COMPREHENSIVE RESEARCH ON RICE
ANNUAL REPORT

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PROJECT TITLE: Genetic and Physiological Determinants of Yield and Quality

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

The overall objective of the research is to integrate conventional and molecular genetics of rice, and thus develop germplasm and breeding methods useful to the California rice industry. The overall objective is being attacked through a series of sub-objectives, arranged in approximate order from conventional procedures through those which provide a bridge between conventional and molecular or genetic engineering techniques:

1. Inheritance and interspecific transfer of aggregate sheath spot (sheath blight) resistance.
2. Evaluation of "Early S-201," early maturing M-201, and double-dwarf M-201 mutants.
3. Use of Asian and African rice to improve germplasm, through male sterile facilitated outcrossing.
4. EMS induction of genetic male steriles in additional varieties.
5. Hybrid rice mechanisms.
6. Seedling screening for herbicide resistance.
7. Somatic tissue culture selection.
8. Anther culture studies.
9. Searching for apomixis in rice.
10. Isolation of transposable elements.

SUMMARY OF 1985 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1. Inheritance and interspecific transfer of aggregate sheath spot (sheath blight) resistance.

Mr. A. O. Bastawesi completed an M.S. thesis study on the inheritance of aggregate sheath spot, using materials jointly provided by the present project, by Dr. R. K. Webster, and by Dr. S. T. Tseng. As in the earlier genetic studies on stem rot resistance, weedy rice species were found to be more resistant than cultivated rice, *Oryza sativa*. The weedy donors of aggregate sheath spot resistance were:

Oryza rufipogon (Accession 100923 from IRRI)
Oryza fatua (PI 239671)

These two donors were used in three crosses:

O. rufipogon/*O. sativa* 78:18347
O. sativa 8147528/*O. fatua*
O. fatua/*O. sativa* 78:18347

Principal findings were:

Evaluation of three disease scoring methods showed that the ratio method (ratio of the average distance from the soil surface to the uppermost lesion, divided by mature plant height) was the best method for scoring aggregate sheath spot resistance in populations segregating for plant height.

Narrow sense heritability estimates, using the ratio scoring method, ranged from 0.51 to 0.83 in the six generation analysis. The predicted gains from selection for resistance ranged from 5 to 11 percent which would be reasonable for interspecific transfer of resistance.

Tall plants tended to be more resistant than short ones. Leaf pubescence or glabrousness had no effect on disease severity.

The *O. fatua* line was a better donor source for aggregate sheath spot resistance than the *O. rufipogon* line. Thus in Figure 1 there was a larger proportion of resistant F_2 plants with low disease scores (left side of Figure 1) in the cross with *O. fatua* than in the cross with *O. rufipogon*.

2. Evaluation of "Early S-201," early maturing M-201, and double-dwarf M-201 mutants.

In 1984 an "Early S-201" mutant was found to be 7 days earlier and 25% higher yielding than its parent, S-201. However, detailed examination of its grain shape showed that it was somewhat intermediate between typical short and typical medium grain types, i.e., like Calpearl. In view of the marketing difficulties that have occurred with this atypical grain shape, "Early S-201" was shelved, except for uses in near-isogenic comparisons of maturity.

In 1984, we selected 649 early maturing plants in an M_2 population of the variety M-201, which had been treated with the chemical mutagen EMS. In 1985 progeny tests we narrowed this material down to 25 lines which ranged from 5 to 13 days earlier than M-201 (Table 1). Seeds were made available to the breeding program.

A double-dwarf mutant of M-201, first identified as a single plant in 1984 (84:3860) was progeny-tested and found to be about 15 cm shorter than M-201. At the resulting height of only 70 cm (28 inches), the double-dwarf mutant is probably too short for water seeding.

