ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 1994 - December 31, 1994

PROJECT TITLE: Rice Genetics and Germplasm Development

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LEVEL OF 1994 FUNDING: $ 35,000

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.

   1994 Experiments:

   1. Crosses were made between California japonica cultivars and exotic varieties/species to introduce useful genes and new genetic variability.

   2. Variability for RAPD and AFLP markers within and between California varieties was assessed.

2. **Identification of useful genes**. The main strategy we use for identifying useful genes 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.

   1994 Experiments:

   1. Genetics of seedling vigor.
   2. Genetics of submergence tolerance.
   3. Genetics of stem rot resistance.
   4. Genetics of cold tolerance.
   5. Genetics of water weevil tolerance.

3. **Hybrid rice**. Hybrid rice production has been spreading in Asia, and interest has grown in the U.S. Genetic mechanisms have now been developed for commercial hybrid rice production. Whether these will be viable in California depends on the cost of seed production and the availability of a hybrid with sufficient yield advantage and acceptable grain quality. Our research focuses on genetic mechanisms of seed production.

   1994 Experiments:

   1. Transfer of cytoplasmic male sterility (cms) and restorer genes into California cultivars.
   2. Transfer of wide compatibility genes into California cultivars.
   3. Development of photoperiod-sensitive genic male sterility (PGMS) for hybrid rice seed production.

SUMMARY OF 1994 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE

1. **Rice genetic resources**

The number of exotic lines evaluated in 1994 was reduced from 1993. Most work concentrated on utilization of lines previously identified. Thirty-seven new crosses were made during the year. Of these, ten were crosses with tropical indica types made to introduce genetic variability into California cultivars. An additional ten were made with exotic japonica cultivars. Last year a cross was made between M-202 and and accessikon (no. 100195) of the wild species *Oryza nivara* (this species is closely related or identical to *O. rufipogon*). The objective was to study introgression of genes affecting agronomic characteristics from an "AA" genome species. The F_1 of this cross was completly male sterile and was backcrossed to M-202. Individual backcross F_1 plants are being backcrossed again to M-202. So far, 15 second backcrosses have been made. Additional BC_1F_1 plants were also produced. The goal is to obtain about 100 BC_2 lines to study the effect of genes from *O. nivara* on agronomic traits.

The survey of U.S. rice cultivars with RAPD (random amplified polymorphic DNA) markers was completed in 1994. The results showed that the popular California cultivars could be differentiated by several bands produced by these markers (Table 1). A new study was initiated to determine whether these markers would be useful to distinguish California cultivars from different seed sources. Several seed samples were collected for five cultivars (Table 2). When plant tissue from several plants were bulked within each seed source, the RAPD patterns for 5 markers agreed with previous results. Thus, these markers seem to provide a reliable means of differentiating cultivars.

We tried a new extraction protocol for small tissue samples using individual plants from the same seed sources (Table 2). The results indicated that individual plants within samples had some variation for the molecular markers used in the above study. It is not clear yet whether these differences are true genetic differences or are artefacts of the method of DNA extraction.

We attempted to use a new type of marker, termed AFLP (amplified fragment length polymorphism) to identify differences between cultivars. The level of polymorphism (genetic differences) was very high for AFLP markers. Unfortunately, there appeared to be differences that were due soley to the method of DNA extraction. We are currently following up these studies to determine if AFLP markers will be useful for DNA fingerprinting.

2. **Identification of useful genes**

Seedling vigor. The F_3 population of the cross betwee Black Gora (indica, high vigor) and Labelle (japonica, low vigor) was anayzed for seedling vigor-related traits (Table 3). RFLP markers were used to identify the QTLs (quantitative trait loci) controlling these characters. QTLs for shoot length, root length, coleoptile length and mesocotyl length were identified at both 18 C and 25 C (Table 4, Fig. 1). Only four of the 16 QTLs identified were expressed at both temperatures. The unusual finding of this study is that, while Black Gora was superior to Labelle for all traits at both temperatures, no positive QTLs were identified in it for shoot length. While it appears to be a good parent based on phenotypic data, this genetic study indicates that it would not be particularly desirable for inroducing useful genes for seedling vigor into japonica cultivars. It does, however, possess some desirable genes for root growth.

