ISOZYME NOMENCLATURE FOR EIGHT ENZYME SYSTEMS IN THREE BRASSICA SPECIES

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INTRODUCTION

From the early work by Nijenhuis (1971) until recent publications (Chen and Heneen, 1990; Mündges et al., 1990), isozyme markers have been increasingly applied in the genetics, systematics and breeding of the Crucifers. General criteria for the assignment of names to enzyme systems, regions of activity, isozymes and allozymes have been lacking, making it difficult in some cases the comparison of results produced by different authors.

In this paper we propose a general nomenclature for eight enzyme systems in the three diploid species of U's triangle: B. campestris, B. nigra and B. oleracea.

MATERIALS AND METHODS

Crude extracts of leaves were prepared with an extraction buffer containing Tris HCl 0.1 M pH 7.5 and 1% w/v glutathione. Gel/electrode buffers for starch gels were Histidine/Tris-citrate pH 7.0 (H 7.0); Morpholine-citrate pH 6.1 (MC 6.1) and Tris-citrate/lithium borate pH 8.3 (TC 8.3). These buffers can be found in Shields et al. (1983) as buffers E (H 7.0), G (MC 6.1) and C (TC 8.3). Polyacrylamide gels (10%, pH 8.9) were prepared as described by Arús and Orton (1983). Staining recipes for leucine aminopeptidase and aconitase are given by Arús and Orton (1983) and Wendel and Stuber (1984), respectively. The remaining six enzymes were stained as specified by Vallejos (1983)

TERMINOLOGY PROPOSED

Enzyme System

Enzyme names and abbreviations can be found in Table 1.

Region of Activity

Gels were divided into regions of activity generally numbered from most to least anodal (i.e., PGI-1 is more anodal than PGI-2). When possible each region was coincident with the zone of migration of the enzymes produced by a single gene. Exceptions were regions of intergenic heterodimers or pairs of duplicated loci when their products migrate to a narrow zone of the gel, both cases in dimeric enzymes.

Isozymes

Since each region of activity corresponds generally to the

zone of migration of an isozyme, the gene that encodes for this isozyme has a similar terminology (i.e., gene Pgi-2 produces the enzymes that migrate to the PGI-2 region). If more than one isozyme occurs in a given region of activity, a special notation has to be given in order to identify each gene (i.e., in region TPI-1, there are two duplicated isozyme genes: Tpi-1 and Tpi-1' from most to least anodal).

Homologous isozyme genes from different species can be distinguished from each other by adding the letter(s) characteristic of each genome (i.e., Pgi-2A, Pgi-2B and Pgi-2C correspond to the gene Pgi-2 of B. campestris, B. nigra and B. oleracea, respectively). This notation is unnecessary when working with only one species.

Allozymes

The following terminology is proposed: Numbers 1, 2 ... n, are given to the different allozymes of each isozyme for in the order of most to least anodal. New allozymes located between previously described ones can be notated as decimal fractions (i.e., 1.6 and 3.4 correspond to allozymes between 1 and 2 and between 3 and 4, respectively; allozyme 0.6 would migrate to a more anodal position than allozyme 1). This system allows the addition of a large number of allozymes to the existing ones, providing at the same time information of the position of a given allozyme relative to others.

Gel/Electrode Buffers

Since results obtained in different gel/electrode buffers may be difficult to compare, we have taken as standard for each enzyme system the buffer that provided the best resolution.

RESULTS

The most important characteristics of the enzymes studied are summarized in Tables 1 and 2. Specific observations not included in the Tables and some bibliographic references with descriptions of each enzyme in different species of the Crucifers can be found in the following paragraphs.

Acid Phosphatase

Only the most anodal region of activity produced by leaf extracts (APS-1L) is considered here. Activity in more cathodal zones of the gel and isozyme patterns of other tissues (seed) have been described elsewhere (Arús and Orton, 1983; Woods and Thurman, 1976). Additional references: Wills and Wiseman (1980), Arús and Orton (1983), Ellstrand and Devlin (1989).

Aconitase

Although evidence on the genetic basis of Aco-1, Aco-3 and Aco-4 was provided by Arús (1989) in B. oleracea, conclusions are still tentative in some cases due to overlapping between zones of activity or differences in staining intensity between different alleles. References: Ellstrand and Devlin (1989), Mündges et al. (1990).

Alcohol Dehydrogenase

In addition to the two regions of activity described in Table 2, a third intermediate zone, named ADH 1/2 was also found

containing the inter-genic heterodimers between the monomers encoded by Adh-1 and Adh-2. Adh-1 is active in seeds, but not in leaves, roots or pollen. Reference: Arús and Orton (1983).

