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Phenotypic polymorphism and allele differentiation of isozymes in fodder beet, multigerm sugar beet and monogerm sugar beet

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Summary. Thirteen enzymes (MDH, SDH, LAP, PGM, PX, IDH, GPI, 6PGD, APH, GOT, GDH, ME and SOD) of 3 cultivated beet (*B. vulgaris* L.) gene pools, comprising 12 accessions of fodder beet, 11 of old multigerm sugar beet and 10 of modern monogerm sugar beet, were investigated using horizontal starch gel electrophoresis. Eleven accessions of primitive or wild *B. vulgaris* were also included for the comparison of isozymes. Variation in isozyme phenotypes was investigated to detect diversity in the three cultivated forms of beet. Phenotypic variation was observed in all except ME and SOD, which were monomorphic. A high degree of phenotypic polymorphism (Pj) was found in GDH, PGM, IDH, APH and MDH. Differences in phenotypic polymorphism in MDH, GPI and PX were recognized between fodder beet and both sugar beet groups. Average polymorphism for 13 enzymes in both sugar beets was significantly higher than that in fodder beet. For 13 enzymes, the existence of high isozyme diversity in both sugar beet gene pools was revealed. Allele frequencies in 13 alleles of five enzyme-coding loci, *Lap, Px-1, Aph-1, Got-2* and *Gdh-2,* were investigated. New alleles, *Px-1*^{1} and *Got-2*^{1}, were found in fodder beet accessions. No significant differences of average allele frequencies of five loci between fodder beet and both sugar beets were recognized. Several unique alleles and different isozyme phenotypes were observed in the accessions of *B. vulgaris* ssp. *macrocarpa* and ssp. *adanensis*. Future utilization of cultivated beet gene pools for sugar beet breeding is discussed from the viewpoint of genetic resources.

Key words: *Beta vulgaris* L. – Isozyme phenotype – Phenotypic polymorphism – Allele frequency – Genetic resources

Introduction

The cultivated beet (*Beta vulgaris* L.) was probably domesticated as a leaf beet in the Mediterranean region from wild forms without swollen roots or hypocotyls (Ford-Lloyd and Williams 1975; Ford-Lloyd 1986; De Bock 1986). Of these, sugar beet has been cultivated for less than 200 years and is a relatively new crop to mankind. Sugar beet is thought to have originated from a Silesian fodder beet population (Bosemark 1979) crossed with either a leaf beet or wild form. Present sugar beet varieties are F_1 hybrids utilizing a common source of cytoplasmic male sterility (Owen 1948). In addition, monogermicity, present in all modern sugar beet cultivars, has a single genetic origin. Therefore, modern monogerm F_1 hybrid sugar beet is often considered to have a narrow genetic base in comparison with fodder beet and older multigerm sugar beet varieties.

The improvement in sugar beet has largely been attained by selection from one common gene pool, representing a limited source of genes for utilization, and arousing fears of genetic poverty. The present source of cytoplasmic male sterility is indeed limited only to S type cytoplasm. Contrarily, however, Bosemark (1979) has suggested that sugar beet still holds much variation in nuclear genes.

Conventionally, root shape, fruit shape and leaf characters have been adopted to estimate the diversity of *Beta* species. However, the limited number of efficient genetic markers (pigmentation gene (R); restorer genes) creates difficulties in understanding the genetic structure of populations. Recently, use has been made of isozymes as genetic markers in *Beta*. Van Geyt and Smed (1984) demonstrated polyacrylamide (PAGE) and starch (SGE) gel electrophoresis for analysis of several enzyme systems, and reported the possible utilization of LAP, ME, GDH,

