ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 1996 - December 31, 1997

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

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1. **Rice Genetic Resources.** Objectives are to maintain and evaluate a diverse set of rice varieties and wild species, import useful new germplasm and introduce useful traits into California varieties.

   1997 Experiments:
   1. Characterization of California rice cultivars with RAPD and microsatellite markers.
   2. Evaluation of DNA sequencer for fingerprinting and genetic analysis of California rice cultivars.
   3. Development and propagation of interspecific rice populations.

2. **Identification of useful genes.** The main strategy is to use DNA markers to "tag" important genes. The markers are linked closely to the genes of interest, and their chromosomal location is known or can be easily determined.

1997 Experiments:
1. Stem rot resistance.
2. Seed dimension traits.
3. Submergence tolerance.
4. Cold tolerance.
5. Heat tolerance.

3. **Hybrid rice**. Hybrid rice production has been spreading in Asia, and interest has grown in the US and elsewhere. Our research focuses on developing improved genetic mechanisms of seed production so that the efficiency of hybrid rice can be increased.

1997 Experiments:
1. Evaluation of the photoperiod response of putative male sterile mutants in an attempt to identify photoperiod sensitive genetic male sterile (PGMS) mutants.

SUMMARY OF 1997 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE

1. **Rice genetic resources**

Use of RAPD markers on California rice cultivars. In previous seasons, we used RAPD (random amplified polymorphic DNA) markers to characterize the genetic variability of California rice cultivars. We then applied RAPD markers to mapping genes for seedling vigor in a cross between two japonica cultivars. In order to determine how consistent the differences in RAPD markers were between different seed samples of the same cultivar, we compared three to five samples of five rice cultivars for a set of RAPD primers that had been used to successfully differentiate cultivars. The seed samples were taken from breeders' seed, yield trials, and from certified seed. Results for a subset of the markers included are shown in Table 1. For some markers, seed samples within a cultivar showed differences. For example, a single source of M-201 differed from the other samples for primer C11 (Table 1). A number of primers were consistent across all seed samples within cultivars. With a set of three primers (e.g. A8, D8, E4) we could reliably differentiate L-203, M-201, M-202 and M-401. M-203, a mutant of M-401, could not be distinguished from its parent cultivar with any of the primers. In the future, the primers identified could be used to reliably distinguish commercial cultivars.

Evaluation of the DNA sequencer. We recently acquired a DNA sequencer from USDA-ARS. The purpose of this machine is the increase our ability to perform gene mapping and marker assisted selection. Our initial emphasis will be on microsatellite markers. These markers are highly polymorphic in rice cultivars, and will be the markers of choice for marker-assisted selection. After many experiments we were able to optimize the procedures to reliably detect microsatellite markers in California rice cultivars (Fig. 1). With three fluorescent dyes, at least 9 markers can be detected in a single gel of 36 rice cultivars or plants. The data is automatically transferred to a computer and can be analyzed directly by genetic mapping software after the gel is run.

Development and propagation of interspecific rice populations. An interspecific "advanced backcross" population between *Oryza nivara* and M-202 was developed in previous years. In 1997 we grew these plants in the field for initial evaluation and advancing the generation. Plants were selected based on agronomic characteristics and propagated in the greenhouse. Tissue samples will be collected during the winter for DNA extraction and seed harvested from the field will be used for a replicated experiment in 1998. In addition, a new cross was made between *Oryza glaberrima* and

M-202. The glaberrima rices are characterized by a high competitive ability against weeds. The F_1 plants were grown in 1997 and a backcross will be made in early 1998.

