ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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Title: ENHANCEMENT OF OSMOTIC (SALINITY) STRESS TOLERANCE IN RICE

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OBJECTIVES AND EXPERIMENTAL PROCEDURES:

(1) Objective: Identify a strongly expressed dehydrin locus in the *L. elongatum* genome with antibodies against dehydrin proteins in two-dimensional western blots of proteins isolated from roots of disomic substitution lines of individual *L. elongatum* chromosomes.

Soluble proteins were isolated from roots and dry endosperms of the wheat x *L. elongatum* amphiploid and disomic substitution lines of individual chromosomes of *L. elongatum* in wheat. Proteins were dephosphorylated with alkaline phosphatase, fractionated by two-dimensional isoelectric focusing-SDS polyacrylamide gel electrophoresis (2D-gels) and electroblotted on nylon membranes. The blotted proteins were challenged with dehydrin antibodies provided by T. J. Close (Department of Plant Sci., UC Riverside). A large number of proteins cross-reacting with the antibodies were detected in 2D-gels. Most of them were found to be salinity-responsive. The proteins were encoded by three chromosomes, 4E, 5E, and 6E, as expected from previous genetic mapping (Dubcovsky *et al.* 1994). No protein showing a dramatically greater accumulation than the rest was found. This finding revealed that the accumulation of dehydrin proteins in the tribe Triticeae and rice is due to induction of a number of genes expressed to a similar degree. We concluded that an attempt to construct a salinity

induced dehydrin gene for rice transformation that would significantly enhance the salinity tolerance in rice would require great deal of additional work.

(2) Objective: Develop rice tissue cultures for transient gene expression.

Calli were induced from embryos of M-103 and M-202. Additionally, a salt-adapted callus line was acquired from I. Winicov (University of Nevada, Reno). Calli were also produced from wheat (Chinese Spring) and *Lophopyrum ponticum* (a highly salt tolerant wheatgrass). A total of 70 rice, 59 wheat and 5 *L. ponticum* callus cultures are now maintained. A number of these cultures were passed through a cell suspension state to produce suspension-ready cultures.

(3) New objective: Develop a high-throughput assay for K/Na selectivity.

To screen a large numbers of lines or plants in a segregating population for K/Na selectivity, it is necessary to rapidly determine concentrations of K and Na in leaves. In the past six months we have developed the following protocol for the assay. Six plants per genotype are grown in solution cultures containing 50 mM Na and 1/4 X modified Hoagland solution (Huang *et al.* 1992) for two weeks. The most recently expanded leaf is collected, dried, ground, and extracted with 0.5 N HCl for three days on a shaker. The concentrations of K and Na are then determined by atomic absorption spectrophotometry. Data are analyzed by analysis of variance for a complete bloc design. It takes about 15 person/days to evaluate 40 lines in a replicated trial. Since September, Karin Deal has evaluated the K/Na selectivity of 120 accessions of *Ae. tauschii* and identified lines with very high (K/Na ratios ranging from 30 to 40) as well as very low (K/Na ratios near 1.0) selectivity. Since the assay is nondestructive, plants with high and low K/Na selectivity could be saved and are now being crossed to produce F_2 mapping populations.

SUMMARY OF 1998 RESEARCH:

(1) Detailed analysis of the genetic structure of the dehydrin protein family revealed that dehydrins are controlled by a large number of genes of similar level of induction by salt. The search for a dehydrin gene that is dramatically more induced than others and which can be targeted for transgenic rice development has been unsuccessful.

(2) A large number of callus and suspension cultures of rice, wheat, and *L. ponticum* have been developed and are now maintained for future use in transient gene expression studies.

(3) A high throughput assay for determination of K and Na concentration in salt stress rice and other grasses has been developed and used to identify accessions with high- and low- K/Na selectivity.

PUBLICATIONS:

Gao, M.J., P.E. Gulick, T.J. Close and J. Dvorak. 1999. Association of dehydrin proteins with specific loci in *Lophopyrum elongatum*. In preparation.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

The expression of genes encoding dehydrin proteins in *L. elongatum* was characterized. The high levels of dehydrin proteins seen in roots of salt stressed rice, wheat and the halophytic *Lophopyrum* were found to be due to salt induction of numerous genes. Each

gene appeared to be induced to a more-or-less equal degree. We conclude, therefore, that it is unlikely that salinity tolerance in rice could be dramatically improved by incorporation of a single *Lophopyrum* dehydrin gene unless the expression of the gene is additionally enhanced by manipulation of the promoter, which is beyond the scope of this project.

Salinity tolerance in rice and other cereals highly correlates with the exclusion of Na and maintenance of the accumulation of K in meristems and the most recently expanded leaves (K/Na selectivity). We refocused our attention therefore on genes that control this physiological trait in rice and other grasses. A prerequisite for searching for variation in this trait in the rice genepool is the availability of a high throughput assay for it. Such an assay was developed and its efficacy was tested in assaying an *Aegilops tauschii* (a salt tolerant goatgrass) germplasm collection for variation in K/Na selectivity. Lines with high- and low-K/Na selectivity were found. Saturation mapping of K/Na selectivity loci, *Kna1, Kna2*, and *Kna3*, previously identified in the *Ae. tauschii* genome, has been initiated with lines contrasting in K/Na selectivity with the ultimate goal of isolating these genes and using them in the development of salinity-tolerant transgenic rice. In a parallel approach, aiming at a moderate enhancement of salinity tolerance in rice, rice germplasm collection will be screened with this high throughput K/Na screening technique to identify accessions with K/Na selectivity superior to that existing in California rice germplasm. This germplasm will be used in marker-assisted introgression of salinity tolerance to California rice cultivars to augment other approaches (see this years proposal).