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

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

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LEVEL OF 1996 FUNDING: $ 47,500

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.

     1996 Experiments:
     1.   Evaluation of introduced germplasm, hybridization, and breeding nurseries.
     2.   Evaluation of temperate and tropical japonica cultivars for adaptation to temperate climatic conditions.

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.

     1996 Experiments:
     1.   Stem rot resistance
     2.   Submergence tolerance.
     3.   Others (seedling vigor, cold tolerance, rice water weevil 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.

1996 Experiments:
1.   Transfer of restorer genes into California cultivars.
2.   Transfer of wide compatibility genes into California cultivars.
3.   Evaluation of new male sterile sources for potential photoperiod-sensitive genetic male sterile (pgms) mutants.

SUMMARY OF 1996 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE

1.   **Rice genetic resources**

Evaluation of introduced germplasm, hybridization, and breeding nurseries. The number of accessions evaluated in the field was lower in 1996 than in previous years. The Regional Uniform Nursery (RUN) from the southern US was grown again this year. Additional backcrosses were made to introgress genes from the wild species *Oryza nivara* into California cultivars. This concluded the backcrossing phase of this project and the resulting lines will be selfed this winter in the greenhouse. An additional cross was made with *Oryza glumaepatula*, which is a source of traits related to outcrossing ability.

Evaluation of the temperate adaptation of temperate and tropical japonica cultivars. One hundred seventeen accessions that were previously analyzed for molecular markers were evaluated for traits related to adaptation to temperate conditions. This included cold tolerance at the seedling stage, extent of panicle exsertion (measured as the distance from the flag leaf collar to the neck node as a percentage of plant height) and threshability (or shattering). Results showed that both temperate and tropical japonica types had superior cold tolerance compared to indica types (Fig. 1). US long grains are classified as tropical japonica cultivars by molecular markers. While these varieties are considered as tropical in adaptation, they possess similar vegetative-stage cold tolerance as the temperate japonicas. They are not as good as temperate japonicas in seedling vigor score, and they have poorer panicle exsertion and are more prone to shattering (Fig. 2). Among the indica cultivars tested, Ai Nan Tsao and the Russian cultivar Zilanica had better cold tolerance (Table 1). The latter had good panicle exsertion, in contrast to the other indicas. Temperate japonica cultivars with superior cold tolerance, panicle exsertion, and/or seedling vigor are listed in Table 1.

2.   **Identification of useful genes**

Stem rot resistance. We have been attempting to "tag" genes controlling stem rot resistance with molecular markers to assist in transferring these genes into agronomically superior medium-grain cultivars. In 1996, we identified molecular markers that appear to be linked to stem rot resistance inherited from 87-Y-550, which derives its tolerance from the wild species *O. rufipogon*. Fourteen putative markers have been identified that show an association with stem rot resistance in progeny of crosses between Y550 and susceptible cultivars (Table 2). It is not clear how many of these represent different genes. We were able to map four of these markers using a different mapping population. Each of these markers mapped to different positions on the rice genetic map (Table 2). We are now in the process of validating these markers in crosses between Y550 and the best medium grain cultivars.

Submergence tolerance. Last year we reported the discovery of the genetic locus *Sub1* that controls submergence tolerance in rice. This gene, derived from an Indian land race, confers tolerance to complete submergence for up to 16 days. We have now identified molecular markers that are more closely linked to this gene (Fig. 3). Our next step is to convert these markers into a more appropriate form to be used in selecting for this gene in segregating populations. In addition, we are backcrossing the *Sub1* gene into the cultivar M-202. This year we will be increasing seed in the Hawaii winter nursery of lines which are homozygous for the *Sub1* gene and have a similar phenotype to M-202. The best of these lines will be available for field testing in 1997. It has been observed that submergence tolerance and leaf elongation are negatively correlated. Lines with strong submergence tolerance generally do not elongate rapidly upon submergence. Those lines which rapidly elongate generally die after prolonged exposure to submergence. For application to the water seeding system practiced in California, it would be desirable to obtain lines which have some leaf elongation in addition to possessing the submergence tolerance gene. We evaluated lines derived from the backcross of a submergence tolerant F_3 plant (IR40931/PI543851) with the cultivar M-202. While most tolerant lines showed little elongation, some lines which were homozygous for *Sub1* did elongate upon submergence (Fig. 4). These lines are being further evaluated for their suitability for water-seeding. An additional backcross has also been made to transfer the *Sub1* gene into M-202.

