ISOZYME VARIATION BETWEEN HELIANTHUS ANNUUS RUST DIFFERENTIAL SUNFLOWER LINES

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Summary

An electrophoretic analysis of variation in soluble enzymes between Helianthus annuus rust differential sunflower lines was completed to provide an isozyme standard for the set of rust differential sunflower lines. Soluble proteins were extracted from seeds of S37388, S37388RR (R_1 gene), Polestar, Canada Morden 69 (R_1 gene) Canada Morden 29 (R_2 gene), 953-102 (R_1 gene) and 953-88 (R_2 gene) lines and were subjected to 12% starch gel electrophoresis in histidine, tris citrate and lithium borate variant buffer systems.

Isozyme banding patterns were obtained for 13 enzyme systems and the variation between 15 loci coded for by H. annuus in seven sunflower rust differential lines was assessed. Eight enzyme loci were invariant-alcohol dehydrogenase locus 1, Adh_1^S/Adh_1^S, amino peptidase locus 1, Lap_1^2/Lap_1^2, aspartate aminotransferase, Got^F/Got^F, glutamate dehydrogenase, Gdh^F/Gdh^F, fumarase, Fum^S/Fum^S, malic enzyme, Mal^S/Mal^S, phosphoglucoisomerase, Pgi^3/Pgi^3, and phosphoglucomutase, Pgm^S/Pgm^S.

For seven enzyme loci variation between rust differential sunflower lines was observed. The common genotypes found among the differential lines were for aminopeptidase locus 2, Lap_2^2/Lap_2^2, esterase, Est^3/Est^3, ß-aconitase, Aco^S/Aco^S, acid phosphatase, Acp^4/Acp^4, alkaline phosphatase, Pal^4/Pal^4, alcohol dehydrogenase locus 2, Adh_2^S/Adh_2^S and glucose-6-phosphate dehydrogenase, $G6PD^S/G6PD^S$.

Using the nine variable enzyme loci, for each differential line there is a unique genotype. This isozyme differentiation enables identification of each differential sunflower line. S37388 can be identified by its faster esterase allele, Est^2, and faster glucose-6-phosphate dehydrogenase allele, $G6PD^F$, 953-88 by the fast acid phosphatase allele, Acp^1, Polestar by ß-aconitase phenotype, Aco^{F+}/Aco^S, faster Lap_2 allele and fastest Est^1 allele, Canada Morden 69 by the phenotype Aco^{F+},Aco^S, Canada Morden 29 by any of the distinctive allele Aco^F, present in homozygous form and S37388RR with variation at the Lap_2, G6PD and Aco loci with phenotype Aco^{F+}, Aco^S and genotypes Lap_2^3/Lap_2^3 and $G6PD^F/G6PD^F$.

A standard set of sunflower differential lines with known isozyme markers will be a useful reference for maintenance of genetic purity among differential sunflower lines, for studies of host-pathogen interactions and in sunflower breeding.

Introduction

To help identify races of sunflower rust (Puccinia helianthi) Sackston (1962) developed differential lines of sunflower. Two dominant genes, R1, from the source 953-102 and gene R2 from the source 953-88 were identified from wild annual sunflowers H. annuus in Renner, Texas (Sackston 1962, Putt and Sackston 1963). These resistance genes were transferred to develop - Canada Morden (Cross) 69 (CM 69) containing the R1 resistance gene, and Canada Morden (Cross) 29 (CM 29) containing the R2 gene.

The rust susceptible inbred differential line S37-388 and the open-pollinated line Polestar are also used as susceptible positive controls. The R1 gene was also introduced into the S37-388 line forming the derivative S37-388RR. Together this set of rust differential lines S37-388, 69-17-8-1-1 (R1 gene), 29-3-1-3-2-1 (R2 gene), and S37-388RR (R1 gene) has been used to determine whether Puccinia helianthi isolates are representatives of races 1, 2, 3 or 4 of rust by the virulence pattern of reaction on the differential host plants (Sackston, 1962).

Seeds of all differential lines were imported from Canada and were subject to the usual plant quarantine process on arrival in Australia. It is therefore desirable that the purity of the differentials be checked by use of an independent marker.

Gel electrophoresis of soluble proteins, particularly isozymes provides an excellent method to identify genetic markers of these differential sunflower lines. Since the biochemical tags are a direct reflection of the genetic code any isozyme differences among differential sunflower lines serve to identify them.

Since isozymes are unaffected by changes in the environment the set of isozyme patterns for each sunflower differential line should constitute a useful reference for maintaining standards of genetic purity in each differential sunflower line.

This study provides an electrophoretic description of soluble enzymes in differential sunflower lines of Helianthus annuus. It thus provides an isozyme standard for the set of differential sunflower lines.

