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COMPREHENSIVE RESEARCH ON RICE

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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 techniques and genetic engineering:

1. Male sterile facilitated population improvement, and germplasm releases.
2. Identification of genetic male steriles with best outcrossing properties.
3. Hybrid rice mechanisms.
4. Tissue culture and other selection for herbicide resistance.
5. Other tissue culture studies.
6. Searching for apomixis in rice.
7. Isolation of transposable genetic elements.

SUMMARY OF 1987 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1. Male sterile facilitated population improvement, and germplasm releases.

   a. Male sterile facilitated population improvement.

   Rather than initiate an additional population in 1987, we decided to concentrate more resources on objective 2, below. That will enable identification of male steriles that show higher outcrossing rates; then we can return to this objective in the future.

   b. Germplasm releases.

   Twelve germplasm lines were released in early 1987, jointly with the California Co-operative Rice Research Foundation. These lines thus are being made available to other public and private rice breeders in the US, for use as parental sources of early maturity, short stature, and marker genes for hull color. Among the most useful lines were one with improved stem rot resistance, and another with improved water weevil tolerance (Table 1).

2. Identification of genetic male steriles with best outcrossing properties.

   Genetic male sterility (ms) is useful as a breeding tool for making hybrids with less effort. We have initiated studies to identify male steriles with best outcrossing rates. Thirty accumulated genetic male sterile mutants were included:

   13 EMS-induced mutants from M-201 (M-201 ms #1 to #13)
   2 streptomycin-induced mutants from M-201 (M-201 NP and M-201 S1)
   10 radiation-induced mutants from M-101 (M-101 ms #1,2,3,5,8,13,14,15,16,17)
   1 tissue culture mutant from Calrose 76 (C76 ms #1)
   4 naturally occurring mutants from 4 older varieties (N1, N2, N3, N4)

   Current and previous studies have shown that each of these 30 male steriles is inherited as a single recessive gene character, but it was not known whether 1 or 30 different genes were involved. Allelism tests, which show whether the same or different genes control sterility, were initiated by making about 300 crosses between plants of different sterile sources. (Since it is not possible to cross two male sterile plants, - two females will not hybridize with each other! - it was necessary to use heterozygous, male fertile, plants of one parent in each cross.) To date 47 crosses have been

evaluated, with the finding that each male sterile gene is different. Many more crosses will have to be evaluated, but these preliminary results indicate that many different genes are involved.

Much of the work in 1987 was on determination of fertility characters and outcrossing rates. Thus laboratory pollen staining was used as an index of male fertility, relying on the fact that pollen stainable with iodine-potassium iodide (IKI) or flourecin diacetate (FDR) is usually viable. Typically, male fertile lines such as M-101 and M-201 parents show 90% pollen staining when examined under the microscope, whereas male sterile lines show much less staining, with some being zero (Table 2). We generally would prefer male steriles which show less than 10% pollen staining, so as to minimize the chance of some self-fertile seed set on the sterile line. We also used a laboratory microscope test, the cleared-pistil technique, to estimate female fertility, measured as percent of normal egg sacs in the floret. It is important to know female fertility in male sterile sources, as the gene for male sterility sometimes can also have the undesired effect of causing female sterility, making such a source worth very little. Such seems to be the case for M-101 ms #17, which showed only 19.3% normal egg sacs in this test (Table 2). That information helps us understand why M-101 ms #17 has not been a very good outcrossing line in previous experiments, i.e., M-101 ms #17 not only is male sterile but also is rather female sterile as well.

Percent seed set in the field was determined in three ways in 1987. First, 10 or more panicles of each sterile source were hand-pollinated with normal pollen. Seed set thus obtained ranged from 2.9 to 56.3% on the steriles (Table 2). Second, percent seed set resulting from open, i.e., wind, pollination in the field, was determined on 5 plants of each sterile source, to measure outcrossing. Seed set thus obtained ranged from 0.2 to 31.7% on the steriles (Table 2). Third, we determined percent seed set under bagged selfing conditions, i.e., foreign pollen excluded by placing bags over 5 male sterile panicles at flowering. Seed set thus obtained ranged from 0 to 14.9% on the steriles (Table 2).

