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Isozyme Studies on the Origin and Evolution of *Puccinia graminis* f. sp. *tritici* in Australia

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Abstract

Isozyme phenotypes for eight enzyme systems were used to assess the origins and evolution of *P. graminis* f. sp. *tritici* (the wheat stem rust pathogen) in Australia. The results obtained by this approach agreed with pathways postulated on the basis of virulence studies, confirming the suggestion that most of the major changes in the wheat stem rust pathogen flora of Australia have resulted from overseas introductions. Moreover, they suggest that, although the more recent of these were from Africa, the first major change detected occurred as a result of an introduction from elsewhere.

Introduction

Australia offers unique opportunities for studies in the variation and evolution of the wheat stem rust pathogen, *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.. Barberry bushes (*Berberis vulgaris* L.), on which the sexual stage occurs, are extremely rare and the region is isolated from other wheat-growing regions of the world. On the other hand, the occasional arrival of new rust types provides variability additional to that occurring through mutation. It has also been suggested that somatic hybridization between two different pathotypes of *P. graminis tritici* was responsible for the production of an important strain (Luig and Watson 1977).

On the basis of pathogenicity patterns produced on standard and supplementary differentials and of resistant infection types produced on hosts carrying single resistance genes, the Australian wheat stem rust pathogen flora can be logically subdivided into a number of major and minor groupings. The most important divisions are those between the pathotypes existing before 1925, those predominating between 1925 and 1954 (standard race 126) and those occurring after 1954 (standard races 21, 34, 326). Within the post-1954 grouping, however, a further three subdivisions have been recognized (Luig 1977; Luig and Watson 1977; Watson 1981). The pathotypes in these different groups or subgroups are postulated to have originated either through introduction from overseas (particularly Africa) or through somatic hybridization of strains already occurring in Australia. It is believed that most of the strains recorded in Australia since 1925 have descended from these few original pathotypes, standard races 126, 21, 34 and 326, by mutation at loci governing pathogenicity (Luig 1977; Watson 1981).

The development of procedures for the electrophoretic analysis of soluble proteins in rust pathogens (Burdon and Marshall 1981) provides an additional set of genetic

markers to check these postulated origins and evolutionary pathways within the Australian stem rust pathogen flora. Such a study is possible because samples of newly detected pathotypes of *P. graminis tritici* are stored in liquid nitrogen as part of the Australian wheat rust virulence survey. Isolates of pre-1925 pathotypes no longer exist in this historical collection. However, representatives of all other groups are available and some of these were subjected to electrophoretic analysis. In addition, four isolates derived from the wheat stem rust pathogen flora of North America and two from Africa were included for comparison.

Materials and Methods

Forty-one Australian, four North American and two African isolates of *P. graminis tritici* were grown separately on *Triticum aestivum* L. Urediniospores of each isolate were collected, dried and then stored at 4°C or in liquid nitrogen until required.

Rapid and synchronous germination of urediniospores was obtained through a modification of the method detailed by Maheshwari and Sussman (1971). 50 mg of urediniospores were suspended in 30 ml of a calcium phosphate–potassium phosphate buffer, pH 7·0 (Maheshwari *et al.* 1967), containing 10^{-4} M nonyl alcohol and 0·01% Tween (polyoxyethylene sorbitan monolaurate). Self-inhibitors of germination were removed by vigorous agitation for 5 min on a reciprocal shaker (150 strokes per minute) in diffuse light at room temperature. The suspension was then centrifuged (2000 rpm for 2 min), the supernatant discarded, and the spores resuspended in another 30 ml of buffer solution. This suspension was then shaken for 80–120 min at room temperature after which virtually all spores had germinated. The suspension was again centrifuged, the supernatant discarded and the remaining spores resuspended in a few drops of 0·05 M phosphate buffer, pH 7·0, containing 1 mg ml^{-1} dithiothreitol. This germinating-spore slurry was then frozen in liquid nitrogen until required.