2. **Identification of useful genes**

Stem rot resistance. Last year we reported the detection of AFLP (amplified fragment length polymorphism) markers linked to resistance for stem rot. We used populations derived from 87-Y-550 developed by Dr. S. T. Tseng and screened for stem rot resistance by Jeff Oster. The resistance is derived from an accession of the wild species *Oryza rufipogon.* This year we expanded our analysis and were able to identify a single marker that explained 50% of the observed variability for resistance in the populations we scored. In one population, the marker was completely associated with stem rot resistance (Fig. 2). This marker was mapped on rice chromosome 2, between the RFLP markers RZ166 and RG139 (Fig. 3). Identifying the map position of this marker will allow us to identify other markers that can be used to screen for this gene. These markers should allow selection for stem rot resistance without the need for laborious screening with the pathogen in each generation.

Seed traits. We mapped genes controlling panicle size and seed size and shape in a population previously used for mapping genes for seedling vigor. QTL (quantitative trait loci) controlling these genes were identifid (Fig. 4). Of particular interest are: (1) a gene for increased panicle size (adding 26 grains per panicle average) on chromosome 3 and (2) loci for grain length and breadth on chromosomes 3 and 7. The loci for grain length and breadth on these two chromosome explain about half of the variablity for grain shape in this population.

Submergence tolerance. The postion of the submergence tolerance locus on chromosome 9 was further refined. In addition, we made a third backcross to transfer this gene into the cultivar M-202. First backcross progeny were evaluated in the field at Davis and Biggs, and in the Hawaiian winter nursery. A few of these lines had improved plant type but they are still relatively unadapted to California conditions. The BC_3F_1 plants will be grown in the greenhouse in 1997-98 to produce F_2 plants for field selection in 1998.

Cold tolerance. We performed the first screen of the population for mapping cold tolerance genes in 1997. Two temperature treatments were used: 14 C and 12 C for five days at the booting stage. Both treatments resulted in lower pollen and spikelet fertilities than the control plants, with the 12 C treatment showing the lowest values (Fig. 5). These plants will be ratooned, replanted and evaluated again under low temperature to obtain a more reliable estimate of cold tolerance. DNA was extracted from all the F_2 plants, and molecular marker analysis is currently underway.

Heat tolerance. A small experiment was conducted to evaluate heat tolerance of California cultivars. Maximum temperatures above 40 C, which occur frequently in California, are known to cause spikelet sterility during the heading stage. N22, an indica cultivar, was used as the heat tolerant check. Surprisingly, S-102 had the highest heat tolerance by far. M-202 also had superior heat tolerance to the check, but L-204 had low tolerance. Previous results indicate that heat tolerance is not necessarily related to geographic origin. These results indicate that California cultivars probably have sufficient heat tolerance for most years.

3. **Hybrid rice**

Photoperiod-sensitive genetic male sterility. In previous years, we have identified male sterile mutants that appear to show increasing sterility under long daylength. This character, designated pgms (photoperiod sensitive genetic male sterility), has been used in China to produce seed for hybrid rice production. Last year an experiment was performed at different planting dates to measure the change of sterility for these mutants. In contrast to commercial cultivars, which show decreased fertility at later planting dates, some of the mutants show increased fertility at later dates (Fig. 6). We are currently evaluating the pollen and spikelet fertility of plants grown in 1997 to determine if the same pattern occurs. Spontaneous mutants which were isolated from a grower's field have been evaluated at Davis and Biggs under long day conditions and in Hawaii under short day conditions. Several of these mutants showed differential reaction under the two daylengths. The mutant M204-MS475 appeared particularly promising, showing sterile plants under long days but partial to complete fertility under the short days in Hawaii. However, in the growth chamber, this mutant appeard to have fertile pollen under long day conditions. The more promising of these mutants are being evaluated in the Hawaiian winter nursery.

Gift of herbicides from United Agri Products is gratefully acknowledged.

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Inukai, T., R. J. Nelson, R. S. Zeigler, S. Sarkarung, D. J. Mackill, J. M. Bonman, I. Takamure, and T. Kinoshita. 1996. Genetic analysis of blast resistance in tropical rice cultivars using near-isogenic lines. p. 447-450. *In* Khush, G. S. (ed.) Rice Genetics III. International Rice Research Institute, Manila.

