ALLOZYME UNIFORMITY BETWEEN RACES OF SUNFLOWER RUST PUCCINIA HELIANTHI SCHW.

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Summary

In Australia, cultivated sunflowers, Helianthus annuus, have been subject to infection by the pathogen Puccinia helianthi. Rust race 1 was present in 1970 and rust race 3 was identified in 1983 (Kochman and Goulter 1984). Studies of the genetic variation in sunflower rust have been predominantly concerned with the virulence genes. An alternative approach to characterize the races of rust involves gel electrophoresis of soluble proteins, especially isozymes. The degree of polymorphism of 21 enzymic loci was tested for the two races of rust. Rust spores were germinated overnight on a monolayer of water. After the spores were ground, the extract was absorbed onto paper chromatography wicks and electrophoresis was carried out on 12% starch gels in a histidine continuous system and in two discontinuous systems, lithium borate and tris citrate pH 8.0 buffers.

It has been possible to detect the following enzymes in Puccinia helianthi: acid phosphatase, b-aconitase, NADH diaphorase, (leucine) aminopeptidase, esterase, aspartate aminotransferase, glucose-6-phosphate dehydrogenase, phosphoglucoisomerase, phosphoglucomutase, peptidase, catalase, indophenoloxidase, and hexokinase. The following enzymes have remained undetected in Puccinia helianthi:- alcohol dehydrogenase, malate dehydrogenease, endopeptidase, lactic dehydrogenase, and shikimate dehydrogenase.

All isozyme phenotypes for the sixteen enzymes detected were invariant for races 1 and 3 of Puccinia helianthi. To date the monomorphic nature of the enzymes in rust race 1 and rust race 3 is distinct from the variation in the virulence of the two physiologic races of rust of sunflower.

Introduction

In Australia cultivated sunflowers Helianthus annuus have been subject to infection by the pathogen Puccinia helianthi, which although identified in 1887 (McAlpine 1906), did not become a problem until an increase in oilseed production after 1970. The rust prevalent in crops in 1979 was race 1, and rust race 3 was identified in commercial crops in 1983 (Kochman and Goulter 1984). Rust can lead to substantial loss of yield and therefore is a disease of considerable economic importance.

Resistance to sunflower rust in the host was shown to be under the genetic control of two dominant genes, R_1 and R_2 (Putt and Sackston 1963; Sackston 1962). These resistance genes were identified in wild annual sunflowers in Renner, Texas, where the R_1 gene came from the source 953-102 and the R_2 gene came from the source 953-88. The R_1 gene has been widely used in Australia since 1972 and controls resistance to races 1 and 2 of rust but the host remains susceptible to races 3 and 4. The occurrence of the R_2 gene in commercial hybrids has not been described however one hybrid (Hysun 33) confers resistance to race 1 and race 3 rust.

Studies of the genetic variation in sunflower rust have been predominantly concerned with the virulence genes. All four rust races were shown to be heterozygous in selfing studies when tested on the Canadian differential hosts

(Jabbar Miah and Sackston 1970). These authors concluded that the basis of genetic control of pathogenicity appeared to vary with the host variety tested and appeared to be influenced by an interaction of chromosomal and nonchromosomal factors. Thus to date the basis of the genetic control of pathogenicity remains undefined.

Rusts have been characterized by one or more of the following features: spore colour, pathogenicity, spore viability, host range, temperature tolerance, urediospore size, shape and echinulation and incubation period (Brown 1940).

An alternative approach to characterise the races of rust involves gel electrophoresis of soluble proteins, especially isozymes. Isozyme markers could be useful to determine genetic variation in soluble enzymes within the Puccinia helianthi species. In this study enzymes present in Puccinia helianthi were determined and the degree of polymorphism at twenty-one enzyme loci was tested for the two races of rust.

