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:

1. To develop new germplasm useful in variety development and in crop physiological studies.

2. To investigate standard and innovative breeding methods which can raise yield levels and/or accelerate variety development.

SUMMARY OF 1984 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1. To develop new germplasm useful in variety development and in crop physiological studies.

   a. Introduction of new genetic diversity through the genetic male-sterile-facilitated crossing program.

   Some 3,000 hybrid plants resulting from the natural outcrossing in 1983 of some 400 world collection entries from Japan, Korea, and Taiwan onto genetic male sterile M-101 were grown and harvested. Seeds from these plants will be grown in 1985 for two purposes: (i) To allow further intermating through natural pollination of male sterile plants by fertile plants in the segregating population. (ii) As source populations in searching for apomixis, which is a form of non-sexual seed production. Discovery and successful application of apomixis would permit production of true-breeding hybrid rice.

   b. Inheritance and interspecific transfer of aggregate sheath spot (sheath blight) resistance. (Ph.D. thesis research of A. O. Bastawesi)

Inheritance of sheath blight (now known as aggregate sheath spot) resistance in crosses between the partially-resistant weedy species O. rufipogon and O. fatua and cultivated rice, O. sativa, was studied in several populations grown both in the greenhouse and the field. Data are still being collected from the several thousand individual plants. Preliminary examinations indicate that the resistance of the weedy parents is useful under field conditions, although the resistance apparently can be overwhelmed by large inoculum loads in high-humidity conditions in the greenhouse (Table 1). Since farm production of rice is in the field (not the greenhouse!), it is hoped that these sources of resistance will be useful in breeding and thus give partial control of the disease. Although the levels of resistance are not as high as we would like, the weedy species nevertheless offer the best sources of resistance we have for aggregate sheath spot. These findings parallel the situation for stem rot, where we demonstrated several years ago that weedy species were the best sources of resistance.

c. Evaluation of rice varieties from China.

The following pairs of crosses between Chinese and US rice varieties were made:

Waxy M-101 x Quin Qun Wang
Quin Qun Wang x Waxy M-101

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

Paired crosses such as the above are known as "reciprocal" crosses and are used to determine whether there is any difference in using a given line as a female parent versus using it as a male parent. Such difference, termed "cytoplasmic effects" are known to be useful in hybrid rice production. Differences sometime occur for agronomic characters such as heading date, height, and yield. The F_1 generations are being grown this winter; further evaluations will be conducted in 1985.

d. Yield test of an early maturing S-201 mutant.

An "Early S-201" mutant (83:10074) was compared to its parent, S-201, in a small-plot, 8-replicate yield test, with 4 rows per plot. Only the two center rows in each plot were harvested, to eliminate border effects. The "Early S-201" mutant was 7 days earlier than its parent, S-201, and yielded 25% more than S-201

(Table 2). Seed size of Early-S-201 was reduced about 3%, and the kernels had a less-rounded shape than S-201. Consequently it also had less white belly than S-201. Bulk seed of this mutant was produced for further evaluations in 1985.

2. To investigate standard and innovative breeding methods which can raise yields and/or accelerate variety development.

   a. Population improvement, through the use of genetic male sterility.

(i) The approximately 300 lines provided by Dr. I. W. Buddenhagen were allowed to pollinate M-101 genetic male steriles. These lines, many of which are from Africa, represent new sources of germplasm which may be useful for increasing genetic diversity in materials adapted to California, especially for larger seed size. Planting dates of these potential male parents and the M-101 genetic male sterile (female) parent were manipulated in order to match flowering times (called "nicking") of the male and female. About 200 males flowered within ± 3 days of the female and thus were considered to have "nicked". The females of these 200 combinations were harvested for 1985 studies on large seed size.

(ii) Induction of genetic male sterility in a second California variety

In 1984 we identified 75 lines of the variety M-201 which were segregating for EMS-induced sterility. EMS is a chemical mutagen; based on the 1984 results it appears to be extremely effective for inducing genetic male sterility. The EMS-induced male sterile lines of M-201 will complement earlier male sterile mutants of M-101 which were previously induced by gamma radiation. For both population improvement and for basic genetic studies, it is important to have several different sources of male sterility, in different background varieties.

