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V. Simonsen · W. K. Heneen

Inheritance of isozymes in *Brassica campestris* L. and genetic divergence among different species of *Brassiceae*

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Abstract Electrophoretic investigations of *Brassica campestris* revealed 8 polymorphic loci that had not been described earlier. The inheritance of 7 of them was determined by crosses between different accessions of *B. campestris*. A phylogeny of *B. campestris*, *B. nigra*, *B. alboglabra*, *Sinapis arvensis* and *S. alba* was estimated from the analysis of 15 isozyme loci. The phylogeny showed a close relationship between *B. nigra* and *S. arvensis* and a less close relationship between *B. campestris* and *B. oleracea*. *S. alba* was remotely related to all of the other species.

Key words *Brassica* species · *Sinapis* species · Isozymes · Inheritance · Phylogeny

Introduction

During the last decade, our understanding of the biology of the *Brassica* species has increased, and efforts to gain more knowledge about their genetics have been intensified. The important crop oilseed rape (*B. napus* L.) is an amphidiploid. This means that the inheritance of allozymes can be complex when both, and possibly partially duplicated, parental genomes are expressed, or when different loci of the parental genomes are silenced (Chen et al. 1989). The problem might be overcome by studying the progenitors of *B. napus*, i.e. *B. campestris* L. and *B. oleracea* L. Previous studies on *B. campestris* have disclosed the inheritance patterns of isozymes glucose phosphate isomerase, leucine aminopeptidase, 6-phosphogluconate dehydrogenase, phosphoglucomutase and shikimate dehydrogenase (Quiros et al. 1987; Chen et al. 1990). The inheritance in *B. oleracea* of isozymes acid phosphatase, alcohol dehydrogenase, glucose phosphate isomerase, glutamate oxaloacetate transaminase, leucine aminopeptidase and phosphoglucomutase has been described by Arús and Orton (1983).

Isozyme analyses have been useful in species identification among *B. oleracea*, *B. campestris* and *B. napus* accessions (Arús et al. 1987), cultivar identification in *B. napus* (Mündges et al. 1990), control of inbreeding in *B. oleracea* (te Nijenhuis 1971; Wills et al. 1979) and estimation of outcrossing rate in *B. napus* (Becker et al. 1992). U (1935) suggested the following genomic relationship among the *Brassica* species: *B. campestris* (genome AA), *B. carinata* A. Br. (BBCC), *B. juncea* (L.) Czer. (AABB), *B. napus* (AACC), *B. nigra* (L.) Koch (BB) and *B. oleracea* (CC). On the basis of the variation in seed albumins obtained by acrylamide electrophoresis in *Brassica* and *Sinapis* species, Vaughan and Denford (1968) produced a dendrogram that showed a closer relationship between *B. oleracea* and *B. campestris* than between any one of these species and *B. nigra*. They also held that *Sinapis* species were distantly related to the *Brassica* species. Their results were supported by subsequent protein and isozyme analyses (Vaughan et al. 1970). In their electrophoretic study of soluble proteins, peroxidase, esterase and catalase in *Brassica* species, Yadava et al. (1979) obtained a result which was in conflict with U's triangle. They suggested that *B. nigra* and *B. campestris* are the parental species of *B. carinata*. This result has not been supported by chromosomal and other types of studies (e.g. Röbbelen 1960).

Recently, Song et al. (1990) used nuclear restriction fragment length polymorphisms (RFLP) for studying *Brassica* taxonomy. Yanagino et al. (1987), Warwick and Black (1991) and Warwick et al. (1992) made similar studies based on chloroplast DNA. A mutual result of these DNA

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V. Simonsen (✉)[1]
National Institute of Animal Science,
Animal Physiology and Biochemistry, Foulum, P.O. Box 39,
DK-8830 Tjele, Denmark

W. K. Heneen
Department of Plant Breeding Research,
The Swedish University of Agricultural Sciences,
S-268 31 Svalöv, Sweden

Present address:
[1] National Environmental Research Institute, Department for Terrestrial Ecology, Vejlsøvej 25, P.O. Box 314, DK-8600 Silkeborg, Denmark

Table 1 List of species, accession number, sample size, origin and further information on the accessions studied for the phylogeny

Species	Accession no.[a]	Sample size	Origin	Remarks
Brassica campestris	21	25	China	Locality: Wu Chang Bei You Cai
	44	32	Sweden	Breeding line Sv 88–39339
	46	32	India	Yellow sarson K-151
Brassica alboglabra	49	32	China	no. 4003
Brassica nigra	55	33	Sweden	
Sinapis arvensis	56	33[b]	Sweden	
Sinapis alba	57	33	Sweden	

