

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE

January 1, 1988 - December 31, 1988

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. Identification of genetic male steriles with best outcrossing properties.
2. Hybrid rice mechanisms.
3. Tissue culture and other selection for herbicide resistance.
4. Anther culture studies for "instant homozygote" production.
5. Other genetic studies.
6. Searching for apomixis in rice.
7. Isolation of transposable genetic elements.

SUMMARY OF 1988 RESEARCH (MAJOR ACCOMPLISHMENTS) BY
OBJECTIVES:

1. 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. As part of a long-term effort to identify useful ms sources, Mr. Jinguo Hu completed his Ph.D. thesis in December 1988, on "Genetics, developmental cytology and fertility characterization of 28 male sterile mutants in rice (Oryza sativa L.)." Each of the 28 male steriles is inherited as a single recessive gene character. Allelism tests, which show whether the same or different genes control sterility, were conducted by making 309 of the possible 378 crosses between 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.) The data indicate that at least 20 different genes are involved, i.e., almost every male sterile gene is different.

From a three-stage series of tests involving pollen stainability (viability), female fertility, and outcrossed seed set rate, we concluded that the two most useful mutants are M-201 no-pollen ms and M-101 ms #2. both are completely pollen sterile (desirable because selfing is eliminated), both have normal female fertility (desirable because they can function satisfactorily as females in crossing), and both show high rates of natural outcrossing (desirable for use in practical crossing studies). M-201 no pollen ms and M-101 ms #2 are coded in Table 1 as NP and G1, respectively.

Seeds of these two male steriles will be distributed to plant breeders as requested.

2. Hybrid rice mechanisms

Although we are not as optimistic about the potential of hybrid rice as China is, we continued 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. Previously 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).

In the 1988 Davis summer nursery (long days), this source of male sterility segregated in the expected recessive gene fashion (286 fertile:108 sterile plants; Chi square for 3:1 ratio = 1.23, non-significant). Seed set on the fertile plants averaged 76%; seed set on sterile plants averaged 16%. Presumably the 16% seed set on the sterile plants arose from outcrossing, but until we get our marker genes into the population we cannot be sure. Therefore, in 1988, crosses were made with a marker gene stock to clarify the outcrossing matter.

In 1988 a second male sterile, M-201 ms-7, was confirmed to be environmentally sensitive, although the environmental factor for this source may be temperature rather than day length. A possible third source, M-101 ms #3, did not prove to be reproducibly sensitive to day length or temperature.

Both the Calrose 76 ms-2 and the M-201 ms-7 are being further evaluated in the 1988/89 Hawaii winter nursery.

b. Apomixis (see Sub-objective 6)

3. Tissue culture and other selection for herbicide resistance

As reported last year, tissue culture mutants selected for resistance to the American Cyanamid herbicide AC499 seemed initially promising but did not hold up under repeated testing. Therefore, we have concentrated on the parallel study of selecting resistant mutants in mutagenized seed populations. In brief, these latter mutants seem to be holding up under repeated testing.

The best candidates for tolerant rice lines have been selected from M2 bulk populations of the rice cultivar M-102. M4 seedlings from three selected lines, HS7, HS17, and HS51, have survived a soak and spray assay and are currently growing in the greenhouse. When available, tillers from these lines will be sent to American Cyanamid for the AHAS enzyme assay. These same individuals have been grown to the flowering stage and crossed to Calmochi-101, a cultivar with two morphological markers, in order to determine segregation patterns of putative tolerant herbicide gene(s). Backcrosses are being made to the M-102 parent cultivar to increase levels of tolerance as was presumably the case with the tissue culture-selected tolerant maize lines of American Cyanamid. In summary, our progress in the past year towards selecting tolerant rice lines has been encouraging, and we expect that our objectives will be met in one more year or less.

4. Anther culture study for "instant homozygote" production

Ms. Shannon Pinson is expected to complete her Ph.D. thesis in May, 1989, on "Anther culture and its use in rice breeding." A useful application of anther culture to plant breeding would be the production of "instant" pure lines from F₁ plants. This could shorten variety development cycles by 2 or 3 years.