From these 1987 studies, the principal conclusion drawn was that the M-201 NP male sterile has the best overall outcrossing properties. It is completely pollen sterile (indeed it has no pollen (NP)), it has normal egg sacs (93.8%), it shows good seed set when hand pollinated (56.3%), shows 27.2% outcrossing, and has no bagged

selfing. Close behind M-201 NP in desirability is M-101 ms #2, a sterile which we have used in previous population improvement schemes. M-201 NP thus provides us with an additional useful male sterile source for the future.

3. 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. Photosensitive genetic male sterility

Photosensitive genetic male sterility would be an extremely useful genetic tool for making hybrid rice. For example, it would reduce hybrid rice breeding from a 3-line to a 2-line process. Last year we reported an apparently photosensitive male sterile, Calrose 76 ms-2, in which sterile plants became fertile when grown in the Hawaii winter nursery (short days of about 12 hours), but remained relatively sterile in the Davis summer nursery (long days of about 15 hours). This year we found two more lines in which sterility/fertility appears to be conditioned by day length, M-101 ms #3 and M-201 ms #7.

Behavior of the first putative photosensitive male sterile, Calrose 76 ms-2, is puzzling. Thus 9 plants, which were sterile in the field in 1986, were ratooned in the fall of 1986, each ratoon was divided into two halves, with one half going into a growth chamber with 12 hour (short) days and the other half going into a chamber with 15 hour (long) days. Averaged over the 9 clones, seed set was 50.1% under 12 hours and 16.7% under 15 hours. These data seem to support the hypothesis that fertility is enhanced by short day length. However, we are concerned about the 16.7% seed set under 15 hours--we expected almost none. This sterile source also was studied again in the 1986/87 Hawaii winter nursery and the 1987 summer nursery, with inconclusive results. Part of the confusing data probably are due to the fact that sterile plants show about 25% seed set under open pollination conditions at Davis--until we get some marker genes incorporated into this source we cannot determine if the 25% seed set is due to outcrossing or to some unusual self-pollination mechanism.

The two new, putative, photosensitive male steriles, M-101 ms #3 and M-201 ms #7, were discovered in summer 1987, as follows. In the greenhouse (short days) in the spring of 1987, fertile plants from populations expected to be segregating, were harvested and planted panicle-to-row in summer 1987 (long days). One fertile panicle of

the M-101 ms #3 source produced a row containing only sterile plants (13 of them), and another fertile panicle also produced a row with only sterile plants (19 plants). Similarly, one fertile panicle of M-201 ms #7 produced a row with 26 sterile and no fertile plants. These are the reactions expected if the plants had photosensitive male sterility.

Pollen staining of the 26 sterile plants of M-201 ms #7 was determined in August 1987 in the field, then all 26 plants were ratooned to the growth chamber under 12 hour days. The growth chamber ratoons flowered in late October-early November and pollen staining was again determined. Considerable fertility conversion, as measured by pollen staining, occurred as the plants were moved from the field (long days) to the growth chamber (short days). Thus under field conditions, pollen staining was only 16.8%, but in the growth chamber, pollen staining rose to 83.8% (Table 3). These results are strongly suggestive of photosensitive male sterility. In the months ahead we will pursue these findings with great interest.

b. Apomixis (see Sub-objective 6).

4. Tissue culture and other selection for herbicide resistance.

a. Tissue culture selection

Last year we selected cells from over 2,000 mature embryos of 5 rice varieties, finally narrowing this down to two regenerated plants of M-202, designated MA1-1 and MA1-2. When these plants were germinated for 10 days on agar containing 0.02 mg/L of AC499, they grew to heights of 32.0 and 28.0 mm, which was 3 to 4 times the height of the control in AC 499 (Table 4). However, when we grew these two plants to maturity and tested their progenies, these were not resistant. We have "gone back to the drawing board" and are trying additional tissue culture selection for AC 499 resistance.

b. Mutagenized seed selection

For the first experiments, mature seeds of M-201 treated in 1986 with 1.25% EMS were used for planting in the field. One thousand random panicles from the M1 plants were used as a screening population. About 2,500 M2 seeds were soaked 48 hours in 3 mg/L AC 499 and planted in shallow flats with soil. In one panicle row, two seedlings emerged which were transferred to the greenhouse and grown to maturity. Forty mature seeds from one selected plant, designated HS25, were placed on

agar with 0.02 mg/L AC 499 and allowed to grow 10 days. Four seedlings, designated HS25-1 to HS25-4, displayed shoot lengths of 4-12 fold greater than that of the M-102 control in AC 499 (Table 4). HS25-1 was transferred to agar for five days with 0.1 mg/L AC 499 and then transferred to soil. The remaining three clones were transferred to agar with 0.04 mg/L for five days and then to soil. These plants will be grown to the flowering stage and crossed with the M-102 check and other tester strains to determine inheritance of tolerance. Segregating progeny from these crosses will be tested up to 1 mg/L on agar and screened using the AHAS enzyme assay.

