PREFACE

Rice is the world's most important staple food crop; it feeds more people than any other. Although rice is adapted to grow with abundant water, only one-half of the land area on which rice is grown is irrigated. In the remaining half, which accounts for one-quarter of total rice grain production, yields are often depressed by drought, and further expansion of the area under rice will be mainly in unirrigated (rainfed) conditions. Most people who consume rice are dependent on local supplies; only about 5% of the crop is traded in international commodity markets. This makes the local distress caused by crop failures particularly acute. The people affected are among the poorest in the world.

The impact of drought on rice production could be reduced by developing varieties better adapted to water deficits. However, drought resistance in rice is hard to address by traditional plant breeding methods. Two important factors limiting breeding progress have been lack of understanding of the fundamental mechanisms of drought injury and resistance in rice, and lack of ready means to interconnect physiological/biochemical knowledge and rice breeding. This situation is changing rapidly: the advances in rice transformation and the development of a rice RFLP (restriction fragment length polymorphism) map have provided timely new tools for both basic research and rice breeding for complex traits.

The conference therefore reviewed major areas of plant water stress research in which progress is currently being made, with emphasis on (a) the particular problems of rice under drought, and (b) building on the biotechnology program already funded by the Rockefeller Foundation. The following overall conclusions emerged.

1. Despite the importance and age of rice as a crop, the physiology of water stress in tropical rice is little known. There is thus an urgent need to understand rice better, in relation to drought at various stages of the life cycle and with respect to the physiological, biochemical and molecular levels of organization.

2. The genetic resources and expert personnel of the International Rice Research Network are a great resource. Building appropriate bridges in key areas of the RF Biotechnology program could help to exploit this resource in two mutually-reinforcing ways: (a) by providing new tools for breeding programs for drought resistance, (b) by advancing basic knowledge of water stress responses in rice.

3. New tools for breeding programs would be provided primarily by incorporating selected physiological traits into the RFLP program. An example here might be the major genes governing deep rooting and penetration of compacted soil layers. Once identified and mapped, these genes (which are extremely difficult to score in the field) could be readily tracked in a breeding program via the associated molecular markers.

4. Advances in basic knowledge of stress responses would be achieved by the use of the RFLP map in an analytical way to dissect major components of drought resistance, and by creating novel genetic variation via gene transfer into rice. An example of the former could be the

identification of independent sets of genes controlling the same complex trait, such as genes regulating pollen viability and embryo abortion, both of which lead to drought stress-induced sterility. Gene transfer provides the power to manipulate individual genes, and so to test hypotheses about their biochemical and physiological function. Conceptually akin to the classical approach of comparisons between isogenic lines, it is in principle faster and much more precise. Although further knowledge is needed to design and produce agronomically useful genotypes by gene transfer techniques, some valuable experimental types can be envisioned at this stage. An example might be the transfer of the two genes for the betaine pathway from another cereal into rice, as rice appears to have lost the capacity to produce this osmo-protective compound.

PROMISING RESEARCH AREAS

The following Table represents a consensus view of topics that hold promise at present. The Table was constructed with the following points in view.

1. The left- and right-hand sides of the Table cover research focused on the RFLP map, and research involving gene isolation and transfer, respectively. Although these are very different research approaches and they will eventually merge and reinforce each other, both should be followed at this time.

2. Certain areas of research seem potentially highly fruitful, but not enough preliminary work has been done to define their significance for rice. These areas have been placed in the "Exploratory" class, in the lower part of the Table. In most cases a modest, short-term project will suffice to establish their importance for rice.

3. Some research areas emerged as promising enough to warrant consideration for longer-term support. These areas are in the upper part of the Table.

4. The pace of advance in the physiology, biochemistry and molecular genetics of water stress in plants is rapid, although such research on rice has been limited. Other promising areas are therefore likely to appear on both sides the Table within the next few years.

## PROMISING RESEARCH AREAS

| RFLP ANALYSIS | GENE IDENTIFICATION, ISOLATION AND MANIPULATION |
|---|---|
| Use of RFLP map as an analytical tool to identify components of drought-resistance. | Molecular basis of osmo-regulation, especially betaine (which appears lacking in rice). |
| Root systems (depth, penetration) | Regulation and function of water stress-induced genes (e.g. dyhydrins) |
| Cuticle thickness | |
| **EXPLORATORY** | |
| Flowering stage stress | Chemistry and physiology of endogenous osmolytes |
| $S^{13}c$ discrimination and water use efficiency | Expression and function of alternative oxidase |
| Genotypic variation for osmotic adjustment | _Agrobacterium rhizogenes_ roots ~~as~~ as a culture system, and a source of genes for novel root phenotypes |
| Natural variation in water stress-induced genes (e.g. dehydrins) | Photoinhibition under water-stress |

## CRITICAL EVALUATIONS OF CONFERENCE SESSIONS

### SCHEDULE OF SESSIONS

Day 1. Modifying water use efficiency by Rubisco engineering
Stress-induced sterility
Stress-induced proteins

Day 2. Osmotic adjustment and cytoplasmic osmolytes
Alternative pathway respiration
Photoinhibition and water stress

Day 3. Molecular basis of stress effects on root and shoot growth
Use of RFLP to map and monitor resistance traits

DAY 1.

