Project No. RB-3

# ANNUAL REPORT
# COMPREHENSIVE RESEARCH ON RICE
January 1, 1989 - December 31, 1989

**PROJECT TITLE:** Genetic and Physiological Determinants of Yield and Quality

**PROJECT LEADER AND PRINCIPAL UC INVESTIGATORS:**


*J. Neil Rutger, Leader, Research Geneticist, USDA-ARS, and Adjunct Professor, UCD
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S. R. Pinson, Graduate Student
Zhikang Li, Graduate Student



**LEVEL OF 1989 FUNDING:** $8,500


*NOTE: Rutger left on January 3, 1989. The 1989 proposal was only to use residual funds of about $8,500 from the CY 1988 allocation. These funds were used to employ two part-time people (Zhikang Li and Linghua Li) to conclude some ongoing aspects of the rice genetics research. Until the ARS position is refilled, Dr. James Oard is conducting the interim activities of the rice genetics project.

## 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 subobjectives, arranged in approximate order from conventional procedures through those which provide a bridge between conventional techniques and genetic engineering:

1. Photosensitive genetic male sterility as a hybrid rice mechanism.

2. Tissue culture and other selection for herbicide resistance.

3. Anther culture studies for "instant homozygote" production.

4. Other genetic studies.

## SUMMARY OF 1989 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1. Photosensitive genetic male sterility as a hybrid rice mechanism.

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 1989 Davis summer nursery, an F2 population was grown of a controlled cross between the Calrose 76 ms-2 and the marker gene line, waxy M-101 (PI 506223). The F2 generation segregated for glabrous leaves (55 pubescent:39 glabrous, $P(3:1) \leq 0.005$), and waxy endosperm (28 normal endosperm:55 heterozyous:ll waxy endosperm, P(1:2:1 = 0.01 - 0.025), but unexpectedly failed to segregate for sterility, i.e., all plants were fertile. Thus, even though the mutant was stable in progenies transmitted by selfing through the R8 generation, at least when the populations were grown at Davis, it may "disappear" in crosses. Such results were dismaying, as the maximum use of a photosensitive genetic male sterile would be dependent upon its transmissibility in crosses. Interestingly, the phenomenon of "disappearing" genes has been noted in another tissue culture-derived mutant of rice, a dwarf mutant which was transmitted through eight generations of selfing, but disappeared in crosses (K. Oono 1985. Putative homozygous mutations in regenerated plants of rice. Mol Gen Genet 198:377384). Oono proposed that "The mechanism responsible for the phenomena may be heritable gene inactivation induced by tissue culture." Since the present male sterile gene apparently was transmitted in a previous natural cross, there still may be other combinations in which it will be transmissible. More controlled crosses are needed to determine the usefulness of this male sterile.

Meanwhile, work on a second environmentally sensitive male sterile, M-201 ms-7, is being continued by Dr. James Oard.

2. Tissue culture and other selection for herbicide resistance.

As previously reported three selections (HS7-3, HS17-1, HS51-7) from EMS-treated M2 bulk populations have been partially characterized for tolerance to American Cyanamid herbicide AC499 (now called "Pursuit"). This material exhibited some tolerance as reported in the 1988 annual report, but replicated trials to quantify the level of tolerance were yet to be conducted. To answer this question, pre-emerge and foliar spray experiments were performed with the three selections, additional rice variants from other M2 populations, and barnyardgrass as a control. For the pre-emerge experiment, data from two different sprayings were combined and averaged as presented in Table 1. The M-102 control exhibited sensitivity (73-97% height reduction) at 75-150 g ai/h. Barnyardgrass was less sensitive (48-87% height reduction) at 75-150 g ai/h. For the selections, HS17-1 displayed the greatest tolerance (45-58% height reduction) at 75-150 g ai/h. The other selections showed less tolerance, although more tolerance than the M-102 control. At the 100 g ai/h rate, the height of HS17-1 was nearly 50% greater than the M-102 control. In addition, HS17-1 showed less height reduction than barnyardgrass at the 100-150 g ai/h range. These data demonstrate that HS17-1 exhibits the greatest potential of the selections, although it is not at a "field use" level (150 g ai/h, Table 1) for barnyardgrass control. One reason for this 'low' tolerance of HS-17 may be due to reduced height, less tillering, delayed maturity, and thin curled leaves when compared without herbicide treatment to the wild type M-102 parental cultivar. To transfer the tolerance of HS-17 to an adapted cultivar, one backcross has been made to M-102. $BC_1F_1$ seeds are currently maturing in the greenhouse and will be screened in a pre-emerge spray experiment for increased tolerance levels over HS-17. Those individuals with increased tolerance will be used in a second round of backcrosses and selection. In addition, crosses with HS-17 have been made recently with Calmochi-101 to determine inheritance of tolerance to AC499.