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CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

Signficant progress this year was made on genetics of stem rot resistance. The identification of a molecular marker on rice chromosome 2 opens up exciting posibilities for imroving the efficiency of selection for stem rot resistance in the rice breeding program at Biggs. Hopefully, this discovery will be translated into a practical marker assisted selection program in the coming year.

The aquisition of a DNA sequencer will greatly facilitate the ability of our lab to map important genes and apply this technology to a practical breeding program. Increased automation is essential to more widescale application of molecular markers in rice improvement. The timely development of a genetic map based on a new DNA marker (called microsatellite marker) will greatly facilitate the use of this information for breeding japonica rice.

New germplasm being developed includes submergence tolerant rice and male sterile mutants with potential use in hybrid rice seed production. This germplasm will be evaluated more widely in the coming year.

Table 1. Representative RAPD fingerprint patterns for California cultivars from different sources. RAPD primer C11 shows a difference in one seed sample (B) for M-201. Otherwise, all primers here show the same pattern within a cultivar.

		L-203				M-201					M-202					M-203			M-401				
Primer	Size	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	A	B	C	D
A8	800	0	0	0	0																		1
C7	500	0	0	0	0						1	0	0	0	0								1
C11	650					1		1	0	0													1
C11	750	0	0	0	0		1				1	0	0	0	0	0	0	0	0	0	0	0	0
D6	2000	0	0	0	0						1	0	0	0	0	0	0	0	0	0	0	0	0
D8	1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0								1
E4	900										1	0	0	0	0	0	0	0	0	0	0	0	0

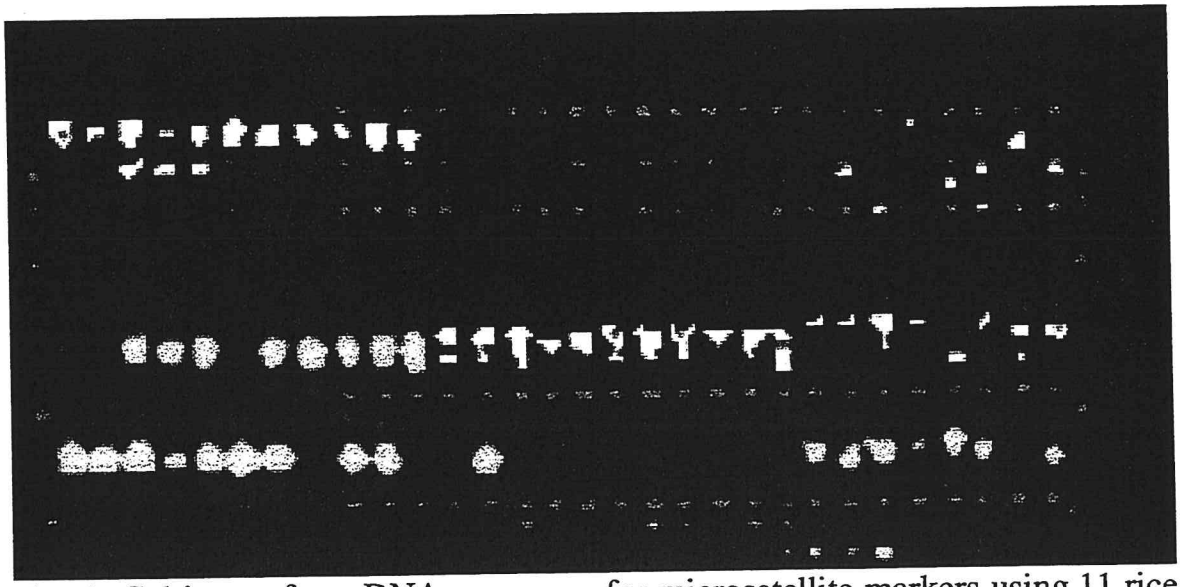

Fig. 1 Gel image from DNA sequencer for microsatellite markers using 11 rice cultivars. At least 10 markers can be run per lane using 3 different dyes and different sized markers.

