Variation in Double-Stranded RNA from Isolates of *Pyricularia oryzae*

Penny L. Hunst, Frances M. Latterell, and Albert E. Rossi

Research associate, Department of Plant Pathology, Physiology & Weed Science, Virginia Polytechnic Institute & State University, Blacksburg 24061, and research plant pathologist and biologist, respectively, USDA-ARS Plant Disease Research Laboratory, Frederick, MD 21701.

Present address of first author: Monsanto, Corporate Research and Development, 800 N. Lindbergh, St. Louis, MO 63167.

Mention of a trade name or proprietary product does not constitute a guarantee or warranty by the USDA and it does not imply its approval to the exclusion of other products that may also be suitable.

We thank S. S. Hurtt for electron microscopy and A. D. Hewings for purification of virus components.

Accepted for publication 6 January 1986 (submitted for electronic processing).

ABSTRACT

Hunst, P. L., Latterell, F. M., and Rossi, A. E. 1986. Variation in double-stranded RNA from isolates of *Pyricularia oryzae*. Phytopathology 76:674-678.

Mycelial extracts of 29 isolates of *Pyricularia oryzae* representing 21 physiologic races from worldwide sources were analyzed for double-stranded RNA (dsRNA) content. Among the 20 isolates found to contain dsRNA, 15 distinct dsRNA electrophoretic patterns were observed. A number of isolates related by host specificity and/or genealogy had common dsRNA bands as well as unique bands. Virus particles about 35 nm in diameter were partially purified from mycelial extracts of isolates 455 (race IA-65) and 793 (IB-49) and were observed by transmission electron microscopy. Upon dissociation, the particles were found to contain dsRNA, which comigrated with the dsRNA extracted from the mycelium. Dot-blot hybridization analysis revealed sequence homology between the dsRNA of isolate 455 of race IA-65 and several other isolates of related races. Evidence of a relationship between dsRNA content and pathogenicity of *P. oryzae*, however, was inconclusive.

Viruses commonly occur in fungi and have been detected in all major fungal taxa (7). Most of the characterized mycoviruses contain segmented double-stranded RNA (dsRNA) genomes (7). Multiple-virus infections are common in fungi; however, knowledge of the relationships among the viruses and of their distribution among fungal isolates is lacking (7,9).

In 1970–1971, viruses were reported to occur in mycelia of *Pyricularia oryzae* Cavara, the pathogen of the blast disease of rice (*Oryza sativa* L.) (6,19,20). Yamashita et al (20,21) purified polyhedral virus particles about 36 nm in diameter from infected mycelia, whereas Férault et al (6) found "paraspherical" virus particles of two sizes: 25 and 32 nm. In 1976, virus was transmitted from "infected" to "healthy" cultures of *P. oryzae* by protoplast fusion (1).

Our isolates of *P. oryzae*, originating worldwide, exhibit many pathogenic, cultural, and metabolic differences (12). Recognition by Latterell et al (13) of differential pathogenicity of isolates from different geographical areas to selected cultivars of rice led to the development of a differential set of rice cultivars (12).

Possible relationships have been suggested among the races of *P. oryzae* primarily on the basis of their pathogenicity to two of the international differential cultivars of rice: Sha Tiao Tsao-S and Caloro (11). On this basis, some 50 races were divided into three groups: race group 1, none of which attack Caloro; race group 2, none of which attack Sha Tiao Tsao-S but all of which attack Caloro; and race group 3, all of which attack both of these cultivars. We analyzed representatives of each group for dsRNA. We selected isolates of races that appeared to be related genealogically by virtue of their ascent, probably through mutation, from a narrow to a broader host range. Other selections included races of similar host specialization patterns from remote geographical areas. A "lysed" culture that had lost capacity to sporulate and grow aerially was compared with a normally sporulating "healthy" culture from the same original isolate.