We also selected one double-dwarf mutant, and several early-maturing mutants, from the same mutagenized population of M-201. These will be evaluated in progeny tests in 1985.

   b. Hybrid rice

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

(i) Induction of cytoplasmic male sterility in adapted California rice varieties by the antibiotics streptomycin and mitomycin. The 35 sterile or near-sterile plants found in antibiotic-treated M-201 were narrowed down to three plants which were sterile in repeated tests:

83:14002-2
83:14020-3
83:14033-2

Plant 83:14020-3, which produces no pollen at all, readily hybridizes with normal plants. The F_1 generation is currently in the greenhouse, but it is too soon to determine if cytoplasm male sterility actually has been induced. If successful, this technique will provide a valuable short-cut to conventional breeding techniques for introducing male sterile cytoplasm into adapted California varieties.

(ii) Inheritance of fertility restoration for the Chinese source of cytoplasmic male sterility. Since US varieties do not show adequate fertility restoring ability (as determined in 1982 and 1983 on this project) for the Chinese cytoplasmic male sterile source, it is important to learn more about the mode of fertility restoration in order to efficiently transfer this characteristic into adapted lines. In 1984, inheritance of pollen fertility and seed set were studied in four crosses between the Chinese cytoplasmic male sterile and US rice varieties L-201, Labelle, and an experimental line from Texas, 803005. Inheritance of pollen fertility restoration appears to involve at least two genes, and is confounded by the different genetic backgrounds of the Chinese versus the US varieties. It appears that considerable breeding work would be required to develop suitable US lines with adequate pollen fertility restoring ability for the Chinese cytoplasmic male sterility source.

(iii) Chemically-induced sterility

The problems described in (i) and (ii) above, could be avoided completely if male sterility would be induced by chemical sprays. Therefore, in 1984 we cooperated with Shell Development Company in greenhouse evaluation of a chemical that they have found promising in wheat. We provided paired lines, the female of which carried the recessive waxy (sweet rice) gene, so that hybrid seeds could be identified at maturity. The spray reduced seed set on the female plants, but very few hybrid seed were found. Further studies are needed in order to adequately evaluate this chemical for rice.

c. Innovative breeding methods

(i) Seedling screening for resistance to the herbicide glyphosate.

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 weeds without damaging the crop. Resistance to this herbicide has been induced in bacteria, so there is at least some chance that resistance could be induced in higher plants. In 1984 we screened mutagenized seedling populations of the variety S-201 for resistance. Some 1,950 X_2-derived lines were germinated on petri dishes containing glyphosate at concentrations of either 0.015 mM or 0.04 mM. After two weeks growth, 121 plants which seemed to be surviving were transplanted to the greenhouse and grown to maturity. Progenies of these 121 plants were again screened in the seedling stage for glyphosate resistance, and 2 plants seemed to have some tolerance. Both plants, which trace back to a single X_2 line, are being grown to maturity for more progeny testing.

(ii) Somatic (seed-derived) tissue culture of rice.

Before we can begin a planned study to select for biochemical mutants in rice tissue culture, we must improve our techniques for large-scale regeneration of plants from callus.

The results to date indicate that rice can be regenerated from many types of tissue culture, but not yet at a high rate. We have regenerated plants from the varieties Calrose 76, M-101, L-201 and L-202, using explant sources of stems, mature seeds and immature embryos. Light is critical for regeneration with the highest light intensity (200 µE) giving the best regeneration. Regeneration usually procedes from sections of embryogenic callus which can be identified by having a different color and morphology than non-embryogenic callus. Occasionally plants regenerate from non-embryogenic areas but it isn't known if they might have originated from small, unidentified pieces of embryogenic callus. L-202 gives the highest regeneration with 34% of the callus cultures derived from mature seeds producing embryogenic callus (n=135) compared with 8% for M-101 (n=48). Immature embryos are better explant sources than mature seeds. They give healthier callus and a higher percentage of cultures with embryogenic callus 41% (n=34) for L-202 vs. 34%. Higher sucrose levels in the initiation medium increases embryogenic callus formation. Callus initiated from immature L-202 embryos on 5% sucrose gave 64% embryogenic cultures (n=17) vs. 41% for 2% sucrose (n=34).