[a] The same numbering as in Simonsen and Heneen (1995)
[b] The seed sample comprised more than one species as inferred from the zymograms. Only 12 individuals assumed to be *S. arvensis* were scored

investigations was that *B. campestris* and *B. oleracea* were found to be more closely related to each other than to *B. nigra* and that *B. nigra* was found to be more closely related to *S. arvensis* L. and *S. alba* L. than to *B. campestris* and *B. oleracea*. Pradhan et al. (1992) compared the variation in chloroplast and mitochondrial DNA of these species and observed an incongruence in phylogeny based on these data. However, the results, by and large, have confirmed the above-mentioned relationships between *B. nigra* and *S. arvensis*, and between *B. campestris* and *B. oleracea*.

The aim of the present study was to determine the inheritance patterns of seven isozymes in *Brassica campestris* and to use isozyme analysis for studying the genetic variation within accessions of *B. campestris*, *B. alboglabra* Bailey (a subordinate of *B. oleracea*), *B. nigra*, *Sinapis arvensis* and *S. alba*, and among these species.

Materials and methods

Plant material

The following accessions of *Brassica campestris* were used for the inheritance study: var 'yellow sarson' (K-151), which is of Indian origin, two Swedish breeding lines (Sv 85–38301 and GJ 2891) and one Chinese landrace (no. 21, see Simonsen and Heneen 1995). Accessions K-151 and Sv 85–38301, which are the same as nos. 44 and 46 in Table 1, were used as parents for an F_1 generation that was selfed in order to obtain the F_2 progeny. Accessions GJ 2891 and no. 21 were used as parents in a similar crossing procedure. The species and accessions used for the phylogenetic study are listed in Table 1.

Sample preparation and electrophoresis

The sample preparation has been described by Chen et al. (1989). The electrophoretic method used was horizontal starch gel electrophoresis. The enzymes analysed were the five described by Chen et al. (1989, 1990) as well as acid phosphatase (ACP, E.C. 3.1.3.2), diaphorase (DIA, E.C. 1.8.1.4), esterase (EST, E.C. 3.1.1.-), fructose-1,6-biphosphatase (FBP, E.C. 3.1.3.11), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), iso-citric acid dehydrogenase (ICD, E.C. 1.1.1.42) and malate dehydrogenase (MDH, E.C. 1.1.1.37). The buffer used for DIA and EST was a histidine buffer pH=5.7 (Adam 1985); for FBP, a TRIS buffer pH=7.0 (Gottlieb 1981); and for ACP, GOT, ICD and MDH, a histidine buffer pH=8.0 (Brown 1980). References for the staining procedures are cited by Simonsen and Frydenberg (1973) and by Richardson et al. (1986).

Isozyme and allozyme nomenclature

The nomenclature followed the outlines given by Chen et al. (1989, 1990).

Statistical analyses

Fit to Hardy-Weinberg proportions was tested with the testator $F\sqrt{N}$ as described by Brown (1970) by using the programme G-FSTAT from the G-STAT package (Siegismund 1992). The phylogeny was estimated using the programme CONTML, and the phylogeny was depicted by the programme DRAWTREE from the PHYLIP package (Felsenstein 1989).

Results

Zymograms of the enzymes DIA, EST, FBP, GOT, ICD, MDH and PGM from *B. campestris* are presented in Fig. 1. The resolution of the enzyme ACP was too poor when starch gel electrophoresis was used, hence no further work was done with this enzyme.

Inheritance patterns in *B. campestris*

Diaphorase (DIA)

One or several bands of DIA were found (Fig. 1a). The fastest migrating anodic band was present in all individuals and represented presumably 1 locus, designated *Dia-1A*, with 1 allele, labelled *a*. A slower migrating anodic band was located either very close to *a*, designated *b*, or at a greater distance from *a*, named *c*. The *c* band was often associated with additional bands that had a slower migrating mobility towards the anode. The *b* and *c* bands were assumed to be products of the *Dia-2A* locus. Segregation in the F_2 is given in Table 2, and the results supported the single-locus hypothesis of inheritance for the monomeric enzyme DIA.