Because the presence of virus has been shown to affect the degree of pathogenicity or toxicity of a fungus to its plant host (2,4,10,14), we undertook this study to determine the presence and heterogeneity or similarity of viruses in different isolates of *P. oryzae* as evidenced by their dsRNA content. We also sought to determine whether any relationship could be established between the presence of mycoviruses and physiologic specialization in this pathogen of rice.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1986 The American Phytopathological Society

MATERIALS AND METHODS

Isolates of *P. oryzae* and cultural methods. The isolates of *P. oryzae* used in this study are listed in Table 1. *Pyricularia* cultures

TABLE 1. Classification, geographic origin, and dsRNA content of *Pyricularia oryzae* isolates studied

Isolate	Race	Geographic origin	dsRNA
Race group 3			
453	IB-33	El Salvador	+
455	IA-65	Philippines	+
455 (aberrant)	IA-65	Philippines	+
479	ID-13	Florida	+
495	IA-65	Taiwan	+
504	IC-17A	Pakistan	−
566	ID-9	Indonesia	−
580	IC-17	Japan	−
640	IG-1	Nicaragua	−
648	IA-65	Hungary	+
649	ID-1	India	+
747	ID-13	Philippines	+
793	IB-49	Louisiana	+
820	IF-1	Japan	+
825-D6	IB-1	Costa Rica	+
825-D6-Ram	IA-1	Costa Rica	+
888	IA-65	India	+
923	IA-109	Philippines	+
1930-Alicia	ID-13	Peru	−
1932 (healthy)	IC-17	Texas	+
1932 (lysed)	IC-17	Texas	+
Race group 2			
367	IC-19	Taiwan	−
603	IH-1	Arkansas	+
633	ID-15	Louisiana	+
1931-Tsukuba	IE-3	Japan	+
Race group 1			
429	IB-54	Louisiana	−
468	IG-2	Cambodia	+
499	ID-8	India	−
712	II-1	Colombia	−

were grown on tubes of rice polish agar (RPA) (17) for use as seeding inocula. Conidial suspensions were harvested from 5-day-old cultures by flooding with sterile distilled water containing Tween 20 (one drop per 100 ml) and abrading with a transfer needle to dislodge conidia. Conidial suspensions (about 1×10^4/ml) were transferred aseptically to 70 ml of yeast extract-dextrose liquid medium (3 g of yeast extract + 15 g of dextrose per liter of water) in 300-ml Erlenmeyer flasks. One nonsporulating "lysed" culture was seeded by mycelial plug transfer. Liquid cultures were grown for 5 days on a shaker (130 rpm) at 25 C with 12 hr of light and 12 hr of darkness.

Conidial transmission tests. To test for mycovirus transmission through conidia, 16 single-conidial isolates were made from isolate 455 (race IA-65). A conidial suspension was spread over a water agar plate and incubated overnight to allow time for germination and evaporation of excess water. Agar blocks bearing single conidia with short germ tubes, observed under a dissecting binocular (×100), were cut aseptically and transferred to RPA tubes.

Isolation of dsRNA. dsRNA was extracted from fungal mycelia according to a modified procedure of Dodds and Bar-Joseph (5). Mycelial mats were separated from liquid medium by vacuum-filtration and frozen (30 min to overnight) at −80 C. Tissue (0.5–10 g) was ground in an extraction medium consisting of 2× STE (50 mM tris, 100 mM NaCl, 1 mM EDTA, pH 7.0) (1 ml/1 g of tissue), equal volume phenol (1 ml/1 g of tissue), one-half volume of chloroform/pentanol (25:1) (0.5 ml/1 g of tissue), 1/10 volume of 10% sodium dodecyl sulfate (SDS) (0.1 ml/1 g of tissue), 1/10 volume of 2-mercaptoethanol (0.1 ml/1 g of tissue), and 0.5 ml of 10% bentonite. The ground mixture was stirred at 4 C for 30–45 min and centrifuged at 8,700 *g* for 15 min in a SS34 Sorvall rotor. The aqueous phase was decanted, and ethanol was added to a final concentration of 15%. A nonionic cellulose powder (Cellex N-1, Bio-Rad, Rockville Centre, NY) (1.5 g) was added, and the mixture was then loaded into a 50-ml glass syringe plugged with two layers of Miracloth (Calbiochem, Inc., San Diego, CA). The column was allowed to pack and was then washed with 80 ml of STE containing 15% ethanol to remove all single-stranded RNA; dsRNA was eluted from the column with 10 ml of STE and precipitated overnight with two volumes of ethanol and 1/10 volume of 2 M sodium acetate, pH 6.0, at −20 C. The precipitate was collected by centrifugation at 12,100 *g* for 10 min, air-dried, resuspended in a minimal volume of sterile distilled water, and stored at −20 or −80 C.