In a separate screening experiment, a second M2 bulk population derived from M-201 was used. The M2 seed were soaked in various experiments at 4, 5, and 10 mg/L AC 499 for 10 days. Individuals that developed roots and shoots were washed with distilled water and planted. To date 200,000 M2 seeds have been screened with 6 surviving seedlings that are currently growing in the greenhouse. As with the other experiments, progeny from these 6 plants will be tested for the level and inheritance of tolerance to AC 499.

5. Other tissue culture studies.

a. Anther culture

A useful application of anther culture to plant breeding would be the production of "instant" pure lines from F_1 plants. This could shorten variety development cycles by 2 or 3 years. Since December 1986, 28,010 anthers have been planted onto agar. Only 9810 of these have had time to produce callus and 7871 have had time to regenerate plants. Actual number of plants regenerated so far is 140, of which only 87 had green shoots (the others were albinos). About 30% of the regenerated plants are spontaneous diploids while the rest are haploids. Colchicine treatments are in progress to double the haploid plants and thus make them into diploids. Inheritance of anther culture regeneration ability is being studied in a diallel cross of seven genotypes.

b. Somaclonal variation

Somaclonal variation is the term which describes the mutations that are induced by tissue culture. Over the last few years it has become evident to many researchers that the tissue culture process is in itself a way of inducing mutations. The question then arises of whether the same spectrum of mutants arises from somaclonal variation as from conventional seed mutagenesis by

irradiation, etc. To do this we are comparing the following mutants from L-202: 73 from seed irradiation, 51 from seed treatment with the chemical mutagen EMS, and 55 somaclonal mutants. There seem to be some small differences in spectra and frequencies of mutants from the three treatments. However, the most interesting mutants, three lines which seem to show dominant genetic male sterility instead of the usual recessive genetic male sterility, all came from seed irradiation. Another season is required to complete this study.

6. 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. One of the approaches in screening for apomixis has been to look for high-frequency twin seedlings, i.e., two shoots per seed. Twin seedlings can be an indicator of apomixis. Mr. Li Yuan Ching, a visiting scientist from China, brought four high-frequency twinning lines with him in May 1986. These lines were grown in the plant quarantine introduction nursery at Beltsville, MD in late 1986, and brought to Davis in early 1987. Frequency of twin seedlings in these four lines ranged from 6 to 32%, which is much higher than the usual frequency of 0.1% or less that is observed in ordinary rice (Table 5). Of great interest is the fact that many of the twin seedlings arise from two separate mesocotyls, indicating the presence of two embryos in the seed. Since many apomictic grasses show high-frequency twinning due to multiple embryos per seed, the present data are encouraging. It must be noted, however, that multiple embryos can result from causes other than apomixis. Cytological analyses are underway to determine the exact origins of the twins. In addition, several crosses have been made to study the inheritance of the twinning phenomenon (Table 6).

7. Isolation of transposable genetic elements.

Transposable genetic elements, or "jumping genes", are useful genetic engineering tools for isolating and moving foreign genes into an organism.

As for sub-objective 6, 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. Among 82 waxy endosperm genotypes screened to date, four show spontaneous reversion to normal pollen at rates of 10^{-5}. We crossed two of these four lines with genetic marker stocks carrying plant color genes, in order to set up progeny tests to detect mosaic patterns caused by transposition. We also sent two lines to a molecular genetics lab in Georgia for molecular screening; some positive evidence of transposition has been reported.

PUBLICATIONS OR REPORTS:

McKenzie, K. S., P. K. Bollich, D. E. Groth, F. Jodari, J. F. Robinson and J. N. Rutger. 1987. Registration of 'Mercury' rice. Crop Sci. 27 (in press).

Oard, J. H. and J. N. Rutger. 1987. Callus induction and plant regeneration in elite U.S. rice lines. Crop Sci. (accepted 11/20/87).