Electrophoretic Analysis

The frozen germinated spores were allowed to thaw, ground with a mortar and pestle and the crude extract absorbed on paper chromatography wicks (6 by 4 mm). These were inserted in a single sample slot in a horizontal starch gel, each sample being duplicated, and electrophoresis was then carried out in one continuous (histidine, pH 8·0) and two discontinuous (borate, pH 7·8, and citrate, pH 8·0) systems. Details of these systems may be found in Broué *et al.* (1977), Moran and Marshall (1978) and Brown *et al.* (1978). In the continuous system, electrophoresis was conducted for 5 h, and in the discontinuous systems it was allowed to proceed until the borate or citrate front had migrated 9 cm from the sample slot. Each gel was then cut horizontally into three slices and both the anodal and cathodal portions of the gel were assayed for the following range of enzymes: arylesterase (EST, EC 3.1.1.2), aminopeptidase (cytosol) (synonym leucine amino peptidase; LAP, EC 3.4.11.1) and dihydrolipoamide reductase (synonym NADH diaphorase; NADHD, EC 1.6.4.3)—assayed on citrate gels; aspartate aminotransferase (synonym glutamate oxalate transaminase; GOT, EC 2.6.1.1) and glucosephosphate isomerase (synonym phosphoglucoisomerase; PGI, EC 5.3.1.9)—assayed on borate gels; glutamate dehydrogenase (GDH, EC 1.4.1.2), malate dehydrogenase (MDH, EC 1.1.1.37) and phosphoglucomutase (PGM, EC 2.7.5.1)—assayed on histidine gels. The staining procedures were similar to those described by Brewer and Sing (1970), Brown *et al.* (1978) and Burdon *et al.* (1980).

Results

GDH and MDH enzyme phenotypes were invariant for all isolates examined. The results obtained with the other isozymes studied varied between groups and consequently they are presented seriatim using the Stakman system of classification of standard races (Stakman *et al.* 1962).

Australian Isolates

1925–1954

Standard race 126. Both pathotypes (see Appendix) showed identical isozyme patterns for the eight enzyme systems examined (Fig. 1).

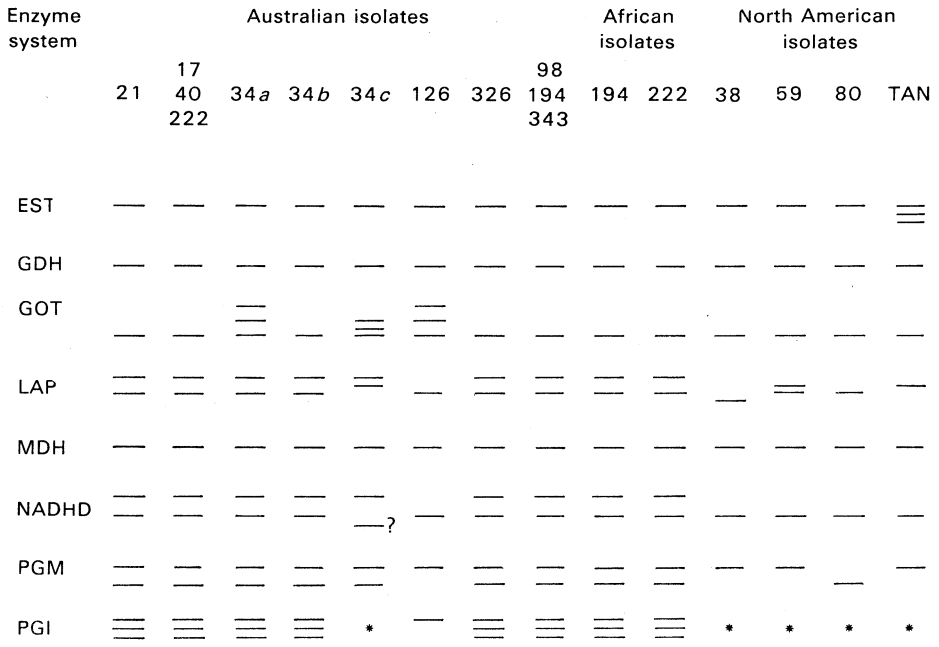

Fig. 1. Isozyme phenotypes for Australian isolates of the Stakman races representing the major types of *P. graminis tritici* present in Australia from 1925 to 1980. Two African and four North American isolates are included for comparison. * Not determined.

Post-1954

Standard race 21. Sixteen isolates, representing thirteen pathotypes, were examined (see Appendix). All isolates possessed the same isozyme patterns (Fig. 1). However, this pattern differed from that of standard race 126 in five different systems (GOT, LAP, NADHD, PGI and PGM).

Standard races 17, 40 and 222 (postulated derivatives of race 21; Watson 1981). The five pathotypes of these races (see Appendix) possessed exactly the same isozyme phenotype as standard race 21.

Standard race 34. Thirteen isolates representing nine different pathotypes were examined (see Appendix) and three different isozyme pattern groupings were observed (Fig. 1). These groups were:

(a) Ten isolates (34-2,4,5,6,7,11; 34-2,5,11; 34-2,11; 34-5,11; two 34-1,2,7,11; four 34-2,4,5,7,11) all with an isozyme phenotype the same as that of standard race 21 except for the enzyme GOT, which resembled that of standard race 126.