The dsRNA samples were treated with DNase at 20 μg/ml in 30 mM $MgCl_2$ at 37 C for 20 min before electrophoresis through 5% T/2.7% C polyacrylamide slab gels (15 × 14 cm × 1.5 mm) at 60V for 18 hr in 40 mM Tris-Cl, 100 mM borate, 2 mM disodium EDTA, pH 8.3. After electrophoresis, the gels were treated with RNase A at 50 μg/ml in 300 mM NaCl for 30 min at 37 C. The gels

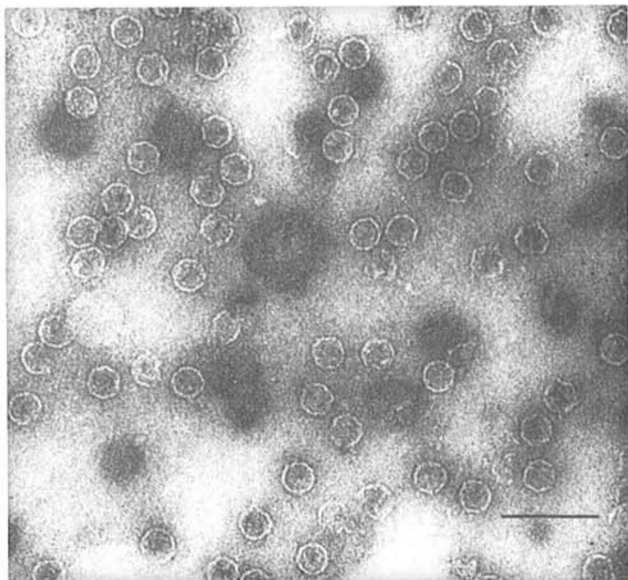

Fig. 1. Electron micrograph of virus particles partially purified from mycelia of isolate 455 (race IA-65) of *Pyricularia oryzae*. Negatively stained with phosphotungstic acid, pH 7.0. Scale bar = 100 nm.

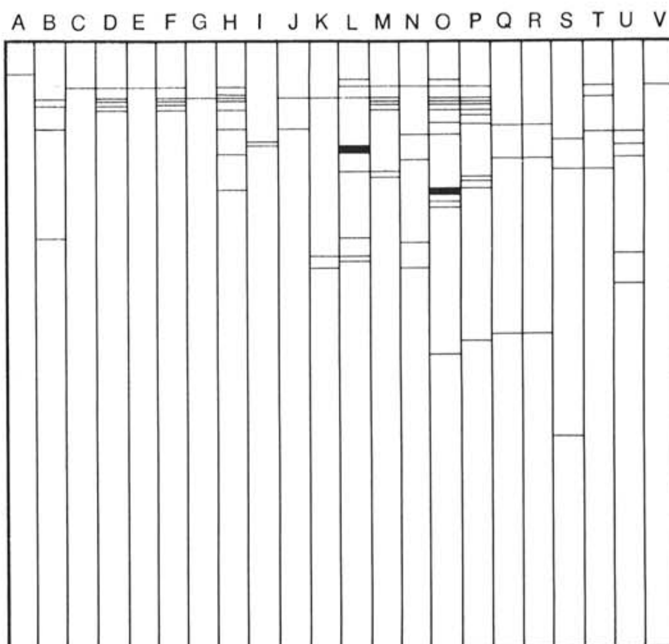

Fig. 2. Graphic summary of the dsRNA electrophoretic profiles detected in isolates of *Pyricularia oryzae*. The lanes show dsRNA profiles: A = tobacco mosaic virus (molecular weight standard), B = brome mosaic virus (molecular weight standard), C = 453 (IB-33), D = 455 (IA-65), E = 455 aberrant (IA-65), F = 495 (IA-65), G = 479 (ID-13), H = 648 (IA-65), I = 649 (ID-1), J = 747 (ID-13), K = 793 (IB-49), L = 820 (IF-1), M = 825-D6 (IB-1), N = 825-D6-Ram (IA-1), O = 888 (IA-65), P = 923 (IA-109), Q = 1932 (IC-17), R = 1932-lysed (IC-17), S = 603 (IH-1), T = 633 (ID-15), U = 1931-Tsukuba (IE-3), and V = 468 (IG-2).