Marker S10/G19$_{170}$ explained almost 50% of the phenotypic variation for stem rot resistance in four crosses

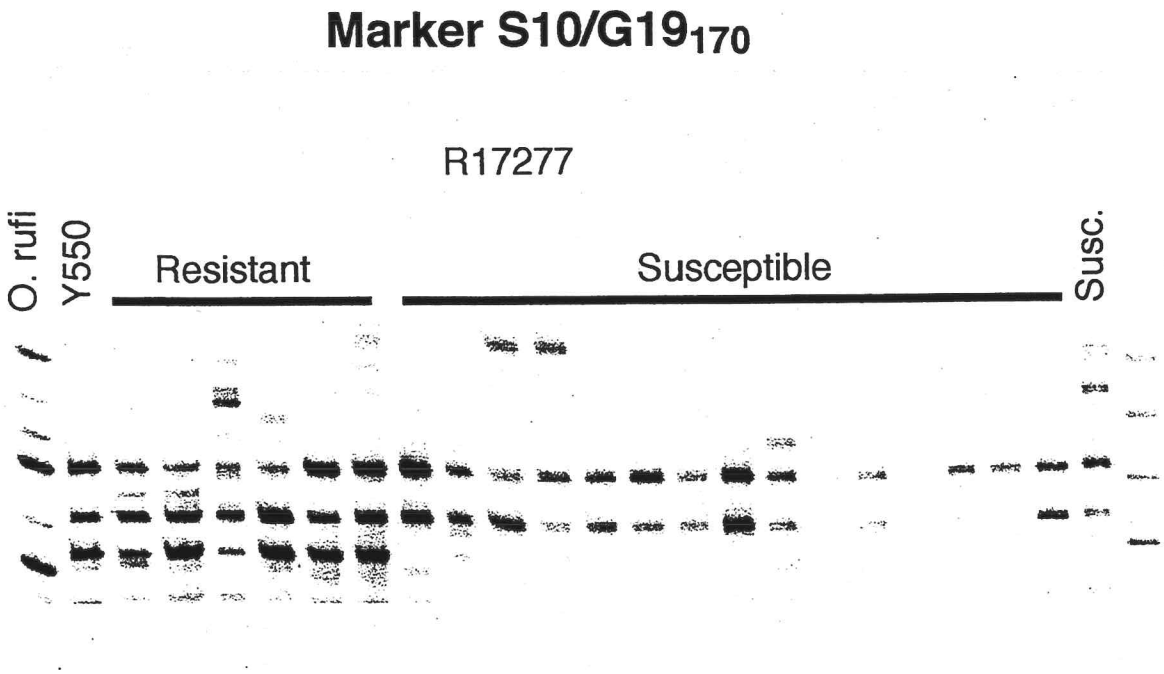

Fig. 2. Bands from the AFLP marker S10/G19-170 are associated with stem rot resistance in the recombinant inbred lines from the cross R17277. The source of resistance is 87-Y-550, which inherits its resistance from *Oryza rufipogon*.