In 1988, an economic analysis of anther culture was expanded to include the culture of F₂ anthers. The use of F₂ anthers would increase the probability of recombination in cases of undesirable linkage. The use of F₂ anthers doubles the chance of recombination when compared to the use of F₁ anthers, but still offers only 2/5 the chance offered by selfing regimes that go to the F₆ generation. When the culture of F₂ anthers is used, it increases the time required for the production of pure lines by 6 months, however, this method is still faster than Single Seed Descent (SSD) (2 generations per year) by as much as a year. If culture of F₂ anthers is coupled with selection on the F₂ population, the number of lines to be produced through anther culture can be reduced. (For instance, if selection is made on F₂ plants for glabrousness, only 1/4 of the plants would be cultured to produce homozygous lines. Yet this smaller number of lines would be equivalent to the production of 4X as many lines from F₁ anther culture.) The

reduction in lines to be produced can make the anther culture process even less expensive than SSD.

The use of anther culture in genetic analyses was also examined in 1988. Fifteen traits were tested for their expressability in haploids, and for segregation frequencies that fit current linkage knowledge. Only 2 of the 15 traits failed to be expressed in haploids, and only 2 failed to segregate in the expected ratios (Table 2).

An inheritance study of regenerability within a 7-parent California germplasm diallel was also continued in 1988. Data will be available for inclusion in Ms. Pinson's thesis.

5. Other genetic studies

In 1981 Rutger and Carnahan reported on a recessive tall mutant designated eui for elongated uppermost internode, which might be useful in hybrid rice seed production. Since that time, Drs. Carnahan, Johnson, and Tseng have found similar-appearing mutants in four additional varieties: M-301, M-202, 83Y45, and Calmochi-101. Panicle and internode measurements of these four new types, together with their parent lines and the original eui source, are given in Table 3. The principal distinguishing features of the eui type are the near-doubling in length of the 1st (uppermost) internode, and the greatly increased length between the panicle collar and the flag leaf. From Table 3 it is evident that all four of the new eui types are phenotypically similar to the original eui type. In order to determine if the new eui types have the same or different gene from the original eui, appropriate crosses were made. To date, 5 of the 8 needed crosses have been studied in both the F_1 and F_2 generations. Although incomplete at this time, the cumulative evidence indicates that all four new sources are 1) recessive tall, and 2) allelic (have the same gene) to the original eui (Table 4). As this study is completed, we also will determine the effect of the eui gene(s) on seed length - a general observation is that seed length is slightly increased by this gene.

Mr. Zhikang Li completed the data collection for his Ph.D. thesis on "Genetic Structure of Cultivated Rice: Morphological, Quantitative, and Electrophoretic Variability". Each of the three types of variability represents only a part of the

total genetic diversity in rice. Therefore, it is important to understand the general pattern of the three types of diversity and their relationships for planning breeding programs and conserving rice genetic resources. The main objectives of this study are: 1) to examine both spacial and temporal patterns of variation in morphological characters, quantitative characters, and isozymes of American rice varieties and world rice collections, which are presumed to represent a significant portion of total genetic diversity of rice; and 2) to study the relationships between the three types of genetic diversity, in order to obtain a clearer picture of genetic organization of the species and give more reasonable classification of rice varieties. A sample of 510 rice varieties from 9 presumed geographical regions and 31 countries was used in the electrophoretic survey: 215 of these were selected for studying morphological and quantitative variations under field conditions (Table 5). Lengthy statistical analyses are underway and will be reported in Mr. Li's thesis about mid-1989.

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 been searching 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 four rice lines, designated AP I, AP II, AP

III, and AP IV, exhibited twin seedling rates of 16.1%, 23.4%, 32.4%, and 5.0%, respectively. Twin seedlings were of two types, one-mesocotyl and two-mesocotyl. Embryo sac analysis indicated that most twin seedlings originated from fertilized two-egg nuclei or multiple egg nuclei. However, there were adventitious embryos in the embryo sacs at frequencies of 2.6-5.1% which were proportional to frequencies of green single seedlings or twin seedlings in F_1 's between twin seedling females and a dominant purple leaf male. Therefore, there may be a low frequency of apomixis in these twin seedling lines, which presumably was derived from integument or antipodal cells. The tendency to produce twin seedlings seemed to be controlled by two recessive genes in three crosses (Table 6), although this needs to be confirmed by progeny tests which will be completed in January 1989.

Another approach used was to attempt to cross rice with known apomictic pearl millet species, Pennisetum flaccidum and Pennisetum squamulatum. In 1988, 10,936 rice florets were pollinated with pollen from these Pennisetum species, but no true hybrid seeds were formed (Table 7). Earlier reports from China suggested such crosses might be possible, but we were not able to reproduce their results.

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 received partial assistance from Rockefeller Foundation to pursue this extremely basic research. Results to date have been inconclusive.

