ISOZYME VARIABILITY IN BRASSICA NAPUS

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INTRODUCTION

Since many years, isozymes have become a useful tool for various applications in plant breeding. In Brassica, they have been used to study phylogenetic relationships (Vaughan et al,1970; Truco and Arus,1987), to identify interspecific hybrids (Chen et al,1989; Mündges et al,1989; Sjödin and Glimelius,1989), to characterize addition lines (Quiros et al,1987; Chèvre et al,1990) or to assess F1 hybrid purity (Arus et al,1985). Isozymes have also been used to appreciate genetic diversity of various cultivated species (Chevallier and Dattée,1984; Tostain and Marchais,1989; Nagamine et al,1989) and try to predict F1 hybrid performance (Frei et al,1986; Peng et al,1988; Smith and Smith,1989).
Genetic analysis of several isozymes has been made for the diploid parental species of rapeseed: B.oleracea (Arus and Orton,1983) and B.campestris (Truco,1986). A nomenclature for 8 isozyme systems in B.oleracea, B.campestris and B.nigra is proposed by Arus et al (1991). Description of different rapeseed varieties were reported by Mündges et al (1990).
In this paper, a study of isozyme variability was carried out on B.napus genotypes from different geographical origins.

MATERIAL AND METHODS

Four groups of genotypes were studied:

- Group I : 10 french winter rapeseed genotypes
- Group II : 5 french spring rapeseed genotypes
- Group III: 7 german winter rapeseed genotypes
- Group IV : 5 japanese rapeseed genotypes.

These genotypes were extracted from the cultivars by self pollinations. Eleven isozyme systems (Table 1) were studied using starch and acrylamide electrophoresis. Young leaves were crushed in a Tris-HCl 0.1M buffer (pH 7.5) containing 1% w/v gluthatione. Gel/electrode buffers for starch gels are given in Shields et al (1983). MDH, IDH, LAP, 6PGD were separated on G buffer system (pH 6.1), GOT, TPI on C buffer system (pH 8.3) and ADH, ACO, PGM on E buffer system (pH 7.0). PGI was studied on the three buffer systems. Acrylamide separation was more efficient for GOT (Tris-HCl gel buffer pH 7.0) and APS (Tris-HCl gel buffer pH 8.9) (Truco,1986). Staining recipes for LAP and ACO were given by Arus and Orton (1983) and Wendel and Stuber (1984),respectively. The other staining procedures were reported by Vallejos (1983).
Thirty plants per genotype were analysed.

RESULTS AND DISCUSSION

Nine systems (ACO, ADH, APS, IDH, LAP, 6PGD, PGI, PGM, TPI) are encoded by 44 loci (22 loci from each diploid rapeseed parent). 6PGD and TPI are encoded by two sets of duplicated loci (Quiros et al,1987): 6Pgd-1 and 6Pgd-1', Tpi-1 and Tpi-1' are expressed in the plastids; 6Pgd-2 and 6Pgd-2', Tpi-2 and Tpi-2' are expressed in the cytosol. Conventionnally, the isozymes encoded by the genes 1' and 2' are the most cathodal ones. Among these 44 loci, 22 were polymorphic (Table 1) with 2.36 alleles/locus.

For the two remaining systems (GOT, MDH), the genetics is still not well known. Truco (1986) reported that GOT was encoded by five genes in B.campestris. The isozymes produced by these five genes are not separated in region of activity and some of these genes interact to form heterodimers. Inheritance data are only available for one GOT gene in B.oleracea (Arus and Orton,1983). Nevertheless, GOT was a very interesting system since nearly all genotypes could be distinguished .

Sixteen loci were studied more precisely: 37 alleles were characterized (Table 2). The six remaining loci were:

- 2 loci of IDH 1 which were difficult to reveal,
- 2 loci of ACO 3 and 2 loci of ACO 4: it was difficult to separate their allozymes.

Table 2 gives allele frequencies within each group of genotypes. Since the genes coming from B.oleracea and the ones coming from B.campestris could not yet be distinguished except in a few cases, it was choosen to call "a" the most anodal and "b" the other one. As proposed by Arus et al (1991), the allozymes (and alleles) were numbered in the order of most to least anodal. No variation was observed within the genotypes except for two of them where one locus was variable.

Concerning the allelic diversity within each group of genotypes, the japanese group appeared to be richer with 1.7 alleles/locus compare to 1.5 for group I, 1.4 for group II and 1.3 for group III. Five genes were monomorphic except for the japanese varieties (Aco-1b, 6Pgd-1'a, Pgm-2b, Pgm-3b and Tpi-1'b).Moreover, eight alleles were specific of this japanese group (Table 2). Two of these alleles need further study: they could be either null alleles or alleles overlapping with alleles of the homeologous genes.