Submergence tolerance. Submergence tolerance may be a useful trait for rice varieties if deep water is used at seeding as a method of weed control. Sources of tolerance have been identified and transferred into improved indica lines, but the genetics is not well understood. We are now tagging the genes controlling submergence tolerance. This should assist us in transferring these genes into California japonica cultivars.

We used an indica breeding line, IR40931 as the tolerant parent. This line derives its tolerance from the traditional cultivar FR13A. It was crossed with PI543841 (an induced male sterile mutant of Calrose 76). Ten-day old F_3 seedlings were subjected to 14-16 days of submergence. Individual plants were rated on a scale of 1 (no damage) to 9 (completely dead). The japonica parent PI543851 had a mean score of 8.5 compared to 1.5 for the tolerant parent. Segregation data (Fig. 2) indicated that at least two genes were responsible for submergence tolerance. We had already identified six molecular markers that are linked to the tolerance gene. Mapping the genes is underway. F_3 lines with the highest level of submergence tolerance were crossed to M-202 to transer the submergence tolerance genes into a California cultivar.

Stem rot resistance. Recombinant inbred populations for studying the genetics of stem rot resistance were developed by Jeff Oster from segregating populations supplied by S.

T. Tseng. Resistant and susceptible F6 lines were identified in each of four crosses (Table 5). We are now surveying the parents for polymorphism, and plan to begin RFLP mapping of the resistance genes soon.

Cold tolerance. Studies on the genetics of cold tolerance were begun in the population M-202 X IR50R. This F_2 population was evaluated for spikelet sterility under normal greenhouse temperatures. Because it is a japonica X indica cross, there was considerable genetic sterility. Plants with more than 50% fertility were selected to study tolerance to low-temperature-induced sterility. Mapping genes controlling cold tolerance at the seedling and booting stages is under progress.

Water weevil tolerance. Based on molecular marker polymorphism, two crosses were made for studying the genetics of water weevil tolerance. These will be advanced to a later generation before analysis will begin.

3. **Hybrid rice**

Cytoplasmic male sterility. Research focused on developing California-based germplasm with the genetic mechanisms necessary for hybrid rice production. Backcrossing of sources of cytoplasmic male sterility (cms) to California cultivars continued. Five backcrosses were completed for several lines with the WA source of cms (Table 6). These lines show approximately 100% sterility when the panicles are prevented from cross pollinating. Only too backcrosses have been made using the Chinsurah Boro source of cms, and a higher seed set rate has been observed. The source of cms from Ringaround company (CMS-RA) is not known. Crosses with most California cultivars seem to show varying levels of fertility, but a backcross with L-203 seems to have a good level of sterility (Table 6).

R (restorer) genes, are necessary to take advantage of the cms system of seed production. R genes for the WA cytoplasm are not present in japonica rice, so we are introducing these genes from indica varieties. We have begun making test crosses to determine which lines carry these genes.

Wide compatibility. The wide compatibility (wc) gene may be useful for development of indica X japonica hybrid rice. It could also be useful for introducing genes from indicas into japonicas as it allows the production of fertile indica X japonica F_1 plants. We are introducing this gene into California medium grain cultivars from the japonica variety Lemont. Molecular markers are being used in collaboration with Dr. Pam Ronald.

Photoperiod-sensitive genetic male sterilility. The cms system of seed production is often referred to as the 3-line method because it relies on three components: a cms line, a restore line (with R genes) and a maintainer line to maintain the cms line. The maintainer line has a normal cytoplasm but it lacks the R genes so its progeny with the cms lines are sterile. A disadvantage of the system is that it require additional resources to maintain the sterile plants. An alternate method, referred to as the 2-line method, makes use of a conditional male sterile plant. This type of plant is sterile under certain conditions, but fertile under others. The most commonly used types are referred to as photoperiod-sensitive genetic male sterile (PGMS) mutants. These plants are sterile under longer day lengths, but produce self-pollinated seed under shorter daylengths.

Two PGMS mutants were identified previously at UCD. We studied the behavior of these two mutants in 1994. Unfortunately, neither appear to be useful for hybrid seed production. The mutant PI543851, identified from Calrose 76, is actually a "leaky" male sterile mutant. Even under long days it has self-pollination rates near 20%. The mutant ST25, identified from M-201, has the opposite behavior. It is sterile even under relatively short day lengths.