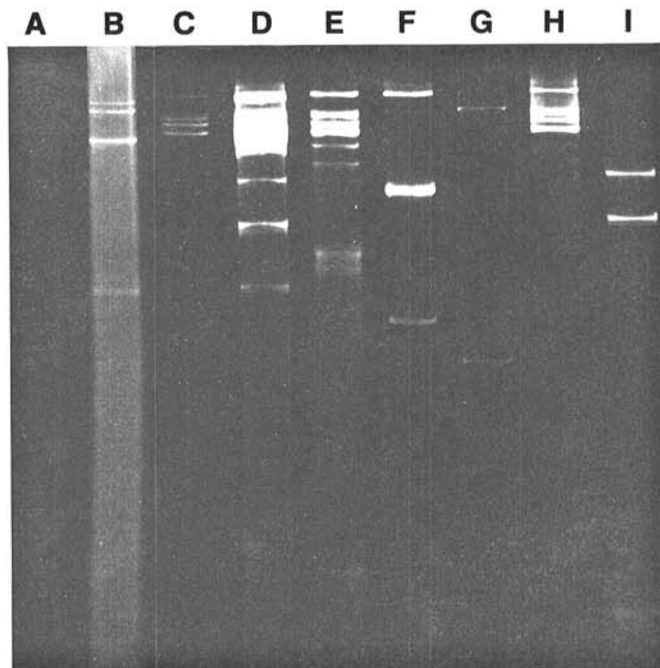

Fig. 3. Electrophoretic analysis of dsRNA extracted from mycelia of isolates of *Pyricularia oryzae*. The dsRNAs were separated on 5% T/2.7% C polyacrylamide slab gels, under nondenaturing conditions, at 60V for 18 hr. After treatment with RNase A, the gels were stained with ethidium bromide. The lanes contain dsRNA profiles: A = tobacco mosaic virus, B = brome mosaic virus, C = 455 (IA-65), D = 648 (IA-65), E = 888 (IA-65), F = 820 (IF-1), G = 793 (IB-49), H = 495 (IA-65), and I = 603 (IH-1).

were stained with ethidium bromide (10 ng/ml) and photographed with Polaroid Type 55 film using a 23A red filter and an ultraviolet light source (302 nm). Tests that were negative for dsRNA content were repeated at least twice.

The dsRNAs of tobacco mosaic virus (mol wt 4.1×10^6) and brome mosaic virus (mol wt 2.2, 2.0, 1.5, and 0.6×10^6), isolated from infected plant tissue, were used as molecular weight standards (8).

End-labeling and dot-blot hybridization analysis. The dsRNA extracted from isolate 455 (race IA-65) was end-labeled with [γ^{32}P] ATP according to a modified procedure of Maniatis et al (15). After DNase treatment and ethanol precipitation, the dsRNA was hydrolyzed in deionized formamide by heating in a boiling water bath for 15 min, quick-cooled on ice, and precipitated with two volumes of ethanol and 1/10 volume of 2 M sodium acetate, pH 6.0. The precipitate was collected by centrifugation at 9,980 *g* for 4 min, air-dried, and resuspended in sterile distilled water. Each 50-μl reaction mixture for end-labeling contained 50 mM Tris-Cl (pH 7.6), 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM EDTA, 10 units of $T4_1$ polynucleotide kinase, 100 μCi [γ^{32}P] ATP, and 3–4 μg of hydrolyzed dsRNA. After incubation at 37 C for 30 min, the reaction was terminated by adding 2 μl of 500 mM EDTA, pH 8.0. The labeled dsRNA was separated from unincorporated [γ^{32}P] ATP by chromatography through a column of Sephadex G-50 (Sigma Chemical Co., St. Louis, MO).