3. Use of Asian and African rice to improve germplasm, through male sterile facilitated outcrossing.

a. Asian rice

In 1985 we progeny tested the 2,214 F_1 hybrid plants selected in 1984, following natural crossing in 1983 of some 400 world collection entries from Japan, Korea, and Taiwan onto genetic male sterile M-101. The two achievements of this study were: i) Following further intermating through natural pollination of male sterile plants by fertile plants in the segregating populations, 374 male sterile, semidwarf, glabrous hull F_2 plants were harvested and bulked to begin another cycle of population improvement. Seeds were provided to the breeding program. (ii) The materials were used as source populations in searching for apomixis (see sub-objective 9, below).

b. African rice

This study was initiated one year after the Asian rice study, and is following the same steps. Thus, in 1984 about 200 African lines were allowed to wind pollinate the M-101 genetic male sterile. The hybrid seeds from this natural-crossing program were planted in 1985, as the next step in introducing additional diversity into California rice. We harvested 222 F_1 plants, which carried marker genes for pubescent hulls and/or tall plant height. These 222 F_1 plants will be progeny tested in 1986 for two purposes: i) To allow further intermating, and (ii) as source populations in searching for apomixis.

4. EMS induction of genetic male steriles in additional varieties.

EMS (ethylmethyl sulfate) appears to be a very efficient mutagen for inducing genetic male sterility. In turn, genetic male sterility is useful as a tool for making hybrids with less effort, and thus is a tool which has applications in subsequent genetic programs (as in number 3, above). It is important to have male sterile genes in different genetic backgrounds in order to maximize opportunities for genetic recombination among diverse parents.

In 1985 we reselected the 75 lines of the variety M-201 which were segregating for EMS-induced sterility. There were 10 lines which met the dual criteria of 1) segregating in the expected 3 fertile:1 sterile ratio, and 2) showed 5 to 10% seed set (presumably from outcrossing) on sterile plants (Table 2). Four additional lines failed to segregate in the expected 3 fertile:1 sterile ratio, but are being saved for progeny testing in 1986 to elucidate inheritance patterns.

In 1985 we also mutagenized four additional varieties, in preparation for screening for male sterility in 1986. The varieties and mutagen treatments are listed in Table 3.

5. Hybrid rice mechanisms.

Although we are not as optimistic about the potentials of hybrid rice as China is, we are continuing basic research on genetic mechanisms which might make large scale hybrid seed production easier.

a. Induction of cytoplasmic male sterility in California rice varieties by the antibiotics streptomycin and mitomycin.

(i) Three male sterile plants previously identified in antibiotic-treated M-201 were found to be genetic male steriles, rather than the desired cytoplasmic type of male steriles, and thus are not useful for hybrid rice production. The best one (83:14020-3) has been added to our genetic male sterile collection for use in population improvement studies (see sub-objective 4, above).

(ii) Three additional varieties, L-202, M-202, and Calmochi-101, were treated with streptomycin and mitomycin in 1985. Only two sterile plants were saved, one each from streptomycin-treated L-202 and M-202. Crosses are being made to determine if the sterility is cytoplasmic or genetic.

b. Inheritance of fertility restoration for the Chinese source of cytoplasmic male sterility.

This work was continued in 1985. Pollen fertility restoration seems to be controlled by two genes in greenhouse studies. The cold susceptibility of the Chinese parents makes field results impossible to interpret. Because of this cold susceptibility, we plan to de-emphasize work with the Chinese hybrids.

c. Cytoplasmic effects on agronomic characters.

Impetus for this research is provided by the observation that only one cytoplasm, the Cina source, is used in many of the the rice varieties in Asia. Most US varieties apparently have other cytoplasms. However, in the southern US, where both types can be grown, the varieties with Asian cytoplasms yield more than the US varieties. Is that due to cytoplasms or to other factors?