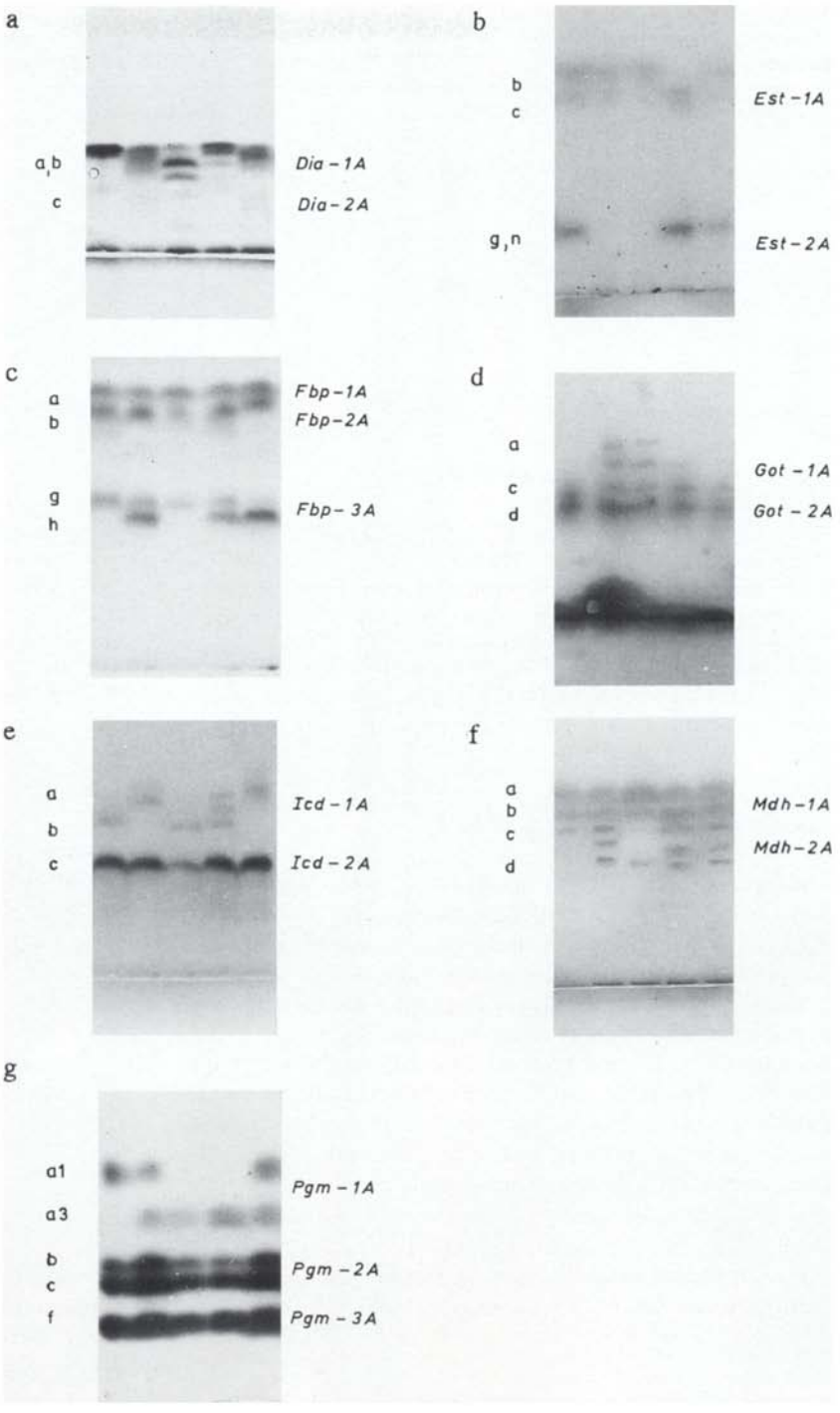

Fig. 1a–g Zymograms of the enzymes DIA, EST, FBP, GOT, ICD, MDH and PGM of *Brassica campestris*. Designation of bands are shown to the *left* of the zymograms and designation of loci to the *right* of the zymograms

Esterase (EST)

Three to four zones of activity were revealed for EST, and presumably each of these was determined by at least 1 locus. Variation was observed in the two fastest migrating anodic zones (Fig. 1b) for this monomeric enzyme. In the *B. campestris* accessions studied, the polymorphism in the fastest migrating anodic zone was most likely caused by 1 locus, designated *Est-1A*, with 2 codominant alleles (Table 3). The crosses did not reveal polymorphism, but additional analyses of about 40 landraces from China support this hypothesis (Simonsen and Heneen 1995). At least 3 codominant alleles were revealed in the landraces. The second fastest migrating anodic zone consisted of one band that was either present or absent. One possible hypothesis for the inheritance was that a locus *Est-2A* with 2 alleles, *g* and *n*, of which *n* is silent, determined the banding pattern in this zone. This explanation was supported by the

