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a copy of his comments re each grant should be with file on that grant

April 10, 1989

Dear Peter:

Thank you for your report on the seven programs. This will be very helpful to us as we evaluate and plan funding during this year.

We appreciate your efforts.

With best wishes.

Sincerely,

Gary H. Toenniessen
Associate Director
Agricultural Sciences

Dr. Peter Quail
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GHT:gtb

REPORT ON SELECTED RESEARCH PROGRAMS

OF ROCKEFELLER FOUNDATION INTERNATIONAL PROGRAM

ON RICE BIOTECHNOLOGY

To Gary H. Toenniessen

From Peter H. Quail

March 22, 1989

University of Ghent. Marc Van Montagu.

The lack of success thus far in using Agrobacterium to transform rice has apparently led this group to focus on three alternative lines of research:

(a) Transient gene expression in electroporated intact tissue. Both 2'-NPT and 2'-GUS constructs have been used to determine whether DNA can be introduced into intact rice seedling tissue (mainly excised coleoptiles and leaves) by direct electroporation of the tissue. Both activities were reported to be detectable in a transient manner following electroporation. I have strong reservations about these data. No proper negative controls were presented. In such experiments it is necessary to have a promoterless construct (i.e., lacking the 2' promoter in this case) to establish specificity. Read-through expression in contaminating electroporated microorganisms is always a possibility in such systems until proven otherwise. Non-specific cleavage of the GUS substrate at acid pH (such as vacuolar pH) is also a concern at cut tissue surfaces where there are large numbers of wounded cells. Finally, many other labs have tried electroporating intact cells, such as suspension culture cells (intact single cells and aggregates) and walled leaf cells released by macerase, without success. In short, more rigorous data are needed before this approach can be considered to have worked.

(b) Isolation of stress-induced genes.

Clones have been isolated for a 14.5 kD protein. Northern analysis showed some induction by salt, but it was not clear to me that this is indeed a stress-induced gene. Study of the heat-shock genes referred to might be useful, as these are more likely to be involved in stress responses.

(c) Pathogen-related proteins. Preliminary data on clones for a root-specific peroxidase have been obtained. However, the role if any of this

protein in host-pathogen interactions is yet to be established.

Overall, I was very underwhelmed by the quality and productivity of this program, especially given the high level of funding. On the other hand, Van Montagu's lab is noted for its innovativeness, especially in the area of plant transformation. I would recommend continuing to support a small effort in the area of stable transformation, if at all. Rice still has a ways to go in that area. The other lines being pursued are not particularly innovative.

University of Leiden. Rob Schilperoort.

This group has made progress in the isolation and partial characterization of tissue-specific and constitutive cDNA and genomic clones from IR36 for future use of the corresponding promoters in transgenic rice. They have also explored several approaches to stable transformation of rice, including protoplast electroporation, the "pollen tube pathway procedure", and Agrobacterium inoculation of cellulase/pectinase digested cells and tissues. All the transformation work has been done with Japonica rice. No unequivocal evidence of transgenic, regenerated plants has thus far been obtained for any of these methods, although GUS-marker activity and drug-resistant cells have been variously observed. Most of these data represented "in progress" experiments awaiting verification of success through Southern analysis. One observation of potentially far-reaching impact was that azocytidine apparently greatly enhanced expression of a 2'-GUS construct in microcalli that had been selected following electroporation of the construct into protoplasts. This result raises the possibility that transformation efficiencies of rice cells could be vastly improved by suppression of methylation of introduced DNA. Although the funding may be a little on the high side, this group appears to

be working fairly actively and systematically with the rice system. Continued support appears justified. Once again my bias would be to deemphasize the gene characterization side and support the transformation side. Perhaps they should be encouraged to branch out into indica lines.

Purdue University Tom Hodges.

The work reported by this group represents a major advance toward the goal of regeneration of transformed indica rice plants. Fertile, mature plants have been regenerated from protoplasts prepared from established suspension culture lines of IR54. Kanamycin-resistant calli have been obtained from IR54 protoplasts transformed with a 35S-NPTII construct.