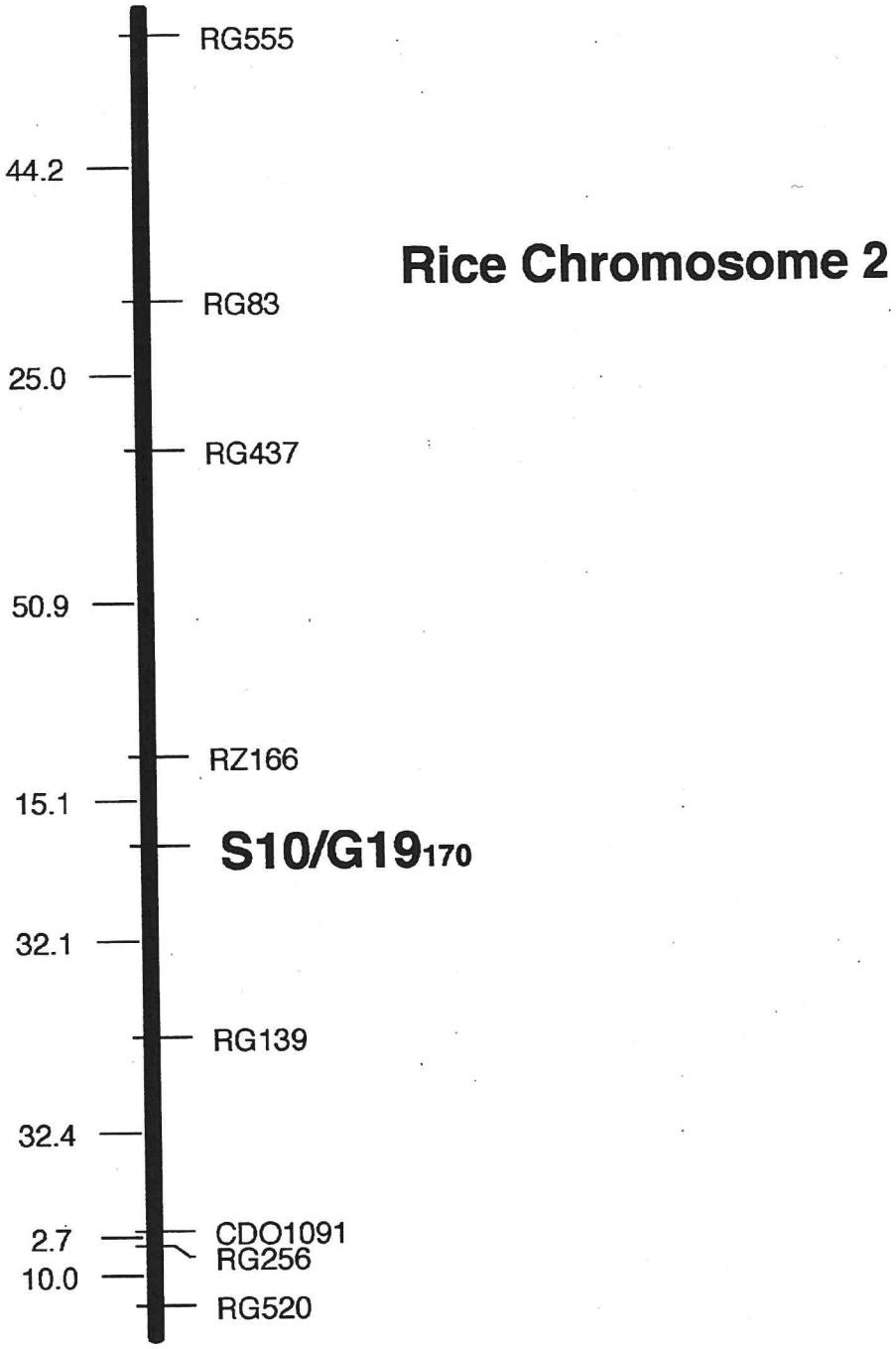

Fig. 3. Map of rice chromosome 2 showing the location of themarker S10/G19-170 which is linked to stem rot resistance.

