Isozymic Variation in *Puccinia graminis* f. sp. *tritici* Detected by Starch-Gel Electrophoresis

J. J. BURDON, Senior Research Scientist, Division of Plant Industry, CSIRO, G.P.O. Box 1600, Canberra, A.C.T. 2601, Australia, and A. P. ROELFS, Senior Plant Pathologist, Cereal Rust Laboratory, Agricultural Research Service, USDA, University of Minnesota, St. Paul, MN 55108

ABSTRACT

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A survey of electrophoretic variation in 65 isolates of *Puccinia graminis* f. sp. *tritici* (the causal organism of wheat stem rust) collected in 13 countries detected 13 loci in 10 enzyme systems. Five loci were monomorphic, whereas two, three, or four alleles were detected at the remaining loci. Most alleles were widely distributed. These results are integrated with those of earlier extensive studies of this pathogen in Australia and North America to provide an overall picture of electrophoretic variation in *P. g.* f. sp. *tritici.*

Studies of the population genetics of cereal rust fungi have been hampered by the limited and special nature of the available genetic markers. Urediniospore color mutants are few in number and, because of the major selective disadvantage associated with light-colored mutants, rare in natural populations. Virulence markers are also selectively highly labile, with pathogen populations responding rapidly to changes in the frequency of resistance genes in agricultural cultivars (7). Recently, starch-gel electrophoretic techniques have been used to uncover a third set of genetically controlled markers (3,4). To date, starch-gel electrophoretic data are available only for populations of *P. g.* f. sp. *tritici* occurring in Australia, North America, and South Africa. Virtually nothing is known about variation in these characters elsewhere in the world, although characters like pathogenicity show similar levels of variation to those found in Australia and North America.

If electrophoretic markers are to be used more extensively in ecopathological studies (e.g., the movement of spores from one crop area to another) or in detailed genetic studies of the pathogen genome, a more complete picture of the extent of variation occurring in these characters is needed. In this paper, we present data concerning electrophoretic variation among 65 isolates of *P. g.* f. sp. *tritici* collected in 13 countries around the world. These results are integrated with previously published information (2,4–6) to provide a catalog of all identified isozyme alleles and their known regional distribution.

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MATERIALS AND METHODS

Origins of collections. The 65 samples examined in this study were collected over the past 10 yr in the following regions: South America—Bolivia, four isolates; Brazil, six isolates; Ecuador, seven isolates; Chile, seven isolates; Europe—France, one isolate; Spain, one isolate; Northern Africa—Morocco, eight isolates; Eastern Africa—Ethiopia, 16 isolates; Kenya, three isolates; Asia Minor—Syria, two isolates; Turkey, three isolates; and Indian subcontinent—India, five isolates; Pakistan, two isolates. These isolates are currently stored in liquid nitrogen at the Cereal Rust Laboratory, University of Minnesota. The isolates were collected by a variety of workers and do not necessarily represent a random sample or the commonest pathogenic races in the country of origin.

Sample preparation and analysis. Before electrophoretic analysis, the purity and racial identity of each isolate was checked by inoculation to a suitable set of differential wheat hosts (8). Urediniospores of each isolate were then collected, dried, and stored at 4 C.

Starch-gel electrophoresis was carried out on enzyme extracts obtained from homogenized, germinated spores. Crude extract was absorbed on paper chromatography wicks, which were inserted in single sample slots in a horizontal starch-gel prepared with 11% hydrolyzed starch. Electrophoresis was then carried out using one of three standard buffer systems. After electrophoresis, each gel was cut horizontally into three slices, and the anodal portion was assayed for aconitate hydratase (ACO, EC 4.2.1.3), arylesterase (EST, EC 3.1.1.2), catalase (CAT, EC 1.11.1.6), glutamate dehydrogenase (GDH, EC 1.4.1.2), glutamate oxalate transaminase (GOT, EC 2.6.1.1), leucine aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), NADH diaphorase (NADHD, EC 1.6.4.3), phosphoglucoisomerase (PGI, EC 5.3.1.9), and phosphoglucomutase (PGM, EC 2.7.5.1). The buffer systems and staining procedures have been described in detail previously (1,2,4–6).