Materials and Methods

Seed of seven sunflower differential lines: S37388, S37388RR (R1 gene), 69-17-8-1-1 (R1 gene), 29-3-1-3-2-1 (R2 gene), Polestar, 953-88-3-1-54 and 953-102-1-1-41 and a test line of suspect purity were spiked and placed on filter paper moistened with distilled water for 20 hours in the dark. The pericarp was removed and the seed contents were squashed using a perspex rod. 0.5 cm^3 of 0.05 M phosphate buffer pH 7.0 containing 1 mg ml^{-1} dithiothreitol were added and the extract was absorbed on paper chromatography wicks (6mm x 5 mm). The 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 Broué 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 and 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), fumarase (fumarate hydratase FUM E.C. 4.2.1.2), glucose phosphate isomerase (phospho glucoisomerase PGI E.C. 5.3.1.9), glucose-6-phosphate dehydrogenase (G6PD), glutamate dehydrogenase (GDH E.C. 1.4.1.2), malic enzyme (MAL E.C. 1.1.1.40), phosphoglucomutase (PGM E.C. 2.7.5.1), and shikimate dehydrogenase (SDH E.C. 1.1.1.25.). The staining procedures were similar to those described by Brewer and Sing (1970), Brown et al. (1978) and Burdon et al. (1980).

Fourteen enzyme systems were selected from a survey of 24 systems for the simplicity of the banding patterns and for the formation of bands of high resolution for one or more loci. To score isozyme phenotypes the locus specifying the allele with the fastest anodal migration was designated 1, the next 2, and so on. Alleles at each locus were designated either F (fast), I (intermediate), S (slow) or 1 (fastest), 2, 3, 4.

Results

Optimum enzyme staining was achieved using the buffer-enzyme combinations and position in the gel indicated in Table 1. Table 1 shows the number of loci scored for each enzyme, the Rf value (relative position of the fast band to the front) obtained for the isozyme and the nature of the heterozygote formed in any enzyme system.

Considerable isozyme variation was encountered in the genotypes at the fourteen loci assayed in the Helianthus annuus rust differential lines. A diagrammatic representation of all of the major isozyme bands detected in the fourteen different enzyme systems examined is presented in Figure 1 with the bands obtained for each enzyme system notated as scored.

Eight enzyme loci were invariant for seven differential lines examined. The differential lines were homozygous for the alleles Adh_1^S, Lap_1^2, Got^F, Gdh^F, Fum^S, Mal^F, Pgi^3, Pgm^S and Sdh^S. For seven enzyme loci variation between differential sunflower lines was observed. The common genotypes found among the differential lines were homozygous Lap_2^2/Lap_2^2, Est^3/Est^3, Aco^S/Aco^S, Acp^4/Acp^4, Pal^4/Pal^4, Adh_2^S/Adh_2^S, and $G6PD^{S2}/G6PD^{S2}$.

In Table 2 the isozyme genotype of each of the sunflower differential lines is indicated. The original sources of the rust resistance genes, sunflower lines 953-102 and 953-88 showed a distinct acid phosphatase allele Acp^1 compared with the slower migrating allele Acp^4 of the other lines. Similar banding patterns occurred in the alkaline phosphatase system. Whereas most lines have genotype Lap_2^2/Lap_2^2 S37388RR has genotype Lap_2^3/Lap_2^3 and Polestar carries the Lap_2^1 allele in some seeds.

The variants in the esterase enzyme system were S37388 with a faster anodal migrating band 2, and Polestar with the fastest band 1 present in homozygous form. Some heterozygous Est^1/Est^3 seeds of Polestar have also been detected.

The predominant ß-aconitase genotype was Aco^S/Aco^S but CM 29 is of Aco^F/Aco^F genotype and CM 69 and Polestar have the phenotype Aco^{F+}, Aco^S, F+ being a very fast form of the isozyme acting as a marker since the band stained is more narrow than the F and S bands. The variants in the glucose-6-phosphate dehydrogenase system were S37388 and S37388RR lines with a faster anodal

Table 1. Isozyme Systems on Starch Gel Electrophoresis in Helianthus annuus

Symbol	E.C. Code	Enzyme	Gel System and Position	Loci Scored	Rf Value	Heterozygote (bands)
ACP	3.1.3.2	acid phosphatase	TC pH 8.0, T	1	0.48	3 + conformer
ACO	4.2.1.3	β-aconitase	His, T	1	0.47	2
ADH	1.1.1.1	alcohol dehydrogenase	TC pH 8.0, T	2	0.55	3 + intergenic
PAL	3.1.3.1	alkaline phosphatase	TC pH 8.0, M	1	0.40	3 + conformer
LAP	3.4.11.1	aminopeptidase	TC pH 8.0, B	2	0.64	2
EST	3.1.1.2	arylesterase	TC pH 8.0, M	1	0.82	2 + 2 conformers
GOT	2.6.1.1	aspartate aminotransferase	LiV, B	1	0.28	3
FUM	4.2.1.2	fumarase	LiV, M	1	0.28	2
PGI	5.3.1.9	glucose phosphate isomerase	LiV, T	1	0.66	3
G6PD		glucose-6-phosphate dehydrogenase	His, B.	1	0.44	3
GDH	1.4.1.2	glutamate dehydrogenase	TC pH 8.0, M	1	0.18	5
MAL	1.1.1.40	malic enzyme	TC pH 8.0, B	1	0.33	2
PGM	2.7.5.1	phosphoglucomutase	His, M	1	0.60	2 + 2 conformers
SDH	1.1.1.25	shikimate dehydrogenase	His, B	1	0.53	2