We are attempting to identify new PGMS mutants that will be suitable for California conditions. We looked for spontaneous male sterile mutants in growers' fields of M-202 and M-204 (Table 7). We also identified male sterile mutants in irradiated populations of Koshihikari and Arborio grown at Biggs by Kent McKenzie. We identifed 800 putative male sterile plants. Fertilities ranged from close to zero to over 60% (Fig. 3). Steriles obtained from the irradiated population had a higher mean sterility percentage. We took plant samples from most of the mutants and transplanted them to the greenhouse. We will take sterility data to determine those that show potential of being PGMS mutants.

PUBLICATIONS OR REPORTS:

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Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R. and Nelson, R. J. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434.

Mackill, D. J. 1994. Genetic deversity and agronomic characteristics of temperate rice germplasm. p. 41. *In* Proceedings of the Twenty-Fifth Rice Technical Working Group. 6-9 Mar 1994. New Orleans, LA. Texas Agric. Exp. Stn., College Station.

Champoux, M. C., Wang, G. L., Sarkarung, S., Mackill, D. J., O'Toole, J. C., Huang, N., McCouch, S. R. 1994. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. p. 33. *In* Proceedings of the Twenty-Fifth Rice Technical Working Group. 6-9 Mar 1994. New Orleans, LA. Texas Agric. Exp. Stn., College Station.

Wang, G. L., Mackill, D. J., Bonman, J. M., McCouch, S. R., Champoux, M. C., Nelson, R. J. 1994. RFLP mapping of complete and partial resistance to blast in a durably resistant rice cultivar. p.60. *In* Proceedings of the Twenty-Fifth Rice Technical Working Group. 6-9 Mar 1994. New Orleans, LA. Texas Agric. Exp. Stn., College Station.

Mackill, D. J., Colowit, P. M., Zhang, Z., Lai, C. 1994. DNA fingerprinting and gene mapping of California rice cultivars. p. 4-5 *In* Rice Field Day, 30 Aug 1993, Rice Experiment Station, Biggs, CA.

Fadel, J. G., Mackill, D. J. 1994. Characterization of rice straw to enhance utilization. p. 7-8. *In* Rice Field Day, 30 Aug 1993, Rice Experiment Station, Biggs, CA.

Redoña, E. D., Mackill, D. J. 1994. Mapping quantitative trait loci for seedling vigor-related traits in rice. Agronomy Abstracts 1994:211.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 1994, we made crosses to introduce new genetic variability from exotic sources into California germplasm. We also began making the backcross of an F_1 between M-202 and a wild species *O. nivara*. We confirmed the accuracy of identifying California rice cultivars with molecular markers. We obtained data indicating that samples from the breeders' nurseries and from seed growers show the same banding patterns. There was some indication of variability within samples, but it is not clear if this was due to the method of DNA extraction or actual genetic differences. We began using a new type of DNA marker, termed AFLP. This maker has problems in terms of DNA fingerprinting applications, but it appears useful for genetic mapping in crosses involving only japonica cultivars.

Quantitative trait loci (QTL) were identified for seedling-vigor related traits in the cross Black Gora (indica, high vigor) X Labelle (japonica, low vigor). Useful genes in Black Gora were identified for root length. No positive shoot length genes were identified in Black Gora. RADP markers linked to genes conferring submergence tolerance were identifed. These should be useful in transferring tolerance from indica into japonica cultivars. Populations have been developed for tagging genes conferring stem rot resistance and cold tolerance. We are beginning the genetic studies on these traits. A population for studying water weevil tolerance is being developed.

Five backcrosses have been made for transferring cytoplasmic male sterility (cms) into California rice cultivars. Initial results indicate the cms lines show stable sterility. Restorer genes are also being transferred. The wide compatibility gene is being transferred into California medium grain cultivars with the aid of a closely linked molecular marker. In 1994, we identified 800 putative male sterile mutants in the field. These are being tested for response to photoperiod, with the aim of identifying new mutants for photoperiod-sensitive genetic male sterility (PGMS).