Four pairs of crosses and their reciprocals were made:

Lemont x Kwang Chang Ai
Kwang Chang Ai x Lemont

Lemont x Quin Qun Wang
Quin Qun Wang x Lemont

Lemont x LA 110
LA 110 x Lemont

Lemont x Nanking Sel. 119
Nanking Sel. 119 x Lemont

The F_1 generations were grown in the greenhouse, with 3 replications at 3 plants per replication (pot). Fertility and yield per plants were significantly lower in the USA (Lemont) cytoplasm than in the Asian cytoplasm (Table 4). Other characters did not differ in the different cytoplasms. Further generations and crosses are needed to answer the question posed above.

d. Chemical hybridizing agent (CHA).

In 1985 we continued a cooperative study with Shell Development Corporation on evaluation of a CHA for making hybrid rice. We provided paired lines, the female of which carried the recessive waxy (sweet rice) gene, so that hybrid seeds could be identified at maturity. Data analyses are incomplete, but two things are evident:

(i) The CHA induces male sterility
(ii) Outcrossing mates are still low, only 1 to 15%.

6. Seedling screening for herbicide resistance.

Resistance in rice to the broad-spectrum herbicide glyphosate (Roundup) would be extremely helpful in weed control, since a resistant rice variety could be sprayed to control weed without damaging the crop. Two plants previously selected for tolerance to glyphosate failed to pass the tolerance on to their progenies. Since work with bacteria now indicates that glyphosate resistant mutants are extremely rare (one in 10^{11} bacteria), we have concluded that it is unlikely that we can ever find a seedling mutant resistant to this herbicide. Hence we are redirecting our work to selecting in tissue culture, and for other, less difficult herbicides (see sub-objective 7, below).

7. Somatic tissue culture selection.

In the past year regeneration from rice callus tissue has increased from an average of 0.03 regenerated plants per explant (from mature seeds) up to nearly 100 regenerants per explant (from immature embryos). Modifications in regenerating media and source of explants account for the increase.

Some 200 plants have been regenerated from tissue culture of L-202. Twenty of the 200 were grown in the field in 1985. A few possible mutants have been seen; the most interesting is one which shows nearly 20% twin seedlings. This line may be useful in searching for apomixis (see sub-objective 9, below).

8. Anther culture studies.

Over 700 plants were regenerated from anther culture in 1985. Sixty percent were green, and 40% were albino (died in seedling stage). Of the green plants, about 12% were diploid, and the remainder haploid. These green, diploid, plants are the ones of greatest interest. As we gain expertise in regenerating green, diploid, plants from anther culture, we may be able to use this technique to beneficially shorten the variety development cycle by 2 or 3 years.

In cooperative studies with Dr. Gideon Schaeffer of USDA-ARS at Beltsville, MD, we discovered a genetic male sterile in his anther culture derived populations of Calrose 76 (Table 5). This provides us with yet another genetic male sterile source for use in population improvement schemes (see sub-objective 3, above).

9. Searching for apomixis in rice.

Apomixis is a form of asexual seed production, which is sometimes called "cloning through seeds." It is currently unknown in rice, but discovery and successful application of apomixis in rice would permit production of true-breeding F_1 hybrids with permanently fixed heterosis. Hybrid rice is grown on 20 million acres in China, and is reported to show 15-20% heterosis for grain yield, but high cost of hybrid seed (10x normal) precludes use of hybrid rice in the U.S.

Thus, apomixis, if it can be found, offers the potential for enabling US rice farmers to economically capture the increased yields of hybrids.

With partial assistance from the Rockefeller Foundation Program on Genetic Engineering in Rice, we have begun an intensive search for apomixis in rice. Four approaches, in increasing order of difficulty, are underway: a) screening for apomixis in world collections of cultivated rice, b) screening for apomixis in weedy relatives of rice, c) intergeneric hybridization between apomictic forage grasses and rice, and d) genetic engineering to transfer genes for apomixis from forage grasses to rice.

In 1985 we began work on approach a, screening for apomixis in world collections. We grew the F_2 generation of 2,214 crosses previously developed in the population improvement program (sub-objective 3, above), and looked for abnormal segregation. The desired abnormal segregation for this case would be for lines producing plants that were the same as the F_1 parent.