The first session highlighted both advances and deficiencies in our understanding of drought stress. The following studies on rice were recommended.

(a) The rice plant has a thin cuticle, suggesting the need for an increase in cuticle thickness of both leaf and reproductive structures to reduce water loss. Efforts should be made to understand the rice cuticle and ultimately to improve its effectiveness.

(b) Several physiological problems specifically pertaining to drought stressed rice should be clarified . These include: i) the physiological basis for the high susceptibility of grain yield to drought at anthesis; ii) the contributions of physiological adaptation and pattern development in root drought response. For i), it is not certain that observations made with vegetative tissues can be extended to events taking place at anthesis. Promising areas for experimentation include the effect of $CO_2$ supplementation during drought, studies on the ability to mobilize and utilize stored carbohydrate and protein reserves, and further analysis of the cause of failure of the reproductive parts. For ii), the lack of physiological and metabolic information for roots is striking for a crop in which there is such a high degree of genetic variability for root development, and whose shoot is so often dehydrated simply by high evaporative demands.

(c) In the long term, it will be advantageous to be in a position to apply to rice the advances being made in the engineering of cellular components such as Rubisco in other systems. In the area of dehydrins and other stress-induced genes, there is a good case for doing pioneer work in rice as valuable probes and promoter regions may be found.

(d) Recent progress in understanding ABA effects on vegetative growth increases optimism that this hormone may play a pivotal role in reproductive tissues. However, it is probably more appropriate to do the groundbreaking work in a crop where current work is already more advanced than in rice.

next page

(e) Considerable progress has been made toward identifying, rice genotypes having superior vegetative performance in water-limited environments. A matching program to select genotypes tolerant to drought during reproductive phases of development would be valuable.

(f) For several crops, measurements of WUE have proven useful when made with the d13C technique, particularly when done in conjunction with RFLP mapping. Although rice will present some significant technological problems, the application of the d13C technique should be tested.

Day 2.

On the second day of the conference, presentations and discussions centered on the three following issues.

(a) Whether osmotic adjustment plays a significant role in protecting rice at times of limited water availability.

Discussion led to the following recommendations:

i) There is a positive correlation between osmotic adjustment and harvest index in a number of crops, although not enough is known in rice to make a projection for this crop. Gap-filling research on osmotic adjustment is therefore needed for rice. This includes basic chemistry to find which osmolytes are important. This is necessary because different solutes have different properties and the type of solutes present may determine the capacity for osmotic adjustment as well as influence protein stability at high temperature - a likely problem for rice. Assessment of the importance of osmotic adjustment in various tissues and developmental stages is required.

ii) It is necessary to examine how much genetic variability for osmotic adjustment exists in cultivated and wild rices. The wild species could yield information on critical stages and responses and ultimately provide useful promoters for genetic engineering.

iii) Cultivated rice apparently does not synthesize betaine, an important osmolyte in all other grass species so far tested. It would therefore be valuable to transfer betaine genes into rice [and a dicot system for comparison] with constitutive or tissue-specific promoters, so that the transgenic plants could be monitored for improvement in osmotic adjustment.

(b) Whether alternative pathway respiration is a metabolic load (or possibly of some advantage) under water stress conditions.

Recommendations were:

(i) Because the alternative pathway of respiration in plants is apparently inefficient, and there is now an isotopic method for measuring the relative contribution of the alternative pathway and the cytochrome pathway in vivo, a biochemical/physiological assessment of the alternative oxidase pathway should be carried out in rice.

(ii) It could prove worthwhile to down-modulate the rice alternative oxidase by antisense gene transfer experiments. This could enable direct evaluation of the importance of the pathway in rice.

(c) Whether photoinhibition reduces the productive capacity of rice at times of water deficit. Photoinhibition appears to be of minor significance in water-stressed sorghum and cotton. This may well be true of rice but the basic physiology of drought-induced photoinhibition is not known for rice. Some basic physiological work to evaluate the importance of photoinhibition in rice is therefore justified.

DAY 3.

The final session dealth with the specific topic of root and shoot growth, and with the much broader area of the opportunities opened up by the rice RFLP program.

(a) Root characteristics -- especially the depth of rooting, root thickness, size of metaxylem elements and ability to penetrate compacted subsoil - seem likely to play a crucial role in drought avoidance in rainfed rice environments . Research is urgently needed on the range of root morphologies available in the rice gene bank. Such research should be greatly facilitated and its impact on existing plant breeding programs greatly enhanced through the use of RFLP mapping. This should, for example, permit effective analysis of which root characteristics are most significant in the various drought-prone environments.

(b) Root culture in vitro using Ri plasmid-transformed roots should also enhance our understanding of root function and morphogenesis, and its exploration is recommended. Ri plasmid genes which alter root phenotype in other species should also be considered for introduction into rice.

(c) Very high priority was given to the use of RFLP mapping both as an aid to more efficient plant breeding procedures for all environments, and also to resolve which individual characters or combinations of them are likely to be most effective in the various rice environments. Although root growth was given highest priority in this context, cuticle thickness, $^{13}C$-discrimination and resistance to dehydration injury at anthesis were all ranked highly, with stress-responsive genes also deserving analysis in conjunction with RFLP mapping. It was noted that most of this work must be done at, or in close collaboration with the International Rice Research Institute and that these additions to IRRI's activities will impose new manpower demands.

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New page

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