RF 87058

THE INTERNATIONAL RICE RESEARCH INSTITUTE
RF Grant for Equipping Plant Pathology
RF - 85058 - # 27

Final Financial Statement
March 31, 1987

Funds Received	104,740.00
Less: Expenditures	101,796.64
Fund Balance	$ 2,943.36

Certified Correct:

Edward N. Sayegh
Director for Finance

THE INTERNATIONAL RICE RESEARCH INSTITUTE
RF Grant for Equipping Plant Pathology
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Schedule of Expenditures
March 31, 1987

Reference	Particulars	Amount
85-05104	UV-Light Box with Polaroid Camera	4,113.14
-05106	X - Ray Film Cassetter	535.82
-07109) -05107)	Electrophoreses Units and Power Supply	2,048.55
-05116	Microwave Oven	343.34
-07071	Vacuum Oven	978.76
-07073	Incubator Shaker	3,695.63
-07112	Flourescent Microscope	8,390.05
-07115	PH Meter and Meter Bath	1,367.54
-07117	Table Top Centrifuge	5,498.04
-07118	Eppendorf Microfuge (2 Units)	3,115.95
-07119	Ultracentrifule plus motor	56,384.26
-07312	Environ-Shaker, Gyratory	3,014.75
87-06073	Comfort Aire Electric Dehumidifier (2 Units)	686.95
-06122	Power Supply Heathkit (4 Units)	1,266.47
-06309	Percival Dew Chamber (1 Unit) and Percival Lighted Incubator (1 Unit)	8,900.57
-09768	Precision Incubator	638.04
-46352	Horizontal Electrophoroses Apparatus (10 Units)	318.78
	TOTAL	$ 101,796.64

RF GRANT ON APPLICATION OF MOLECULAR TECHNIQUES IN RICE PATHOLOGY
1987 PROGRESS REPORT TO THE ROCKEFELLER FOUNDATION

OVERVIEW

In 1987, the primary focus of the program has been on the genetics and molecular characterization of the blast fungus, the bacterial blight pathogen, and rice tungro virus complex. Considerable progress has been made in defining the genetic control of virulence in the blast fungus and in identifying genes that are important in the pathogenesis pathway. A genetic system has been developed for the bacterial blight pathogen to conduct molecular analysis of pathogenesis. We have successfully applied non-radioactive labelling techniques to analyze the bacterial genome and we are in the process of developing diagnostic DNA probes for both the bacterial and fungal pathogens. For rice tungro virus complex, we have analyzed the proteins and nucleic acids of RTSV and RTBV with the aid of specific antisera.

By the end of 1987, the EM laboratory was operational and sufficiently equipped to conduct experiments involving recombinant DNA techniques. With this facility, IRRI research staff and visiting scientists from national programs have received training in genetics, recombinant DNA technology, and serology. In addition, a teach-in workshop on molecular techniques in Xanthomonas will be held in this laboratory on March 17-18 in conjunction with the First International Workshop in Bacterial Blight of Rice supported by the Belgium Government.

PROJECT UPDATE

A. Blast fungus

Pathogenesis

We have used the sexual stage of the blast fungus to determine the genetic control of virulence on rice. Random spore and tetrad analysis showed that two loci Pos1 (pathogenicity on Oryza sativa) and Pos2 conditioned virulence to rice lines 51583 and Sha-tiao-tsao. From a cross between two field rice isolates, we identified two additional loci - Pos3 and Pos4 - which controlled virulence on rice lines K59 and Kinandang Patong, respectively. Joint segregation analysis showed an excess of parental types with respect to virulence. We are determining whether this reflects genetic linkage of virulence genes or segregation of other genetic factors epistatic to virulence. The virulence genes are being mapped by restriction fragment length polymorphism analysis.

We have identified a buff pigment mutation which acts epistatically to virulence. The buff gene controls melanin synthesis in the fungus; melanin production is essential for successful penetration of host cells. Buff mutants cannot infect rice even though they have the correct combination of virulence genes. Thus, the buff gene represents a target to which control strategy can be directed. We will continue to analyze the interaction between the buff gene and the rice plant in order to design control measures.