Of the 2,212 lines observed, 87 gave abnormal segregation which might be indicative of apomixis. Since there are other possible explanations for such abnormal segregation, progeny tests are underway to sort things out.

10. Isolation of transposable genetic elements.

Transposable genetic elements, or the "jumping genes" of corn for which Dr. Barbara McClintock was recently awarded the Nobel Prize, are useful genetic engineering tools for isolating and moving foreign genes into an organism.

As for sub-objective 9, above, we are receiving partial assistance from Rockefeller Foundation to pursue this extremely basic research. We are pursuing this by looking for a high reverse mutation rate of the waxy gene to normal translucency. We do this by observing pollen grains under the microscope. When stained with dilute iodine solution, waxy pollen grains are distinguishable from normal grains. To date, among about 25 waxy lines examined, we have found one intersting line, which showed 13 reverse mutations among 130,000 pollen grains. Although such low frequencies (1 in 10,000) may seem small, they are actually high for mutation rates, and may be suggestive of transposable genetic elements. Progeny tests will tell the story.

PUBLICATIONS OR REPORTS:

Lu, Yong-Gen and J. N. RUTGER. 1984. Cytological observations on induced genetic male sterile mutants in rice (_Oryza sativa_ L.). Scientia Sinica (Series B) 27:482-493.

RUTGER, J. N. 1985. Plant breeding problems needing solutions - conventional or biotechnological! pp. 87-90. _In_ Proc. 1985 California Plant and Soil Conf., Fresno, CA, Jan. 30-Feb. 1, 1985.

RUTGER, J. N., L. E. Azzini and P. J. Brookhouzen. 1985. Inheritance of semidwarf and other useful mutant genes in rice. To appear in Proceedings of the International Rice Genetics Symposium, Int. Rice Res. Inst., May 27-31, 1985.

RUTGER, J. N. and C. N. Bollich. 1985. Public-sector research on hybrid rice in the USA. To appear in Proceedings of the International Rice Research Conference, Int. Rice Res. Inst., June 1-5, 1985.

Bastawisi, A. O. 1985. The inheritance of aggregate sheath spot resistance in interspecific crosses of _Oryza_. M.S. Thesis, UCD. 63 pp.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

Results encompass a range of procedures, from conventional breeding techniques to those involving genetic engineering:

1. As in the case of stem rot, weedy relatives of cultivated rice (*Oryza sativa*) were found to be good sources of resistance to aggregate sheath spot. The best weedy donor was *Oryza fatua* entry PI 239671.

2. Twenty five early-maturing mutants were selected from M-201. The mutants ranged from 5 to 13 days earlier than M-201.

3. In male sterile facilitated outcrossing studies, 374 F_2 plants, derived from crosses between Asian rice and male sterile M-101, were composited for another cycle of population improvement. Also, 222 F_1 plants were saved from a similar series of crosses between African rice and male sterile M-101.

4. Fourteen genetic male sterile mutants were selected from M-201. This is part of a program to produce male sterile genes in different genetic backgrounds (first M-101, now M-201, etc.) in order to maximize opportunities for genetic recombination among diverse parents.

5. The development of genetic and other mechanisms for hybrid rice production continued, with further attempts to induce cytoplasmic male steriles in California varieties, with studies on inheritance of fertility restoration, and with studies on use of chemical hybridizing agents. To date, none of these is an adequate solution by itself for hybrid rice production in California.

6. Induction of seedling mutants for resistance to the herbicide glyphosate has not been successful. Induction of mutants resistant to other grass-killing herbicides is being attempted.

7. Some 200 plants have been regenerated from tissue culture of L-202. One regenerated line shows nearly 20% twin seedlings, which might be an indication of apomixis (see #9, below).

8. A genetic male sterile mutant was recovered from an anther culture-derived population of Calrose 76. Otherwise, we are concentrating on techniques in increase regeneration of plants from another culture.