Table 2 Single-locus goodness-of-fit tests for seven polymorphic A-genome loci in the F_2 of various *B. campestris* crosses

Enzyme locus	Family	F_1 genotype	F_2 genotype and frequency	Expected ratio	df	χ^2	*P*
Dia-2A	K-151 × SV 85–38301	bc	bb:bc:cc 15 23 12	1:2:1	2	0.68	0.71
Est-2A	K-151 × SV 85–38301	gn	g-:nn 42 8	3:1	1	2.16	0.14
	GJ 2891 × 21	gn	g-:nn 21 4	3:1	1	1.08	0.30
Fbp-3A	K-151 × SV 85–38301	gh	gg:gh:hh 9 29 12	1:2:1	2	1.64	0.44
Got-1A	K-151 × SV 85–38301	ac	aa:ac:cc 8 26 16	1:2:1	2	2.64	0.27
Icd-1A	GJ 2891 × 21	ab	aa:ab:bb 10 28 12	1:2:1	2	0.88	0.64
Mdh-2A	K-151 × SV 85–38301	cd	cc:cd:dd 13 20 17	1:2:1	2	2.66	0.26
Pgm-1A	GJ 2891 × 21	a1a3	a1a1:a1a3:a3a3 16 26 8	1:2:1	2	2.64	0.27

segregation data obtained in the F_2 generation (Table 2). The inheritance patterns of the bands in the other zones were impossible to explain on the basis of the results.

Fructose-1,6-biphosphatase (FBP)

The zymogram revealed three zones of activity (Fig. 1c). The fastest migrating anodic zone consisted of one band that was found in all of the individuals analysed. The zone was assumed to be determined by 1 locus, *Fbp-1A*, with 1 allele, *a*. The second fastest migrating anodic zone expressed variation, but the interpretation of the zone was not unambiguous; at least 1 locus (*Fbp-2A*) might determine this zone. The third anodic zone showed either a single band with two different locations on the gel or a two-banded pattern, obviously comprising the two single bands seen for a heterozygote of a monomeric enzyme. This zone was assumed to be due to a locus, *Fbp-3A*, with 2 codominant alleles, *g* and *h*. This hypothesis was supported by the segregation data obtained in the F_2-generation (Table 2).

Glutamate oxaloacetate transaminase (GOT)

This enzyme revealed two zones of activity (Fig. 1d); the faster migrating anodic zone was faint in intensity, whereas the slower migrating anodic zone expressed a high activity. The faster migrating zone showed the variation expected for a dimeric enzyme with a three-banded phenotype for heterozygous individuals and might be interpreted as 1 locus *GOT-1A* with 2 codominant alleles, *a* and *c*. Our hypothesis was supported by the segregation obtained in the F_2 generation (Table 2). The other zone was determined at the very least by 1 monomorphic locus in *B. campestris*, named *Got-2A*, with the *d* allele. No variation in *Got-2A* was obtained in this study or in the survey of other accessions of *B. campestris* (Simonsen and Heneen 1995).

Iso-citric acid dehydrogenase (ICD)

Again two zones were revealed, one with faint activity and one with strong activity (Fig. 1e). The faster migrating anodic zone expressed the variation expected for a dimeric enzyme with a three-banded phenotype for heterozygous individuals. This zone was interpreted as the product of 1 locus, *Icd-1A*, with 2 codominant alleles, *a* and *b*. This hypothesis was supported by the results presented in Table 2. The second zone was invariable and so far has been assumed to be determined by 1 locus, *Icd-2A*, with 1 allele, *c*.

Malate dehydrogenase (MDH)

The zymogram showed two zones (Fig. 1f). The faster migrating anodic zone consisted of two bands, named *a* and *b*, which were present in all of the individuals analysed. The two bands were determined by at least 1 locus, *Mdh-1A*. A slower migrating anodic zone expressed variation in conformity with a dimeric enzyme. The variation might be explained as 1 locus, *Mdh-2A*, with 2 codominant alleles, named *c* and *d*. The segregation of the alleles for the F_2 at the locus *Mdh-2A* is presented in Table 2.

