RF Bd of Trustees 3/92 RF 93001

Basic and Applied Rice Biotechnology

RF 92001

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GUIDELINE: Applying Biotechnology to Developing-Country Food Crops

GRANTEE: For allocation by the officers

OBJECTIVE: To support research and training at industrialized-country laboratories participating in the Foundation's international program on rice biotechnology.

AMOUNT: $2,600,000 in addition to RF 91001

DURATION: Period ending December 31, 1993

STRATEGY: The Foundation's rice biotechnology program has two goals: providing improved varieties that will enable production in rice-dependent countries to keep pace with population growth, and building up those countries' scientific capacity as an essential ingredient for economic development. To accomplish this an international network now composed of roughly 200 senior scientists and 300 trainee scientists has been established to develop and apply powerful new tools of genetic engineering to the genetic improvement of rice. The network combines the skills of many of the world's leading plant molecular biologists with those of scientists responsible for rice improvement in developing countries. The industrialized-country laboratories in the network undertake basic and applied research on the program's more complex high-priority research objectives, provide training for third-world scientists, and through a chain of research collaborations transfer new knowledge and materials to rice improvement programs in developing countries. Scientific progress has been excellent, the first of the trainees are returning home to establish their own research programs - often in partnership with the laboratory where they received training - and the work at the industrial-country institutes is becoming more sharply targeted.

DESCRIPTION: A majority of the world's poor people are dependent upon rice as their primary source of food. During the 1960s and 1970s a system of national and international rice research institutions was built that successfully applied existing technology to the genetic improvement of tropical rice. In Asia widespread adoption of the resulting rice varieties led to higher yields, increased per capita production, and a reduction in the real price paid for rice by consumers. The massive famines which had been predicted for Asia did not occur.

By the mid-1980s, however, most of the gains possible using existing technologies had already been achieved and growth in rice yields virtually ceased. Since the scope for increasing the area planted to rice in Asia is also extremely limited, projections of rice demand and supply into the next century again predict significant shortfalls.

In the 1980s scientific discoveries in molecular and cellular biology led to the development of the new field of plant genetic engineering. This promised to allow further expansion in the genetic potential and productivity of food crops, but the industrialized world dominated the field and re-

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search was focused on crops important there. The Foundation's rice biotechnology program, launched in December 1984, seeks to counter this in two ways: by enlisting plant molecular biology laboratories in the industrialized world to undertake basic and applied research relevant to the needs of developing countries, and by building the capacity of third-world scientists and research institutions to utilize the knowledge. The expected products are improved varieties that can solve the rice production problems judged most critical in rice-dependent countries. This appropriation supports continuing and new research and training programs at industrialized-country laboratories, collaborative in nearly all cases with institutes in the developing world.

In the initial years, much of the research focused on generating the knowledge base, techniques, and protocols required for genetic manipulation of rice at the cellular and molecular level. Progress has been rapid. Workshops have been and continue to be held to transfer promising technologies to participating developing-country laboratories and rice breeding programs. More recently funding has been concentrated on identifying and formulating gene constructs that, when introduced into the rice genome via genetic engineering, may instill the top priority traits in rice. Appendix A lists the developed-country laboratories currently receiving support.

This past October the program's Scientific Advisory Committee* participated in the three-day annual meeting, held in Tucson, and met with the officers for an additional two days. They reviewed progress to date, helped identify opportunities for support of more focused research in the future, and evaluated new and renewal research proposals within this context. The status of the work at the developed-country laboratories can be summarized as follows.