Each isolate of *P. g.* f. sp. *tritici* was subjected to electrophoretic analysis on at least two separate occasions, whereas two to four North American isolates were used routinely as markers of particular isozyme phenotypes. In no case was any intraisolate variation detected.

RESULTS AND DISCUSSION

Number of loci and alleles detected. A total of 13 loci were detected in the 10 enzyme systems assayed. Single loci were found for ACO, CAT, GDH, GOT, LAP, MDH, and NADHD, whereas two loci were detected using the EST, PGI, and PGM enzyme assays. Multiple loci in any one enzyme system are numbered sequentially starting with the most anodal zone of activity. The number of alleles detected varied considerably between isozyme loci (Table 1). All alleles were labeled alphabetically in order of decreasing electrophoretic mobility, "a" being the most anodal. This labeling system incorporated alleles previously detected in Australian and North American populations of *P. g.* f. sp. *tritici* in their correct sequential position (Table 1).

For all 65 samples assessed, only one allele was detected at the *Aco*, *Est*1, *Gdh*, *Mdh*, and *Pgi*1 loci. In contrast, four alleles were found for *Lap*, *Pgm*1, and *Got*. When data for Australia and North America were included (2,4–6), the locus with the most alleles was *Got*, where five alleles were identified. Averaged over all geographic regions the mean number of alleles per locus was 2.31. Three alleles that have not previously been detected were found in this survey (*Cat* a, *Got* e, and *Nadhd* a). All three were detected in only a few isolates (two, one, and three, respectively) from Asia Minor, Europe, or South America (Table 1).

Pathogenic identities (Cereal Rust Laboratory race designations [8]) and storage code numbers of representative cultures of *P. g.* f. sp. *tritici* possessing at least one copy of the particular isozyme allele designated are as follows:

ACO: *Aco* a TNMK 74.21.1409A
CAT: *Cat* a TTMT 81.SA.BZ.SA
Cat b TNMK 74.21.1409A
EST: *Est*1 a TNMK 74.21.1409A
*Est*2 a TNMK 74.21.1409A
*Est*2 b GBCS 76.45.585B
GDH: *Gdh* a TNMK 74.21.1409A
GOT: *Got* a LCCN 75.10.1744C
Got b 34-4,7(57096)
Got c TNMK 74.21.1409A
Got d QCMJ 75.45.1378B
Got e RKCT 1983
LAP: *Lap* a QDCS 75.45.1376A
Lap b TNMK 74.21.1409A
Lap c HDLQ 80.39.512C
Lap d TNMK 74.21.1409A
MDH: *Mdh* a TNMK 74.21.1409A
NADHD: *Nadhd* a TNMK 74.21.1409A
Nadhd b TNMK 74.21.1409A
PGI: *Pgi*1 a TNMK 74.21.1409A
*Pgi*2 a TNMK 74.21.1409A
*Pgi*2 b TNMK 74.21.1409A
PGM: *Pgm*1 a BCCS 76.45.1214A
*Pgm*1 b LCCN 75.10.1744C
*Pgm*1 c RCCN 75.45.1650C
*Pgm*1 d QBMN 75.45.1811C
*Pgm*2 a QBCS 75.45.1766C
*Pgm*2 b RCCN 75.45.1650C
*Pgm*2 c RCCN 75.45.1650C
*Pgm*2 d BCCS 76.45.1214A

All cultures are stored in liquid nitrogen at the Cereal Rust Laboratory, St. Paul, MN, except *Got* b, which is stored at the Plant Breeding Institute, Castle Hill, Australia.