Others. We currently have projects underway to map genes related to cold tolerance at the booting stage and resistance to rice water weevil. We are also developing populations to evaluate molecular markers that were previously shown to be linked to seedling vigor.

3.   **Hybrid rice**

Restorer genes. The restorer genes (R) are required for producing fertile hybrid seed when using the cytoplasmic male sterility (cms) system for hybrid seed production. In 1996 we evaluated F_6 lines derived from crosses with the indica source for restorer genes, IR50R. These lines will be evaluated for their restoring ability and will be further selected in 1997. The cms trait has already been transferred into California cultivars.

Wide compatibility. The wide compatibility (wc) trait is controlled by the S_5 gene. Cultivars with the S_5^n allele, which is present in many tropical japonica cultivars, can produce fertile hybrids when crossed with either indica or japonica cultivars. This allele is not found in temperate japonica cultivars. We are now transferring this gene into the temperate japonica background of the California medium-grain cultivars. This should facilitate the transfer of genes into California cultivars from indica germplasm. In addition, is may make the production of indica/japonica hybrid rice possible in California.

Photoperiod-sensitive genetic male sterility. The so-called 3-line method of hybrid seed production depends on the use of cms and restorer lines described above. While quite reliable, this system requires additional time and labor to develop the necessary components and to produce the hybrid seed. The use of the 2-line system has recently been implemented in China. In this system, a male sterile mutant that is sensitive to photoperiod allows seed production under short daylength (i.e. fertility conditions) and is used as a male sterile line under long days (i.e. sterilizing conditions) for hybrid seed production. Last year we reported the isolation of over 600 male sterile mutants from a grower's field. These mutants have been evaluated under different daylengths in both

growth chambers and in the field. Some the most promising are shown in Fig. 5. The ideal mutant would have low fertility (10-30%) under long daylengths, which are common in California during the normal growing season. These lines would be completely male sterile, and the seed production would be the result of cross pollination from adjacent male fertile plants. Under shorter daylengths, the plants would have higher fertility (>40%), which would allow production of the male sterile seed stock without the use of a special maintainer line. An additional advantage of the 2-line system is that there is no requirement for restorer genes, thus eliminating the need to transfer these genes into California japonica cultivars. Some of the lines in this study showed the opposite trend from that expected (lower points in Fig. 5). These plants showed a high fertility under long days, but a lower fertility under short days. If confirmed, these mutants could also be interesting for hybrid seed production. These promising mutants are being evaluated under short daylength in the field at the winter nursery in Hawaii.

PUBLICATIONS OR REPORTS:

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CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 1996, progress was made on identification of useful donors for new traits in the California breeding program. To ensure future progress from plant breeding, a supply of useful genetic diversity is essential. Since California cultivars already possess many desirable traits, it will be necessary to use backcrossing to introduce these useful traits from exotic cultivars into the California japonica gene pool. Recent research has indicated that backcrossing combined with the use of DNA markers is a powerful method to identify and transfer genes controlling quantitative traits (quantitative trait loci or QTL) from exotic cultivars or wild relatives into elite lines. We are currently evaluating this method to introduce new genetic variability into California cultivars.

QTL controlling seedling vigor have already been tagged with molecular markers. We have made crosses to attempt introducing these QTL into improved cultivars. Further progress has been made in identifying QTL controlling stem rot resistance, the most important rice disease in California. While a useful level of resistance has been introduced from *O. rufipogon*, it has been difficult to transfer it into improved medium-grain lines. With the identification of markers that appear to be linked to stem rot QTL, we are closer to being able to use the marker to transfer the QTL into California medium grains. This should increase the precision and reduce the time required for transferring the QTL. It should also allow us to determine why it appears to be so difficult to obtain the desired level of resistance in a medium grain type.