Aminothiophenol (APT) paper was made according to the procedure of Masters et al (16). The dsRNA extracted from the *P. oryzae* cultures, which had previously been used in electrophoresis, was applied to the activated APT paper in 2-μl aliquots and allowed to air-dry. The dot blots were then prehybridized in a solution of 5× SSCP (0.9 M NaCl, 0.09 M sodium citrate, 50 mM sodium phosphate, pH 7.0) containing 50% formamide and 125 μg denatured calf thymus DNA at 42 C for 30 min. Before hybridization, the dsRNA of end-labeled isolate 455 (IA-65) was denatured by heating at 100 C for 5 min. The blots were then hybridized with the probe at 42 C for 24 hr. After hybridization, the blots were washed successively in 5× SSCP, 0.1% SDS; 2× SSCP, 0.1% SDS; and 0.1× SSCP, 0.1% SDS at 42 C, air-dried, and autoradiographed at −80 C using Kodak SB-5 film and Dupont Cronex Lightening Plus intensifying screens for 24 hr.

RESULTS

dsRNA extracted from *P. oryzae* isolates. Among the 29 isolates examined, representing 21 physiologic races, 20 isolates contained dsRNA. Fifteen distinct dsRNA electrophoretic patterns were detected from these 20 positive isolates. Virus particles about 35 nm in diameter were purified from two race group 3 isolates (455 and 793) (Fig. 1), which had distinctly different dsRNA patterns. The nucleic acids within the particles were found to be dsRNA and comigrated with dsRNA isolated from the respective mycelia. Mycelial extracts showed no dsRNA bands that were not present in preparations from the particles.

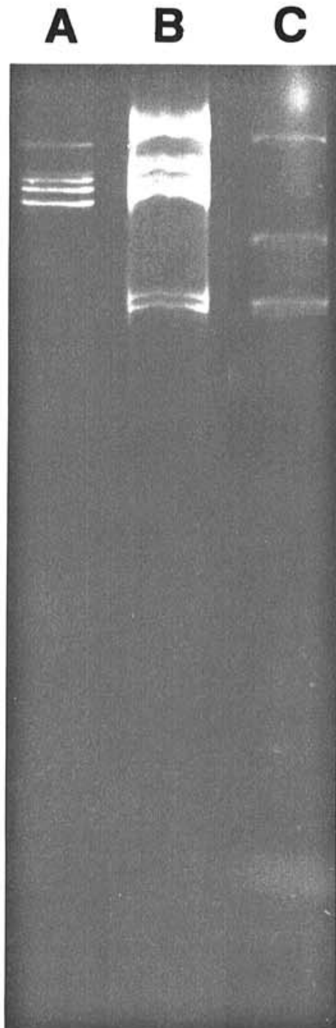

Fig. 4. Electrophoretic analysis of the dsRNA extracted from mycelia of isolates of *Pyricularia oryzae*. The dsRNAs were separated on 5% T/2.7% C nondenaturing polyacrylamide slab gels for 18 hr at 60V and stained with ethidium bromide after treatment with RNase A: A = 455 (IA-65), B = 825-D6 (IB-1), and C = 825-D6-Ram (IA-1), a cultural variant of 825-D6 (IB-1).

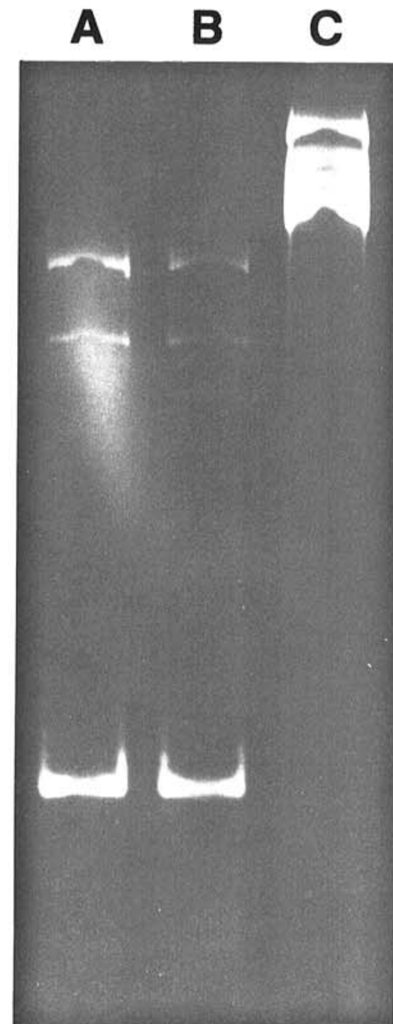

Fig. 5. Electrophoretic profiles of dsRNA extracted from mycelia of isolates of *Pyricularia oryzae*: A = 1932, a normal culture of race IC-17, B = a "lysed" variant (1932 lys), and C = 495 (IA-65). The 5% T/2.7% C polyacrylamide gel was run for 18 hr at 60V at room temperature.