Materials and Methods

Puccinia helianthi race 1 and race 3 were grown on the set of sunflower differential lines S37388, 69-17-8-1-1, 29-3-1-3-2-1, and Polestar in separate glasshouses. Approximately 50 mg of urediospores of each race was dispersed on a monolayer of distilled water and allowed to germinate overnight. The germinated spores were collected and ground with a mortar and pestle in 0.5 cm^3 of 0.05 M phosphate buffer pH 7.0 containing 1 mg ml^{-1} dithiothreitol. The crude extract was absorbed on paper chromatography wicks (6mm x 5 mm). Wicks were inserted in slots in a horizontal 12% starch gel, each sample in duplicate, and electrophoresis was carried out in one continuous (histidine buffer pH 8.0) and two discontinuous (lithium borate variant buffer pH 8.2 and tris citrate buffer pH 8.0) systems. Details of these systems are given in Broue et al. (1977), Moran and Marshall (1978) and Brown et al. (1978). In the continuous system, electrophoresis was conducted for 5 hours and in the discontinuous systems electrophoresis was carried out until the borate or citrate fronts had migrated 10 cm from the sample slot. Each gel was then cut horizontally into three slices. The anodal portion of the gel was assayed for the following range of enzymes: acid phosphatase (ACP E.C. 3.1.3.2), beta-aconitase (ACO E.C. 4.2.1.3), alcohol dehydrogenase (ADH E.C. 1.1.1.1), alkaline phosphatase (PAL E.C. 3.1.3.1), aminopeptidase (cytosol) (leucine amino peptidase LAP E.C. 3.4.11.1), arylesterase (EST E.C. 3.1.1.2), aspartate aminotransferase (glutamate oxaloacetate transferase GOT E.C. 2.6.1.1), catalase (CAT E.C. 1.11.1.6), endopeptidase (ENDO E.C. 3.4.22.9), b galactosidase (b GAL E.C. 3.2.1.23), glucose phosphate isomerase (phospho glucoisomerase PGI E.C. 5.3.1.9), glutamate dehydrogenase (GDH E.C. 1.4.1.2), hexokinase (HEXO E.C. 2.7.1.1), indophenoloxidase (IPO), lactic dehydrogenase (LDH E.C. 1.1.1.27), malic enzyme (MAL E.C. 1.1.1.40), malate dehydrogenase (MDH E.C. 1.1.1.37), NADH diaphorase (NADHD E.C. 1.6.4.3), peptidase (PEP), phosphoglucomutase (PGM E.C. 2.7.5.1), and shikimate dehydrogenase (SDH E.C. 1.1.1.1.25). The staining procedures were similar to those described by Brewer and Sing (1970) and Brown et al. (1978) and Burdon et al. (1980).

Results

It was possible to detect the following 16 different enzyme systems in Puccinia helianthi:- Acid phosphatase, beta-aconitase, NADH diaphorase, alkaline phosphatase, aminopeptidase, esterase, aspartate amino transferase, glucose 6-phosphate dehydrogenase, phosphoglucoisomerase, phosphoglucomutase, peptidase, b galactosidase, malic enzyme, catalase, indophenoloxidase, and hexokinase.

Table 1 shows the number of loci scored for each enzyme system, the buffer system in which the enzyme was detected, the position in the gel, and the Rf values of each of the bands considered. In Puccinia helianthi all loci were homozygous for the two races of rust. All isozyme phenotypes for the 16 enzymes detected were invariant for races 1 and 3 of Puccinia helianthi. To date the monomorphic nature of the enzymes in rust race 1 and rust race 3 contrasts with the variation in the virulence of the two physiologic races of rust of sunflower.

Discussion

In Puccinia helianthi differences between temperature of germination of urediospores have been determined for races 1 and 3 compared with races 2 and 4 (Sood and Sackston 1972) and suggests that races may differ in some of their enzymes. The availability of starch gel electrophoresis provides an approach to identify variability in the enzyme systems of the two identified races of Puccinia helianthi in Australia; race 1 and race 3. These reference sources of rust were isozymically identical.

Other Puccinia species have demonstrated isozyme uniformity in Australia. No variation was detected within 58 isolates of Puccinia graminis tritici (wheat stem rust) representing seven pathotypes and 55 isolates of Puccinia recondita tritici (wheat leaf rust) representing six pathotypes collected in the field (Burdon et al. 1983). Similarly two races of Puccinia graminis tritici in Czechoslovakia were invariant for six enzyme systems studied (Macko et al 1967).

Relative to the isozymes of the host Helianthus annuus the corresponding isozymes in Puccinia helianthi are slower migrating forms in most enzyme systems examined. However aminopeptidase, LAP, and aspartate aminotransferase, GOT, isozymes of rust have increased Rf values compared with the sunflower isozymes (Dry 1985a).