(b) Two isolates (34-1,2,3,7 and 34-2,3,7,11) with isozyme phenotypes indistinguishable from that of standard race 21.

(c) One isolate (34-4,7) with a distinctive isozyme phenotype for the enzymes GOT and LAP and possibly NADHD.

Standard race 326. The only available isolate of this race possessed an isozyme phenotype identical to that of standard race 21.

Standard races 98 and 343 (postulated derivatives of race 326; Watson 1981) and standard race 194, a close relative. The four isolates of these races (see Appendix) possessed exactly the same isozyme phenotype as standard races 21 and 326 (Fig. 1).

African Isolates

Two African isolates (standard races 194 and 222) of *P. graminis tritici* were examined (see Appendix). These isolates had exactly the same isozyme phenotypes as the Australian standard race 21 isolates (Fig. 1).

North American Isolates

Four North American isolates (standard races 38, 59, 80 and TAN) of *P. graminis tritici* were examined (see Appendix). These isolates showed a diversity of isozyme phenotypes, which are illustrated in Fig. 1.

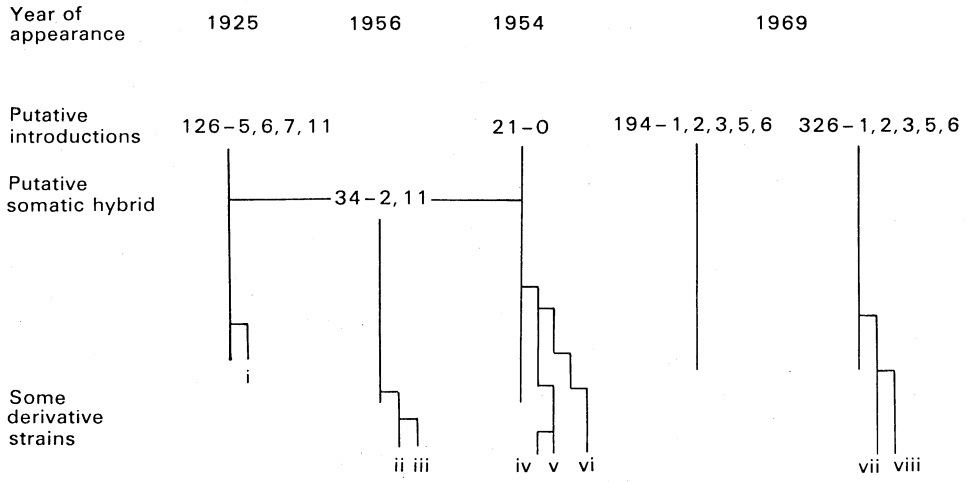

Fig. 2. Diagrammatic representation of the possible origins and evolution of selected strains of *P. graminis tritici* in Australia from 1920 to 1980 (based on virulence studies). Only some of the derivative strains used for isozyme analysis are shown. (i) 126-1,5,6,7,11; (ii) 34-2,5,11; (iii) 34-2,4,5,11; (iv) 17-1,2; (v) 21-1,2; (vi) 34-1,2,3,7; (vii) 343-1,2,3,5,6; (viii) 98-1,2,3,5,6.

Discussion

The main postulated evolutionary pathways of the Australian wheat stem rust pathogen flora are illustrated in Fig. 2. Of the groupings illustrated, we determined the isozyme phenotypes of the pre-1954 pathotypes 126-5,6,7,11 and 126-1,5,6,7,11; those of the original pathotype of race 21 (21-0) first detected in 1954, and twenty of its putative descendant strains (other race 21 isolates, races 17, 40 and 222 and some of the race 34 isolates); the phenotype of a putative somatic hybrid (34-2,11) between race 21 and race 126, and five of its putative descendant pathotypes; and finally the phenotype of pathotype 326-1,2,3,5,6 first detected in 1969, and three of its putative descendant strains (98-1,2,3,5,6; 194-1,2,3,5,6 and 343-1,2,3,5,6).

Despite the profound differences in the post-1954 isolates, with respect to pathogenicity, year of occurrence and place of collection, no isozyme differences were detected between standard races 21, 194 and 326, and the differences between these groups and race 34 are, with the exception of the single isolate included in 34(*c*), restricted to a maximum of a single difference in the GOT enzyme system (Fig. 1). By contrast, the two pre-1954 pathotypes of standard race 126 have distinctly different isozyme phenotypes from the post-1954 isolates examined (Fig. 1). The lack of variation between the post-1954 pathotypes of race 21 and race 194 of 1969 is especially surprising since it is thought that these two groups were derived from two separate introductions from overseas some 15 years apart (Luig 1977; Watson 1981).