The relevance of the submergence tolerance gene *Sub1* in California is still not clear. The effects of this gene in conferring resistance to short-term submergence are dramatic. The trait is thought to be more useful in low-lying areas of the tropics under conditions where short-term inundation occurs after heavy rainfall. The observation that some degree of leaf elongation occurs in plants homozygous for *Sub1* is an indication that the trait could be useful under water seeding. The development of agronomically improved japonica lines with this gene should allow its evaluation for water-seeded conditions. Alternate management options will be explored in collaboration with agronomists and physiologists.

The exciting developments in hybrid rice research were highlighted at the recent Third International Hybrid Rice Symposium in India, where it was announced that India has released 14 new F_1 hybrid cultivars in the last couple of years. They are targeting 2 million ha (5 million acres) under hybrids by the year 2000. Significant progress has also been made in developing hybrids for the southern US by the company RiceTec. It has also been announced that a Japanese company has developed high-yielding japonica hybrids with superior grain quality. These developments indicate that the barriers to the development of hybrid rice in California may not be as formidable as once thought. Our work has concentrated on development of the necessary components for hybrid seed production in a California germplasm background. This will allow the production of prototype hybrids that can be evaluated under field conditions to determine the feasibility of F_1 hybrids adapted to the California environment.

Table 1.   Notable entries among 117 rice accessions evaluated for cold tolerance at the seedling stage (CT, 1=most tolerant, 9=most susceptible), extend of panicle exsertion as a percentage of plant height (Exs/Ht), days to heading (DH), plant height (Ht), threshability (Thr, 1=most difficult to thresh, 9=easiest to thresh or shattering), seedling vigor (1=most vigorous, 9=least vigorous).

Designation	Source	Origin	CT	Exs/Ht (%)	DH	Ht (cm)	Thr	SV
Indica cultivars								
Ai Nan Tsao 1	PI 412423	China	5.2	-3.3	81	56	7	6
Zilanica	PI 266123	Russia	6.4	6.0	93	100	5	5
Temperate japonica cultivars								
Akceltik	PI 172526	Turkey	5.5	4.6	96	93	3	3
Daegwanbyeo		Korea	1.9	4.8	94	84	1	5
Fukunishiki	PI 344090	Japan	4.5	9.0	106	112	5	5
Hirayana	PI 224822	Japan	2.9	4.8	103	88	4	5
Italia Agostano	PI 265108	Poland	4.6	2.8	78	84	4	3
Italica Livorno	PI 291478	Italy	3.9	3.9	81	93	7	1
Kagawa	PI 202965	Japan	4.6	6.2	99	106	7	3
M-204	PI 559472	USA-Ca	2.3	4.4	101	87	6	6
Michikogane		Japan	2.3	5.2	83	70	1	6
Norin 33	PI 224871	Japan	5.0	11.9	66	70	1	5
Omirt 168	PI 266163	Hungary	5.9	4.8	81	84	8	3
Penbegobek	PI 164986	Turkey	4.3	-0.4	83	98	6	3
Seolagbyeo	PI 464624	Korea	2.6	1.3	95	67	2	8
Shiranui	PI 331435	Japan	3.8	9.8	103	105	5	5
Sigadis	PI 224605	Indonesia	3.2	13.8	105	99	7	5
Su Bio	PI 162297	Korea	2.9	5.4	95	108	6	5
Sung Pan Tao	PI 161049	China	2.4	8.4	92	128	3	5
Szaniszlo 1	PI 271889	Hungary	2.9	6.6	78	97	8	5
Tominishiki	PI 224655	Japan	5.0	9.8	82	77	1	6
Uz Ros 269	PI 348905	Russia	4.6	7.4	87	102	3	3
WC 1403	PI 162254	Korea	2.5	5.2	94	107	6	4
Yukihikari		Japan	2.8	6.3	81	82	1	5

Table 2.   Putative markers linked to stem rot resistance in crosses with 87-Y-550 (resistance derived from *O. rufipogon*) and long-grain lines. Resistant and susceptible plants with (1) or without (0) the specified band were used to compute the chi-square values and determine the probability values. Probabilities below 0.05 are considered evidence for an association. Markers were mapped on a separate population.