The variability intra and intergroup was then assessed by calculating a percentage of dissimilarity between two genotypes:

$$D = \frac{\text{Number of variable loci between the two genotypes}}{\text{Total number of polymorphic loci}} \times 100$$

The variability inside the german group was significantly lower than in the other ones. The difference between the japanese group and the others was significantly higher than the ones between the two french or the german group (Table 3).

The difference between the japanese genotypes and the european ones could be related to the good complementarity reported between these two groups by Lefort-Buson et al (1987) in European x Asiatic F1 hybrids. A strong correlation between population structure and their geographical origin has also been reported in maize (Chevallier and Dattée,1984; Salanoubat and Pernes,1986).

Exploiting the variability and the complementarity of japanese varieties could be interesting in a F1 hybrid breeding program. But they have first to be improved for their quality characteristics (erucic acid and glucosinolates contents) and disease resistance. Isozymes could then be used to try to keep the specificity of the japanese group during this improvement.

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Table 1: Main features of the enzyme systems studied

Isozyme system	Abrev.	E.C. number	Region of activity	Number of loci	Polymorphic loci
Aconitase	ACO	4.2.1.3	ACO 1	2	1
			ACO 2	2	1
			ACO 3	2	2
			ACO 4	2	2
Alcohol dehydrogenase	ADH	1.1.1.1	ADH 1	2	0
			ADH 2	2	0
Acid phosphotase	APS	3.1.3.2	APS 1L	2	2
Isocitrate dehydrogenase	IDH	1.1.1.42	IDH 1	2	2
			IDH 2	2	0
Leucine aminopeptidase	LAP	3.4.11.1	LAP 1	2	2
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	6PGD 1	4	1
			6PGD 2	4	1
Phosphoglucoisomerase	PGI	5.3.1.9	PGI 1	2	0
			PGI 2	2	2
Phosphoglucomutase	PGM	2.7.5.1	PGM 1	2	0
			PGM 2	2	1
			PGM 3	2	2
Triosephosphate isomerase	TPI	5.3.1.1	TPI 1	4	2
			TPI 2	4	1
Malate dehydrogenase	MDH	1.1.1.37			
Glutamate oxalo-acetate transaminase	GOT	2.6.1.1			

Table 2: Allele frequencies in the four groups of genotypes for the polymorphic loci

Loci	Alleles	Group I	Group II	Group III	Group IV
Aco-1b	1	-	-	-	0.20
	2	-	-	-	0.20
	3	1	1	1	0.60
Aco-2b	1	0.91	0.57	0.60	1
	2	0.09	0.43	0.40	-
Aps-1La	1	0.50	-	1	0.20
	2	0.50	1	-	0.80
Aps-1Lb	2	0.92	0.86	0.80	-
	3	0.08	0.14	0.20	1
Lap-1a	1	-	-	-	0.20
	2	0.92	1	1	0.60
	3	0.08	-	-	0.20
Lap-1b	2	-	-	0.40	-
	3	0.43	1	0.60	1
	4	0.57	-	-	-
6Pgd-1'a	1	-	-	-	0.20
	2	1	1	1	0.80
6Pgd-2a	1	0.07	0.14	0.50	0.17
	2	0.93	0.86	0.50	0.83
Pgi-2a	1	1	1	0.80	1
	3	-	-	0.20	-
Pgi-2b	2	0.23	-	0.60	-
	3	0.77	1	0.40	1
Pgm-2b	2	1	1	1	0.40
	3	-	-	-	0.60
Pgm-3a	1	0.92	1	1	1
	2	0.08	-	-	-
Pgm-3b	1	-	-	-	0.20
	2	-	-	-	0.40
	3	1	1	1	0.40
Tpi-1a	1	0.43	0.75	0.60	-
	2	0.57	0.25	0.40	1
Tpi-1'b	1	1	1	1	0.80
	2	-	-	-	0.20
Tpi-2'b	1	0.93	0.50	1	-
	2	0.07	0.50	-	1

Table 3: Comparison of the average percentage of dissimilarity between and within each group of genotypes.

	Group I	Group II	Group III	Group IV
Group I	17.2 b	27.3 c	16.7 b	41.2 de
GroupII		26.1 c	26.9 c	45.6 e
Group III			6.6 a	38.9 d
Group IV				23.6 bc

The letters indicate the means which are not significantly different (Duncan's method, P = 0.05%)