# ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: Rice Genetics and Germplasm Development

PROJECT LEADER: David J. Mackill, Research Geneticist, USDA-ARS
Agronomy & Range Science, UCD

PRINCIPAL UC INVESTIGATORS

David J. Mackill, Research Geneticist, USDA-ARS, Agronomy & Range Science
Teresa Erickson, Biological Technician, USDA-ARS, Agronomy & Range Science
Xiaomao Lei, Staff Research Associate, Agronomy & Range Science
Edilbeto D. Redoña, Graduate Student, Agronomy & Range Science
Satoshi Masaki, Visiting Scientist, Agronomy & Range Science

COOPERATORS:

Carl W. Johnson, Plant Breeder, Rice Experiment Station, Biggs
Kent S. McKenzie, Plant Breeder, Rice Experiment Station, Biggs
S. T. Tseng, Plant Breeder, Rice Experiment Station, Biggs
Jeffrey J. Oster, Plant Pathologist, Rice Experiment Station, Biggs

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1. **Rice Genetics Laboratory**. In 1992 we developed the rice genetics lab in the Agronomy & Range Science Department at UCD for application of molecular markers to rice. We now have the capability to take advantage of this technology for gene tagging, and eventually we should be able to use the approach of marker-assisted selection for rice breeding.

   We optimized a genomic DNA extraction protocol which reproducibly yields large quantities of good quality DNA and does not require use of phenol. An initial set of 100 DNA "probes" cloned into plasmids were obtained from Cornell University. These were transformed into *E. coli* bacteria, and are being stored at -70°C. The plasmids were reisolated and the inserts amplified by PCR for use to detect RFLPs (restriction fragment length polymorphism). We attempted to optimize chemiluminescent (non-radioactive) labeling but with limited success. This would allow us to minimize use of radioactivity. We will continue work on chemiluminescent labeling but will rely on radioactive probes for the near future.

2. **Rice Genetic Resources.** Objectives are to maintain and evaluate a diverse set of rice varieties and wild species, import useful new germplasm and introduce useful traits into California varieties.

Of the 378 rice accessions introduced from the International Rice Research Institute (IRRI), Philippines, 76 were grown in quarantine at Davis and seed was obtained. A further 135 were grown in quarantine at the USDA facility in Glenn Dale, Maryland, and seed was received at Davis. The remainder are to be processed through quarantine in Maryland. Crosses were made from some of the accessions grown out at Davis.

Over 600 varieties from the local collection were grown out at Davis in 1992 and agronomic data were collected. Selected varieties were chosen for breeding and genetic studies.

A set of 140 rice varieties was selected for studies on the use of molecular markers to assess genetic variability. The major objective is to determine the genetic diversity of California varieties in comparison with japonica varieties from other countries, and with the rice germplasm as a whole. Most rice researchers feel that japonica varieties are less diverse than indica varieties, and pedigree analysis indicates that California varieties have a particularly narrow germplasm base. Molecular markers provide a more objective basis for determining the level of diversity. The results will have important implications for California rice breeding. Diversity may provide a means of protection against potential biological pests. It is also necessary for the exploitation of hybrid vigor if hybrid rice becomes possible. Molecular markers offer a means of identification of varieties, which is becoming increasingly important for plant variety protection.

In 1992, we grew the 140 varieties and extracted DNA from the leaf tissues. These were digested with restriction enzymes, run by electrophoresis agarose gels, and transferred to nylon membranes. We have begun making filters for probing with RFLPs. We have been initially using a non-radioactive labeling method, but have not had success yet. We are now in the process of preparing filters for radioactive probing. The results should be available in 1993. We have also been using a PCR (polymerase chain method) based method to assess genetic diversity. We have adapted a protocol for using this procedure in our lab and are now using it to characterize the varieties. We plan to compare these DNA-based methods of varietal characterization with the previously-used isozyme method.

3. **Identification of useful genes.** The main strategy we use for identifying useful genes is to use DNA markers, as described above for the germplasm survey. The following are the major ongoing projects:

**Stem rot.** Mapping populations are being developed by the breeders and pathologist at Biggs. $F_2$ populations were grown and scored for stem rot resistance in the summer of 1992. $F_3$ populations will be increased during the winter, and DNA will be extracted at Davis from the most promising crosses. In the summer of 1993, disease resistance will be measured on the $F_3$ and $F_4$ lines. Assuming genetic diversity is high enough, we will use molecular markers to identify the genes conferring resistance.

**Water weevil.** The best source of resistance, CI1403, and resistant lines developed from it at Biggs are being characterized with DNA markers to determine if there are sufficient differences to identify the genes. Populations from CI1403 are being developed for gene mapping.

**Cold tolerance.** Cold tolerance at the booting stage will be studied both with indica X japonica and japonica X japonica crosses. While the latter are more relevant for California conditions, it may be difficult to use molecular markers to tag genes due to the lack of genetic diversity within japonica varieties. Indica X japonica crosses have high genetic differences, so use of molecular markers is much easier. It will be useful to know what genes in japonica varieties give them higher cold tolerance than indica varieties. This should assist in transfer of traits from indicas into japonicas, and also help in increasing the level of diversity in the California japonicas. Cold tolerance at the seedling stage can also be mapped with these populations. In 1992 we made the crosses for these genetic studies. $F_1$ plants will be grown in the winter, and $F_2$ populations should be available by the summer of 1993.