Name	Primers	Size (bp)	Resistant 1	0	Susceptible 1	0	X^2	Prob.	Mapping[a]
SR1	B+3/G01	105	17	10	3	22	12.172	0.00049	chrom2
SR2	B+3/G01	110	13	8	2	19	10.37	0.00128	chrom11
SR3	S03/G08	200	21	9	8	23	10.233	0.00138	monomorphic
SR4	S9/F38	75	11	18	25	5	10.942	0.00094	chrom7
SR5	S10/G19	150	27	2	8	23	25.218	< 0.00001	unlinked
SR6	S10/G19	215	22	7	11	20	8.3062	0.00395	unlinked
SR7	S5/G27	450	18	10	4	21	10.772	0.00103	monomorphic
SR8	R03/G06	90	21	9	8	23	10.233	0.00138	unlinked
SR9	S06/G06	310	16	14	24	7	2.9237	0.08729	unlinked
SR10	S7/F7	130	12	2	3	24	19.018	0.00001	chrom7
SR11	S9/G5	250	11	6	1	9	5.5767	0.01820	monomorphic
SR12	S6/G27	450	21	8	7	24	13.015	0.00031	1;0
SR13	P3/D1	180	17	12	7	24	6.6769	0.00453	monomorphic
SR14	P1/G3	320	11	3	7	20	8.3477	0.00386	1;0