Rutger, J. N., R. A. Figoni, R. K. Webster, J. J. Oster and K. S. McKenzie. 1987. Registration of early maturing, marker gene, and stem rot resistant germplasm lines of rice. Crop Sci. 27:1319-1320.

Tseng, S. T., C. W. Johnson, A. A. Grigarick, J. N. Rutger and H. L. Carnahan. 1987. Registration of short stature, early maturing, and water weevil tolerant germplasm lines of rice. Crop Sci. 27:1320-1321.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

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

1. In cooperation with the California Cooperative Rice Research Foundation Inc., 12 improved germplasm lines were released.

2. Thirty different genetic male sterile mutants were studied, in order to identify ones with best outcrossing rates. One new mutant, M-201 NP, and one older mutant M-101 ms #2, were found to be the best ones; both show outcrossing rates of 20% or higher.

3. In studies on genetic mechanisms for hybrid rice, three different genetic male steriles have been identified in which sterility apparently can be turned on and off by adjusting day length. If confirmed, these would be valuable genetic tools for producing hybrid rice.

4. Tissue culture and other selection for rice mutants resistant to a grass-killing herbicide continues. Some previous selections have been discarded but some new ones are coming along.

5. In other tissue culture studies, low rates of regeneration of plants from anther culture continues to be a bottleneck. In a study involving comparison of somaclonal variations and regular induced mutation, three possible dominant genetic male sterile mutants have been found. Most male sterile mutants are recessive; dominant mutants are more desirable.

6. With assistance from Rockefeller Foundation we have introduced four lines from China which show high-frequency twin seedlings (6 to 32% compared to perhaps 0.1% in normal lines). High-frequency twinning is often associated with apomixis, which would be desirable for hybrid rice breeding.

7. Also with Rockefeller Foundation assistance, we have identified four lines which display some of the symptoms expected of materials with transposable genetic elements ("jumping genes"). Transposable genetic elements are useful genetic engineering tools for isolating and moving foreign genes into an organism.

Table 1. Improved germplasm lines released as parental breeding materials in 1987.

Line	Source	Unique Attributes
PI 506219	S-201 mutant	7 days earlier than S-201
PI 506220	M9 mutant	7 days earlier than M9
PI 506221	M-101 mutant	Light green panicle
PI 506222	ESD7-3 mutant	Yellow green panicle
PI 506223	M-101 mutant	Waxy endosperm
PI 506224	M-101 mutant	Goldhull
PI 506225	CI 11032 mutant	Short stature
PI 506226	CI 11032 mutant	Short stature
PI 506227	CI 11032 mutant	Short stature
PI 506228	CI 11032 mutant	Short stature
PI 506229	Interspec. cross	Stem rot resistance
PI 506230	Complex cross	Water weevil tolerance

Table 2. Fertility characters of 30 ms sources in 1987.

Source	Pollen Staining, % IKI	Pollen Staining, % FDR	Normal egg sacs, %	Seed set, % Hand pollination	Seed set, % Out-crossing	Seed set, % Bagged selfing
M-201 ms#1	40.1	36.0	94.6	49.4	21.3	7.2
M-201 ms#2	31.3	9.9	93.5	56.2	8.9	0.1
M-201 ms#3	9.2	6.9	20.8	13.3	4.9	0.5
M-201 ms#4	0	0	92.7	47.9	5.3	0
M-201 ms#5	12.9	19.4	61.9	27.8	14.2	0
M-201 ms#6	65.1	50.8	94.0	35.3	14.5	14.9
M-201 ms#7	20.2	20.0			15.5	0.8
M-201 ms#8	16.8	10.2	94.8	46.8	14.0	1.28
M-201 ms#9	16.4	9.3	56.7	13.7	25.5	13.6
M-201 ms#10	29.1	21.5	95.8	31.7	13.2	4.2
M-201 ms#11	1.2	3.8	54.5	33.4	11.3	0.1
M-201 ms#12	9.6	13.6	18.3	8.4	6.9	0.2
M-201 ms#13	4.3	3.1	37.2	26.1	3.6	1.0
M-101 ms#2	0	0	97.6	28.9	20.7	0
M-101 ms#1	7.6	3.4	89.6	35.2	19.0	0.1
M-101 ms#1	0.6	0	19.3	5.7	9.3	0.6
M-101 ms#3	4.6	5.3	29.3	19.0	13.2	2.1
M-101 ms#5	0.5	0.7	50.0	18.0	19.0	1.3
M-101 ms#8	85.1	95.8				
M-101 ms#13	2.6	2.2	27.2	7.4	11.4	0.1
M-101 ms#14	0	0	46.7	8.5	5.5	0
M-101 ms#15		92.6				
M-101 ms#16	1.3	0.6	60.2	23.4	31.7	3.0
N1	0.3	0.3	30.5	14.2	13.2	1.3
N2	2.7	3.1	71.3		0.2	0
N3	0	0	82.4	15.2	0.4	0
N4	1.5	6.7	87.8	12.3	3.5	0
M-201 NP	0	0	93.8	56.3	27.2	0
M-201 S1	0.1	0.1	47.5	7.5	2.0	0
C76 ms#1	0	0	36.1	2.9	2.6	0
M-101 parent	91.7	72.7	94.5		84.0	55.71
M-201 parent	90.0	90.0	93.5			