Phosphoglucomutase (PGM)

Three zones were disclosed in the zymogram (Fig. 1g). The region determined by *Pgm-1A* represented 1 locus with 2 codominant alleles, *a1* and *a3*, that resulted in phenotypes that were in agreement with the monomeric structure

Table 3 Allelic frequencies for 15 loci analysed in different species and accessions

Locus	Allele	Frequency in accession no.						
		21	44	46	49	55	56	57
Est-1	*b*	0.06	0.81	0.68	0.00	0.00	0.00	0.00
	c	0.94	0.19	0.32	0.77	0.00	0.00	0.45
	d	0.00	0.00	0.00	0.23	0.00	0.00	0.55
	f	0.00	0.00	0.00	0.00	1.00	1.00	0.00
Fbp-1	*a*	0.00	0.00	0.00	0.00	0.23	0.00	0.00
	b	0.00	0.00	0.00	1.00	0.77	1.00	1.00
	c	1.00	1.00	1.00	0.00	0.00	0.00	0.00
Fbp-3	*e*	0.00	0.00	0.00	0.00	0.00	0.00	0.29
	f	0.00	0.00	0.00	0.00	0.00	0.00	0.71
	g	0.33	0.30	0.00	0.00	0.23	0.00	0.00
	h	0.65	0.70	1.00	0.94	0.77	1.00	0.00
	i	0.02	0.00	0.00	0.06	0.00	0.00	0.00
Got-1	*a*	0.22	0.08	0.05	0.00	0.00	0.28	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	c	0.78	0.92	0.95	1.00	1.00	0.72	0.00
Got-2	*d*	1.00	1.00	1.00	0.00	0.00	0.00	0.45
	e	0.00	0.00	0.00	1.00	1.00	1.00	0.55
Gpi-2	*a*	0.00	0.00	0.00	0.00	1.00	0.28	1.00
	b	0.82	1.00	0.31	0.23	0.00	0.67	0.00
	c	0.18	0.00	0.69	0.15	0.00	0.05	0.00
	d	0.00	0.00	0.00	0.63	0.00	0.00	0.00
Icd-1	*a*	0.34	0.86	1.00	1.00	0.36	0.67	1.00
	b	0.66	0.14	0.00	0.00	0.64	0.33	0.00
Icd-2	*c*	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	d	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Lap-1	*a*	0.42	0.92	0.89	0.00	0.00	0.72	0.00
	c	0.58	0.08	0.11	1.00	1.00	0.22	0.00
	d	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	e	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Mdh-1	*a*	1.00	1.00	1.00	1.00	1.00	1.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Mdh-2	*c*	0.72	0.86	0.75	1.00	1.00	0.00	–
	d	0.28	0.14	0.25	0.00	0.00	0.61	–
	e	0.00	0.00	0.00	0.00	0.00	0.39	–
6-Pgd-1	*a*	1.00	1.00	1.00	0.50[a]	0.77	0.00	0.00
	a1	0.00	0.00	0.00	0.00	0.00	0.28	0.00
	b	0.00	0.00	0.00	0.50	0.00	0.00	0.66
	f	0.00	0.00	0.00	0.00	0.23	0.00	0.34
	f1	0.00	0.00	0.00	0.00	0.00	0.72	0.00
6-Pgd-2	*c*	1.00	1.00	1.00	1.00	0.00	0.00	0.00
	g	0.00	0.00	0.00	0.00	0.47	0.33	0.00
	h	0.00	0.00	0.00	0.00	0.53	0.67	0.00
	i	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Pgm-1	*a1*	0.66	0.00	0.00	0.00	–	1.00	0.00
	a2	0.20	0.90	1.00	1.00	–	0.00	0.00
	a3	0.14	0.10	0.00	0.00	–	0.00	1.00
Pgm-2	*c*	0.70	0.30	1.00	0.00	0.17	0.00	0.89
	d	0.30	0.70	0.00	0.08	0.00	1.00	0.11
	e	0.00	0.00	0.00	0.92	0.83	0.00	0.00
Pgm-3	*f*	1.00	1.00	1.00	0.00	0.42	0.78	1.00
	g	0.00	0.00	0.00	1.00	0.58	0.22	0.00
Sdh-1	*a*	0.10	0.26	0.00	1.00	0.00	0.00	1.00
	b	0.90	0.66	0.02	0.00	0.70	1.00	0.00
	c	0.00	0.08	0.98	0.00	0.21	0.00	0.00
	d	0.00	0.00	0.00	0.00	0.09	0.00	0.00