Table 1. Number of bands difference between pairs of California cultivars. A total of 20 RAPD primers produced 43 polymorphic bands.

	Number of bands difference between varieties														
	Akit.	Kosh.	CM101	S101	S201	S301	M103	M201	M202	M203	M204	M401	A301	L202	L203
Akitakomachi	0	3	9	10	11	10	10	15	15	14	14	14	13	11	11
Koshihikari		0	6	9	8	7	7	14	12	11	11	11	12	14	12
CM-101			0	9	4	3	1	8	6	7	5	7	14	16	14
S-101				0	11	10	10	7	11	10	8	10	19	19	17
S-201					0	3	5	10	10	9	7	9	16	18	16
S-301	Short grain					0	4	9	7	8	6	8	15	17	15
M-103							0	9	7	8	6	8	15	17	15
M-201								0	6	5	3	5	22	18	16
M-202									0	5	5	5	18	18	16
M-203										0	4	0	17	17	15
M-204											0	4	19	17	15
M-401							Medium grain					0	17	17	15
A-301													0	10	8
L-202														0	4
L-203													Long grain		0

Table 2. Seed source of California rice varieties included in RAPD survey.

Sample	Variety	Source	Description
1	L-203	Biggs	92 Foundation, can
13	L-203	Biggs	HR/panicles
23	L-203	Davis	92:3269-72
24	L-203	CalCrop	Registered
29	L-203	CalCrop	Certified
6	M-201	Biggs	HR/diff pan/91Fnd
18	M-201	Davis	Box47, 86:8022
21	M-201	Davis	92:849
25	M-201	CalCrop	Registered
30	M-201	CalCrop	Certified
2	M-202	Biggs	92 Foundation, can
7	M-202	Biggs	HR/diff pan/92Fnd
19	M-202	Davis	Box47, 86:8042
26	M-202	CalCrop	Registered
31	M-202	CalCrop	Certified
12	M-203	Biggs	HR:1 seed/row
17	M-203	Biggs	92HR
27	M-203	CalCrop	Registered
11	M-401	Biggs	HR:1 seed/row
22	M-401	Davis	92:852
28	M-401	CalCrop	Registered
33	M-401	CalCrop	Certified

Table 3. Differences (mm) in seedling vigor-related traits for two parents (Black Gora and Labelle) at two temperatures.

Trait	18°C		25°C	
	Black Gora	Labelle	Black Gora	Labelle
	mm	mm	mm	mm
Shoot length	66	52	180	136
Root length	121	102	185	148
Mesocotyl length	12	8	9	3
Coleoptile length	29	23	29	23

Table 4. Quantitative trait loci (QTL) identified in the cross Black Gora X Labelle. The percent contribution of each QTL towards the total variation is indicated along with the cultivar possessing the positive allele (BG = Black Gora, Lble = Labelle, Het = heterozygote superiority).

RFLP marker	Chromosome	% of trait/parent with positive allele	
		18°C	25°C
Shoot length			
RZ730	1	16%/Het+12	35%/Het+15
RZ452	3	17%/Lble+18	16%/Lble+26
RG435	5	8%/Lble+12	
Root length			
RZ730	1	30%/Lble+26	39%/Lble+46
RG171	2	6%/BG+10	
RG450	3	8%/BG+13	
RZ811	10		8%/BG+11
Mesocotyl length			
RG811	1		13%/BG+5
RG448	3	18%/BG+7	12%/BG+7
RG13	5		9%/BG+6
RZ711	7	9%/BG+5	
RG341	12	6%/BG+3	
Coleoptile length			
RZ448	3		25%/BG+6
RG360	5	7%/Lble+3	
RG716	6		15%/Lble+5
RG971	8	18%/Het+4	

Table 5. Populations developed by Jeff Oster at Biggs for tagging genes conferring resistance to stem rot.

Cross	Total F_2 plants	Selections	
		Resistant	Susceptible
R17277	231	29	58
R17278	231	29	9
R17281	227	21	32
R17636	230	45	10

Table 6. Lines incorporating cytoplasmic male sterility (cms) into California cultivars.