Initial results were consistent with the hypothesis that the viruses of these isolates might influence their pathogenic specialization. Two isolates of race IA-65 (455 and 495) from different geographical sources appeared to contain dsRNA of identical number and molecular weight (Figs. 2 and 3). Some isolates of different races that were unrelated pathogenically had quite different dsRNA bands; e.g., 603 (IH-1) and 468 (IG-2) (Fig. 2). Isolate 825-D6 (IB-1) and a variant of this isolate, 825-D6-Ram (IA-1), differentiated by its pathogenicity to rice cultivar Raminad-3, possessed very different dsRNAs (Fig. 4). This suggested that the presence of different dsRNAs within two related isolates might influence their pathogenicity. In other instances, the presence of dsRNA appeared to be unrelated to pathogenicity of the fungus. Isolate 1932 of IC-17 from Texas possessed dsRNA, but 580, an isolate of the same race from Japan, had none. Here we should note, however, that these racial identities are based only on the differential reactions of eight cultivars of rice. We know that these cultivars do not contain all the available resistance genes and that tests using additional selected cultivars might reveal that these two isolates do represent different races. We tested only one isolate that showed abnormal growth habit; this "lysed" variant (1932 lys) and its normal parent (1932) (received from M. A. Marchetti, USDA, Beaumont, TX) contained identical dsRNA patterns (Fig. 5). Thus at least in this instance, the presence of dsRNA appeared to be unrelated to deterioration of cultural growth habit.

Certain dsRNA bands in common were observed among isolates from race group 3. Isolates 455, 495, 453, 923, 648, and 888 all had one or more dsRNA bands with a common mobility (Figs. 2 and 3). Because all the isolates except 453 possess multiple dsRNA bands, the presence of common bands suggests that the isolates may be infected with more than one virus or viruslike agent, which is consistent with previous data for other fungi (7,9). A more extensive testing of isolates within the race groups may yield more information on the relationships between the dsRNAs found in the isolates.

Conidial transmission. Among 16 single-conidial isolates of 455 (race IA-65), 14 showed dsRNA banding identical to that of the parent isolate; one had no dsRNA, and one ("aberrant 455") contained only one dsRNA of the parent. All 16 isolates showed the same pathogenic specialization on differential rice cultivars as the original isolate.

Hybridization analyses. Dot-blot analyses were made between nine isolates of race group 3 and two isolates of race group 2 using labeled dsRNA of isolate 455 of race group 3 (Fig. 6). The dsRNA of isolate 455 hybridized with the dsRNA of six of nine isolates in race group 3 and one of the two isolates in race group 2. Several isolates had dsRNA bands of the same molecular weight as those in 455 as shown by electrophoretic analysis; however, not all of these isolates hybridized to the 455 probe. This may be preliminary evidence for the existence of different viruses. The hybridization results indicate some sequence homology between the dsRNAs of the isolates. However, the intensity of hybridization in these tests was not quantitative because only the presence and not the amounts of dsRNAs used on the blots were verified by dsRNA electrophoresis.

DISCUSSION

In previous studies on viruses in *P. oryzae* (1,2,6,10,20,21), no information concerning the viral nucleic acids or the distribution of virus among identified physiologic races of diverse origins has been reported. The results of our study showed that viruses were prevalent among *P. oryzae* isolates. By analyzing a number of isolates from many sources, we were able to show the heterogeneity of viruses (dsRNA) in *P. oryzae*. Only one of the isolates tested showed unusual cultural characteristics. The one "lysed" culture we tested had the same dsRNA as its normal parent. This confirms previous reports that the presence of mycoviruses is not necessarily associated with atypical growth of fungal cultures (7,9,18).