PUBLICATIONS OR REPORTS:

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- Rutger, J. N. and T. R. Anderson. 1988. Evaluation, inheritance, and gibberellin response of induced semidwarf mutants of rice. pp. 125-133. In: Semi-Dwarf Cereal Mutants and Their Use in Cross-Breeding III. IAEA-TECDOC-455.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

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

1. A study on 28 genetic male sterile mutants was concluded. Mutants M-201 NP and M-101 ms #2 were confirmed to be the best; both show outcrossing rates of 18%.
2. In studies on genetic mechanisms for hybrid rice, two genetic male steriles have been identified in

which sterility apparently can be turned on and off by adjusting day length and/or temperature. If confirmed, these would be valuable genetic tools for producing hybrid rice.

3. Three rice mutants which are tolerant to a grass-killing herbicide have been identified. Progeny tests to determine inheritance of tolerance are underway.
4. In anther culture studies, the use of anthers from F_2 plants was considered. Otherwise, anther culture was tested as a tool for genetic analysis.
5. In other genetic studies, elongated uppermost internode (eu1) mutants appear to carry the same gene as the original eu1 mutant. A study on electrophoretic variation was continued.
6. Four lines from China which show high-frequency twin seedlings (5 to 32% compared to perhaps 0.1% in normal lines) were crossed to normal lines. High-frequency twinning is often associated with apomixis, which would be desirable for hybrid rice breeding.
7. Studies on lines which display some of the symptoms expected of materials with transposable genetic elements ("jumping genes") have been inconclusive. Transposable genetic elements are useful genetic engineering tools for isolating and moving foreign genes into an organism.

Table 1. Fertility characterizations of 28 male sterile mutants and the two normal cultivars M-101 and M-201.

| ID Code | Pollen Stainability (%) | | | Female Fertility (%) | | | Seed Set Rate (%) | | |
|---------|-------------------------|---------------|-----------|----------------------|------|------|-------------------|------------------------|--|
| | 1987 (IKI & FCR) | 1988 (IKI) | Mean | 1987 | 1988 | Mean | Bagged Selfing | Natural Outcrossing | |
| | | | | | | | | | |
| M-201 | 90.0 | 93.5 | 91.8 | 93.5 | 96.8 | 95.2 | 74.6 | 82.9 | |
| E1 | 38.1 | 10.1 | 24.1 | 54.6 | 44.9 | 49.8 | 7.3 | 18.6 | |
| E2 | 20.7 | 32.4 | 26.5 | 93.5 | 96.7 | 95.1 | 0.1 | 13.0 | |
| E3 | 8.1 | 0.5 | 4.3 | 20.8 | 17.0 | 18.9 | 0.6 | 3.1 | |
| E4 | 0.0 | 0.3 | 0.2 | 92.7 | 97.1 | 94.9 | 0.0 | 6.3 | |
| E5 | 16.2 | 13.5 | 14.9 | 61.9 | 65.8 | 63.9 | 1.9 | 10.9 | |
| E6 | 58.0 | 42.8 | 50.4 | 94.0 | 96.6 | 95.3 | 14.9 | 9.7 | |
| E7 | 20.1 | 18.5 | 19.3 | 95.3 | 97.0 | 96.2 | 0.8 | 17.1 | |
| E8 | 13.6 | 23.9 | 18.7 | 94.8 | 97.1 | 96.0 | 1.3 | 9.7 | |
| E9 | 12.9 | 46.8 | 29.8 | 56.7 | 63.6 | 60.2 | 13.6 | 23.0 | |
| E10 | 25.3 | 10.4 | 17.9 | 95.8 | 93.9 | 94.8 | 4.2 | 7.5 | |
| E11 | 2.6 | 3.3 | 2.9 | 54.5 | 42.7 | 48.6 | 0.2 | 6.9 | |
| E12 | 11.7 | 9.0 | 10.3 | 18.3 | 18.9 | 18.6 | 0.3 | 8.1 | |
| E13 | 3.7 | 3.5 | 3.6 | 37.2 | 27.1 | 32.1 | 1.1 | 5.0 | |
| * NP | No Pollen | No Pollen | No Pollen | 93.8 | 94.9 | 94.4 | 0.0 | 34.5 | |
| S1 | 0.1 | 0.1 | 0.1 | 47.5 | 42.6 | 45.1 | 0.0 | 2.0 | |
| T1 | No Pollen | No Pollen | No Pollen | 36.1 | 32.6 | 34.3 | 0.0 | 1.7 | |