Cross no.	Pedigree	cms source	Comments
DX191	Wu 10A/M-202eui//M-202	CB II	Some fertility
DX192	Wu 10A/M-202//M-204	CB II	Some fertility
DX193	IR68289A/M-202*5	WA	High sterility
DX194	IR58025A/M-204*5	WA	High sterility
DX195	IR58035A/M-204*3//M-202///M-204	WA	High sterility
DX196	IR58025A/M-202*5	WA	High sterility
DX197	CMS-RA/L-203//Michikogane///L-203*2	Unknown	High sterility

Table 7. Putative male sterile plants identified in the field during 1994.

Cultivar	Type	Location collected	Date collected	Number of plants	Mean fertility (%)
M-202	Spont.	Erdman 102G	8/18/94	101	37
M-202	Spont.	Erdman 102G	8/23/94	201	31
M-204	Spont.	Erdman 115	9/8/94	175	28
Koshihikari	Irrad.	Biggs	9/20/94	62	32
Arborio	Irrad.	Biggs	9/20/94	75	23
Koshihikari*	Irrad.	Biggs	9/25/94	186	24
Total				800	29

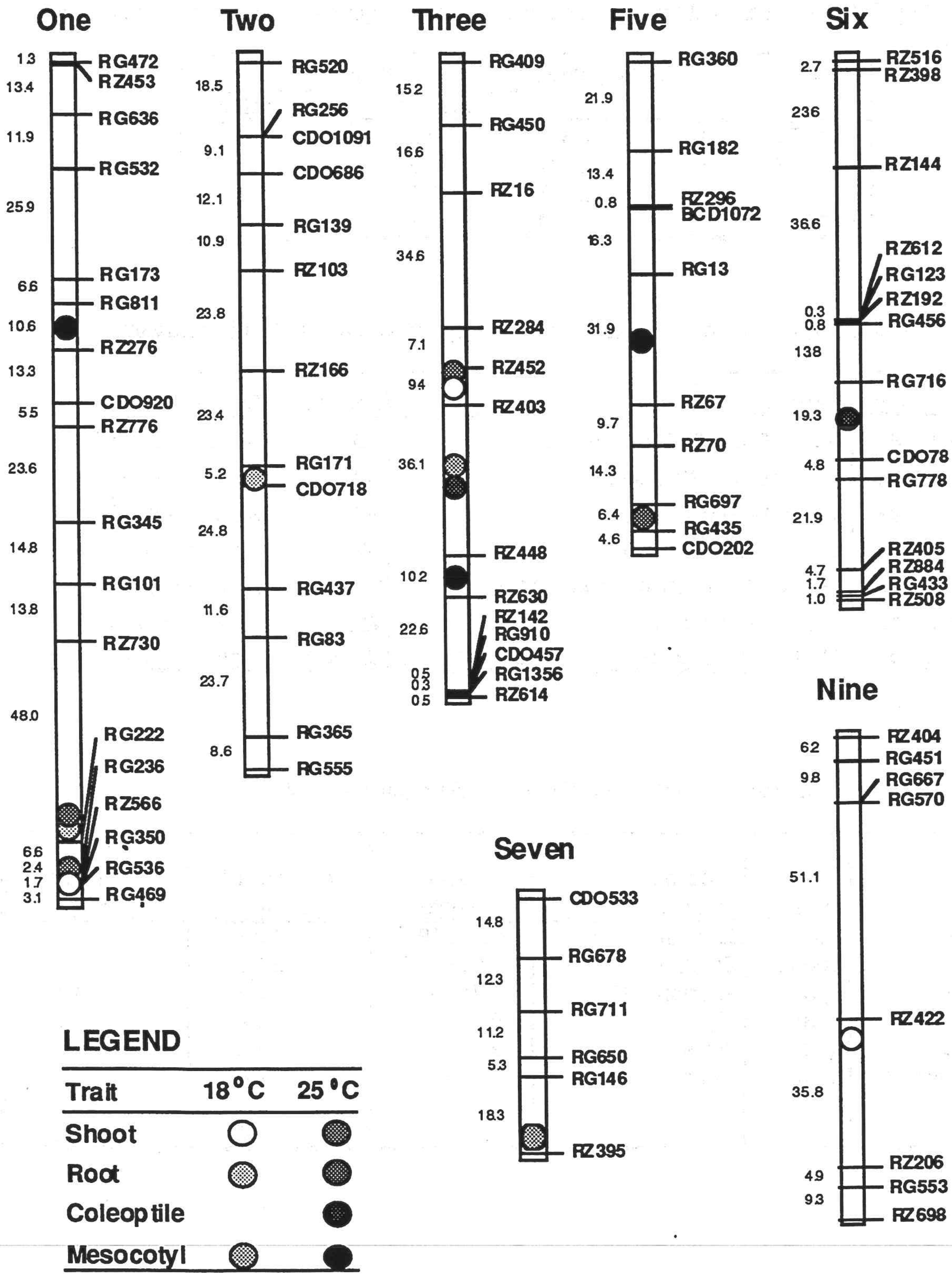

Fig. 1. Location of QTL controlling seedling vigor-related traits in the cross Black Gora X Labelle. QTL were identified in 7 of the 12 rice chromosomes.

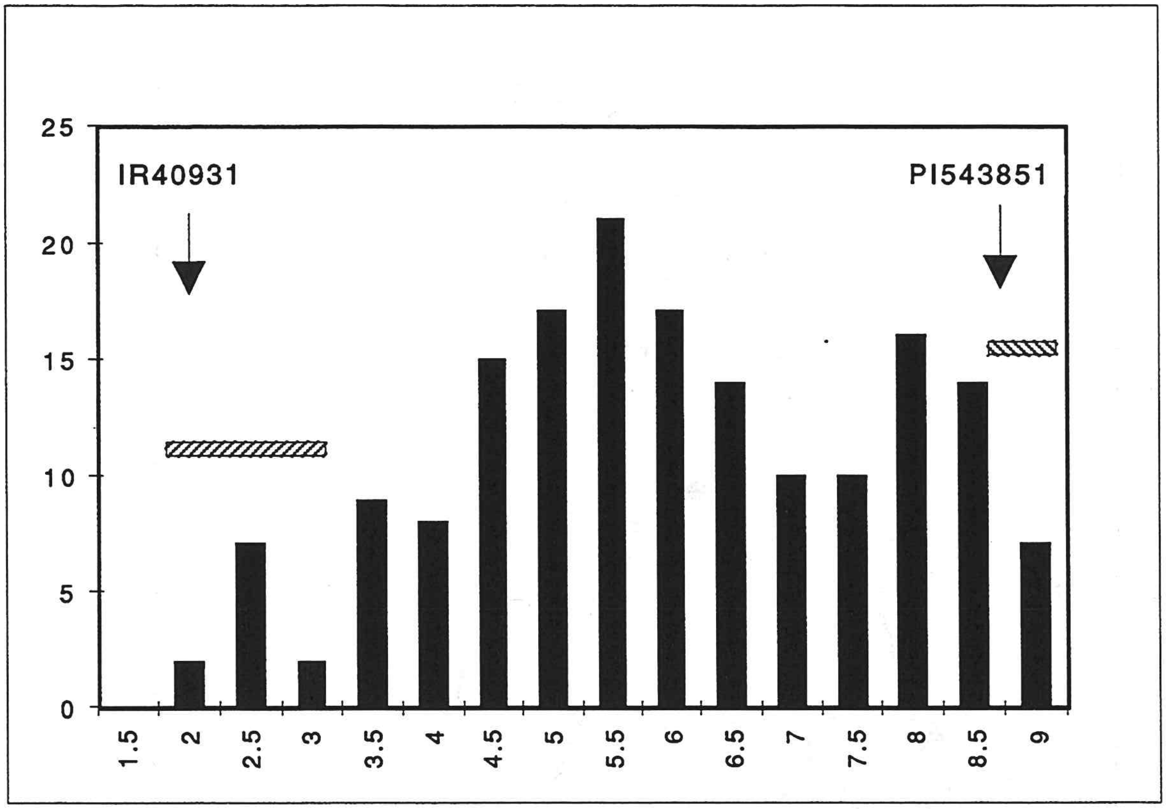

Fig. 2. Distribution of mean submergence tolerance scores for the F_3 population of the cross IR40931 X PI543851. Ten-day old seedlings were submerged for 14-16 days. Individual plants were scored on a scale of 1 = all plants alive to 9 = all plants dead. Horizontal bars are the most resistant and susceptible F3 lines that were used in identifying linked molecular markers.