[a] This locus is duplicated in *B. alboglabra* (accession no. 49), which means that all individuals perform as heterozygotes. In this case the individuals were scored as heterozygotes in order to make benefit of the identical locations of the alleles when estimating the phylogeny

of the enzyme. This hypothesis was supported by the segregation of the alleles for the F_2 individuals as shown in Table 2. A third allele of *Pgm-1A*, named *a2*, was found when analysing more specimens of the parent, no. 21, see Table 3. No variation in the second zone (*Pgm-2A*) was seen in present work. The study of 43 accessions from China (Simonsen and Heenen 1995) revealed two or three bands for each individual in this zone. The bands of the second zone were named b, c and d. The inheritance of this locus was assumed to be as follows. Each allele determined two bands, allele *c* consisted of a faint b band and strong c band, allele *d* of a faint c band and a less strong d band. The phenotypes shown at Fig. 1g are homozygous for the *c* allele. The slowest migrating anodic band was at the very least determined by 1 locus with 1 allele; no variation was seen in *B. campestris*.

In summary, at least 5 monomorphic loci and 8 polymorphic loci were described in *B. campestris*. The inheritance of 7 of the polymorphic loci was verified by segregation observed in the F_2 generation.

Genetic variation within *Brassica* and *Sinapis* accessions

The loci described above as well as those reported by Chen et al. (1989, 1990) were included in this part of the investigation. These loci consisted of *Est-1*, *Fbp-1*, *Fbp-3*, *Got-1*, *Got-2*, *Gpi-2*, *Icd-1*, *Icd-2*, *Lap-1*, *Mdh-1*, *Mdh-2*, *6-Pgd-1*, *6-Pgd-2*, *Pgm-1*, *Pgm-2*, *Pgm-3* and *Sdh-1*. *Mdh-2* and *Pgm-1* were not found in *S. alba* and *B. nigra*, respectively, but were present in the other species. *Dia-1*, *Dia-2*, *Fbp-2* and *Gpi-1* were not included due to problems in interpreting the zymograms in all of the accessions. *Est-2* and *Sdh-2* were not used due to the prevalence of silent alleles. *Lap-2* and *Sdh-3* were very often too faint to score and hence not included in this analysis.

The allelic frequencies are listed in Table 3. Tests for fit to Hardy-Weinberg proportions were conducted when possible. Forty-four test values were obtained, and 3 of these deviated significantly, which was more than the expected 5%. The small sample size might explain the deviation. When the ratio of samples with an excess of heterozygotes was compared to those with a defiency of heterozygotes, a distribution of 25:19 was obtained, which was a good fit to the expected ratio 1:1 (χ^2=0.82 with 1 df, P=0.37).

The observed heterozygosity values (H_{obs}) for the different accessions are listed in Table 4. The loci included in

Table 4 Heterozygosity (H_{obs}) in the accessions studied

Species	Accession no.	Number of loci	H_{obs}
B. campestris	21	17	0.20
	44	17	0.13
	46	17	0.11
B. alboglabra	49	17	0.07
B. nigra	55	16	0.22
S. arvensis	56	17	0.24
S. alba	57	16	0.14

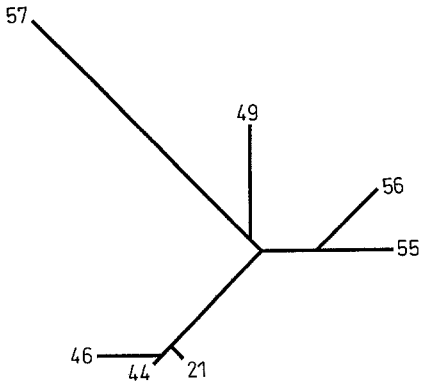

Fig. 2 Phylogeny of *Brassica campestris* (no. 21, landrace from China; no. 44, breeding line from Sweden; no. 46, cultivar from India), *B. alboglabra* (no. 49), *B. nigra* (no. 55), *Sinapis arvensis* (no. 56) and *S. alba* (no. 57) based on 15 isozyme loci estimated with the programme CONTML from Felsenstein (1989) and depicted with the programme DRAWTREE

Table 4 are the 17 mentioned above, excluding *Mdh-2* in *S. alba* and *Pgm-1* in *B. nigra*. The observed heterozygosity values were very similar to the ones expected. This was in accordance with the fact that the genotypic distribution in most of the accessions fit Hardy-Weinberg proportions. The heterozygosity of the *B. campestris* accessions differed from the lowest value by a magnitude of two, and *B. alboglabra* also had a low level of heterozygosity.