[a] Chromosome is indicated for markers already mapped. "Unlinked" indicates that the bands did were not linked to other markers on the genetic map and "monomorphic" indicates that markers could not be mapped because they were not segregating in the mapping population.
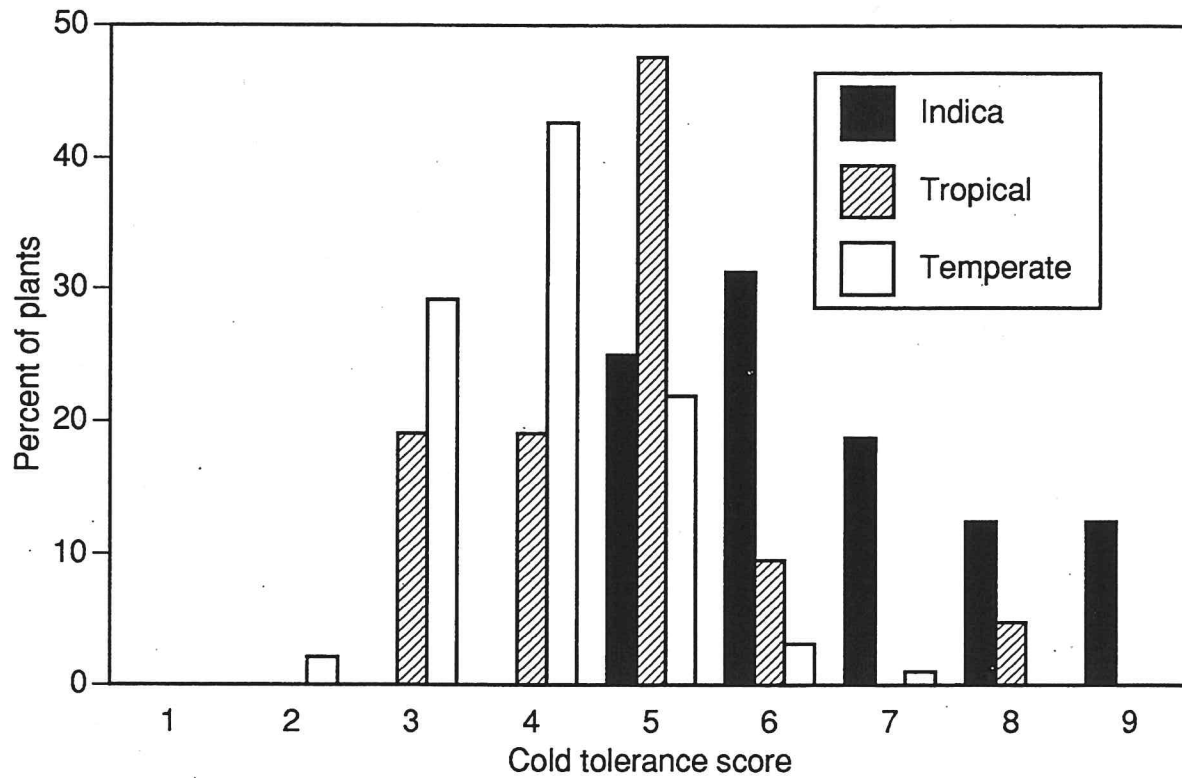
Fig. 1. Distribution of mean cold tolerance scores for three rice cultivar groups (indica, tropicaljaponica and temperate japonica). Overall mean score includes two temperature regimes (9 and 13°C with four sampling dates per temperature (10, 14, 21 and 28 d after