The lack of isozyme variation between the two races of rust present in Australia may be explained by the common origin of the two existing races of rust. Race 3 may have developed from race 1 by mutation in the virulence genes since only race 1 was detected in the early 1970's Commercial hybrids were introduced in 1972 exhibiting resistance to the existing race of rust but this resistance has since been overcome by race 3 of the pathogen. There is now a wide distribution of both race 1 and race 3 throughout sunflower growing regions of Eastern Australia (Dry unpublished data). Alternatively race 3 may be an introduction to Australia and the isozymes, being selectively neutral markers, were identical to the isozymes of race 1.

Isozyme variability has been reported in a Puccinia species, in the wheat stem rust population of the Pacific North West USA (Roelfs and Groth 1980). In wheat stem rust populations (Puccinia graminis f.sp. tritici) in Australia the sexual stage of the life cycle has apparently never occurred (Watson 1981). Watson found that the virulence structure of the population changes greatly when a new pathogen race is introduced and a high level of diversity in virulence is maintained through mutation at loci governing virulence. However for selectively neutral isozyme markers in asexual populations the isozyme phenotype remains uniform (Burdon et al. 1983). In contrast in North America where the sexual cycle was important until the eradication of common barberry in the late 1920's and an asexual population now occurs, moderate diversity in both isozyme and virulence characters possibly reflects clonal lines descended from biotypes prevalent in the pathogen population in the late 1920's. Additional diversity in virulence may be derived from mutation within the clonal lines at loci

controlling virulence (Roelfs and Groth 1980). In the sexual population of the Pacific North West, USA (Roelfs and Groth 1980) there is extensive diversity in virulence and isozyme phenotypes and no association was detected either between individual isozyme alleles or between isozyme and virulence phenotypes (Burdon and Roelfs 1985).

The extent of the sexual cycle in Puccinia helianthi in Australia is not well documented. A large number of isolates of rust from commercial crops and wild populations were collected in the Eastern region of Australia in 1984. Considerable virulence variation was found (Dry, unpublished data). If sexual recombination does maintain variability within pathogen populations, as suggested in studies of the wheat stem rust populations (Burdon and Roelfs 1985) and this applies to Puccinia helianthi, it will be useful to determine whether isozyme variation exists among these isolates. If however low levels of heterozygosity within individual enzyme systems prevail, a common origin hypothesis might then explain the isozyme uniformity.

Conclusions

Sunflower rust (Puccinia helianthi) race 1 and race 3 show allozyme uniformity.

Acknowledgements

I would like to thank Mrs E. Lewis for technical assistance, Dr J. Burdon for useful discussions and Dr R. Downes for comments on the manuscript.

Table 1. Isozyme system on starch gel electrophoresis in sunflower rust Puccinia helianthi

Symbol	E.C. Code	Enzyme	Gel System and Position	No. of Loci	R_f Value
ACO	4.2.1.3	β-aconitase	His, T	1	0.10
ACP	3.1.3.2	acid phosphatase	TC pH 8.0, B	1	0.02
NADHD	1.6.4.3	NADH diaphorase	TC pH 8.0, M	2	0.32
PAL	3.1.3.1	alkaline phosphatase	TC pH 8.0, T		0.02
LAP	3.4.11.1	amino peptidase	TC pH 6.5, M	1	0.72
EST	3.1.1.2	esterase	TC pH 8.0, M	1	0.63
GOT	2.6.1.1	aspartate amino-transferase	Li V, B	2	0.42
G6PD		glucose-6-phosphate dehydrogenase	His, B	1	0.55
PGI	5.3.1.9	phosphoglucoisomerase	Li V, T	2	0.40
PGM	2.7.5.1	phosphoglucomutase	His, B	1	0.08
PEP		peptidase	TC pH 8.0, B	1	0.51
GAL	3.2.1.23	β-galactosidase	TC pH 6.5, M	1	0.04
MAL	1.1.1.40	malic enzyme	His, B	1	0.06
CAT	1.11.1.6	catalase	TC pH 8.0, B	1	
IPO		indolphenoloxidase	Li V, T	1	0.70
HEXO	2.7.1.1	hexokinase	Li V, M	1	

TC - tris citrate pH 8.0; Li V - lithium borate variant; His - histidine; T - top; M - medium; B - bottom.

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