9. With assistance from the Rockefeller Foundation, we have begun searching for apomixis in rice. Apomixis, or asexual seed production, is potentially useful for developing true breeding F_1 hybrids with permanently fixed hybrid rigor.

10. Also with Rockefeller Foundation assistance, we have begun searching for transposable genetic elements ("jumping genes") in rice. Transposable genetic elements are useful genetic engineering tools for isolating and moving foreign genes into an organism.

Table 1. Heading times of 25 early maturing M_2-derived mutants of M-302.

1985 row number	Days earlier than M-201*
9206	13
9296	5
9306	7
9217	5
9324	6
9355	6
9364	11
9372	6
9382	5
9404	7
9428	11
9477	6
9490	11
9496	8
9504	12
9511	11
9516	7
9528	6
9618	13
9654	13
9668	8
9687	8
9773	11
9828	11
9870	11

* M-201 headed in 104 days.

Table 2. Segregation for fertility and sterility in the M_4 generation of 14 EMS-induced M-201 genetic male steriles.

1985 row	Number of plants Fertile	Sterile	Chi square value*
4494	34	22	6.09*
4805	42	32	13.13*
4854	26	15	1.47
4985	13	13	8.67*
5011	6	4	1.20
5236	34	24	8.29*
5487	39	15	0.23
5547	55	22	0.52
5747	26	15	2.93
5761	12	4	0.00
5774	38	16	0.61
5851	22	9	0.27
5931	54	28	3.65
5955	24	14	2.84

* Values greater than 3.84 indicate that the observed segregation did not fit the expected 3 fertile:1 sterile ratio.

Table 3. Varieties treated and mutagens used in preparation for screening for additional genetic male steriles.

Variety	Mutagen Treatment	Number of M_1 panicles harvested
L-202	EMS, 1%	500
L-202	Gamma rays, 25 kR	1000
L-202	Control	100
Quin Qun Wang	EMS, 1%	500
Quin Qun Wang	Control	50
M-202	EMS, 1%	500
M-202	Gamma rays, 25 kR	1000
M-202	Control	50
Calmochi-101	EMS, 1%	500
Calmochi-101	Gamma rays, 25 kR	500
Calmochi-101	Gamma rays, 25 kR	800*
Calmochi-101	Control	50

* 800 plants harvested in bulk

Table 4. Comparison of 4 F_1 crosses and their reciprocals, expressed in terms of cytoplasms of female parents.

Cytoplasm	Fertility %	Days to heading	Plant height, cm	No. panicles /plant	Panicle length, cm	Yield/ plant, g
Asian	83	134	122	5.63	22.18	8.54
USA (Lemont)	70	138	125	4.75	23.22	6.29
Difference	**	NS	NS	NS	NS	*

Table 5. Selection of a no-pollen type genetic male sterile from anther culture derived regenerant of Calrose 76.

1984 Davis

Observed progenies of D84-1-117 (AC-Z/Dwarf from anther culture x Calrose 76) to be segregating 23 fertile:14 sterile (X^2 for 3:1 segregation = 3.25^{NS}).

1984-85 Hawaii

11 fertile plants (from 1984 Davis) segregated into 4 all fertile:7 segregating families for fertile to sterile plants (X^2 for 1:2 segregation = 0.05^{NS}).

Among the 7 segregating families, observed segregation was 52 fertile:17 sterile plants (X^2 for 3:1 segregation = 0.01^{NS}).

1985 Davis

40 fertile plants (from 1984-85 Hawaii) segregated into 10 all fertile:30 segregating families for fertile and sterile plants (X^2 for 1:2 segregation = 1.25^{NS}).

Among the 30 segregating families, observed segregation was 264 fertile:109 sterile plants (X^2 for 3:1 segregation = 3.55^{NS}).

Male sterile plants determined, by cytological observation, to be of the no-pollen (NP) type.