Mutability

Besides its important role in pathogenicity, the buff mutation has proved to be a useful phenotype for studying variability in the blast

fungus. In crosses of certain strains, we observed a high frequency of the buff mutation in the progeny. We hypothesize that this strain-dependent mutability is analogous to the induction of mutation in hybrid dysgenesis in Drosophila. With this system, we aim to analyze the genetic basis of variation in the fungus.

In 65 crosses between wild-type (wt) isolates with normal pigmentation, we observed an average of 6.2% buff progeny depending on the isolate background. A cross between a field rice isolate Guyll and a laboratory strain 2539 consistently gave 25% buff progeny. Of 15 asci examined, 6 asci were 8 wt:0 buff, 5 were 4 wt:4 buff, and 4 were 6 wt:2 buff. Test crosses between wild types (presumably with wild type alleles at 2 loci) and buff mutants gave 1:1 instead of a 3:1 digenic gene ratio; thus, the buff phenotype is not under two-gene control. When UV-induced buff mutants of Guyll and 2539 were backcrossed to the wild type parents, there was an excess of buff progeny significantly deviating from the expected 1 wt:1 buff ratio. Also, tetrads with 8 buff progeny were recovered, suggesting that genetic factors independent of the buff locus were responsible for giving buff progeny. These results suggest that there are complementary elements in certain rice isolates that can interact to cause buff mutation. The genetic mechanism for this mutability might be similar to that causing pathogenic variation.

B. Bacterial blight pathogen

We have successfully applied the genetic techniques developed at the laboratory of Jan Leach to Xanthomonas campestris pv. oryzae

(Xco). Transconjugants of Xco race 2 were obtained by triparental matings using pLAFR5 and pRK2013 plasmids. A cosmid library of race 2 was constructed for genetic analysis of virulence. A series of Xco nonpathogenic mutants were developed by nitrosoguanidine mutagenesis. Nonpathogenic mutants are being used as recipients of the Xco cosmid library to identify pathogenicity genes by complementation.

Due to problems in obtaining radioactive isotopes, we adopted a nonradioactive biotin-labelling technique for genetics analysis and race diagnosis. Biotin-labelled DNA probes were as sensitive as ^{32}P-labelled probes in the analysis of bacterial genomes. Polymorphisms were detected among different pathovars of Xanthomonas campestris and also within and between races of Xco (Fig. 1).

C. **Rice Tungro Virus Complex**

1. Purification and serology

Rice seedlings were inoculated by rice green leafhoppers, Nephotettix virescens, that had fed on rice plants infected with both rice tungro bacilliform (RTBV) and rice tungro spherical virus (RTSV). RTSV-infected plants were identified and selected using antiserum to rice waika virus which is very closely related if not identical to RTSV. RTSV was propagated by inoculating rice seedlings using leafhoppers. To multiply RTBV, seedlings were inoculated by leafhoppers which had fed first on plants infected with both RTBV and RTSV, second on anti-RTSV immunoglobulin through membrane, and then on RTBV-infected plants. RTBV and RTSV were purified separately from their respectively infected plants

by heating sap 1 hr at 40 C, driselase treatment, PEG precipitation, differential centrifugations, and sucrose density gradient centrifugation. Purified RTBV fractions contained bacilliform particles 30-35 nm in width and 160-220 nm in length. Purified RTSV fractions contained isometric particles 30 nm in diameter. Both fractions had UV absorption spectra typical of nucleoprotein. Rabbit antisera obtained had titers of 1/2560 for RTBV and 1/640 for RTSV by the ring-interference precipitin test. The latex test and ELISA specifically detected RTBV and RTSV in leaf extracts. The antisera were virus specific.

2. Proteins of RTBV and RTSV

Molecular weights of proteins from purified RTBV and RTSV preparations were determined by SDS-PAGE using the Protein Molecular Weight Standards 3K to 43K (Bethesda Research Laboratory, USA). Proteins from RTSV particles were resolved into two major and one minor components by PAGE. The estimated molecular weights of the major components were 29K and 22K and that of the minor component was 23K. Proteins from RTBV particles were resolved into two components which had estimated molecular weights of 31K and 29K.