We cooperated with American Cyanamid to evaluate tolerance of the rice selections using foliar spray treatments. Results of these experiments are presented in Figs. 1 and 2. Experimental conditions were as follows: Three replications were used for each treatment combination. Each of the selected lines was treated with five rates of AC499 (also called PURSUIT). The selection HS17-1 appeared to have increased tolerance as compared to the wild-type M-102. The AC499 rate which caused about 50% reduction in growth was between 5 and 10 g ai/h for most selections, but was between 10 and 20 g ai/h for HS17-1. The lethal dose of AC499 for most selections was 10 or 20 g ai/h for all genotypes except HS17-1 where the lethal dose was 80 g ai/h. By these comparisons, HS17-1 appears to be four-fold more tolerant than the control cultivar M-102. Results from these foliar spray experiments again suggest that HS17-1 exhibits greater tolerance than the other selections, but levels must be increased approximately five-fold for field use foliar spray rates. It is expected that the backcrossing program described previously will improve foliar spray tolerance.

In other experiments 14 Oryza species were sprayed with AC499, but none of the accessions showed any promise. A preliminary trial was also conducted with AC087 herbicide which is less toxic than AC499 on rice. No damage was detected using a pre-emerge application up to 400 mg/L pure powder form of the herbicide. Foliar spray treatments will be conducted to evaluate tolerance of M-102 and the selected lines to AC087.

3. Anther culture study for "instant homozygote" production.

Ms. Shannon Pinson completed her Ph.D. thesis in July 1989, on "Applications and limitations of anther culture in rice breeding and genetics programs." A useful application of anther culture to plant breeding would be the production of "instant" pure lines from Fl plants. This could shorten variety development cycles by 2 or 3 years.

The genetic control of callus induction, total shoot regeneration and green shoot regeneration, in California germplasm, was determined to be highly additive. M101, 78:18347, and S-201 were identified as having high GCA for anther culture capability. Use of these lines in the production of materials for use in AC programs can allow researchers to work with otherwise nonregenerable materials.

Observation of gametic rather than disomic F2 segregation can facilitate genetic analyses using smaller population sizes. Although gametic ratios can be obtained through AC, segregation data on 22 marker traits in AC-derived populations indicated that use of AC in genetic studies is limited by the following: 1) AC techniques are labor intensive and costly making it difficult to produce sufficient population sizes, 2) most, but not all characters could be observed in haploid plants, 3) mutations occurred in approximately 5% of the AC-derived n and 2n lines studied, 4) six of 22 AC-derived ratios differed from F2-derived ratios previously reported by others, and 5) there is a high probability that all plants from a single callus were derived from a single microspore. Seven AC-derived spontaneous diploids derived from Fl's were determined to be heterozygous. The nature of the heterozygosity was studied and its most probable origin was determined to be regeneration from diploid microspores resulting from aberrant meioses.