seeding). Plants were scored on a scale of 1 (most tolerant, green leaves) to 9 (most susceptible, dead leaves) based on SES (IRRI, 1988).
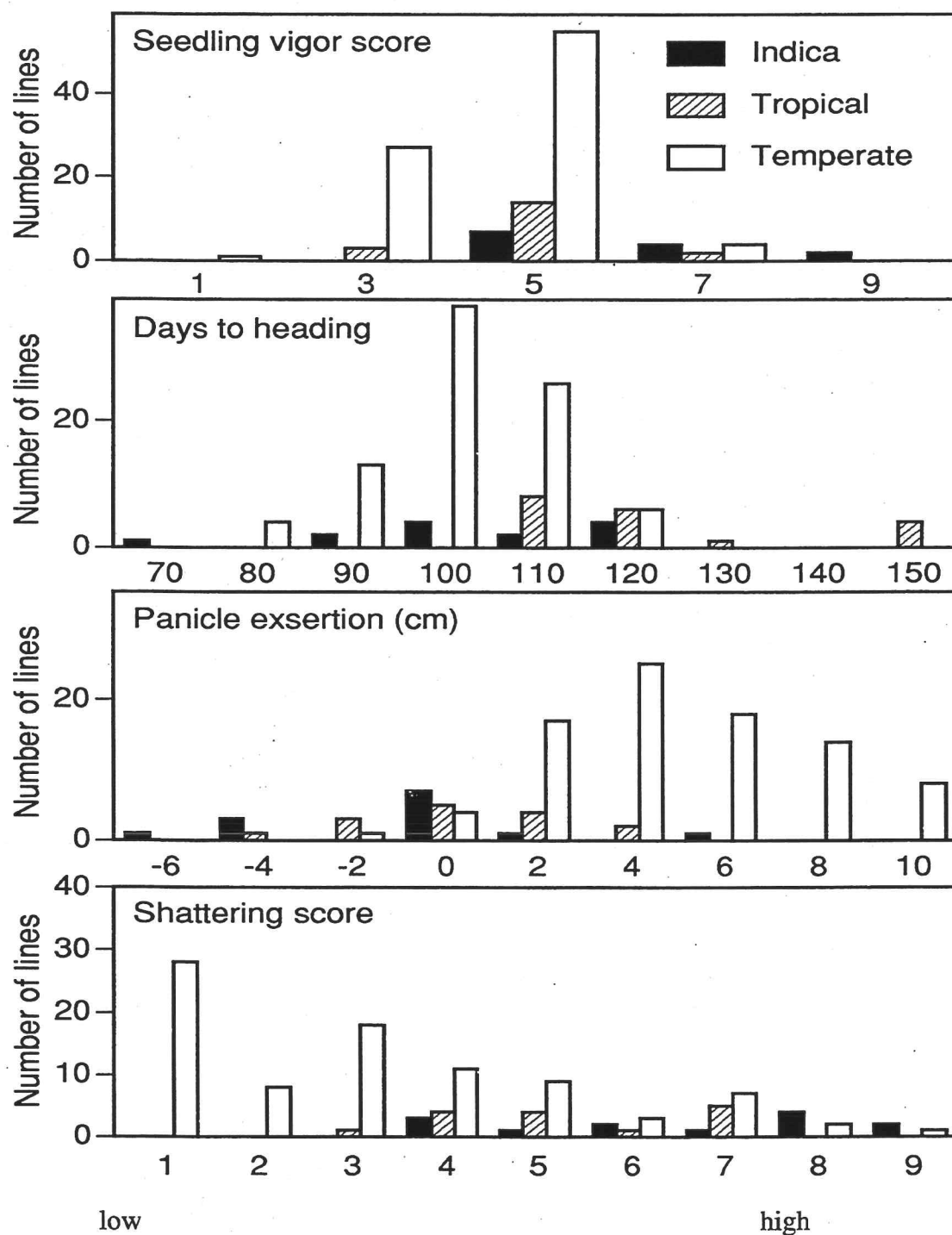
Fig. 2. Distribution of seedling vigor score, days to 50% heading, panicle exsertion and threshability for three rice groups (indica, tropical japonica and temperate japonica). For seedling vigor, scores ranged from 1 = most vigorous to 9 = least vigorous. For threshability, scores ranged from 1 = most difficult to 9 = easiest to thresh.
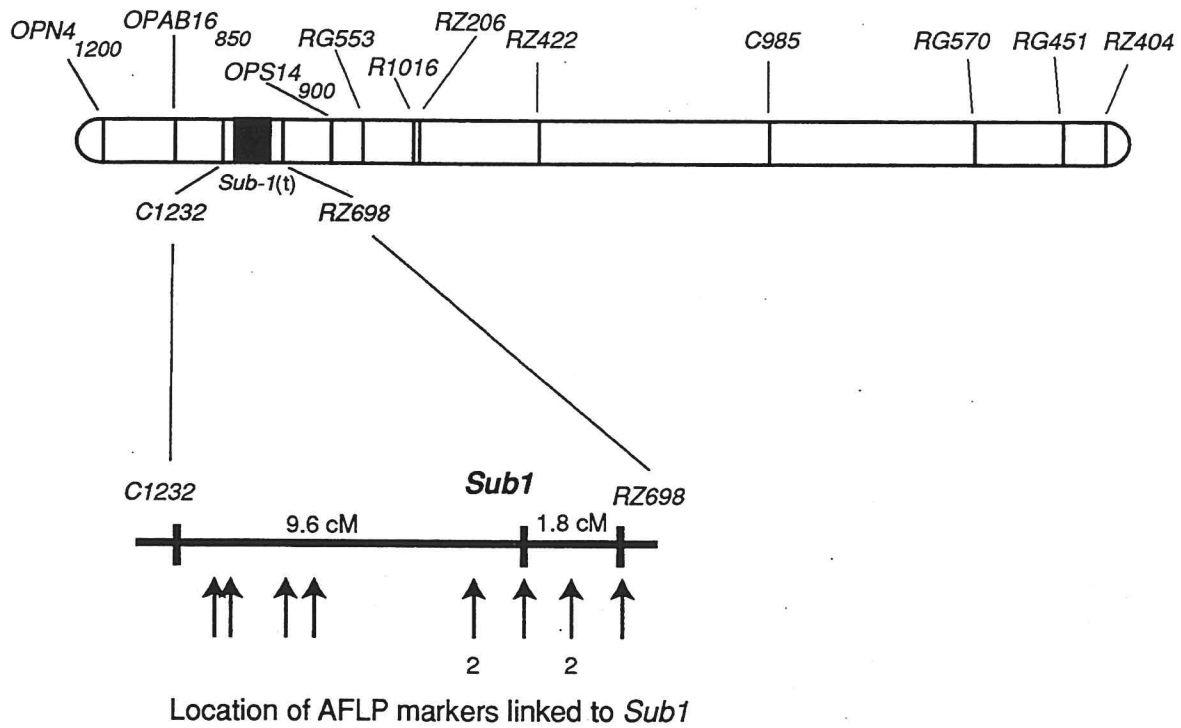
RFLP map of rice chromosome 9