Table 3. Fertility conversion as measured by IKI pollen staining of M-201 ms#7.

87 Row & Plant No.	As of August in the field			As of Oct-Nov in growth chambers		
	Unstained No.	Stained No.	Stained %	Unstained No.	Stained No.	Stained %
21555-01	45	261	17.2	518	57	90.0
21555-02	165	369	44.7	458	95	82.8
21555-03	9	204	4.4			
21555-04	25	328	7.6			
21555-05	154	513	30.0	376	138	73.1
21555-06	198	479	41.3	580	87	86.9
21555-07	87	359	24.2			
21555-08	15	227	6.6	431	76	85.0
21555-09	7	240	2.9	355	170	67.6
21555-10	77	325	23.6	429	98	81.4
21555-11	117	372	31.4	347	60	85.2
21555-12	40	251	15.9	525	67	88.6
21555-13	38	175	21.7	554	151	78.5
21555-14	9	152	5.9	808	95	89.4
21555-15	38	283	13.4	590	73	88.9
21555-16	30	234	12.8	306	61	83.3
21555-17	92	409	22.2	590	53	91.7
21555-18	57	295	19.3	681	134	83.5
21555-19	58	404	14.3			
21555-20	3	262	1.1	624	116	84.3
21555-21	76	618	12.3			
21555-22	8	266	3.0	456	88	83.8
21555-23	33	368	9.9	449	81	84.7
21555-24	45	219	20.5	445	104	81.0
21555-25	66	281	23.4	549	90	85.9
21555-26	21	281	7.4			
Total	1512	8175		10071	1894	
Average			16.8			83.8
Std. Dev.			11.4			5.7

Table 4. Shoot and root length of seedlings exposed 10 days to AC 499.

Genotype	Shoot length, mm	Root length, mm
M-102 control (no AC 499)	151.6	63.8
M-102 control (0.02 mg/L AC 499)	8.4	9.0
MA1-1 (tissue culture selection)	32.0	19.0
MA1-2 (tissue culture selection)	28.0	29.0
HS25-1 (mutagenized seed selection)	99.0	25.0
HS25-2 (mutagenized seed selection)	48.0	30.0
HS25-3 (mutagenized seed selection)	93.0	22.0
HS25-4 (mutagenized seed selection)	73.0	31.0

NOTE: All plants had multiple roots except the M-102 control with herbicide.

Table 5. Frequency of twin seedlings in four Chinese rice lines grown at Davis in 1987.

Line	Number of seeds germinated	Number of twin seedlings: One mesocotyl*	Number of twin seedlings: Two mesocotyls	Rate of twinning
				%
AP I (japonica)	554	47	42	16
AP II (japonica)	208	29	9	18
AP III (indica)	530	64	108	32
AP IV (indica)	110	0	7	6

* Mesocotyls are the first true internode of a seedling.

Table 6. Crosses made between normal and twin seedling lines.

Cross	F_1 plants, number	F_2 plants, number
83:11542/API	6	828
PI 373761/AP II	4	667
Guang 36/AP III	1	365
PI 408449/AP III	8	
AP III/PI 408449	20	
PI 408449/AP IV	49	
AP IV/PI 408449	46	
AP I /PI 4	12	
AP II/PI 3	76	