PGM, GPI and IDH as efficient biochemical markers. Jung et al. (1985) analyzed GOT, aconitase and alkaline and acidic esterases with PAGE and horizontal isoelectric focusing, for the detection of monosomic addition lines with full nematode resistance. Oleo et al. (1986) applied LAP, POD (cathodal peroxidase), PGM, SDH, EST, SOD, GDH and MDH, to investigate enzyme expression in parental species and their hybrids. Abe et al. (1987) demonstrated the availability of isozyme markers for studying species relationships in *Beta* using APH, PX and EST. Abe and Tsuda (1987) determined five enzyme-coding loci, *Lap, Px-1, Aph-1, Got-2* and *Gdh-2,* and analyzed differences in allele frequencies between fodder beet and sugar beet. However, compared with other important crops such as maize, wheat, barley and rice, the utilization of isozyme markers in *Beta* species is still limited and the number of loci and alleles determined very small. This is ascribed mainly to the breeding system of cultivated beets and to the small number of genetic marker lines available. Since there is little information about isozyme composition of cultivated beets, our research has focused on phenotypic polymorphism and allele differentiation of 13 enzymes using SGE. We have attempted to examine variation within and between three groups of cultivated beet which may be said to represent these distinct gene pools, namely multigerm fodder beet, old multigerm sugar beet and modern hybrid monogerm sugar beet. Furthermore, differences in isozymes in several wild taxa allied to *B. vulgaris* and cultivated beets have been assessed from the viewpoint of broad comparison.

Materials and methods

Material representing three cultivated beet (*Beta vulgaris* L.) gene pools, namely 12 accessions of multigerm fodder beet, 11 of old multigerm sugar beet and 10 of modern monogerm sugar beet, was chosen (Table 1). All accessions were advanced cultivars or breeding lines, and a number of tetraploid accessions was included as well as diploid ones. The monogerm sugar beet accessions were all triploid F_1 hybrids. In addition, 11 accessions of primitively cultivated or wild *Beta* species, *B. vulgaris, B. vulgaris* ssp. *asiatica,* ssp. *maritima,* ssp. *macrocarpa* and ssp. *adanensis,* were used for comparison of isozymes occurring in more divergent material. For further reference, ssp. *macrocarpa* (an accession from Imperial Valley of California) and ssp. *adanensis,* the genotypes of which were clarified for five enzyme-coding loci by Abe and Tsuda (1987), were included in every electrophoresis run for comparative purposes.

After sowing 30 seeds from each of the 44 accessions, 10 plants were chosen randomly and assigned individual numbers. A fully developed leaf 24–56 days after germination was sampled for the extraction of enzymes. A crude extract from approximately 10 mg fresh leaf weight was taken using 40 µl of extraction buffer (50 m*M* Tris-HCl, pH 6.8 containing 1% mercaptoethanol). After complete maceration of the leaf, the extract was absorbed by wick (Whatman 3MM chromatography paper), which was then inserted into 12% starch gel containing

Table 1. Plant materials used

No.	Acc. no.	Species	Variety name	Ploidy
Multigerm fodder beet				
1	B1006	*Beta vulgaris*	Rouge d'Eckendorf	2N
2	B1011	*Beta vulgaris*	Jaune Ovoide Barres	2N
3	B1029	*Beta vulgaris*	Orange Barres	4N
4	B1030	*Beta vulgaris*	Long Greentop	2N
5	B1032	*Beta vulgaris*	Ovale Greentop	2N
6	B1035	*Beta vulgaris*	Long Rose	4N
7	B1037	*Beta vulgaris*	Long Cylindric Rose	2N
8	B1038	*Beta vulgaris*	Greentop	2N
9	B1040	*Beta vulgaris*	Red Cylindric	4N
10	B1055	*Beta vulgaris*	Yellow Globe	?
11	B1058	*Beta vulgaris*	Red Gatepost	?
12	B1064	*Beta vulgaris*	White Knight	?
Old multigerm sugar beet				
13	B0079	*Beta vulgaris*	Sharpe's Klein E	2N
14	B0081	*Beta vulgaris*	Sharpe's Klein Megapoly	Mix
15	B0112	*Beta vulgaris*	Unknown	2N
16	B0113	*Beta vulgaris*	Unknown	2N
17	B0115	*Beta vulgaris*	Unknown	2N
18	B0116	*Beta vulgaris*	Unknown	4N
19	B0117	*Beta vulgaris*	Unknown	4N
20	B0788	*Beta vulgaris*	Janacscz III	2N
21	B1043	*Beta vulgaris*	Hilleshog N	2N
22	B1044	*Beta vulgaris*	Kleinwanzleben Z	2N
23	B1075	*Beta vulgaris*	Camkilt	?
Modern monogerm sugar beet				
24	B0076	*Beta vulgaris*	Hilleshog Monotri	3N
25	B0078	*Beta vulgaris*	Sharpe's Klein Monobeet	3N
26	B0378	*Beta vulgaris*	Vytomo	3N
27	B1259	*Beta vulgaris*	Amethyst	3N
28	B1260	*Beta vulgaris*	Bush Mono G	3N
29	B1261	*Beta vulgaris*	Hilma	3N
30	B1262	*Beta vulgaris*	Monoire	3N
31	B1263	*Beta vulgaris*	Novagemo	3N
32	B1264	*Beta vulgaris*	Ovatio	3N
33	B1265	*Beta vulgaris*	Primahill	3N
Wild or primitive forms				
34	B0151	*B. vulgaris*		
35	B0176	*B. vulgaris*		
36	B0640	*B. vulgaris*		
37	B0269	*B. vulgaris* ssp. *asiatica*		
38	B0270	*B. vulgaris* ssp. *asiatica*		
39	B0095	*B. vulgaris* ssp. *maritima*		
40	B0300	*B. vulgaris* ssp. *maritima*		
41	B0334	*B. vulgaris* ssp. *maritima*		
42	B0577	*B. vulgaris* ssp. *macrocarpa*		
43	B0584	*B. vulgaris* ssp. *macrocarpa*		
44	B0148	*B. vulgaris* ssp. *adanensis*		