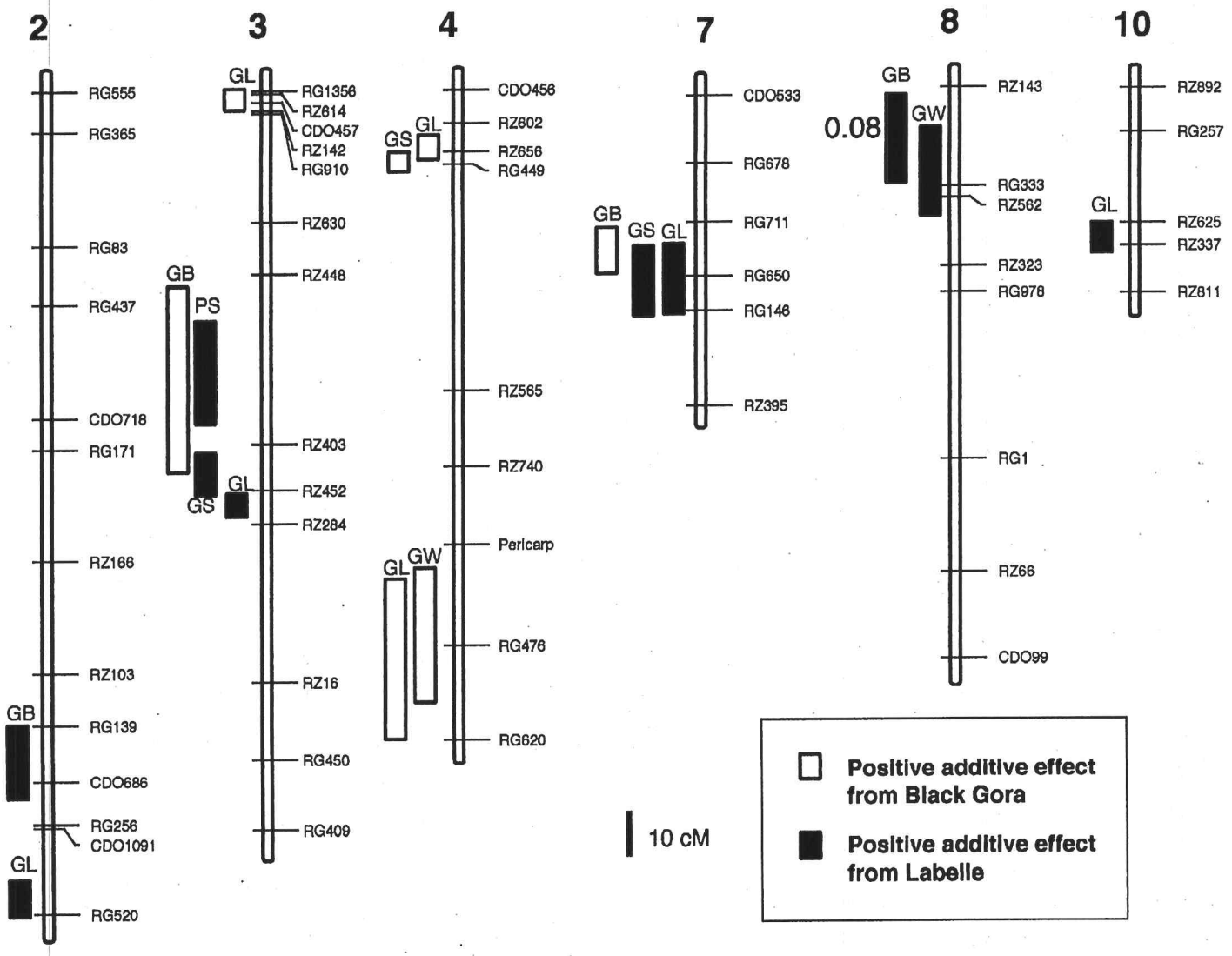

Fig. 4. Genetic map of the cross Labelle X Black Gora showing location of genes controlling seed dimensions and panicle size.