The main difference between 21-0 (of 1954) and 194-1,2,3,5,6 (of 1969) is their array of alleles for virulence and avirulence. Standard race 194 differs from standard race 21 by its avirulence for *Sr9g*, present in Acme and Kubanka. On the 11 Australian supplementary differentials, 194-1,2,3,5,6 is virulent for *Sr6* (for which the designation '-1' indicates virulence), *Sr11* (-2), *Sr9b* (-3), *Sr17* (-5) and *Sr8* (-6), whereas 21-0 is avirulent on each. In 1954, the pathotype 21-0 was new for the Australian–New Zealand geographical area and in 1969 the virulence combination '-1,2,3,5,6' was similarly novel, although by then many pathotypes of standard race 21 possessing *some* of these five virulences existed. The five corresponding host genes have been common in Australia cultivars for some time and may have exerted strong selection pressure on the pathogen during the last three decades.

Pathotypes 21-0 and 194-1,2,3,5,6 are avirulent for *Sr* genes *5*, *7a*, *9e*, *10*, *12*, *13*, *15*, *16*, *21*, *22*, *23*, *24*, *25*, *26*, *27*, *29*, *30*, *31*, *32*, *33*, Tt_1 and Tt_2; of which *7a*, *10*, *12*, *13*, *15*, *23* and Tt_1 are known to confer a range of avirulent responses to different pathogen cultures. However, only in the case of *Sr15* are the infection types produced by 21-0 and 194-1,2,3,5,6 different (Luig 1977): at average temperatures of 18°C, 21-0 gives infection type 'X' while 194-1,2,3,5,6 gives a more resistant ';1++3='. These results, together with the lack of isozyme variation between races 21-0 and 194-1,2,3,5,6, suggest that these two races have a common origin.

There has been some doubt as to the origin of standard race 21 in Australia. However, there is good evidence to support the hypothesis that standard race 194 arrived as wind-borne urediniospores from Africa. Thus, two isolates (194-1,2,3,5,6 and 222-1,2,3,5,6,11) from Angola have exactly the same isozyme phenotypes as those of the Australia pathotypes (98-1,2,3,5,6 194-1,2,3,5,6, 326-1,2,3,5,6 and 343-1,2,3,5,6) derived from, or the same as, an African introduction (Fig. 1). Also international virulence gene surveys (Luig, unpublished data) have shown the presence of standard race 194 forms in Mozambique resembling those found in Australia, and comparative studies of spore size, pathogenicity and growth in axenic culture (de Sousa 1975) have demonstrated that four rusts present in Australia (194-1,2,3,5,6, 194-1,2,3,5,6,7, 343-1,2,3,5,6 and 222-1,2,3,5,6) closely resemble races present in Angola and possibly Zimbabwe. Taken together, this provides very strong evidence for an African origin for the Australian forms of standard races 194, 326 and their derivatives. Moreover, the total isozyme congruence of race 21 with these African isolates strongly suggests that race 21 also originated from the African continent.

Although standard races 21 and 194 and their derivatives have been the most important types of *P. graminis tritici* present in Australia since 1954, from 1957 until the early 1970s another smaller but parallel evolutionary development occurred after the appearance in 1957 of pathotype 34-2,11. After extensive studies, Luig and