Continued

Table 1. Continued.

| ID Code | Pollen Stainability (%) | | | Female Fertility (%) | | Seed Set Rate (%) | |
|---------|-------------------------|---------------|-----------|----------------------|------|-------------------|------------------------|
| | | | | | | | |
| | 1987 (IKI & FCR) | 1988 (IKI) | Mean | 1987 | 1988 | Bagged Selfing | Natural Outcrossing |
| M-101 | 82.3 | 95.6 | 88.9 | 94.5 | 98.6 | 55.7 | 86.2 |
| *G1 | 0.0 | 0.1 | 0.1 | 97.6 | 97.9 | 0.0 | 18.7 |
| G2 | 5.5 | 14.6 | 10.1 | 89.6 | 74.8 | 0.2 | 18.9 |
| G3 | 0.3 | 0.6 | 0.4 | 19.3 | 22.4 | 0.7 | 8.9 |
| G4 | 5.0 | 1.6 | 3.3 | 29.3 | 35.0 | 2.1 | 15.5 |
| G5 | 0.7 | 0.0 | 0.3 | 50.0 | 42.3 | 1.3 | 11.2 |
| G6 | 84.3 | 83.9 | 84.1 | 94.0 | 95.3 | 0.0 | 19.5 |
| G7 | 2.4 | 9.9 | 6.1 | 27.3 | 23.0 | 0.1 | 18.7 |
| G8 | No Pollen | No Pollen | No Pollen | 46.7 | 51.0 | 0.0 | 3.5 |
| G10 | 1.0 | 1.3 | 1.2 | 60.2 | 53.9 | 3.0 | 27.1 |
| N1 | 0.3 | 0.5 | 0.4 | 30.6 | 27.6 | 1.4 | 9.4 |
| N3 | 0.0 | 0.0 | 0.0 | 82.4 | 88.4 | 0.0 | 0.4 |
| N4 | 4.1 | 0.0 | 2.1 | 87.8 | 95.6 | 0.0 | 2.9 |

* Two best male steriles for rice in outcrossing.

Table 2. Expression of 15 qualitative traits in regenerated haploids and their segregation.

| Anatomical traits | Detectable in haploids | Segregation as expected | χ^2 | Plants regenerated |
|--------------------|------------------------|-------------------------|----------|--------------------|
| Cl | yes | yes | 0.35 | 45 |
| lg | yes | no(excess lg) | 0.45** | 20 |
| nl | ? | yes | 2.5 | 20 |
| Sd | (not yet) | yes | 0.13 | 30 |
| gh | yes | yes | 0.55 | 29 |
| G1 | yes | no | 4.9* | 128 |
| la | yes | yes | 0.13 | 31 |
| g | ? | (not yet) | 1.38 | 18 |
| An | no | yes | 0.04 | 36 |
| Plant color traits | | | | |
| C | yes | yes | 0.01 | 83 |
| P | yes | yes | 1.06 | 71 |
| Pl | yes | yes | 1.2 | 56 |
| Pn | yes | yes | 1.07 | 38 |
| Ps | yes | yes | 0.35 | 26 |
| Pr | yes | yes | 0.38 | 24 |

Table 3. Panicle and internode length of the original eui mutant and four additional mutants and their parent varieties.

| Genotype | Panicle length, cm | Panicle collar to flag leaf, cm | Internode length, cm | | | |
|-------------------------|--------------------|---------------------------------|----------------------|------|------|-----|
| | | | 1st | 2nd | 3rd | 4th |
| Original <u>eui</u> * | 22.8 | 17.7 | 49.2 | 17.0 | 12.7 | 5.8 |
| M-301 <u>eui</u> | 21.0 | 23.4 | 54.1 | 20.8 | 15.2 | 5.4 |
| M-302 | 19.9 | 2.0 | 30.9 | 18.9 | 11.6 | 4.2 |
| M-202 <u>eui</u> | 18.6 | 17.3 | 44.0 | 14.5 | 11.8 | 7.0 |
| M-202 | 15.9 | 1.2 | 24.0 | 14.8 | 11.2 | 6.0 |
| 83Y45 <u>eui</u> | 20.2 | 13.5 | 40.4 | 14.3 | 12.5 | 7.5 |
| 83Y45 | 18.9 | 1.3 | 25.4 | 13.8 | 11.6 | 6.6 |
| Calmochi-101 <u>eui</u> | 19.3 | 16.9 | 45.1 | 18.8 | 12.1 | 5.8 |
| Calmochi-101 | 18.6 | -0.3 | 26.4 | 15.3 | 10.2 | 4.8 |

*Average of pubescent and glabrous versions.