An economic analysis comparing AC with two forms of single seed descent (SSD) indicated that although AC is the most expensive method, it offers tremendous time savings, and can be economical at regeneration rates as low as 0.7%. AC costs decreased exponentially as regeneration rates increased and leveled off between 15 and 20% regeneration. The culture of anthers from F2 plants, coupled with selection in the F2 population can decrease AC related costs to a level below those for SSD as well as increasing the opportunity to break unfavorable linkage.

4. Other genetic studies.

Mr. Zhikang Li completed his Ph.D. thesis in December 1989, on "Genetic structure of cultivated rice: Isozymes and morphology."

Genetic variability at both morphological and isozyme levels in cultivated rice (Oryza sativa L.) is remarkably extensive, and the differentiation of the cultivar groups indica and japonica is well known. However, the nature and the causes of the differentiation have long been controversial subjects. In this study, a random sample of 485 rice cultivars from 10 geographical regions and 31 countries was surveyed in terms of their variation at 14 polymorphic isozyme loci (Idhl, Sdhl, Pgd2, Pgil, Pgi2, Lapl, Lap2, Estl, Est2, Est3, Est4, Acp3, Me2, and Gotl) by starch gel electrophoresis. A subsample of 213 cultivars (from five regions) out of the 485 cultivars was subjected to a survey of phenotypic diversity for 18 qualitative characters and 16 quantitative traits.

Three distinct cultivar groups, two of them corresponding to the indica and japonica rices and one new group, were distinguished by cluster analyses based on their isozyme allelic composition. Within the japonica group, two subgroups, corresponding to traditional japonica type and javanica type (including most US bulu types), appeared well differentiated from each other at both phenotypic and isozyme levels.

## PUBLICATIONS OR REPORTS:

Johnson, C. W., H. L. Carnahan, S. T. Tseng, J. J. Oster, J. E. Hill, J. N. Rutger, and D. M. Brandon. 1989. Registration of 'S-lOl' rice. Crop Sci. 29:1090-1091.

Rutger, J. N. 1989. Rice. pp. 326-331. In The World Book Encyclopedia, 1989 edition. World Book, Inc. Chicago.

Rutger, J. N. and G. W. Schaeffer. 1989. An environmentally-sensitive genetic male sterile mutant in rice. p. 98. In Agron. Abs., Ann. Meeting Amer. Soc. Agron. Las Vegas, NV.

Rutger, J. N. and C. N. Bollich. 1989. Use of introduced germplasm in U.S. rice improvement. p. 98. In Agron. Abs., Ann. Meeting Amer. Soc. Agron. Las Vegas, NV.

Rutger, J. N. 1989. Filling the rice bowl. Agricultural Research 37(6):2.

Ching, Li Yuan, Yuan Long Ping and J. N. Rutger. 1990. Characterization and inheritance of twin seedlings in rice (Oryza sativa L.). Manuscript prepared for Crop Sci.

Pinson, S. R. M. 1989. Applications and limitations of anther culture in rice breeding and genetics programs. Ph.D. Thesis, UCD.

Li, Zhikang. 1989. Genetic structure of cultivated rice: Isozymes and morphology. Ph.D. Thesis, UCD.

**CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:**

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

1. The photosensitive genetic male sterile line Calrose 76 ms-2 generally is sterile in long days, i.e., Davis in the summer, and fertile in short days, i.e., Hawaii in the winter. However, the male sterile gene unexpectedly "disappeared" in a controlled cross. More crosses are needed to determine the usefulness of this male sterile.

2. When three previously-selected rice mutants which are tolerant to the herbicide "Pursuit" were further tested, the best selection, HS17-1, showed more tolerance to the herbicide than the rice control or barnyardgrass. Backcrosses to the normal parent (M-102) are underway, to determine if the tolerance can be genetically transmitted and/or enhanced.

3. The genetic control of callus induction, total shoot regeneration and green shoot regeneration, in California germplasm, was determined to be highly additive. M-101, 78:18347, and S-201 were identified as having high GCA for anther culture capability.