(iii) Anther culture of rice

We have been developing anther culture methods for japonica rice, for selecting useful mutants and for shortening the breeding cycle. Callus and regeneration have been obtained from M-101 and S-201 and from reciprocal crosses between Calrose and waxy M-101.

In cold treatment of panicles vs. cold treatment of plated anthers, -cold treatment of anthers still on the panicles was found to be preferable for callus induction (Table 3). Some 474 plantlets have regenerated from anther culture, of which 200 were albino and of no value (Table 4). The green plantlets will be grown to maturity for further studies.

(iv) Evaluation of Dr. Schaeffer's anther culture-derived lines of Calrose 76.

Dr. Gideon Schaffer, USDA-ARS at Beltsville, MD, reported the regeneration from anther culture of Calrose 76 of a dwarf that was about 15-30% shorter than Calrose 76. Since rice cannot be grown in the field in Maryland, we grew two experiments for Dr. Schaeffer at Davis. The first experiment was an F_3 population of a cross between Schaeffer's dwarf and Calrose 76. Surprisingly, no short lines were observed in the population--all were the same height as Calrose 76. In the second experiment we measured small-plot yields of lines which Dr. Schaeffer felt had higher yield potential than Calrose 76. These lines were grown in a 3 replicate trial, with 3 rows per plot. Only the center row of each plot was harvested. One line, DR84-1-272, appeared to yield significantly more than the Calrose 76 check, but yield of this line was inflated because it was bordered by a double dwarf check (DD1) which provided less competition (Table 5). The DD1 check was used as a border because of insufficient seed of the test genotype. It was anticipated that some of the 18 experimental genotypes would be shorter than Calrose 76, but no differences were visible, so height was not measured.

Aside from the upwardly-biased yield of line DR84-1-272, only the S6/M7//M-301 double dwarf check from Dr. Carnahan yielded significantly more than Calrose 76.

Thus, although the successful regeneration of a dwarf from anther culture was an interesting achievement, the progenies from crosses with the regenerated source seem to have no immediate practical use.

PUBLICATIONS OR REPORTS:

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Mese, M. D., L. E. Azzini and J. N. Rutger. 1984. Isolation of genetic male sterile mutants in a semdwarf japonica rice cultivar. Crop Sci. 24:523-525.

Lehman, W. F., J. N. Rutger, L. E. Robinson, and M. Kaddah. 1984. Value of rice characteristics in selection for resistance to salinity in an arid environment. Agron. J. 76:366-370.

Lu, Yong-Gen and J. N. Rutger. 1984. Anther and pollen characteristics of induced genetic male sterile mutants in rice (*Oryza sativa* L.). Environmental and Experimental Botany 24:209-218.

Rutger, J. N. 1984. Genetic and agronomic evaluation of induced semidwarf mutants of rice. pp. 125-133. *In* Semi-Dwarf Cereal Mutants and Their Use in Cross-Breeding II. IAEA-TECDOC-307. IAEA, Vienna.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

Progress in development of useful germplasm included:

1) The introduction of additional genetic diversity through the production of some 3,000 F_1 hybrid plants from natural crossing of 400 world collection entries with the M-101 genetic male sterile.

2) The finding that weedy species donors of aggregate sheath spot resistance may have adequate field resistance even though they can be made susceptible under large inoculum loads of the pathogen in the greenhouse.

3) The development of an "Early S-201" mutant that is 7 days earlier than S-201, yielded 25% more in a small plot test, and has smaller kernels and less white belly than S-201.