10% sucrose. Horizontal SGE was performed at 4 °C. Wicks were removed from the gel after tracker dye moved 1 cm from the origin. Three different buffer systems were used: 5 m*M* L-histidine monohydrochloride pH 7.0 (gel buffer), and 200 m*M* trisodium citrate pH 7.0 (electrode buffer); 9 m*M* Tris, 3 m*M* citric acid pH 7.0 (gel), and 135 m*M* Tris, 47 m*M* citric acid pH 7.0 (electrode) (Van Geyt and Smed 1984); and 46 m*M* Tris, 7 m*M* citric acid, 3 m*M* LiOH, 2 m*M* boric acid pH 8.0 (gel),

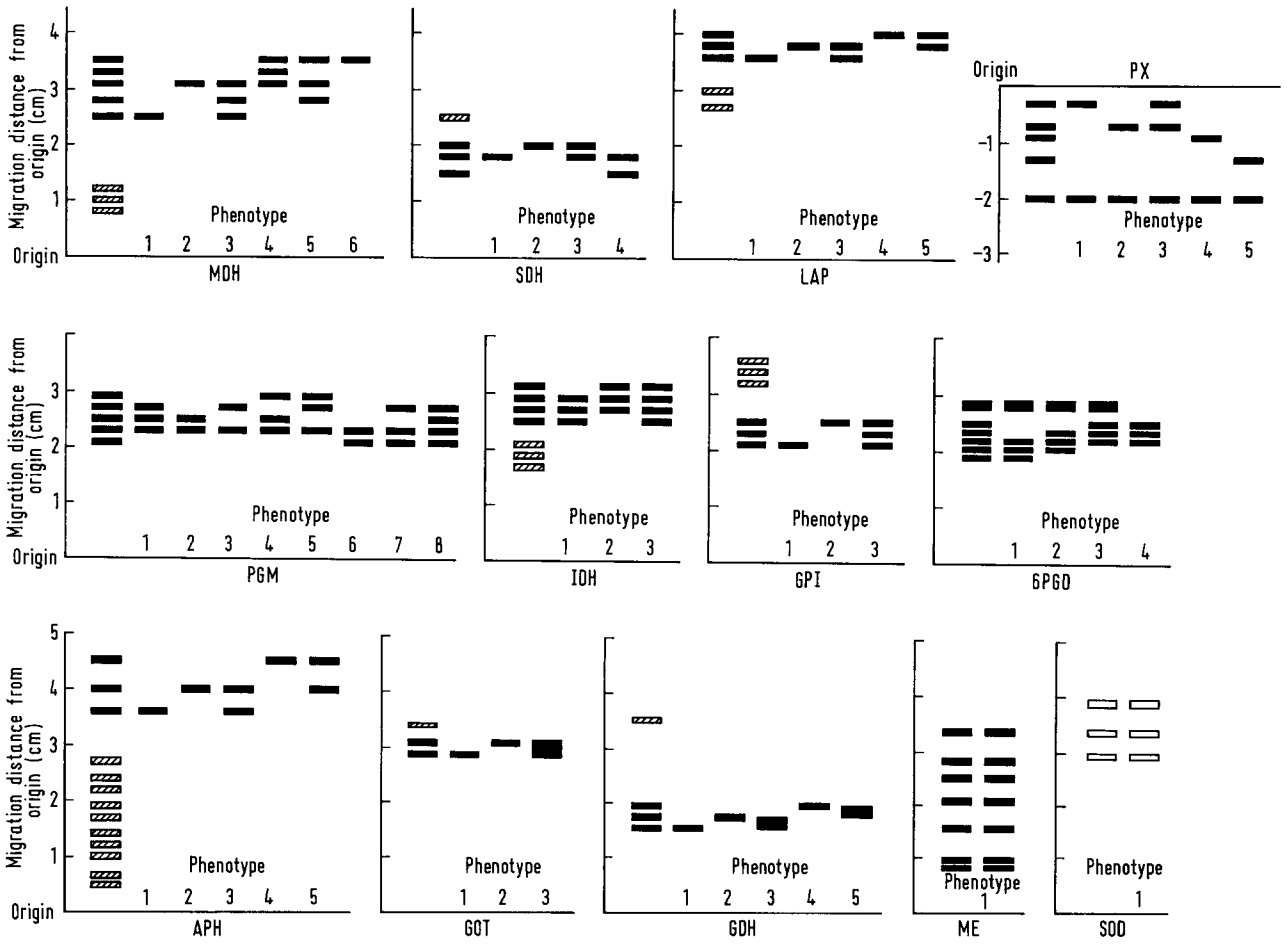

Fig. 1. Schematic diagrams of phenotypes observed for 13 enzymes

and 29 m*M* LiOH 19 m*M* boric acid, pH 8.0 (electrode) (Shaw and Prasad 1970). Electrophoresis was conducted for 7 h with a constant electric current of 30 mA initially until wicks were removed, and then at 20 mA in the first system, for 6 h with 35/25 mA in the second system and for 7 h with 60/45 mA in the third system.

With the first system, malate dehydrogenase (MDH), shikimate dehydrogenase (SDH), leucine aminopeptidase (LAP), phosphoglucomutase (PGM), cathodal peroxidase (PX), malic enzyme (ME) and superoxide dismutase (SOD) were assayed according to Brewer and Sing (1970), Neuman and Hart (1983), Smith and Rutenberg (1966), Tanksley and Orton (1983), Shaw and Prasad (1970) and Baum and Scandalios (1982), respectively. With the second system, isocitric dehydrogenase (IDH), glucose phosphate isomerase (GPI) and 6-phosphogluconate dehydrogenase (6PGD) were analyzed according to Longo and Tanksley (1968), Golding et al. (1985) and Shaw and Prasad (1970), respectively. With the third system acid phosphatase (APH), glutamate-oxaloacetate transaminase (GOT) and glutamate dehydrogenase (GDH) were assayed according to Brown et al. (1978) and Shaw and Prasad (1970), respectively.

Genetic interpretation of isozyme bands was completed for the five enzymes, LAP, PX, APH, GOT and GDH. Only phenotypic variation was investigated for all 13 enzymes. Possible phenotypes of each enzyme were determined by the number of bands and the migration differences from the origin compared with the reference controls, and then designated phenotype numbers. The magnitude of phenotypic polymorphism (Pj) and of weighted phenotypic polymorphism (P̄) were calculated according to Kahler et al. (1980). Furthermore, for the five enzymes specified above, allele frequencies and heterozygosity (He) were calculated (Nei 1972) for five loci, *Lap, Px-1, Aph-1, Got-2* and *Gdh-2*.