1. *Rice genetic maps and markers.* The research on development of rice genetic maps and markers and their application in rice breeding is one of the strong points of the program. The rice map now has over 500 markers, a number more than adequate to tag and follow the inheritance of important genes. Several workshops have been held to transfer the map and associated technology to developing countries. Already, the number of breeding programs using the rice map is greater than the number of such programs using genetics maps developed for other crops. Species-specific DNA markers that can facilitate the use of wild relatives of rice in breeding programs are now also available. Current needs are research focused on making this technology less expensive and more usable under field conditions in developing countries and improved coordination of the various

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*Benjamin Burr, Brookhaven National Laboratory; Michael Gale, Cambridge Laboratory, John Innes Institute, England; James Peacock, CSIRO Plant Science Division, Australia; Peter Quail, USDA Plant Gene Expression Center, California; Ralph Quatrano, Department of Biology, University of North Carolina, and Ralph Riley, former Secretary to the Agricultural Research Council, London.

gene-tagging experiments. Further development of the map as a tool for gene cloning is a longer-term goal.

2. *Techniques for foreign-gene transfer.* Development of techniques for the transfer of foreign genes into the rice genome, i.e., transformation, was one of the early and most significant accomplishments of the program. This was a first for any cereal, and the field testing of transgenic rice plants during 1990 marked another first. But progress toward refining these techniques into routine procedures which could be used in any laboratory has been disappointing. While efforts to refine and simplify the protocols continue, three laboratories in the U.S. and two in Europe are supported in part to provide rice genetic transformation services for others in the program.

3. *Mechanism of gene regulation in rice.* Many of the more sophisticated and powerful uses of rice genetic engineering will involve turning genes on or off in particular cells, tissues, and/or organs at particular stages of plant development and/or in response to particular environmental stimuli. The relatively fundamental research aimed at understanding these control processes is proceeding well. Until recently, most of it involved repeating, in rice, investigations similar to ones conducted with model plant systems. However, the knowledge base and techniques for working with rice are now such that rice itself is becoming a model system for research on gene regulation in plants. Support for research in this area continues at approximately the same level.

4. *Useful traits.* Most of the advanced laboratories added to the program over the past few years are concentrating on the identification and cloning of genes that can instill useful traits assigned high priority. Priorities were established by a comprehensive, quantitative analysis that incorporated estimates of the production, equity, and environmental effects of each, as well as the likely feasibility of successfully finding and introducing a gene for each. Candidate genes for virus resistance, insect resistance, and improved nutritional quality are being tested. Engineering more complex traits - such as drought tolerance - which are controlled by many genes, will be a long-term process, but promising research strategies have been identified and are being pursued.

Attached (Appendix B) is a copy of the remarks with which the chairman of the Scientific Advisory Committee, Sir Ralph Riley, concluded the program's 1991 meeting.

RISKS/EVALUATION: In the field tests of genetically engineered crops that have been conducted so far, the new traits derived from "alien" genes - genes taken from other species - have almost all been effective, and the officers no longer see a risk that such alien genes will not work. However, the costs of transformation are still high and will need to be lowered for routine use of the technology in developing countries. Although in all network activities the officers stress the principles of ecology and population biology which can enhance and prolong the usefulness of

the new traits, there remains a risk that those designing and using genetically engineered plants will not give these principles adequate attention.

Evaluation will be based on the officers' assessments, assisted by the Scientific Advisory Committee, of progress by the advanced laboratories in generating the tools for genetic manipulation of rice and identifying genes or genetic modifications that can add useful new traits to rice, and on their effectiveness in collaborating with and training researchers from developing countries as appropriate.

BUDGET: The appropriation, plus $60,000 remaining in RF 91001, would be used as follows to renew support for several laboratories currently part of the program and to support a few new research projects:

Cornell University	$ 600,000/3 yrs.
Texas A&M University	450,000/2 yrs.
Purdue University	450,000/3 yrs.
University of Nottingham	160,000/2 yrs.
Swiss Technical Institute	250,000/2yrs.
Kansas State University	200,000/3 yrs.
Agracetus, Inc.	150,000
University of California, Berkeley	150,000/2 yrs.
University of California, Davis	150,000/3 yrs.
New projects	100,000
Total	$2,660,000

FURTHER SUPPORT: The officers expect to recommend further support for the rice biotechnology program at roughly the present level.