Genetic variation among *Brassica* and *Sinapis* accessions

The relationship between the accessions studied, as inferred from the isozyme analysis, is depicted in Fig. 2. Our phylogenetic tree is based on the 15 loci that were common to all the species studied. The tree was estimated using the restricted maximum likelihood method (Felsenstein 1989). The *B. campestris* accessions clustered as expected, and *B. alboglabra* seemed to be more closely related to *B. nigra* and *S. arvensis* than to *B. campestris*, whereas *S. alba* had a remote relationship to the other species.

Discussion

Inheritance

The hypotheses of inheritance of the enzymes DIA, EST, FBP, GOT, ICD, MDH and PGM in *Brassica campestris* are discussed below.

Diaphorase (DIA)

Our hypothesis for the inheritance of DIA was that DIA was determined by at least 2 loci, *Dia-1A* and *Dia-2A*; the first locus monomorphic and the second polymorphic with 2 alleles. Two loci determining DIA have been found in maize by Stuber et al. (1988) and in barley by Brown et al. (1978). The DIA zymogram obtained by Becker et al. (1992) for *B. napus* is different from the one we obtained for *B. campestris* in this study. The presence of the *B. oleracea* genomes in *B. napus* might influence the pattern. Furthermore, these authors used a different buffer for the electrophoresis, which makes it difficult to compare the two zymograms. The DIA isozyme was not so easy to score, and the genetic model proposed might not be sufficient to explain all of the variation seen. In order to clarify the inheritance of this enzyme we need to make more crosses.

Esterase (EST)

Previous investigations of EST in *B. campestris* have mainly been carried out on seed extracts by means of polyacrylamide electrophoresis (Vaughan et al. 1970; Khalatkar 1976; Yadava et al. 1979; Iwasaki 1983). Furuya and Ikehashi (1991) used cotyledon or leaf extracts as material for the electrophoresis. As a result of the two different electrophoretic methods used, it was problematic to compare the obtained patterns. However, Furuya and Ikehashi (1991) found 2 *Est* loci with null alleles, and at least 1 of these was revealed in our study. Only the inheritance of the zone with the null allele determined by *Est-2A* in our notation was supported by current results from the F_2 generation.

Fructose-1,6-biphosphatase (FBP)

To our knowledge no previous work had been carried out on this enzyme. Our hypothesis for the inheritance of the locus *Fbp-3A* was supported by the results obtained.

Glutamate oxaloacetate transaminase (GOT)

The fastest anodic migrating zone, observed for *B. campestris* in the present study, is similar to the one documented for *B. oleracea* by Arús and Orton (1983), who interpreted this part of the zymogram as a product of 2 loci. This might be due to the dense staining of this region when analysing leaf material, which made it difficult to interprete the banding pattern. The slower migrating zone was determined by at least 1 monomorphic locus *Got-2A*. The corresponding locus in *B. oleracea* was named *Got-2C* in our nomenclature and is equivalent to *Got-3* in the study of Arús and Orton (1983), who found variation at this locus in *B. oleracea*. Gotoh and Ikehashi (1992) also interpreted the GOT zymogram in *B. oleracea* as a product of 3 loci. Further crosses are needed to elucidate the inheritance of GOT in *B. campestris*. How the observed variation for GOT in *B. campestris* corresponds to the variation

described by McGrath and Quiros (1990, 1991) and by Chevre et al. (1991) requires a side-by-side electrophoresis to solve the problem.

Iso-citric acid dehydrogenase (ICD)

The results obtained in this study support the hypothesis that at least 2 loci determine the ICD zymogram, 1 locus polymorphic with 2 alleles and the other monomorphic. Previous studies of ICD by Khalatkar (1976), McGrath and Quiros (1990) and Chevre et al. (1991) did not document any variation in ICD in neither *B. campestris* nor in *B. napus*.

Malate dehydrogenase (MDH)

Khalatkar (1976) used polyacrylamide electrophoresis to reveal two zones of activity in *Brassica* species, and no variation was detected. This observation is in contrast to the findings made by McGrath and Quiros (1991), who indicated that the pattern of MDH was complex. However, McGrath and Quiros (1991) interpreted a fast migrating band of MDH as a product of a dominant locus with 2 alleles. We assume that the locus *Mdh-1A* described in the present work matches the variable locus found by McGrath and Quiros (1991).