From two of the isolates, 455 and 793, spherical particles 35 nm in diameter were purified and found to contain dsRNAs of the same molecular weight as the dsRNAs extracted from the respective mycelia. The differences in the dsRNAs within the particles from the two isolates, as shown by their electrophoretic patterns, would indicate that the viruses are distinct, albeit morphologically similar. The dot-blot hybridization analyses supported this concept, because the 455 dsRNA probe did not hybridize to the 793 dsRNA. Until particles can be purified from such isolates as 888 and 923, which had numerous dsRNAs, it is not possible to know how many morphologically similar viruses might be present.

Transmission of dsRNA through conidia occurred in 15 of 16 single-conidial isolates of culture 455. The partial transmission in one isolate (one dsRNA band of the five normally found) suggests that the dsRNAs may be individually encapsidated or that there is a multiple-virus infection. Such complexes are common among mycoviruses (3). Also, because virus transmission through conidia was not 100%, it is possible to obtain a virus-free isolate from an infected culture. This would be advantageous for transmission, biochemical, and pathogenicity studies.

Difficulties in obtaining highly purified preparations of mycoviruses have presented problems in establishing serological relationships (7). Even when antisera have been prepared to mycoviruses, they have been found to contain antibodies to host constituents (3). This has hindered studies using ELISA (enzyme-linked immunosorbent assay) for establishing mycovirus relationships (3). In this study, hybridization analyses using ^{32}P-labeled dsRNA were found useful for elucidating rudimentary relationships among some of the dsRNAs extracted from *P. oryzae* isolates. Viruses isolated from race group 3 members hybridized with 455 (IA-65), a member of that race group, which may indicate a relationship among the viruses. Further hybridization studies are needed to clarify relationships among *P. oryzae* viruses. For the future, hybridization analyses will be a useful method for establishing relationships because dsRNA is easily purified from mycelia.

We found only one instance in which the presence of dsRNA may have affected the pathogenicity of two isolates. The cultural variant 825-D6-Ram differed in its host range and dsRNA content from its parent culture, 825-D6. Even though we could not make any further correlations between the presence of dsRNA and pathogenicity of *P. oryzae* isolates, this does not necessarily imply that the viruses are latent or avirulent. Viral symptomatology may be manifested on the molecular level rather than the macroscopic level. A greater awareness of the diversity of dsRNA within *P.*

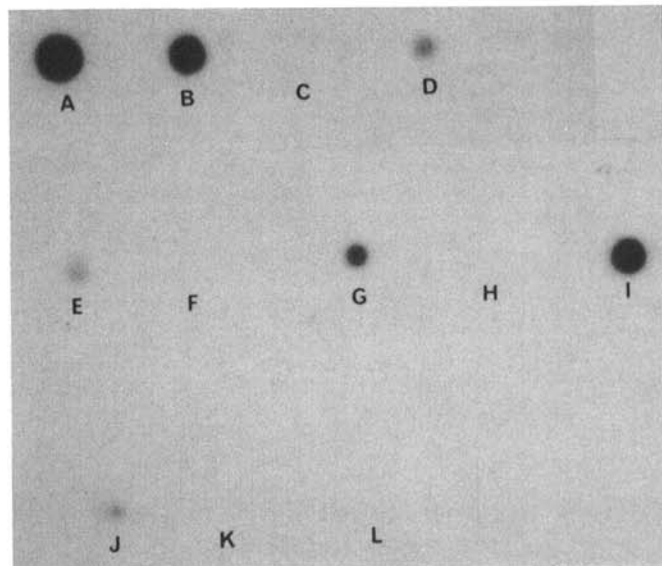

Fig. 6. Dot-blot hybridization analysis of dsRNA extracted from mycelia of isolates of *Pyricularia oryzae*. The hybridization probe was [γ^{32}P]-end-labeled dsRNA of 455 (IA-65): A = 455 (IA-65), B = 495 (IA-65), C = 453 (IB-33), D = 648 (IA-65), E = 888 (IA-65), F = 820 (IF-1), G = 479 (ID-13), H = 1931-Tsukuba (IE-3), I = 825-D6 (IB-1), J = 603 (IH-1), K = 793 (IB-49), and L = lambda phage DNA cut with the restriction endonuclease Hind III.

oryzae as presented in this study should be an important consideration in future molecular and genetic studies of this fungus.