3. Nucleic acids of RTBV and RTSV

Nucleic acids were extracted in 1% SDS and 0.005 M EDTA by heating a pure virus suspension for 10 min at 37 C. Nucleic acid was also extracted by a phenol-SDS method. Extracts were run on acrylamide gel with TMV-RNA and ribosomal RNA as standards.

Electrophoresis of nucleic acids from RTSV resolved into two components. After electrophoresis, the gel was treated with 10 µg/ml RNase A in 2 x SSC for 2 hr at 37 C, then with 10 µg/ml RNase A in 0.1 x SSC for 2 hr at 37 C, followed by treatment with 5 µg/ml of DNase I in 5 mM $MgCl_2$ for 1 hr at 37 C. The two bands on the gels disappeared after treatment with RNase in 2 x SSC. The results indicated RTSV had single-stranded RNA (ssRNA). The estimated molecular weights of the ssRNA species were 2.5 and 2.3 x 10^6 dalton. Electrophoresis of nucleic acids from RTBV revealed only one component. This component was not digested after the treatment with RNase at 2 x SSC and 0.1 x SSC, however, it was digested by treatment with DNase.

WORKPLAN FOR 1988

1. The genetic study of pathogenicity of the blast fungus will be continued at the University of Wisconsin. This will include the mapping of pathogenicity genes using RFLP analysis and genetics analysis of mutability using the buff mutation and other marker genes. At IRRI, we will explore the possibility of identifying metabolic pathways which may affect melanin biosynthesis in the fungus.

2. The genetic system available in Xco will be used to identify genes important in pathogenesis and to study the relationship between the pathogenicity genes and resistance genes in rice.

3. We will continued to analyze nucleic acids from RTBV by gel electrophoresis and to study proteins from RTBV and RTSV by immunoblotting. Also, we will explore the use of a rice protoplast system in studying the interactions between the rice and virus genomes.

4. More efficient and sensitive non-radioactive labelling techniques will be sought for routine analysis of pathogen genomes and disease diagnosis.

COLLABORATION

For both the blast and bacterial blight projects, experiments have been conducted in close collaboration with the University of Wisconsin and Kansas State University. Research objectives and approaches are discussed jointly and materials and information are shared. We expect this highly satisfactory collaboration to continue. For the tungro virus project, we have been providing purified RTBV and RTSV viruses and antisera to Dr. R.N. Beachy and antisera to Dr. R. Hull. We anticipate this collaboration to be strengthened in 1988.

PERSONNEL

	Title	Join IRRI
Dr. Rebecca Nelson	Postdoctoral Fellow	February 1988
Mr. Noel Chua	Research Aide	April 1987
Ms. Alicia Amante	Research Aide	April 1987
Mr. Nestor Amoloza	Laboratory Aide	July 1987

PUBLICATION

Abstract

Ardales, E.Y., N. Chua, J. Leach, and H. Leung. 1988. Isolation of chemically-induced mutants of Xanthomonas campestris pv. oryzae with changed race-specificity. Fifth International Congress of Plant Pathology, August 20-27, Kyoto, Japan.

Hibino, H. and P.Q. Cabauatan. 1988. Purification, serology and properties of rice tungro-associated viruses. Fifth International Congress of Plant Pathology, August 20-27, Kyoto, Japan.

Leung, H., E.S. Borromeo, and M.A. Bernardo. 1988. Detection of possible mutator elements in Magnaporthe grisea. Fifth International Congress of Plant Pathology, August 20-27, Kyoto, Japan.

Skinner, D., H. Leung, and S.A. Leong. 1988. Molecular mapping and electrophoretic karyotyping of Magnaporthe grisea. Fifth International Congress of Plant Pathology, August 20-27, Kyoto, Japan.

Paper

Leung, H., E.S. Borromeo, M.A. Bernardo, and J.L. Notteghem. 1988. Genetic analysis of virulence in the rice blast fungus. Phytopathology (submitted).

