to an RFLP marker RG543, potentially explaining 66% of the phenotypic variance at LOD = 13.6. For these cold tolerance-related traits, screening at more severe temperature seems better in discriminating between families. We have tried screening the materials at 13°C for three weeks but the effects of those loci are not as dramatic. So far, this is the first report of QTLs for seedling tolerance being mapped on chromosome 12.

4. Male sterile mutants and interspecific gene introgression

Male sterile mutants

We have been studying the behavior of male sterility genes in the effort to identify those that are sensitive to photoperiod and can be used in hybrid rice seed production. The mutants identified exhibit marked differences in sterility when grown at Davis (more sterile) and Hawaii (more fertile). However, based on data collected this season, it appears that the pollen of these mutants is fertile. Therefore, we suspect that these mutants may be female sterile. If this is the case, they will not be useful for hybrid seed production, but might be more suitable for basic genetic studies. We will conduct some additional studies this year to confirm our findings, but work on the male sterile mutants will be phased out over the next year.

Interspecific crosses

This experiment involves the analysis of genes in the wild species *Oryza nivara* as a source of useful traits for introduction into the California cultivar M-202. Development of the initial population was very laborious and involved crossing this wild species with M-202, making a backcross of the F_1 to M-202, and then backcrossing individual BC_1F_1 plants again to M-202. The resulting BC_2F_1 plants were selfed for three generations and then studied for agronomic traits. Plants were analyzed for yield and yield components in 1998 and 1999 in the field at Davis. Additional data collected included seedling vigor traits, grain dimensions and weight, and heading date.

The molecular analysis of this population is nearly complete. Fifty-six microsatellite markers have been analyzed on the 198 families. A preliminary analysis of this data has revealed that QTL can be identified for most of these traits. This analysis should be

completed in 2000. Breeding lines with useful characteristics are being increased in the Hawaii winter nursery.

Gift of herbicides from United Agri Products is gratefully acknowledged.

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CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

We have collected useful information on use of microsatellite markers for genetic studies and DNA fingerprinting of California rice varieties. This will be supplemented in 2000 with additional markers received from Cornell University and Dupont. Our results indicate that these markers are extremely useful for applications in japonica rice breeding. We hope to apply these markers to selecting for disease resistance (stem rot and blast) in the near future. The work on cold tolerance indicates that this complicated trait can be influenced by major genes. It will be interesting to observe the effect of the major gene identified on chromosome 12 when it is transferred into otherwise cold-susceptible varieties, such as indica varieties. As yields increase to higher levels, it becomes increasingly difficult to make further advancements. Breeders must look to new sources for genes that enhance yield and its associated agronomic traits. The use of wild species in improving cultivated rice has been demonstrated in the past for resistances to diseases and insects. Our preliminary results indicate that these species can also be of use in improving agronomic traits. This new variability introduced into cultivated rice should provide valuable genes that otherwise would not be available to rice breeders.

Table 1. Microsatellite alleles for California cultivars and some exotic cultivars for microsatellite markers on four rice chromsomes. All cultivars can be differentiated with this set of markers. Different numbers within a column represent different alleles (forms) of that particular marker.

Cultivar	Alleles for microsatellite markers on specific rice chromosomes																																			
	Chromosome 1												Chromosome 2									Chromosome 10												Chr 11		
IR40931				1	13	8	7	3	8	9	3	3	8	14	5	15	4	4	14	1	1		4	2	14	1	19	4	9								
M-103	6	7	4	5	4	1	1	3	8	7	11	3	3	8			4	2	3	1	6		14	14	9		7	6	7								
M-201	4		4	5	4	1	7	3		10	10	3	3	8	5	3	4	1		2	3		3	13	9	9	7	4								
M-202	4	10	4	5	4	1	7	3	8	9	10	3	3	8	6	3	4	1	1	2	3	1	8	13	9	6	20	9	11							
M-203	4	10	4	5	4	1	7	3	10	10	10	3	3	8	5	3		2	1	2	3		8	13	9	6	20	9	11							
M-204	4		4	5	4	1	7	3	8	10	10	12	3	8	6	3	4	1	1	2	3	1	8	13		6	2	9	10							
M-401	4		4	5	3	1	7	3		9	10	10	3	8	5	3	10	1	1	2	3	1	8	13	9	6	20	8	10							
Koshihikari	1	10	4	5	3	9	7	3	7	5	11	3	6	8	6	3	4	1	1	4	3	1		14		6										
Akitakomachi	1		4	5	4	9		3	8	5	11	3	6	1	6	3	4	1	1	4	3		8	13	11	6	2	9								
Italica Livorno	1	9	4	5	5	10	7	3	9	7	11	8	6	8	5	3	4	1	3	4	1	1	4	13	11	6	2	6								
WC 1403		10	4	5	4	11	7	3	11	9	11		3	8	5	3		1	1	1	3		7	13	9	6	4									
L-202	5	7	4	5	4	1	7	3		9	11	3	3	8	6	3	4	1	1	4	5	1	17	2	2	10	21	9	6							
L-203	5	7		5	3	1	7	3	9	9	11	3	3	8	5	3	4	1		4	3	1		13	2	10	21	9	6							