Developed-Country Laboratories Participating in the Foundation's International Program on Rice Biotechnology

University of California at Berkeley	Barbara Baker	Site-directed gene transfer
University of California at Davis	Bill Lucas	Genetic transformation
Commonwealth Scientific, Industrial, and Research Organization, Australia	Wayne Gerlach	Resistance to ragged stunt virus
	Merv Ludlow	Drought tolerance
Cornell University	Steve Tanksley	Genetic maps and markers
	Ray Wu	Genetic transformation/insect resistance
University of Durham	Don Boulter	Insect resistance
University of Georgia	Gary Kochert	Genetic markers
	Sue Wessler	Transposable elements
Jacques Monod Institute	Anne-Lise Haenni	Resistance to hoja blanca virus
John Innes Institute	Roger Hull	Resistance to tungro virus
Kansas State University	Jan Leach	Bacterial-blight resistance
	S. Muthukrishnan	Sheath blight resistance
University of Kyoto	Kunisuke Tanaka	Improved quality of rice storage protein
Michigan State University	Mark Whalon	Insect resistance
University of Missouri	Perry Gustafson	Genetic markers
	Roy Morris	Yield enhancement
University of Montreal	Andrew Hanson	Drought tolerance
National Institute of Agronomic Research, France	David Tepfer	Drought tolerance
Northeast Missouri State University	Brent Buckner	Carotenoid synthesis in endosperm
University of Nottingham	E. C. Cocking	Protoplast techniques
	Alan Alda	
University of Ottawa	Illimar Altosaar	Tissue specific gene expresssion for insect resistance
University of Pennsylvania	Joe Ecker	Genetic mapping
Purdue University	Thomas Hodges	Genetic transformation
	John Hamer	Blast resistance
	Ray Bressan	Drought tolerance
Rockefeller University	Nam-Hai Chua	Gene regulation/drought tolerance
Salk Institute	Chris Lamb	Defense genes
Scripps Research Institute	Roger Beachy	Resistance to tungro and yellow mottle viruses
Stanford University	Virginia Walbot	Mitochondria genes/cold tolerance
Swiss Federal Institute of Technology	Ingo Potrykus	Genetic transformation system for *indica* rice
Texas A&M University	Tim Hall	Rice transformation service
Texas Tech University	Henry Nguyen	Drought tolerance
University of Tokyo	H. Uchimiya	Blast resistance
University of Washington	Eugene Nester	*Agrobacterium*-mediated transformation of rice
Washington State University	Gyn An	Yield enhancement
	Tom Okita	Storage proteins/starch synthesis
	Hei Leung	Blast resistance
University of Wisconsin	Sally Leong	Blast resistance

Comments on The Rockefeller Foundation International Program on Rice Biotechnology

Presented at the conclusion of the 1991 meeting of the program participants by Sir Ralph Riley, FRS, Chairman of the Scientific Advisory Committee, Rockefeller Foundation International Rice Biotechnology Program

All of the leaders of the more than 80 research groups which have participated or are currently participating in the Program, often accompanied by colleagues, assembled in Tucson, Arizona, for the Annual Meeting in October 1991. Together with Foundation staff, Scientific Advisory Committee members, and observers there were in excess of 300 people to take part in more than 60 hours of presentations and discussions. This therefore constituted a major assembly of currently active rice scientists.

The Scientific Advisory Committee concluded, without question, that the level of understanding of the molecular biology of rice is, as a result of the Foundation's investment, in advance of that in any crop. The presentations made and the posters displayed at the meeting were of a high order and displayed research of great competence and often of elegant sophistication.