I think the Rockefeller should definitely continue to fund this project. This is the only group in the program thus far to have succeeded in regenerating indica protoplasts, and is on the way to obtaining the first transformed indica plants. There are, however, still a number of obstacles to be overcome before the system will be as useful as it might be. Thus far IR36 has proven difficult to work with. The group should be encouraged to continue to explore the use of IR36. Hopefully what is learned from IR54 will be at least partially transferable. In addition, it would obviously be useful to short-circuit the cumbersome protocols which require growing suspension cultures for several months before regenerable protoplasts can be produced. This group reports one experiment where IR54 plants were regenerated from protoplasts prepared from primary callus. They should be encouraged to invest some energy in pursuing this issue. In short, this group are in a strong position, and have the experience, to refine and capitalize on their basic success to produce transgenic indica plants. It seems that in the future they

could function at the hub of the wheel interacting with others in the program who have various interesting genes they wish to introduce into indica rice.

Max Planck Institute. Horst Lörz.

The productivity of this group appears to be relatively low given the level of funding. Attempts to regenerate indica rice from protoplasts have been unsuccessful, and data on possible transformation using the "pollen tube method" and direct imbibition of dry, mature embryos in DNA-containing solution were unconvincing. Likewise the production of putatively disease-resistant plants using somaclonal variation needs further work. It is my impression that this project may not have been afforded the attention it deserves. I agree with your assessment that funding should lapse when Lörz moves to Hamburg.

Iowa State. Don Robertson.

The attempts thus far by this group to clone the YI gene from maize by transposon tagging with Mu have failed. The candidate 5.9 kb fragment they had cloned and had been working with proved not to correspond to the YI locus. Transposon tagging is by its very nature a long, time-consuming process in maize and can lead to such blind alleys. My basic feeling is that this is an experienced group and if anyone can do it they should be able to. Nevertheless, two questions occurred to me concerning the basic strategy. First, is there evidence supporting the central premise on which the project is based, namely, that the lack of carotenoid synthesis in rice endosperm results from a lesion in the gene that is the analog of the maize YI gene? Second, it is not entirely clear to me why it is useful to use ts-sensitive

inhibition of carotenoid synthesis in leaves as a secondary screen indicating that the YI locus has been tagged. It seems to be that a Mu insertion that would simultaneously eliminate carotenoid synthesis in the endosperm and allow synthesis in leaves at normal temperatures but not at 37°C would be a very low probability configuration. Perhaps these questions reflect my own ignorance of the system, but it would be nice to be reassured. If these concerns are misplaced, then my guess is that it is probably just a matter of time until they clone the YI gene, and should be supported to continue. Perhaps the level of support should be decreased until they find the gene. I suspect that the costs of this screening phase of the project may not be that high.

University of Wisconsin. Sally Leong.

The major contributions this group has made to an understanding of the molecular genetics of Pyricularia are: (a) The beginnings of an RFLP map (36 markers thus far). (b) The resolution of large chromosomal DNA fragments (up to 12 megabases) using CHEF technology. They observe 6 major bands (possibly one per chromosome?) and are in the process of generating libraries for each band. (c) Have successfully implemented a transformation protocol with the generation of hygromycin-resistant transformants.

It appears to me that a number of the major tools needed to pursue the ultimate goal of cloning and characterizing genes involved in pathogenicity are in place or in progress. I was impressed by the achievements thus far, especially given the relatively modest funding level, and would recommend continued support.

Kansas State University. Gerald Reeck.

A modest start has been made on an approach to isolating clones for inhibitors of rice weevil digestive enzymes, especially α-amylases and proteinases. The P.I. seems to be sensitive to the need to target inhibitors that inhibit weevil, but not human, enzymes. Amino acid sequences of two wheat endosperm inhibitors of weevil α-amylase have been determined. It was not entirely clear to me why a rice cDNA with some sequence similarity to wheat inhibitors was being used to screen the wheat endosperm cDNA library, rather than synthetic oligonucleotide probes based directly on the wheat inhibitor sequences that they have determined. There appears to be no reason why the basic mechanics of this project shouldn't move very rapidly. Isolation and characterization of these clones should be very straightforward, given the tools in hand. The P.I. should be encouraged to look beyond this preliminary phase to generating strategies and constructs for introduction of the relevant inhibitors into transgenic rice. He could presumably isolate rice endosperm specific promoters himself or get them from others (Tom Okita?) for this purpose. Because the project is only just getting under way, I would recommend continued support, but the rate of progress should be monitored closely.