**Seedling vigor.** This is actually a sub-category of cold tolerance. Italica Livorno is the major source of high seedling vigor. Tissue has been harvested from $F_2$ populations with Italica Livorno and DNA will be extracted in 1993. A few exotic varieties are also being studied as potential sources of strong seedling vigor. Some tropical upland varieties like 'Black Gora' have very strong seeding vigor if the temperature is not too low. We have made crosses with this and other sources of seedling vigor.

4. **Hybrid rice.** Genetic mechanisms have now been developed for commercial hybrid rice production. Whether these will be viable in California depends on the cost of seed production and the availability of a hybrid with sufficient yield advantage and acceptable grain quality. Research is focusing on development of seed production components, transferring the necessary genes into California cultivars and assessing the hybrid vigor of potential parent varieties.

In 1992, crosses were made for transferring the following characters:

| **Trait** | **Source** |
|---|---|
| (a) CMS (cytoplasmic male sterility) | IR58025A<br>IR62829A<br>Wu 10A<br>CMS-RA |
| (b) R (restorer gene) | IR50R<br>IR72R |
| (c) WC (wide compatibility) | Lemont<br>Moroberekan |
| (d) *eui* (elongated uppermost internode) | M-202eui |

The CMS sources were crossed with M-202, M-202eui, M-204, and L-203. Previous studies indicate that most California cultivars should be maintainers - they lack the restorer gene so the $F_1$ plants should be completely sterile. Sterile plants will be backcrossed again to transfer the CMS cytoplasm into the California cultivars.

The WC gene allows production of fertile hybrids between indica and japonica varieties.

The *eui* gene was previously identified at Davis. It has been used elsewhere in the female CMS parent to increase panicle exsertion, thereby avoiding application of gibberellic acid to the female plants. It was proposed at Davis for use in the male parent, where it should increase fertilization by allowing the male plants to shed pollen above the female plants.

$F_1$ plants will be grown during the winter. There is sufficient seed for growing some of the CMS crosses in the field in 1993.

The use of both restorer and maintainer lines can be eliminated if a source of conditional male sterility can be employed. Two mutants conditioning environmentally sensitive male sterility were previously identified at Davis. In 1992, seed of both of these mutants were increased. The mutant designated ST25 was increased at Davis. The harvested seed segregates for male sterility. Sterile plants in the field were propagated in the greenhouse as a source of homozygous sterile stocks for 1993 experiments.

The mutant from Calrose 76, designated PI543851, is potentially very useful in that it is always fertile when grown in the southern U.S., but sterile when grown at Davis. (It is fertile in the winter greenhouse at Davis, and in the winter Hawaii nursery.) With this mutant, the sterile lines can be maintained by increasing the seed in the southern U.S., where the plants are fertile. In 1992, seed from putative homozygous sterile plants was increased at Stoneville, MS. This will serve as a source for future experiments at Davis. However, at Davis in 1992 these lines were not completely sterile as expected. Bagged panicles showed varying degrees of seed set. We expect this is because plants flowered later than usual (late August), when the daylength is shorter. A study is planned for 1993 to determine the influence of temperature and planting date/daylength on sterility.

In order for hybrid rice to be viable a hybrid with sufficient yield advantage needs to be identified. This depends on genetic divergence of the two parents used. It is unlikely that any two presently available California varieties would have sufficient hybrid vigor. (Previous reports for japonica X japonica hybrids indicate yields of 10% or less above the highest-yielding parent.) Efforts must be made to developed adapted varieties with maximum divergence from currently grown California varieties. Sources of diversity include indica (tropical) varieties which can produce seed under California conditions and tropical japonica cultivars. Both types are currently under evaluation and appropriate crosses have been made. This aspect of hybrid rice research ties in with the study on characterization of rice varieties with molecular markers mentioned above under Genetic Resources.

PUBLICATIONS OR REPORTS:

Mackill DJ, Erickson TM, Redoña, ED (1992) Use of molecular markers for rice genetics and breeding in California. p. 14. *In* Rice Field Day, 9 Sep 1992, Rice Experiment Station, Biggs, CA.

Mackill DJ (1992) Rice biotechnology programs in California. p. 39-40. *In* Proceedings of the Twenty-Fourth Rice Technical Working Group. 23-26 Feb 1992. Little Rock, AR. Texas Agric. Exp. Stn., College Station.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

Our efforts this year focused on developing the rice genetics laboratory for application of molecular markers to rice breeding and genetics, and on setting up populations for future research on gene tagging and hybrid rice research. Molecular genetics research is now underway in the lab, and we are in the process of using RFLPs and PCR-based strategies to map genes of importance for California conditions. We are also using these techniques to characterize California rice cultivars in comparison with the species as a whole. We are setting up the populations for tagging genes conferring stem rot resistance, water weevil tolerance, cold tolerance, and seedling vigor. We are transferring genes necessary for hybrid seed production into California-adapted rice lines. We are evaluating the potential of various types of rice cultivars for production of high-yielding $F_1$ hybrid varieties.