migrating band F compared with the slow G6PD band of the other lines. Shikimate dehydrogenase was the least reliable enzyme staining system scored but when a satisfactory level of staining occurred results were reproducible.

When the genotype for each sunflower differential line for the seven variable loci was considered a unique genotype was revealed for each line. This isozyme differentiation enables identification of each differential sunflower line.

In addition a line of suspect purity was assessed for its isozyme genotype. For the variable loci it contained Lap_2^3/Lap_2^3, Est^1/Est^3 and Aco^{F+}/Aco^S. The test line had been labelled as S37388RR yet it showed susceptible reaction of 4 to Race 1 rust. The isozyme pattern was distinct from S37388RR by the presence of Est^1 allelle found only in the Polestar differential line with Est^1/Est^1 genotype. Thus the test line was not a differential by both virulence or isozyme studies.

Discussion

An analysis of the isozymes of the rust differential sunflower lines shows them to have considerable variation among them. They thus provide biochemical genetic markers to identify each of the differential lines.

The universal suscept S37388 can be identified by its faster esterase allele Est^2 and the fast form of glucose-6-phosphate dehydrogenase $G6PD^F$ and lines 953-102 and 953-88 by the fast form of acid phosphatase, genotype Acp^1/Acp^1. Canada Morden 69 is distinguished by the very fast Aco^{F+} marker in the ß aconitase system as well as the Aco^S allele probably in homozygous form.

The Puccinia helianthi race 1 and race 3 resistant differential line, Canada Morden 29 showed homozygous fast alleles in the ß-aconitase enzyme system giving it the distinctive genotype of Aco^F/Aco^F, unlike other differential sunflower lines.

S37388RR was the only line with the slower Lap_2 allele Lap_2^3, and the fast G6PD allele, $G6PD^F$, both present in homozygous form. The variation in the test line with 'S37388RR' label suggests the line may have been subjected to an extraneous gene introduction of the Est^1 allele.

The sunflower line with most variation compared to the other lines was Polestar. Polestar is distinguished by the fast form of the esterase alleles Est^1, the fast Lap_2^1 allele and F^+S phenotype in β-aconitase where F^+ is a marker rather than part of the Aco locus. Polestar is an open-pollinated bird seed cultivar developed in Russia whereas the differentials were developed in Canada so more variation in isozymes might be expected. The method of inheritance of the Aco^{F+}, Aco^S phenotype is unknown. It is proposed to determine whether the Aco^S allele is present in homozygous form and whether the F^+ marker segregates from Aco^S in self pollination of the CM69, S37388RR and Polestar lines or whether it represents an allele at another locus. Alternatively the Aco^{F+}, Aco^S phenotype may represent heterozygosity at the b-aconitase locus. If this is the case, even with inbreeding complete homozygosity has not been achieved at all loci. CM69 on selfing segregated for rust resistance to susceptibility in a 3:1 ratio (Jabbar Miah and Sackston 1970) and some progeny demonstrated resistance to race 3 and race 4 rust in addition to resistance to races 1 and 2 observed in the parent plant. This suggests a more complex situation than a single dominant gene R1 controlling resistance to race 1 and race 2 of rust. Together this variation in rust reaction suggests a non-homozygous state for the resistance gene as well as heterozygosity in isozyme alleles.