Leucine Aminopeptidase

Activity for this enzyme is also present in regions cathodal to LAP-1. References: Arús and Orton (1983), Quiros et al. (1987, 1988), Ellstrand and Devlin (1989), Mündges et al. (1989, 1990), Chen and Heneen (1990).

6-Phosphogluconate Dehydrogenase

Two isozymes are included in each of the two regions of activity of the zymogram of this enzyme. Intergenic heterodimers are formed only between monomers of the enzymes located within the same region of activity. References: Quiros et al. (1987, 1988), Ellstrand and Devlin (1989), Chen and Heneen (1990).

Phosphoglucoisomerase

References: Arús and Orton (1983), Quiros et al. (1987, 1988), Mündges et al. (1988, 1989), Ellstrand and Devlin (1989), Chen and Heneen (1990).

Phosphoglucomutase

Three regions of activity: PGM-3, PGM-1 and PGM-2 from most to least anodal correspond to genes Pgm-3, Pgm-1 and Pgm-2 respectively. Pgm-1 and Pgm-2 were first described and genetically analyzed in seed extracts of B. oleracea (Arús and Orton, 1983). Pgm-3, which is present only in green tissues was studied later (Truco, 1986, Arús, 1989). This is the reason for the change in the usual order of activity region numeration. Additional references: Coulthart and Denford (1982), Quiros et al. (1987, 1988), Ellstrand and Devlin (1989), Chen and Heneen (1990).

Triosephosphate Isomerase

The distribution of regions of activity is analogous than in 6PGD, with two isozymes that form intergenic heterodimers in each of the two regions of activity. References: Quiros et al. (1987, 1988), Ellstrand and Devlin (1989).

DISCUSSION

A high level of resemblance has been found among the patterns of isozyme variation in the three species studied. The number of isozymes per enzyme system as well as the differential tissue expression of some enzymes (ADH and PGM) was identical in B. campestris, B. nigra and B. oleracea.

Although polymorphism was the rule (only one (Pgi-1) of the 21 genes studied was monomorphic in all species), important differences were observed among species in the level of polymorphism and position of the allozymes of each isozyme.

While a general and accurate description of regions of activity and isozymes can be done when using a specific method of electrophoresis, gel/electrode buffer and staining recipe, the situation is more complex when trying to characterize individual allozymes. Rf values are not sufficiently precise to unequivocally describe alleles that migrate to close positions.

The best approach would be to use as controls individuals of known isozyme phenotype. For that purpose it will be necessary to construct populations containing different sets of allozymes, easy to keep and reproduce and available to all researchers working on Brassica isozymes.

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Table 1. Main features of the enzyme systems studied in B. campestris, B. nigra and B. oleracea.

Enzyme system	E.C. number	Abbrev.	Quaternary structure	Standard gel/electrode buffer
Acid phosphatase	3.1.3.2	APS	dimeric(*)	Polyacrylamide 10%, pH 8.9
Aconitase	4.2.1.3	ACO	monomeric	H 7.0
Alcohol dehydrogenase	1.1.1.1	ADH	dimeric	H 7.0
Leucine aminopeptidase	3.4.11.1	LAP	monomeric	MC 6.1
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGD	dimeric	MC 6.1
Phosphoglucoisomerase	5.3.1.9	PGI	dimeric	H 7.0
Phosphoglucomutase	2.7.5.1	PGM	dimeric	H 7.0
Triosephosphate isomerase	5.3.1.1	TPI	dimeric	TC 8.3

(*) A monomeric APS was found in seed extracts by Woods and Thurman (1986)

Table 2. Regions of activity, isozyme genes and alleles for eight enzyme systems.

Enzyme	Region of activity	Isozyme gene	B. campestris	B. nigra	B. oleracea
APS	APS-1L	Aps-1L	5	3	5
ACO	ACO-1	Aco-1	2	4	3
	ACO-2	Aco-2	3	3+n(*)	2
	ACO-3	Aco-3	3	1	2
	ACO-4	Aco-4	3	3	2
ADH	ADH-1	Adh-1	4	3	3
	ADH-2	Adh-2	2	1	2
LAP	LAP-1	Lap-1	4	4	4
6PGD	6PGD-1	6Pgd-1	1	2	2
		6Pgd-1'	1	1	1
	6PGD-2	6Pgd-2	2	5	2
		6Pgd-2'	1	3	1
PGI	PGI-1	Pgi-1	1	1	1
	PGI-2	Pgi-2	5	5	5
PGM	PGM-3	Pgm-3	4	1	2
	PGM-1	Pgm-1	3+n	4+n	4
	PGM-2	Pgm-2	1	2+n	4
TPI	TPI-1	Tpi-1	2	2	3
		Tpi-1'	2	2	2
	TPI-2	Tpi-2	2	2	1
		Tpi-2'	2	2	3

(*) n: presence of a null allele detected