Fig. 3 Molecular map of rice chromosome 9 showing the location of the submergence tolerance locus *Sub1* relevant to RFLP and RAPD markers. The arrows in the lower diagram indicate the location of AFLP markers identified through fine-scale mapping around the *Sub1* locus.
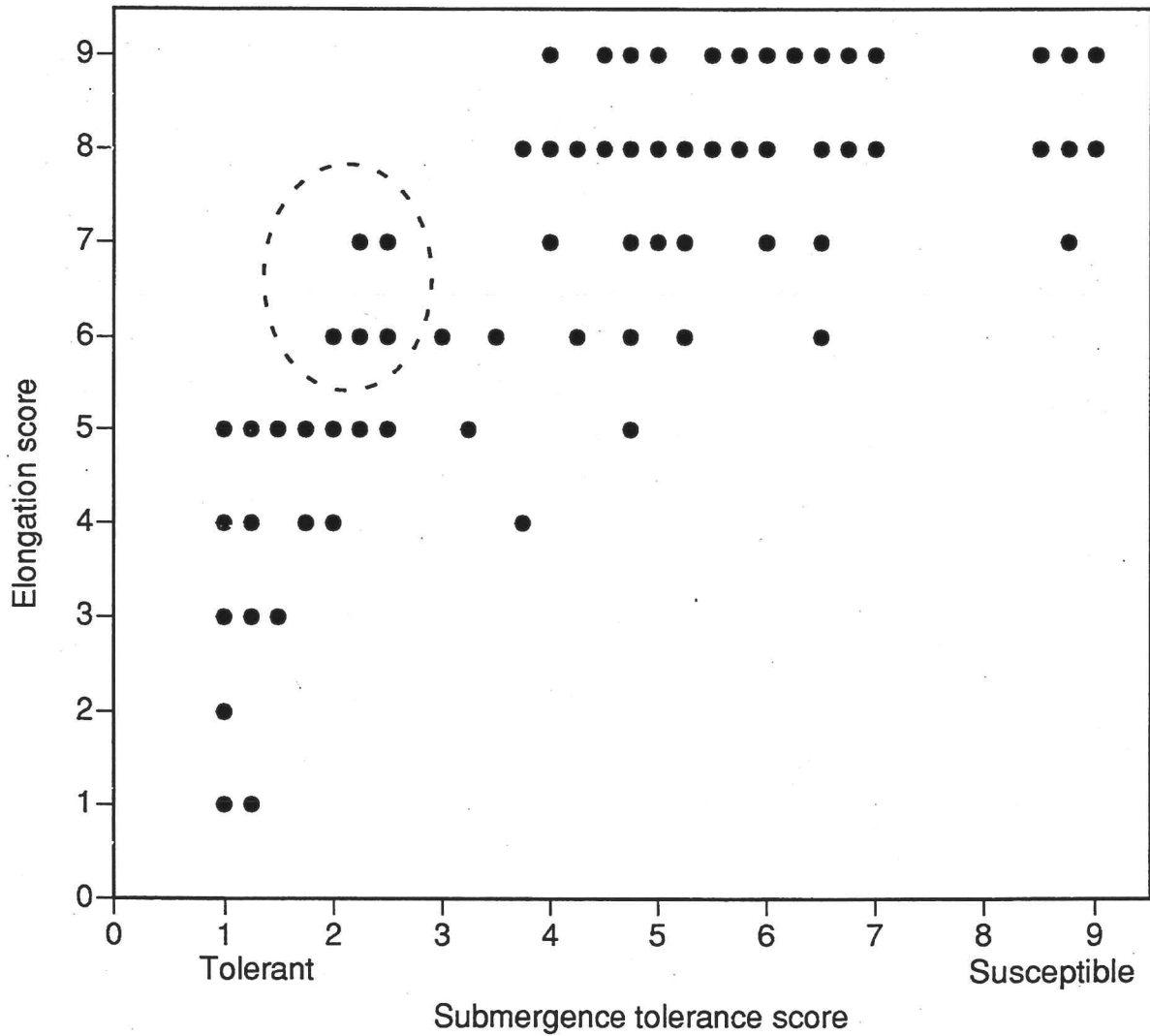
Fig. 4. Relationship between submergence tolerance score and leaf elongation score for lines segregating for the submergence tolerance locus. The circle indicates lines homozygous for the *Sub1* gene that show more leaf elongation during submergence.
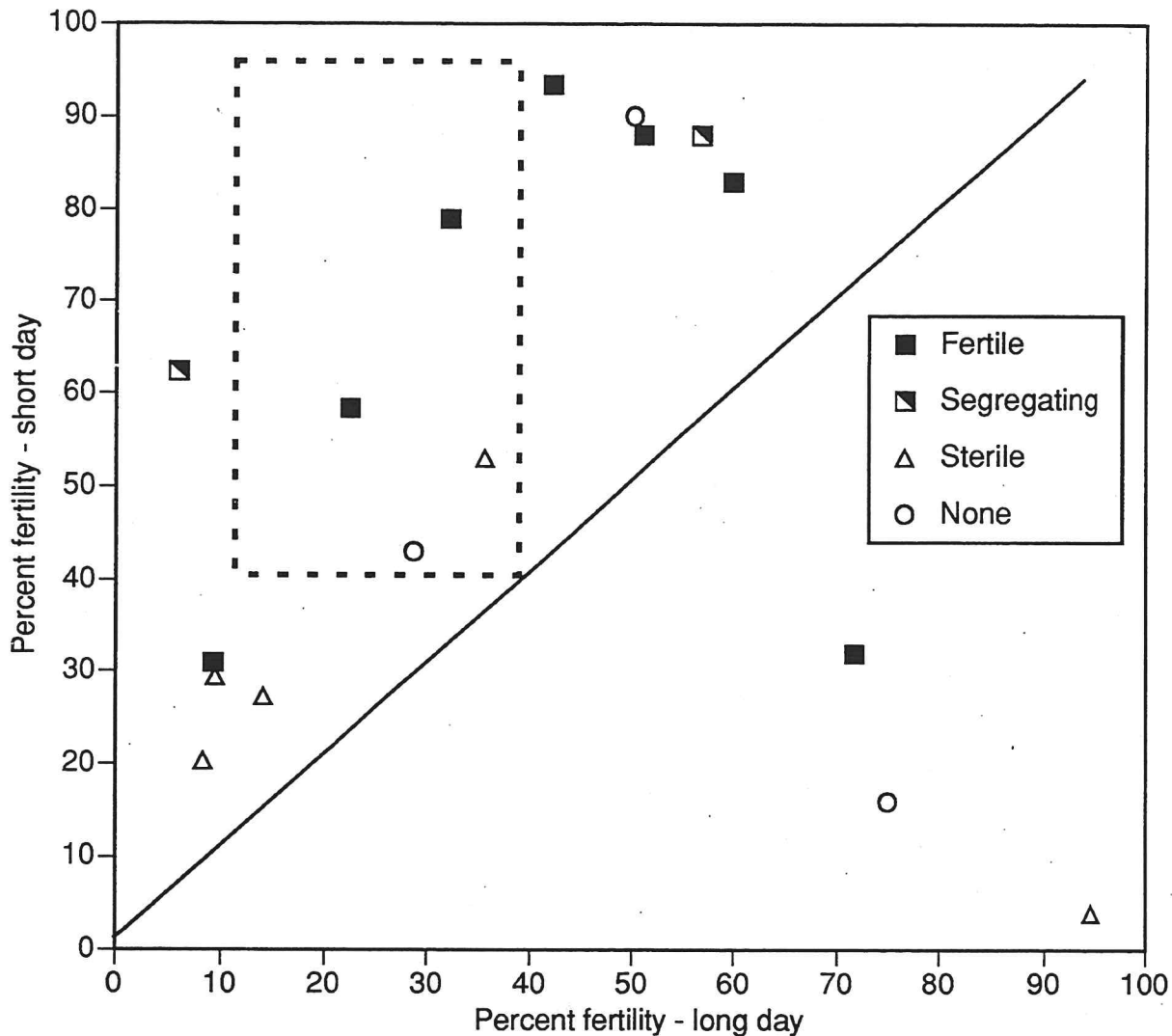
Fig. 5 Relationship between percent spikelet fertility under long day (14 h) and short day (10 h)
for putative pgms mutants. The smaller box indicates those pgms mutants with the
desired characteristics of low fertility (10-30%) under a long day length with higher
fertility (>40%) under a short day length. Mutants are labeled according to whether the
seed harvested from the male steriles produced fertile, segregating or sterile progeny
rows (none refers to those without this data). Under normal cross-pollinated conditions
the progeny rows would be expected to be fertile or possibly segregating if the mutants
were completely male sterile and were cross pollinated by nearby fertile plants.