LITERATURE CITED

1. Boissonnet-Menes, M., and Lecoq, H. 1976. Transmission de virus par fusion de protoplastes chez *Pyricularia oryzae* Briosi et Cav. Physiol. Veg. 14:251-257.
2. Bousquet, J.-F. 1973. Variation du taux de production de pyriculol dans les filtrates de culture de souches saines ou virosées de *Pyricularia oryzae* Cav. Ann. Phytopathol. 5:285-288.
3. Bozarth, R. F. 1979. Physiochemical properties of mycoviruses: An overview. Pages 48-61 in: Fungal Viruses. H. P. Molitoris, M. Hollings, and H. A. Wood, eds. Springer-Verlag, Berlin.
4. Dodds, J. A. 1980. Association of Type 1 virus-like dsRNA with club-shaped particles in hypovirulent strains of *Endothia parasitica*. Virology 107:1-12.
5. Dodds, J. A., and Bar-Joseph, M. 1983. Double-stranded RNA from plants infected with closteroviruses. Phytopathology 73:419-423.
6. Férault, A.-C., Spire, D., Rapilly, F., Bertrandy, J., Skajennikoff, M., and Bernaux, P. 1971. Observation de particules virales dans des souches de *Piricularia oryzae* Briosi et Cav. Ann. Phytopathol. 3:267-269.
7. Ghabrial, S. A. 1980. Effects of fungal viruses on their hosts. Annu. Rev. Phytopathol. 18:441-461.
8. Gildow, F. E., Ballinger, M. E., and Rochow, W. F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infection of oats. Phytopathology 73:1570-1572.
9. Hollings, M., and Stone, O. M. 1971. Viruses that infect fungi. Annu. Rev. Phytopathol. 9:93-118.
10. Lapierre, H. D. 1975. Virus-like particles in fungi. Pages 359-361 in: Proc. Intersectional Congr. Int. Assoc. Microbiol. Soc., 1st. Vol. 3. Infection and Antimicrobial Agents. T. Hasegawa, ed. Science Council of Japan, Tokyo.
11. Latterell, F. M., and Marchetti, M. A. 1968. Relationships among races of *Pyricularia oryzae*. (Abstr.) Phytopathology 58:1057.
12. Latterell, F. M., Tullis, E. C., and Collier, J. W. 1960. Physiologic races of *Pyricularia oryzae*. Plant Dis. Rep. 44:679-683.
13. Latterell, F. M., Tullis, E. C., Otten, R. T., and Gubernick, A. 1954. Physiologic races of *Piricularia oryzae*. (Abstr.) Phytopathology 44:495.
14. Lemke, P. A. 1975. Biochemical and biological aspects of fungal viruses. Pages 380-395 in: Proc. Intersectional Congr. Int. Assoc. Microbiol. Soc., 1st. Vol. 3. Infection and Antimicrobial Agents. T. Hasegawa, ed. Science Council of Japan, Tokyo.
15. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982 Labelling the 5′ ends of DNA with T4 polynucleotide kinase. Pages 122-123 in: Molecular Cloning—A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
16. Masters, B. S., McCarty, D. M., and Hauswirth, W. W. 1983. High-resolution gel and hybridization analysis of plant mitochondrial RNA. Plant Mol. Biol. Rep. 1:125-130.
17. Tuite, J. 1969. Plant Pathological Methods. Burgess Publishing, Minneapolis, MN.
18. Wood, H. A. 1973. Viruses with double-stranded RNA genomes. J. Gen. Virol. 20:51-85.
19. Yamashita, S., Doi, Y., and Yora, K. 1970. A polyhedral virus found in rice blast fungus, *Pyricularia oryzae* Cavara. (Abstr.) Ann. Phytopathol. Soc. Jpn. 36:372-373.
20. Yamashita, S., Doi, Y., and Yora, K. 1971. A polyhedral virus found in rice blast fungus, *Pyricularia oryzae* Cavara. Ann. Phytopathol. Soc. Jpn. 37:356-359.
21. Yamashita, S., Doi, Y., and Yora, K. 1975. Electron microscopic study of several fungal viruses. Pages 340-350 in: Proc. Intersectional Congr. Int. Assoc. Microbiol. Soc., 1st. Vol. 3. Infection and Antimicrobial Agents. T. Hasegawa, ed. Science Council of Japan, Tokyo.