Watson (1977) suggested that this pathotype originated as a somatic hybrid between standard races 21 and 126. In Fig. 1, the isolates of race 34 examined in the present study have been divided into three homogeneous groups on the basis of their isozyme phenotypes. In the first of these groups containing nine isolates (34*a*, Fig. 1), the isozyme phenotypes resemble those of race 21 for all enzymes except GOT which is similar to that of race 126. It is clear that this phenotype is consistent with the hypothesis of somatic hybrid origin for race 34-2,11, and, in fact, provides a valuable marker for identifying races of this type, independent of pathogenicity. Of the remaining two groups of standard race 34, the isolates in group *b* have exactly the same isozyme phenotypes as those of race 21 and appear to have arisen through a simple process of spontaneous mutation at loci governing pathogenicity. (A change from standard race 21 to standard race 34 requires only a single mutational step *viz*, virulence for *Sr5*. This host gene is present in several Australian cultivars.) One of these pathotypes, 34-2,3,7,11 (76131), is of particular interest as it represents the only standard race 34 variant examined that possesses virulence for differential No. 11 that was not derived via the somatic hybridization pathway. Similarly, all isolates of standard race 21 carrying this virulence gene apparently acquired it through spontaneous mutation. According to these isozyme data, there are no examples where a race 34 pathotype derived from the somatic hybridization pathway has mutated to give rise to a standard race 21 pathotype. This is not surprising as a change from race 34 to race 21 represents a loss of virulence and would consequently require a gain of function. Moreover, *Sr5* is present in many modern Australian cultivars, hence encouraging pathotypes with virulence for this gene (race 34 types). The final subgroup [34*c*, Fig. 1] is represented by a single isolate, 34-4,7 (57096), which was collected on the cultivar Bencubbin at Tamworth in 1957. Since that time it has never been re-isolated. On the basis of pathogenicity characteristics involving genes for resistance derived from *Secale cereale*, Luig (1978) suggested that this isolate was not a true strain of *P. graminis tritici* but, rather, had its origin in somatic hybridization between *P. graminis tritici* and *P. graminis secalis*. However, 34-4,7 possesses distinctive isozyme phenotypes for both GOT and LAP not found in Australian isolates of *P. graminis secalis* (Burdon *et al.* 1982) nor, for that matter, in other Australian forms of *P. graminis tritici*. This isolate cannot then be a simple somatic hybrid of any two known Australian forms of *P. graminis tritici* or *P. graminis secalis*. It seems possible, therefore, that it was an accidental introduction from overseas that failed to establish itself.

In Fig. 1, the isozyme phenotypes of four North American isolates of *P. graminis tritici* are included for comparative purposes. In no case is there complete correspondence of phenotype between these and any Australian race. The greatest similarities are between races 21 and 59 and between races 126 and 38, 59 and 80. However, when race 126 (the most distinctively different Australian race) is compared with both Australian race 21 and with this group of North American races, we find that the latter group contains bands different from those in race 126 on average in 1·25 out of 7 enzyme systems and race 21 differs in a similar way from race 126 in four out of eight systems. Because of this similarity, it is tempting to suggest that race 126 derived originally from North America. However, for this to be the case, it is necessary to assume that this pathotype was among the commonest pathotypes in North America around the time it first appeared in Australia. This was not so, nor

has a race resembling it pathogenically ever been found there (I. A. Watson, personal communication). Moreover, this pathotype was first recorded in Australia in the Perth district of Western Australia. This tends to suggest an origin to the north or west rather than to the east.

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Appendix

List of P. graminis *f. sp.* tritici *Isolates Examined*

All material was supplied by the Plant Breeding Institute, University of Sydney, except those isolates marked with an asterisk, which were supplied by the Queensland Wheat Research Institute, Toowoomba, Queensland. (The first two numbers for Sydney University cultures indicate the year of isolation.)

The North American isolates are all laboratory strains chosen for their arrays of genes for avirulence and are not necessarily representative of field populations in North America. TAN is an untyped isolate characterized by colour only.

Australian Isolates

Single isolates of:

17-0	71-L-1
17-1,2	66643
17-1,2,3,7	64404
21-0	54129
21-1,2	62352
21-2,3,4,5,7	68016
21-2,3,7	751354
21-2,3,7,11	73404
21-2,3,7+Sr13	76170
21-2,4,5,7	SS467A*
21-2,5	73405
21-4,5	631088
21-7,9	71178
34-1,2,3,7	761201
34-2,3,7,11	76131
34-2,4,5,6,7,11	72611
34-2,5,11	73323
34-2,11	73560
34-4,7	57096
34-5,11	67363
40-2,4,5,11	68237
126-1,5,6,7,11	7316
126-5,6,7,11	334
194-1,2,3,5,6	S837*

Australian Isolates (contd)

222-2,3,7,8,9	S717
326-1,2,3,5,6	69822
343-1,2,3,5,6	S1205*

Multiple isolates of:

21-1,2,3,7,8,9	731422 and S881*
21-2,4,5	67034 and S511*
21-5	S212B* and S553*
34-1,2,7,11	621038 and 621042
34-2,4,5,7,11	64231; 76785; S212* and S479*
98-1,2,3,5,6	781219 and S1581*

African Isolates
(collected before 1975)

Single isolates of:

194-1,2,3,5,6	Angola 2
222-1,2,3,5,6,11	Angola 4

North American Isolates
(collected before 1960)

Single isolates of:

38
59
80
TAN

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