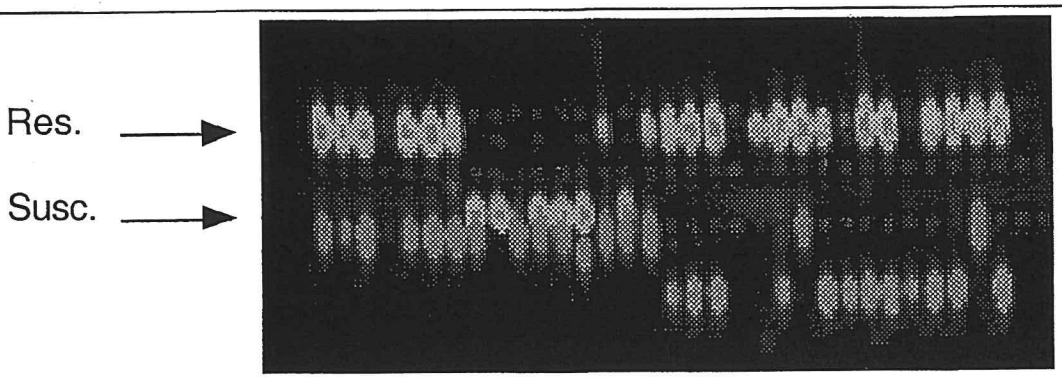

Fig. 1. Microsatellite marker RM 232, showing association with stem rot resistance in long grain crosses.

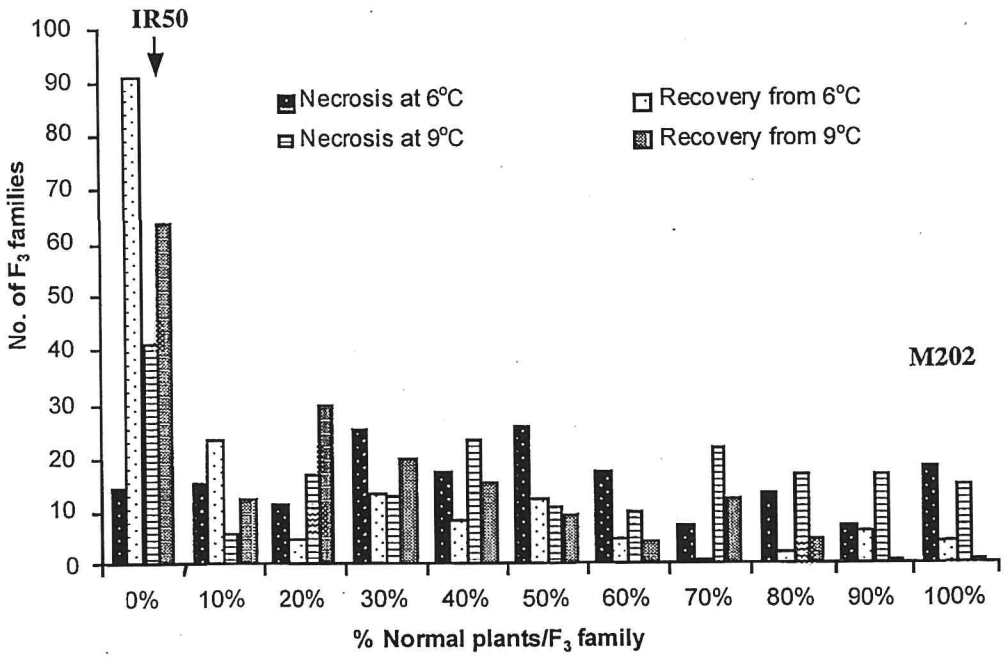

Figure 2. Distribution of % unaffected plants per F_3 family in terms of cool-temperature induced necrosis at 6oC and 9°C temperatures and % recovery of plants per family on those temperature regimes.