4. In other genetic studies, isozyme analysis of 485 rice cultivars revealed the presence of three distinct cultivar groups, two corresponding to the traditional indica and japonica rices, and one new group.

Table 1. Percent height reduction of rice selections sprayed with AC499. Data presented were averaged over two separate experiments. Treatments were replicated three times within each experiment.

| | Percent Height Reduction as Compared to Untreated Control | | | | | |
|---|---|---|---|---|---|---|
| AC499 g a.i./h | M-102 | Barnyard-grass | HS17-1 | HS33 | HS7-3 | HS51-7 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 75 | 73.5 | 48.2 | 44.8 | 66.4 | 61.5 | 68.7 |
| 100 | 88.8 | 61.0 | 46.4 | 67.7 | 61.7 | 64.7 |
| 150 | 97.4 | 87.0 | 58.0 | 63.1 | 65.7 | 69.3 |

Figure 1. Mean plant growth (28DAT) of putative resistant rice after being sprayed postemergence with PURSUIT

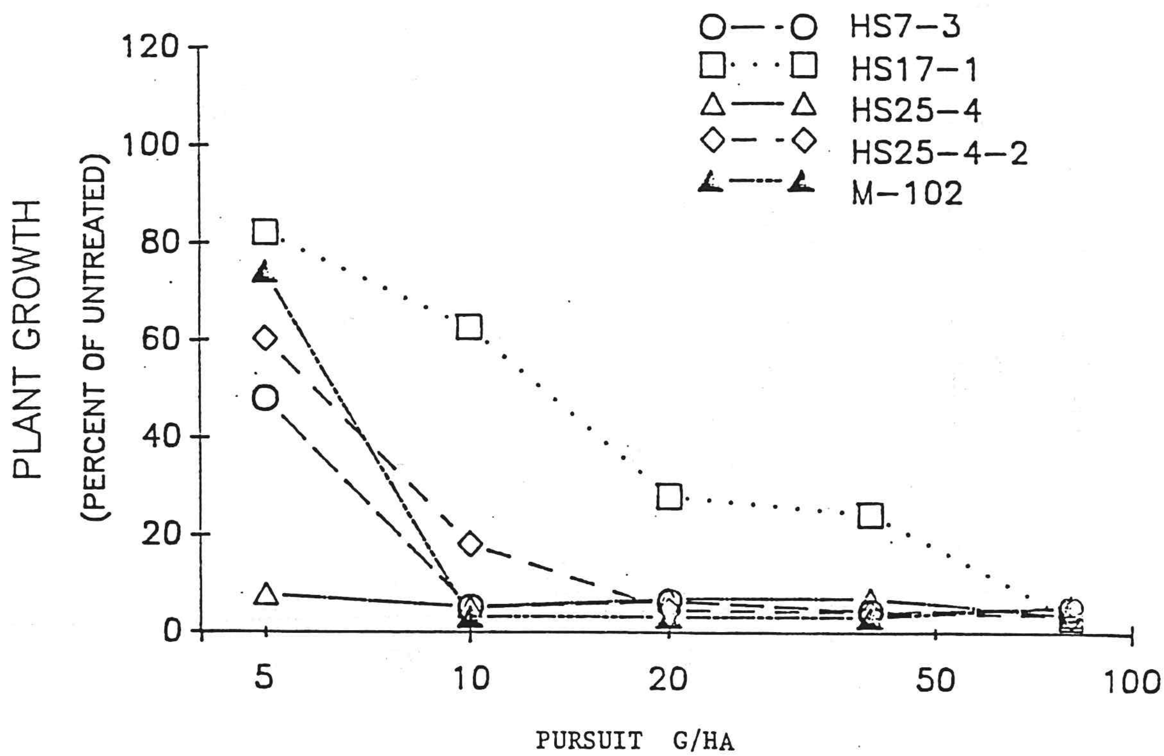

Figure 2. Mean plant growth (28DAT) of putative resistant rice after being sprayed postemergence with PURSUIT