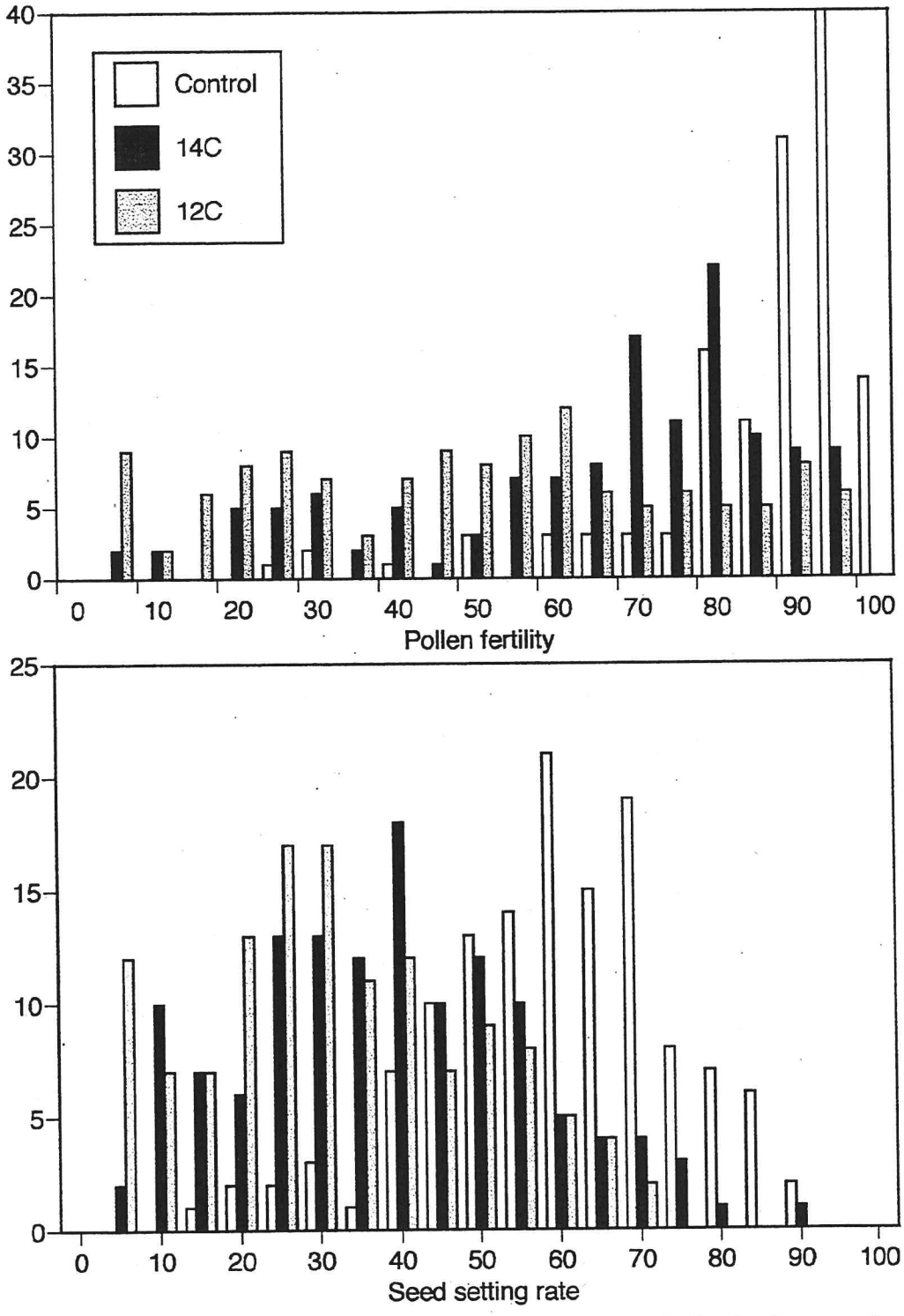

Fig. 5. Segregation for pollen fertility and spikelet fertility in the mapping population M-202 X IR50R. F2 plants were treated under low temperature in the growth chamber.