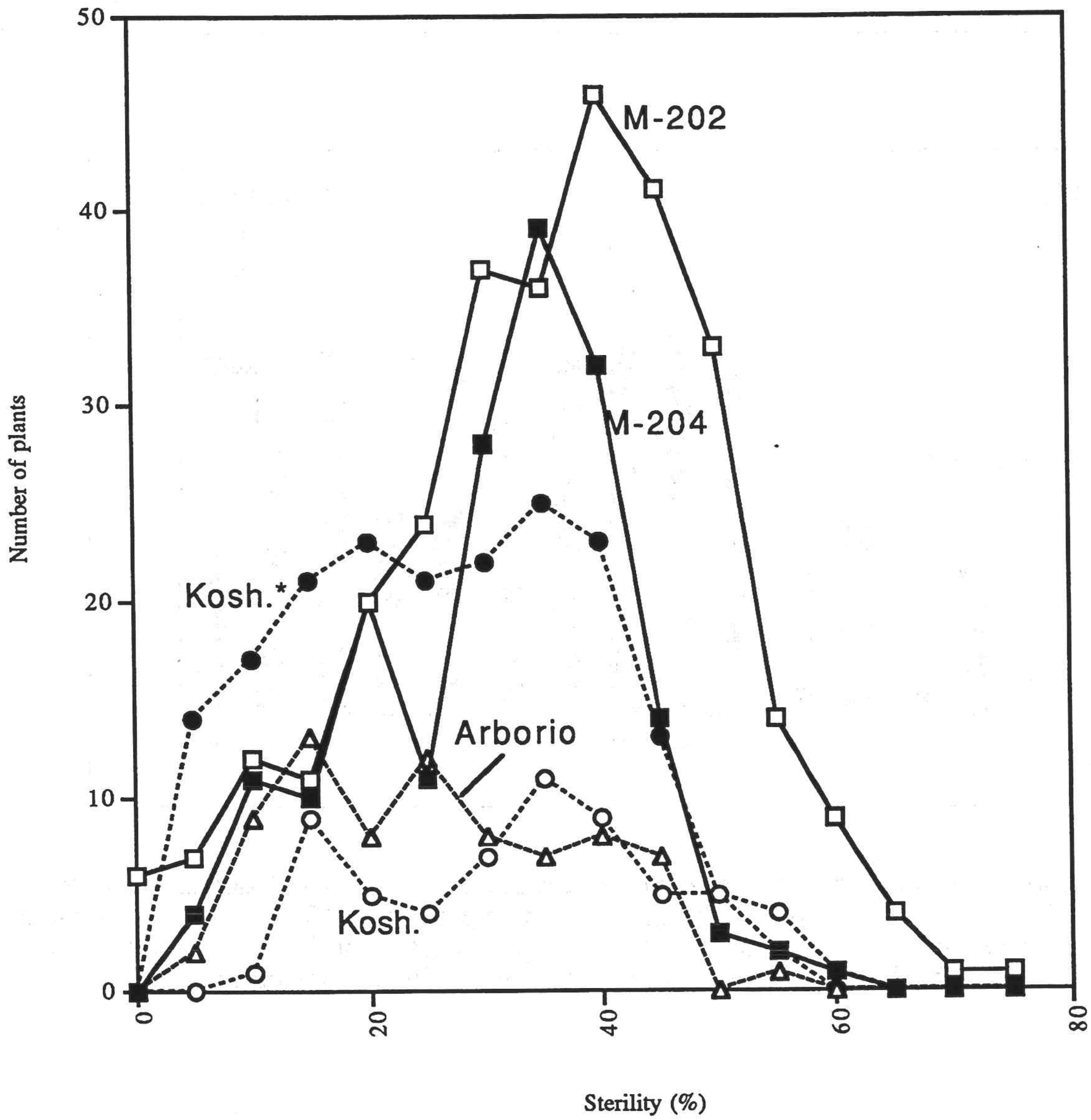

Fig. 3. Distribution of sterility in putative male-sterile mutants isolated from California and exotic rice cultivars in 1994.