Table 4. Inheritance of four new eui sources relative to their parents and to the original eui sources.

| Genotype | Behavior in crosses to: | | | |
|-------------------------|-------------------------|----------------------------|-----------------------|----------------------------|
| | Short parent | | Original <u>eui</u> | |
| | F ₁ height | F ₂ segregation | F ₁ height | F ₂ segregation |
| M-301 <u>eui</u> | short | 3 short:1 tall | -- | -- |
| M-202 <u>eui</u> | -- | -- | tall | none |
| 83Y45 <u>eui</u> | short | 3 short:1 tall | tall | none |
| Calmochi-101 <u>eui</u> | -- | -- | tall | none |

Table 5. Composition of the rice variety samples used for studies of electrophoretic, morphological, and quantitative variation.

| Region | Country | <u>No. of varieties</u> | |
|-----------------|---------------|-------------------------|-----|
| North Asia | Japan | 53 | 72 |
| | Korea | 19 | |
| East Asia | China | 120 | 139 |
| | China Taiwan | 19 | |
| South-East Asia | Burma | 9 | 33 |
| | Thailand | 10 | |
| | Vietnam | 4 | |
| South Asia | Indonesia | 12 | 51 |
| | Philippines | 33 | |
| | Malaysia | 6 | |
| Middle Asia | India | 36 | 62 |
| | Sri Lanka | 1 | |
| | Pakistan | 7 | |
| | Bangladesh | 3 | |
| | Iran | 5 | |
| | Turkey | 10 | |
| Europe | France | 8 | 55 |
| | USSR | 16 | |
| | Spain | 7 | |
| | Bulgaria | 5 | |
| | Hungary | 5 | |
| | Italy | 13 | |
| | New Caledonia | 1 | |
| | Egypt | 7 | 7 |
| South America | Argentina | 7 | 22 |
| | El Salvador | 1 | |
| | Peru | 3 | |
| | Brazil | 10 | |
| | Venezuela | 1 | |
| | California | 16 | 69 |
| North America | Texas | 18 | |
| | Louisiana | 13 | |
| | Arkansas | 16 | |
| | Others | 5 | |
| TOTAL | | | 510 |

Table 6. Segregation of progenies of three F₂ combinations between conventional and twin seedling lines.

| Combination | Total No. of F ₂ plants progeny tested | No. of genotype aapp (with twins) | No. of genotype A- P- (no twins) | P |
|------------------|---|--|---|-------------|
| 83:11542 /AP I | 306 | 24 | 282 | 0.10 - 0.25 |
| PI 373761 /AP II | 314 | 25 | 289 | 0.10 - 0.25 |
| Guang 36 /AP III | 365 | 27 | 338 | 0.25 - 0.50 |

Table 7. Cross combinations attempted in 1988 between rice females and Pennisetum flaccidum and P. squamulatum males.

| Female | Male | Pollinated florets, no, |
|----------------------------|--|----------------------------|
| 14020-3 NP ms | <u>P. flaccidum</u> | 3200 |
| Tetraploid M-101 ms | <u>P. flaccidum</u> | 1023 |
| Waxy NP ms | " | 573 |
| Waxy ms | " | 475 |
| Waxy M-101 | " | 103 |
| Gold Hull L-202 | " | 551 |
| 15173 | " | 97 |
| Bahia 309 | " | 246 |
| 14222-7 | " | 126 |
| G158 | " | 142 |
| Liso 392 | " | 98 |
| Cal Pearl 22 | " | 267 |
| 373241-7 | " | 78 |
| AP 2 | " | 127 |
| V47 | " | 53 |
| (M-101 ms/PI161020) F1 | " | 87 |
| 14020-3 NP | <u>P. squamulatum</u> | 2750 |
| Tetraploid M-101 ms | " | 175 |
| Tetraploid M 3 | " | 176 |
| " SD 7 | " | 132 |
| S 202 | " | 248 |
| Tetraploid M-101 ms. | <u>P. flac.</u> + <u>P. squa.</u> | 89 |
| (14020-3 NP ms/PI295697) / | F1 / <u>P. flac.</u> + <u>P. squa.</u> | 145 |
| Tswrugiba | <u>P. flac.</u> + <u>P. squa.</u> | 75 |
| Total | | 10,936 |