Results

Phenotypic variation of 13 enzymes

Malate dehydrogenase (MDH, EC 1.1.1.37). Two zones of MDH were revealed (Figs. 1 and 2). Both single-band and three-band phenotypes were detected in the second zone close to the origin. However, expression was weak, and therefore only the zymograms in the first zone were analyzed. Four isozyme phenotypes consisting of one or three bands were found in all three gene pools (Figs. 1 and 2), with phenotype 2 being most common. This was particularly the case in fodder beet, except that several individuals of cv Long Rose (B1035) showed phenotype 4. Phenotypes 3 and 2 were also common in both sugar beet gene pools, with phenotype 1 being only occasional-

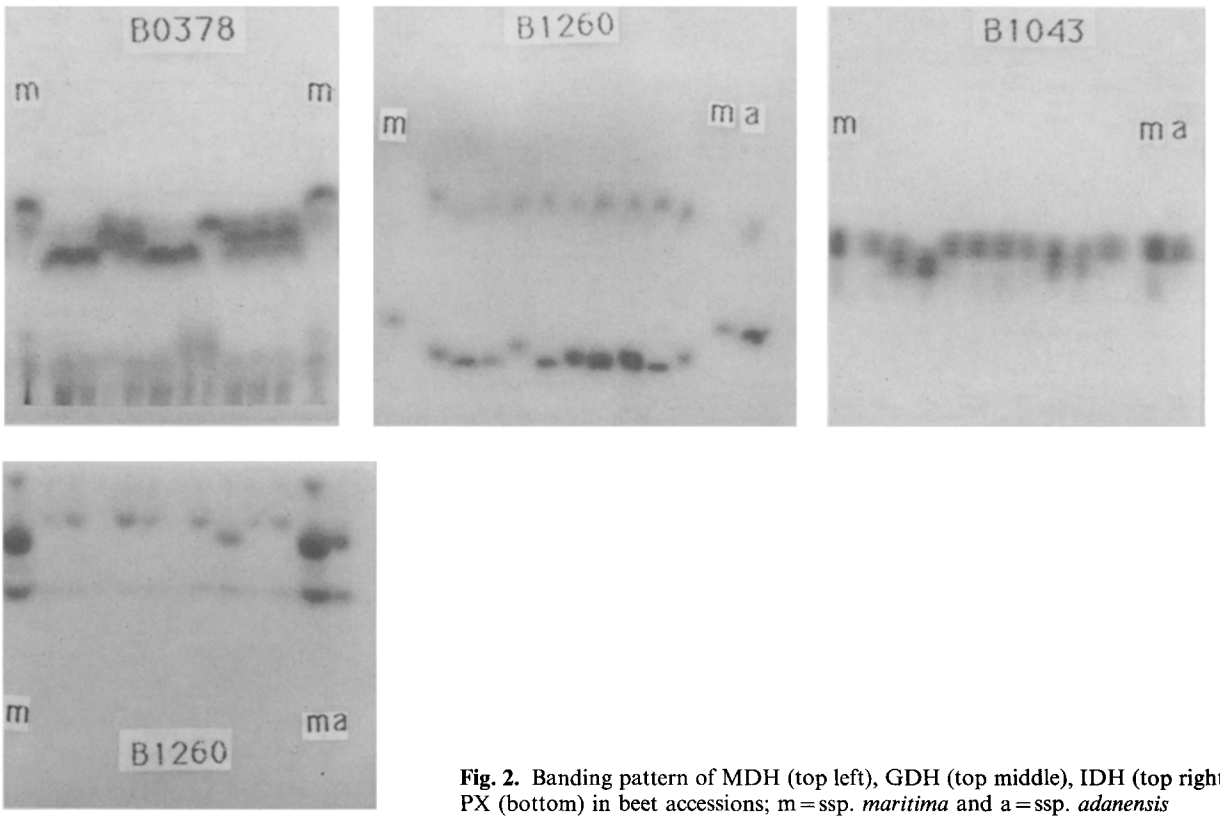

Fig. 2. Banding pattern of MDH (top left), GDH (top middle), IDH (top right) and PX (bottom) in beet accessions; m = ssp. *maritima* and a = ssp. *adanensis*

ly detected. Accordingly, both sugar beet gene pools showed greater polymorphism in MDH than the fodder beet gene pool.