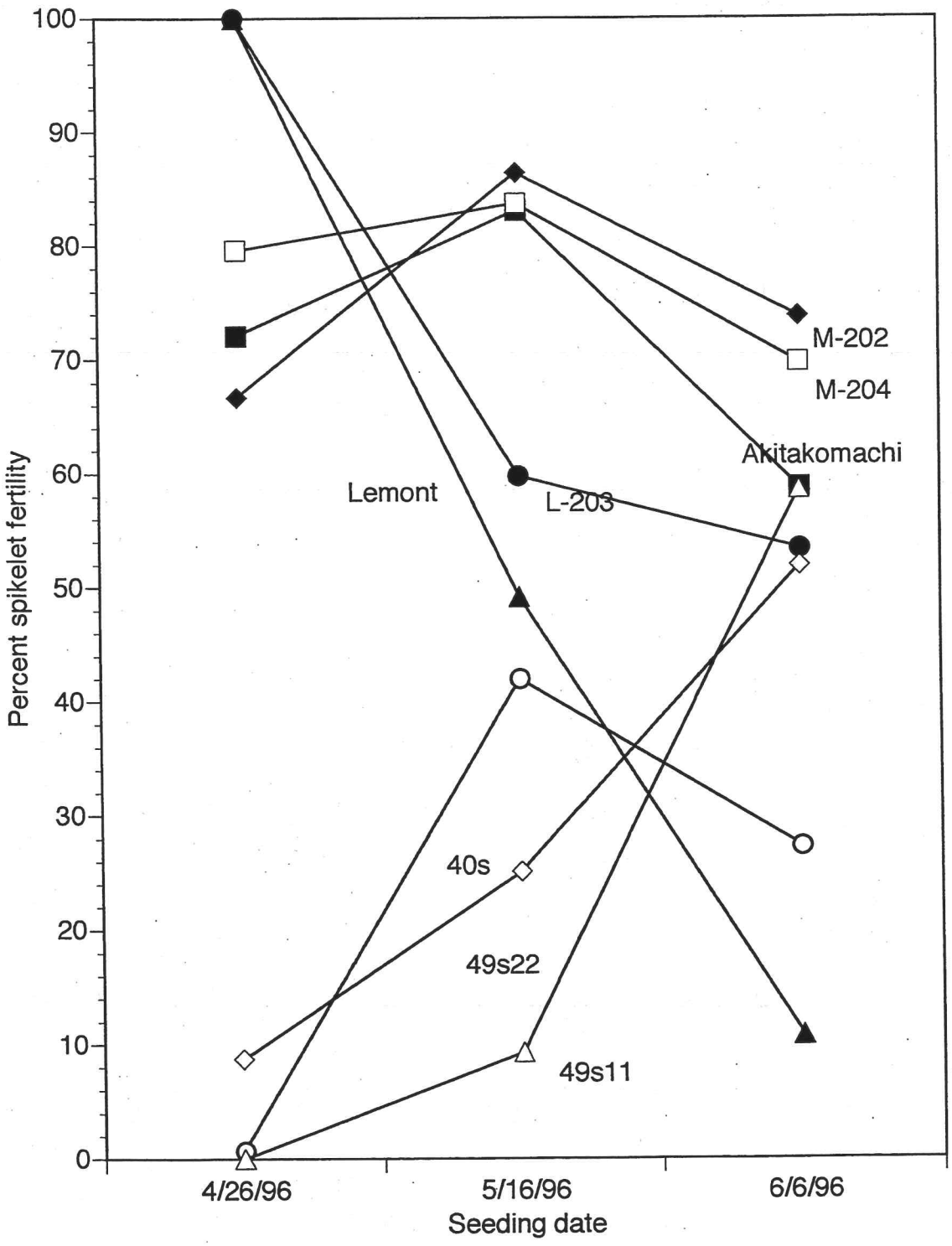

Fig. 6. Spikelet fertility of cultivars and putative pgms mutants at three seeding dates. Davis, 1996.