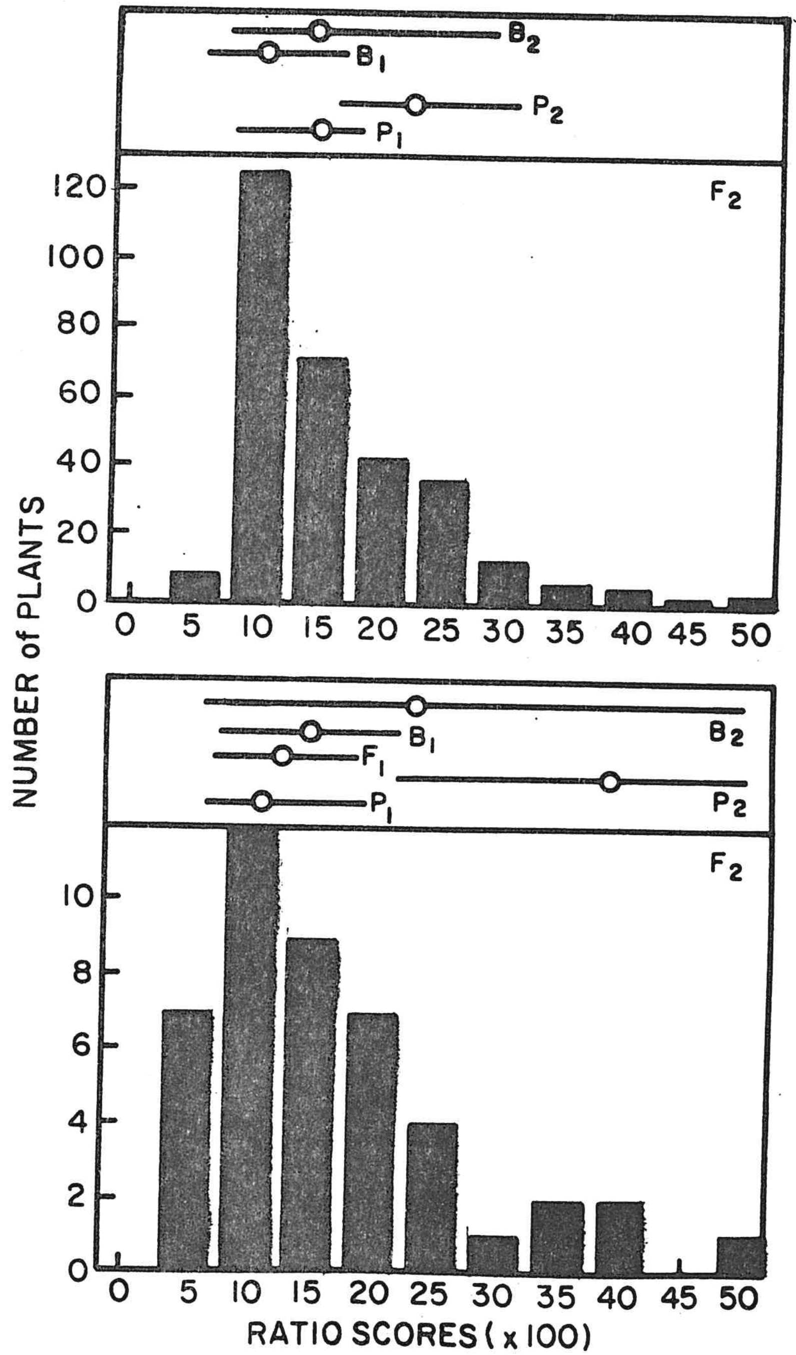

Figure 1. Distribution of aggregate sheath spot ratio disease scores (lower values indicate resistance) for the parent (P_1 & P_2), F_2, and backcross generations (B_1 & B_2) of:

Top: O. rufipogon/O. sativa 78:18347

Bottom: O. fatua/O. sativa 78:18347