Figure 1. Bands formed by major isozyme alleles of Helianthus annuus

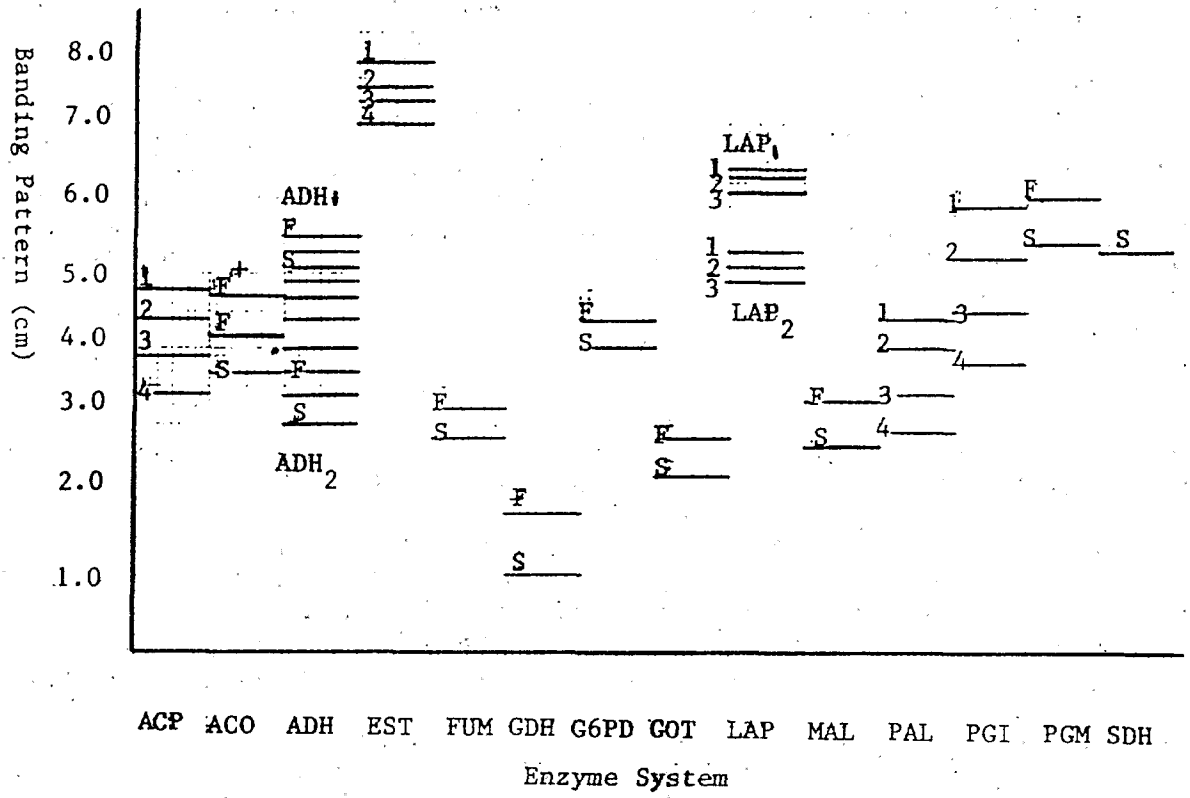

Table 2. Isozyme Phenotypes for Rust Differential Sunflower Lines (Helianthus Annuus).

Differential Line	Enzyme System ACP	PAL	LAP2	EST	ACO	G6PD
S37388	4,4	4,4	2,2	2,2	S_+S	F,F
69-17-8-1-1	4,4	4,4	2,2	3,3	F^+,S	S,S
29-3-1-3-2-1	4,4	4,4	2,2	3,3	F_+F	S,S
S37-388RR	4,4	4,4	3,3	3,3	F^+,S	F,F
953-88-3-1-54	1,1	1,1	2,2	3,3	S,S	S,S
953-102-1-1-41	1,1	1,1	2,2	3,3	S_+S	S,S
Polestar	4,4	4,4	1,2	1,1	F^+,S	S,S

Invariant Phenotypes

ADH_1-S,S	FUM-S,S	GDH-F,F	PGI-3,3	SDH-S,S
LAP_1-2,2	GOT-F,F	MAL-F,F	PGM-S,S	

The isozyme results for the differential sunflower lines were in contrast to the suggestion that very limited variation occurred among cultivated sunflower varieties based on the lack of variation found in the alcohol dehydrogenase and acid phosphatase enzyme systems (Torres 1983).

The isozyme variation between host rust differential lines, of Helianthus annuus contrasts to the isozyme uniformity observed in the obligate pathogens races 1 and 3 of Puccinia helianthi for which the differential lines were developed to detect by differential virulence patterns (Dry 1985b).

A simple explanation is not readily available to interpret the derivation of some of the differential lines based on their isozyme genotypes. Given the manipulation which has occurred to the differential lines imported from Canada it would be useful to confirm the isozyme patterns of differential sunflower lines at their source of origin. Given the Aco^F/Aco^F genotype of Canada Morden 29 which carries the R2 gene and that the 953-88 line was the source of R2 gene the absence of the Aco^F/Aco^F genotype in this line suggests the lack of association between resistance genes and isozyme markers. Alternatively genetic changes may have occurred in the 953-88 line since the host resistance behaviour of the 953-88 line available in Australia is not as definitive as the 29-3-1-3-2-1 differential line.

A standard set of sunflower differential lines with a known catalogue of isozyme markers should be a useful reference for maintenance of genetic purity among differential sunflower lines, for use in virulence studies, for studies of host pathogen interactions and in sunflower breeding.

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