Phosphoglucomutase (PGM)

A description of PGM in *B. campestris* was given by Quiros et al. (1987), who interpreted the zymogram to be determined by 2 loci in a way similar to that in *B. oleracea* (Arús and Orton 1983). Chen et al. (1989) observed a like zymogram for *B. campestris*. In another study Chen et al. (1990) found a minor band, determined by the locus *Pgm-3A*, in *B. campestris* and in *B. campestris-alboglabra* aneuploids, which was interpreted as being 1 locus with 2 alleles, 1 of which was silent. Analyses conducted on Chinese landraces of *B. campestris* (Simonsen and Heneen 1995) did not support our hypothesis (Chen et al. 1990) on the third locus, *Pgm-3A*. This band, labelled d, was most likely a product of locus *Pgm-2A*, but due to the very strong activity of this locus in the *B. campestris* accession K-151, it was difficult to judge if the faint d band had to be included in the region determined by *Pgm-2A* (Chen et al. 1990). Our notation *Pgm-4A* has to be changed to *Pgm-3A*. The hypothesis at present is that PGM in *B. campestris* is determined by 3 loci, *Pgm-1A*, *Pgm-2A* and *Pgm-3A*. Products of 3 codominant alleles, *a1*, *a2* and *a3* were observed at *Pgm-1A*. The bands designated b, c and d were linked to *Pgm-2A* and were most likely determined by 2 codominant alleles, *c* and *d*, as described previously in this work. The band f was a product of the *Pgm-3A* locus. Further crosses need to be made for final confirmation of the inheritance of the enzyme PGM in *B. campestris*.

Genetic variation within accessions

The number of samples which deviated from Hardy-Weinberg proportions was higher than expected. This result might be due to the small sample size or to the propagation regimes of the different accessions. However, the deviation was not due to an excess or a deficiency of heterozygotes, as the ratio between samples with an excess and samples with a defiency was close to the expected 1:1. Sample size seemed then to be the most likely explanation for this observation.

The level of heterozygosity in *B. campestris* clearly indicated that the intensively cultivated accessions, nos. 44 and 46, had less genetic variation than landrace no. 21. The cultivars of *B. alboglabra* and *S. alba*, nos. 49 and 57, respectively, also had a reduced level of genetic variation compared to *B. campestris* landrace no. 21. Propagation regimes are certainly of great significance for maintaining or reducing genetic variation (Lande and Barrowclough 1987), and the reduced level found in the cultivars of this study might be due to propagation. However, it was surprising that *B. nigra* (no. 55) still had a high level of genetic variation despite its status as a cultivar. The estimation of the heterozygosity level of *S. arvensis* (no. 56) has to be accepted with precaution as only 12 individuals were analysed due to the the seed sample being polluted with *B. napus*.

Genetic variation among accessions

Phylogenetic trees for *Brassica* species and related genera were obtained previous to this study by Song et al. (1990) using RFLP on nuclear DNA and by Yanagino et al. (1987), Warwick and Black (1991) and Warwick et al. (1992) using RFLP on chloroplast DNA. Our results agree roughly with the ones found by these authors. The principal divergence between the investigations is that the DNA-based methods revealed a closer relationship between *B. campestris* and *B. oleracea* than between any of these and *B. nigra*, while the isozyme-based method, presented in this work, showed a closer relationship between *B. nigra* and *B. alboglabra* than between any of these species and *B. campestris*. This discrepancy might be explained by the fact that the DNA-based methods included many more species of *Brassicinae* and more genetic markers, which again permitted a distinct separation of the three species (Yanagino et al. 1987; Song et al. 1990; Warwick and Black 1991; Warwick et al. 1992). The work on proteins and isozymes by Vaughan and Denford (1968) and Vaughan et al. (1970) indicated that there exists a closer relationship between *B. oleracea* and *B. campestris* than between any of these and *B. nigra*. Chromosomal studies also support the closer relationship between *B. campestris* and *B. oleracea* (Attia et al. 1987).

The close relationship observed between *B. nigra* and *S. arvensis* and the observation that *S. alba* is remotely postioned relative to the other species agree with the results obtained by Yanagino et al. (1987), Warwick and Black

(1991) and Warwick et al. (1992). Further analyses of other *Brassica* species and genera related to *Brassica* might change the phylogenetic tree based on isozymes in a way that makes it similar to the trees based on DNA analyses as all trees are more or less biased consequential to the analytical method used.

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