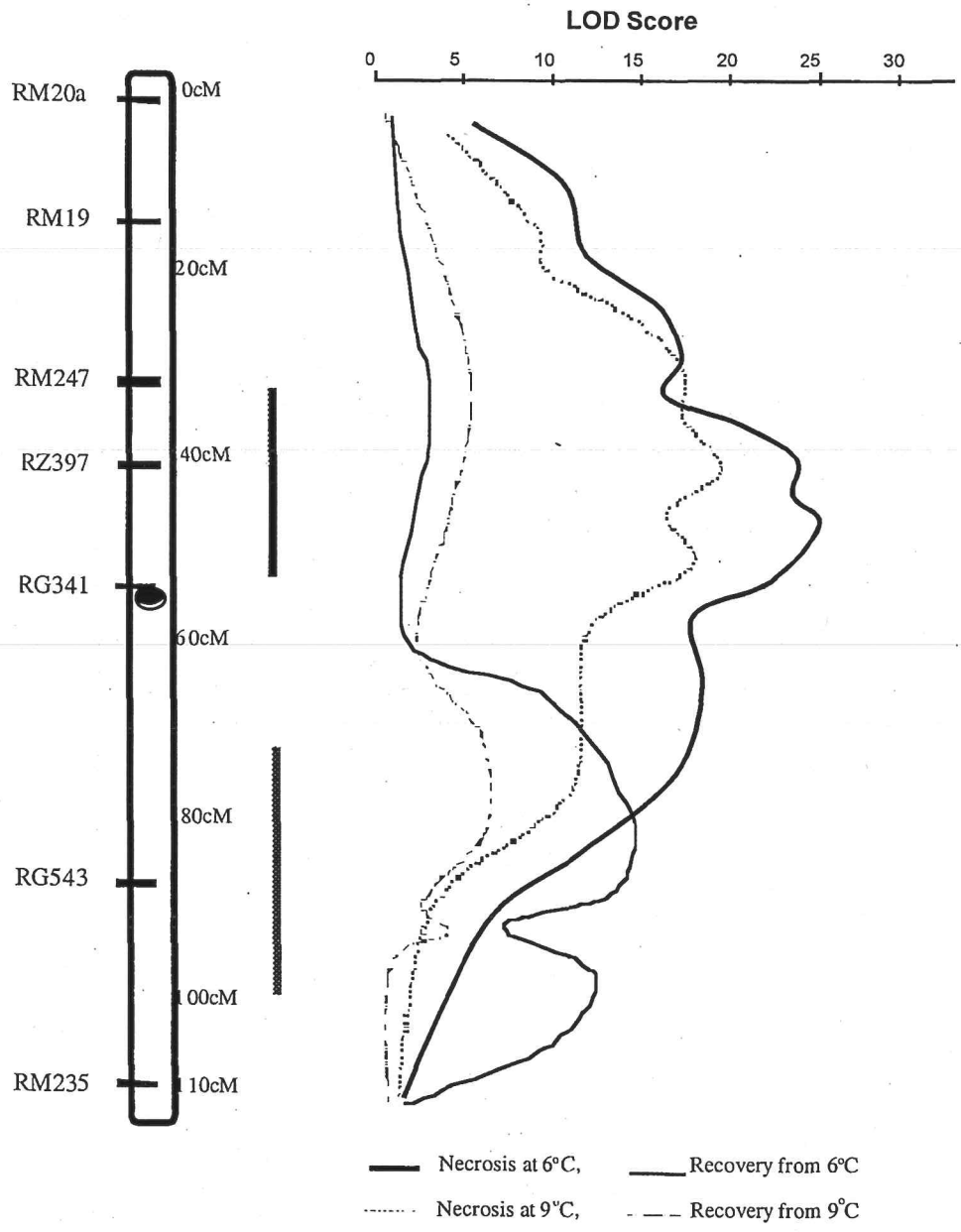

Figure 3. The LOD scores for cool-temperature induced necrosis at, and recovery from, 6°C and 9°C temperatures are plotted against all markers spanning about 110 cM of rice chromosome 12.