However, it must be emphasized that the purpose of the Program is to improve rice production and particularly to assist poor farmers and the underprivileged peoples of developing countries in which rice is a staple. Scientists may become engrossed in the intellectual challenge of their work and fascinated by the sequence of discovery on which they are engaged. This must not happen in ways which cause neglect of the supreme social purposes of their projects. It must be rare for this to happen significantly, nevertheless from time to time, as in Tucson, the prime purpose of the Foundation's Program should be re-emphasized.

Further, at intervals the agricultural and social priorities of the Program must be re-evaluated. At Tucson, Lin Justin Yifu drew to our attention that many of the constraints limiting rice production in Zhejiang were caused by physical factors of the environment. Most, but by no means all, of the Foundation's research program is directed to removing or diminishing biological limitations to rice production. Soon a revision of priorities may be needed.

Research leaders in the Foundation's program must ensure for themselves that crop genotypes and experimental technologies are passed on to other groups to assist in their investigations. The initiative for this must come from within groups themselves and it is particularly important that within developing countries different research groups should communicate as freely as possible but this does not always happen. The importance of the annual meeting of participants in the Rice Biotechnology Program is that it enables interactions between research groups to occur and this may not be easy otherwise even for groups from within the same large developing country who could be a thousand or more miles apart in conditions where travel is difficult.

Impressive progress was reported in transforming rice by the introduction of alien genes. However, it is important to recognize that we are still at the stage of experimentation in transformation and do not as yet have methods that can be used routinely in plant breeding. While there is clear evidence of success using protoplast this system needs to be developed further and research should continue on the feasibility of delivering DNA into rice on projectiles or by *Agrobacterium*. An admirable service is provided by Dr. Tim Hall of Texas A&M in transferring

to rice genes isolated by other groups. However, when the experimental phase has passed in rice transformation and it becomes a routine plant breeding technology we shall need to address questions related to "scale up." For example, how many primary transformants will be needed to ensure that a sufficient proportion of them can be employed in variety development because the introduced DNA has been inserted stably and appropriately in the recipient DNA and there is the optimum level of expression in the target tissue of the host. Finally, referring to the present experimental program on transformation, those supported by the Foundation should always be required to provide unequivocal evidence of transformation by Southern blots ensuring that no exogenous vector remains in the material.

In gene and RFLP mapping there has been tremendous progress under Steve Tanksley of Cornell and with a significant input from Gary Kochert of the University of Georgia. A map is now available which will already allow plant breeders to track agronomically important genes without the need to expose rice plants to hazards against which the genes afford no protection. Now workers in a number of rice-growing developing countries should be encouraged to develop similar maps using locally important varieties and maps that are relevant in relation to the agricultural problems of their countries. This work will be greatly aided by the Cornell map and by the use of probes provided by Dr. Tanksley. Continued encouragement should be provided for the collation of the information derived from conventional genetic, RFLP and cytological maps. In the latter case work on *in situ* hybridization mapping by Perry Gustafson of the University of Missouri, although at an early stage, could become useful. A further sophistication of the mapping process is now necessary if the map is to be safeguarded and made permanently available to rice breeders. This arises because of the risks to which the probes are exposed that identify the restriction sites on the Tanksley map. Probes may be lost, misplaced or confused and clearly, as with genetic stocks, when the originators move on for whatever reason there is no natural inheritance of the responsibility for continued maintenance. On the other hand, determination of the nucleotide sequences surrounding each restriction site would always enable that site to be identified. These sequences could be stored on a computer data base or simply be published. The Foundation should alone, or preferably with others, promote work to determine those sequences and create the relevant data base.

Tissue and protoplast culture has been very important in the development of rice science. However, some targets have been now been achieved. For example, anther culture is now used routinely in numbers of breeding programs and, just as the Foundation leaves the support of rice breeding to NARS and IARCs, it must now be asked whether such work should continue as part of the Rice Biotechnology Program. Similar views may be taken of tissue culture studies aimed at producing somaclonal-variation. In the case of protoplast culture and regeneration this now seems to be routine work with Japonica rices but should still be supported in Indica rices until it is equally routine. These comments imply that a controlled phase-out of tissue, cell and protoplast culture research may be appropriate. However, it can still be argued that where interspecific protoplast culture fusion appears to be the most likely route the exploitation of alien genes (e.g., salt tolerance from *Portersia coarctata*) then protoplast culture must continue to have prominence.