Progress in investigation of standard and innovative breeding methods included:

1) The natural crossing of some 200 African lines onto the M-101 genetic male sterile as the initial step in a recurrent selection program for increased seed size.
2) Use of the chemical mutagen EMS to induce genetic male sterility in the California variety M-201.

3) Studies on hybrid rice which involved:

   a) Selection of one streptomycin-induced male sterile mutant--whether the sterility is the desired cytoplasmic type or merely another genetic type is yet to be determined.

   b) The finding that pollen fertility restoration for the Chinese cytoplasmic male sterile source is controlled by at least two genes, and is further confounded by genetic background of US varieties.

   c) Attempts to cause male sterility by spraying with a proprietary compound. Although some sterility was induced, almost no outcrossing occurred, making the value of this treatment uncertain.

4) Studies on innovative selection techniques which involved:

   a) Seedling screening for resistance to the herbicide glyphosate, in which 1,950 lines were first reduced to 121 plants, then to 2 plants which may have some resistance, although further progenies tests are required to confirm this.

   b) Somatic tissue culture, in which callus derived from immature embryos of the variety L-202, produced embryogenic (plant regeneration) rates of up to 64%. Other varieties produced lower rates.

   c) Anther culture regeneration in which 274 green plantlets have been regenerated in the last 4 months. This technique may yet be useful for shortening plant breeding cycles.

d) Evaluation of Dr. Schaeffer's anther culture-derived lines of Calrose 76. However, materials from this program had no advantage over the original parent, Calrose 76.

Table 1. Aggregate sheath spot reaction of two weedy rice species, O. rufipogon and O. fatua, and four cultivated rice lines in the greenhouse and field at Davis in summer 1984.

Genotype	Average Lesion Length, cm*	
	Greenhouse	Field
O. rufopogon A100923	20.1	10.1
O. fatua PI239671	15.8	7.7
O. sativa 78:18237	30.1	25.1
O. sativa 81:47528	29.5	10.3
O. sativa 82-Y-297	36.2	23.8
O. sativa 82-Y-396	24.1	10.9

* Large values indicate susceptibility; smaller values indicate resistance.

Table 2. Days to heading, yield, and 100-kernel weight of an "Early S-201" mutant and its parent, S-201.

Genotype	Days to heading	Grain yield, grams/13.4 ft^2	100-kernel weight, grams
Early S-201	93	1300	2.34
S-201	100	1043	2.42
$LSD_{.05}$	1	82	0.04

Table 3. Effect of chilling treatment (17C for 7-10 days) on production of callus by rice anthers.

	Total # anthers	# Anthers/ callus	% Anthers/ callus
Chilled as panicles	236	17	5.1
Chilled after planting into solid media	243	3	1.2

Table 4. Numbers of regenerated rice plants from anther culture in late 1984.

	Regenerated Plantlets		
Anther Source	Albino plantlets, No.	Green plantlets, No.	Green plantlets, %
M-101	26	73	74
S-201	125	129	49
Waxy M-101 x Calrose	21	42	67
Calrose x waxy M-101	28	30	52
Totals	200	274	58

Table 5. Yield and days to heading of 18 F_3 lines of Dr. Schaeffer's dwarf/Calrose 76, together with two semidwarf checks, Calrose 76 and 83:2444, and a double dwarf check, S6/M7//M302 from Dr. Carnahan.

Genotype	Yield grams/6.7 ft^2	Days to heading
DR-84-1-19	452	116
-24	430	116
-28	343	116
-79	422	115
-83	488	116
84	451	116
96	430	115
97	429	115
149	501	116
154	431	116
163	383	117
203	444	118
207	468	115
209	507	115
241	414	116
245	438	116
261	453	116
272	640*	117
Calrose 76 (check)	444	116
83:2444 (check)	507	117
S6/M7//M301 (check)	551	106
$LSD_{.05}$	91	1

* Inflated yield due to bordering of this genotype with a double-dwarf check, because seed supply of this line was limited.