Geographic distribution of loci. The small number of cultures screened from many of the different parts of the world and the nonrandom nature of the original sampling prevents the use of statistical techniques and makes comparisons among regions difficult. The mean number of alleles per locus showed little variation among the East African, North African, European, South American, Indian, North American (asexual), and Asia Minor regions (range 1.50–1.85, mean 1.68; Table 1). The mean number (1.31) detected in the existing populations in the Australian and South African regions was lower than this range and higher in the North American sexual population (2.08). In the former regions, the pathogen population reproduces asexually only, and for long periods of time, only one multilocus isozyme phenotype is present (4). This contrasts with the asexual population occurring in North America, where nine multilocus isozyme phenotypes have been identified (5). The high level of allelic diversity of the North American sexual population was undoubtedly due in part to extensive sampling of this population (157 isolates examined [6]).

Although most alleles were widely distributed (for example, *Got* c, *Lap* a and c, *Pgm*2 b and c), a few appear to have much more restricted distributions (for example, *Got* b and e, *Pgm*2 a and d). Indeed, the *Got* e allele has been found only in a single isolate of *P. g.* f. sp. *tritici* collected in Turkey, whereas the *Got* b allele is known only from a single pathogen isolate collected in Australia in 1957 (2). In regions where geographically restricted alleles did occur, they were generally very rare.

The widespread distribution of most of the isozyme alleles detected reduces the possibility that variation in individual electrophoretic markers alone may provide an indisputable means of tracking the intercontinental dispersal of *P. g.* f. sp. *tritici*. However, it is possible that individual multilocus combinations may be distinctive of particular regions and will be sufficient to identify immigrant races. This may particularly be the case where such races enter pathogen populations that do not undergo periodic sexual recombination. Furthermore, when combined with simultaneous consideration of the pathogenicity of isolates from different regions, they may contribute substantially to an assessment of such movement. Even among geographically highly dispersed alleles, marked differences may occur between adjacent populations. Such alleles could be used in measurements of the extent of interaction between populations.

The data presented here provide a comprehensive catalog of the allelic variation currently known to occur in *P. g.* f. sp. *tritici*. These genetic markers could be used to good effect in population genetic studies of this pathogen in many parts of the world.

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Table 1. Geographic distribution of isozyme alleles in *Puccinia graminis* f. sp. *tritici*

Enzyme locus	Alleles (no.)	Geographic occurrence of alleles									
										N. America[v]	
		Australia[u]	S. Africa[u]	E. Africa	N. Africa	Indian subcont.	Asia Minor	Europe	S. America	Asexual	Sexual
Cat	2	b	b	b	b	b	b	b	a,b	b	b
Aco	1	a	a	a	a	a	a	a	a	a	a
*Est*1	1	a	a	a	a	a	a	a	a	a	a
*Est*2	2	a	a	a,b	a,b	a,b	a	a,b	a,b	a	a,b
Gdh	1	a	a	a	a	a	a	a	a	a	a
Got	5	a,[w]b,[w]c	c	a,c	a,c	a,c,d	c,d,e	a,c,d	c,d	a,c,d	a,c,d
Lap	4	a,b,[w]c	a,c	a,b,c,d	a,b,c,d	a,b,c,d	a,b,c	a,b,c	a,b,c,d	b,c,d	a,b,c,d
Mdh	1	a	a	a	a	a	a	a	a	a	a
Nadhd	3	b,c	b,c	b,c	b,c	b,c	b,c	a,b	a,b,c	b,c	b,c
*Pgi*1	1	a	a	a	a	a	a	a	a	a	a
*Pgi*2	2	a,b	a,b	a,b	a,b	a,b	b	b	a,b	a,b	a,b
*Pgm*1	4	a,b,c	c	c,d	a,b	...[x]	...	...	b,d	a,b,c	a,b,c,d
*Pgm*2	4	b,c	b,c	b,c	b,c	b,c	b,c	b,c	b,c	b,c	a,b,c,d
Total no. samples		98	2	19	8	7	5	2	24	110	174
Mean no. alleles/locus		1.31(1.69)[y]	1.31	1.69	1.69	1.75[z]	1.50[z]	1.58[z]	1.85	1.60	2.08

[u] Data derived from Burdon et al (2,4).
[v] Data derived from Burdon and Roelfs (5,6).
[w] These alleles occurred in this geographic region before 1960, not extant now.
[x] Not assessed.
[y] Including all alleles.
[z] Mean of 12 loci only.

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