Work on wide-hybridization has been very successful at IRRI and varieties of *O. sativa* with BPH resistance genes derived from *O. offinalis* will shortly be in cultivation by farmers whose crops are at risk to these insects. Other beneficial introgressions have also been made in this program and will soon become available to farmers. The joint work of IRRI and the University of Georgia has shown by RFLP analysis the changed nature of the DNA in rice with sequences introgressed during the wide hybridization process. However, the causes of these

genetic changes are still not understood although work to determine the causes would clarify the means by which alien introgression could be made a more effective tool in rice breeding.

In recent annual meetings of the Program concern has been expressed that insufficient attention was being given to the improvement of insect resistance in rice. At the Tucson meeting one entire session was devoted to discussion on attempts to incorporate insect resistance derived from *Bacillus thuringiensis*. There can be no doubt that this approach is worthy of the considerable effort devoted to it. Nevertheless, too much concentration on this single source of insect resistance could be dangerous and it would be wise to extend the range of genetic systems from which resistance may be obtained. The first steps to examine other forms of resistance have been made in the support of work of Durham university and elsewhere.

Much useful research has been carried out on several rice viruses such as tungro, hoja blanca, rice stripe, yellow mottle and ragged stunt. Work is under way to attempt to incorporate genes from the viruses in rice to obtain resistance. It is readily possible to understand the exploitation of such resistance when it depends upon the action of the first products of the genes and there seems every prospect that agriculturally valuable forms of rice will be obtained.

The development of biotechnology for resistance to bacterial and fungal diseases is of a different order of complexity because of its dependance upon the products of intermediary metabolism. However, it is clear that the Foundation's support for Chris Lamb's (Salk Institute) dissection of generalized disease resistance is very worthwhile from this point of view. Nevertheless it may be desireable to assess how an agriculturally useful technology might be created in this complex work.

An area that is beginning to open and which probably, as mentioned earlier, needs greater priority, is the genetic analysis of the components of resistance to such physical limitations of the environment as drought or high salinity against which conventional plant breeding has had only modest success. Initially, we shall be in an exploratory phase. When this is approaching completion, an evaluation will be necessary to determine whether practical agricultural technologies can be created.

A very effective rice science community has been developed as a result of the work of the Rockefeller Foundation. It operates in a synergistic way to accelerate rapidly the understanding of the molecular biology of rice and to advance the time when farmers will benefit from the understanding. The present state in the exploration of technologies in which the Foundation has invested is as follows:

Already producing benefits for farmers

1. Anther culture
2. Somaclonal variation
3. Wide hybridization

On the verge of producing benefits

1. RFLP mapping in accelerating conventional breeding and increasing its precision.

Still experimental

1. Production of genetically engineered organism in the following expected order in time of agricultural exploitation:
   - i. Virus resistance
   - ii. Insect resistance

iii. Bacterial and fungal resistance
iv. Resistance to physical limitations to production
v. Nutritional improvement of rice grain for human consumption

Finally, it will be noted that the use of RFLP mapping to enable breeders to track quantitative trait loci affecting yield will have a direct effect on the yield potential of rice. In addition, work on cytokinin at the University of Missouri may lead to increased sink capacity in the panicle. All the remaining work funded by the Rockefeller Foundation has the purpose of ameliorating the damage caused by limiting factors whether biological or physical. Ultimately, if from 10 billion to 14 billion people are to be fed on earth, it will be necessary to advance yield potential by improving the efficiency of carbon fixation. The long term aims of those supporting research in crop production must be to enhance biological productivity through intervention in the photosynthetic process.