Leach, J., S. Kelemu, E.Y. Ardales, and H. Leung. 1988. Genetics of Xanthomonas campestris pv. oryzae. First International Workshop on the Bacterial Blight of rice. March 14-18. IRRI, Philippines (submitted).

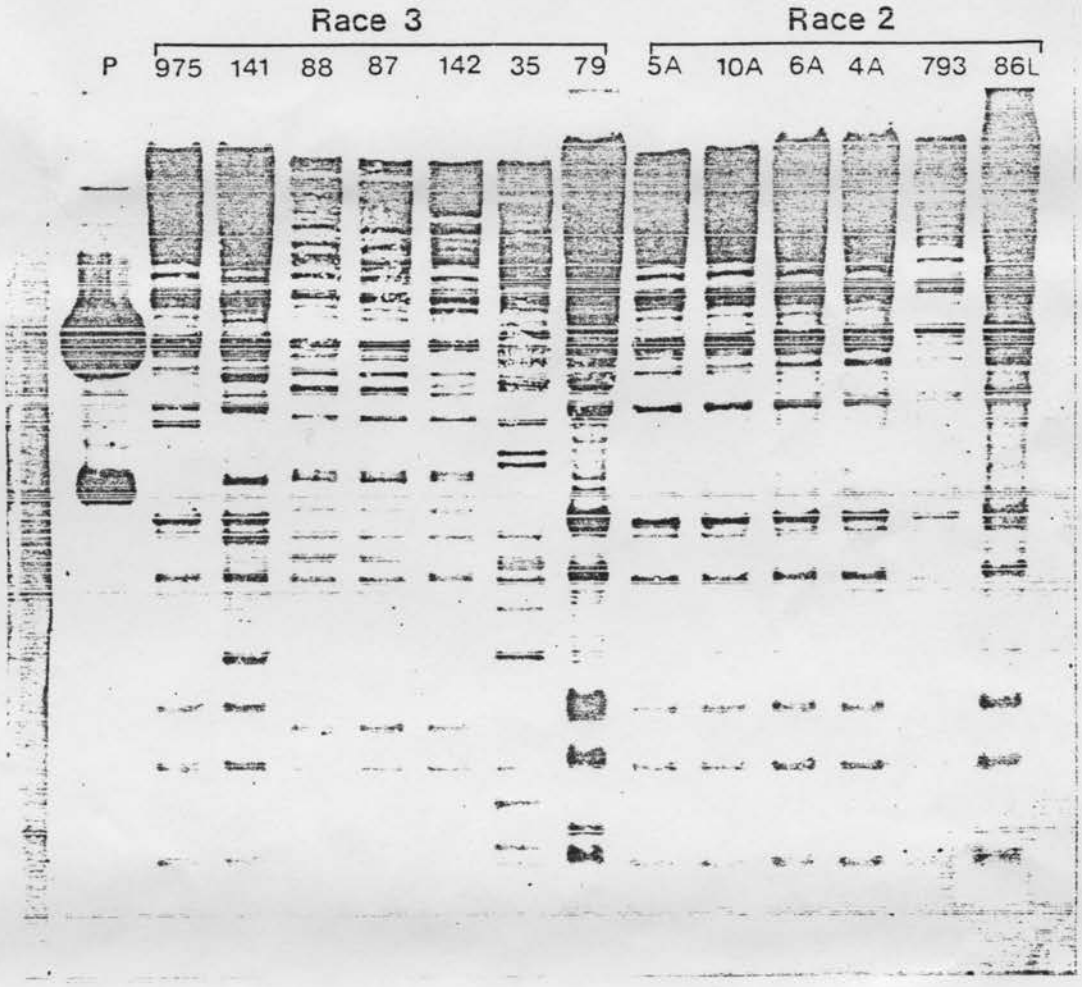

Fig. 1. Southern blot analysis of EcoR1-digested DNA of Xanthomonas campestris pv. oryzae with biotin-labelled plasmid pJEL101. Note the polymorphisms detected among isolates of race 3. Strain 5A, 10A, 6A, and 4A are nitrosoguanidine-induced pathogenic mutants of strain 793. No change in banding patterns is detected in these mutants. P stands for plasmid pJEL101.