In primitive and wild *Beta* species, three additional zymograms were identified. The majority of plants of *B. vulgaris, B. vulgaris* ssp. *asiatica,* ssp. *maritima* and ssp. *adanensis* showed phenotype 2. Phenotypes 4, 5 and 6 were unique to ssp. *macrocarpa.*

Shikimate dehydrogenase (SDH, EC 1.1.1.25). Two zones of SDH activity were detected, the first zone furthest from the origin was not analyzed due to weak expression. Two single-band phenotypes and two double-band phenotypes were revealed in the second zone (Fig. 1), according to the report that SDH in the genus *Beta* is monomeric (Oleo et al. 1986). Three phenotypes were recognized in cultivated beets. Phenotype 1 was the major type, phenotype 3 was rare and phenotype 2 was detected only in the fodder beet gene pool (B1035). It can be concluded, therefore, that SDH was not highly polymorphic.

An additional phenotype 4 was recognized in primitive *B. vulgaris* and wild ssp. *macrocarpa.*

Leucine aminopeptidase (LAP, EC 3.4.1.1). There were two zones of activity. As staining in the second zone was weak, only the first zone was analyzed. Three types of zymogram in the first zone were detected in the cultivated beets (Fig. 1) and two additional phenotypes were recognized in wild *Beta* species. The LAP enzyme has already been analyzed genetically by Abe and Tsuda (1987). According to their interpretation, LAP enzyme in beet is controlled by a single locus with three codominant alleles, Lap^1, Lap^2 and Lap^3. The bands in the first zone analyzed by us correspond to the alleles determined by Abe and Tsuda (1987). The corresponding genotypes of each phenotype described in Fig. 1 were as follows: phenotype $1-Lap^{3/3}$, $2-Lap^{2/2}$, $3-Lap^{2/3}$, $4-Lap^{1/1}$ and $5-Lap^{1/2}$, respectively. Phenotype 2 was of major occurrence in fodder beet, with 7 of the 12 fodder beet accessions being monomorphic. In sugar beet accessions, on the other hand, phenotype 3 was found as well as phenotype 2, and phenotype 1 was rare. Therefore, both sugar beet gene pools had a high phenotypic polymorphism for LAP.

Phenotypes 2 and 3 were also observed in *B. vulgaris, B. vulgaris* ssp. *asiatica,* ssp. *maritima* and ssp. *adanensis.* However, phenotypes 4 and 5 were recognized only in ssp. *macrocarpa,* and therefore allele Lap^1 could be regarded as being unique to ssp. *macrocarpa* as suggested by Abe and Tsuda (1987).

Phosphoglucomutase (PGM, EC 2.7.5.1). Two closely located zones were found. The first zone close to the

anodal front was faint, therefore, only the second zone was studied. Out of the 13 enzymes analyzed, PGM showed the greatest range of phenotypes. Five phenotypes could be recognized within the three cultivated beet gene pools (Fig. 1). Phenotype 1 predominated, followed by phenotype 3, while phenotype 2 was rare. Phenotypes 4 and 5 were extremely rare and were detected in only the fodder beet Long Greentop and Ovale Greentop (B1030 and B1032). Most accessions of cultivated beets showed high phenotypic polymorphism for PGM.

Two additional phenotypes were revealed in primitve and wild *B. vulgaris* (Fig. 1). Most individuals in *B. vulgaris, B. vulgaris* ssp. *asiatica,* ssp. *maritima* and ssp. *adanensis* expressed phenotypes 1, 2 and 3 as with the cultivated beet accessions.

Cathodal peroxidase (PX, EC 1.11.1.7). Bands of PX were both cathodal and anodal. Since the PX anodal bands were too faint to distinguish, only the cathodal bands were analyzed. There were two zones for cathodal PX. One double-band and four single-band phenotypes were observed in the first zone close to the origin. Only single bands were commonly detected in the second zone furthest from the origin (Figs. 1 and 2). Phenotype 1 showing a single band was predominant in all cultivated beet accessions, including most of the accessions of fodder beet. Phenotype 3 could be found occasionally in both sugar beet gene pools. Phenotype 2 was rare in cultivated beets.

PX was investigated genetically by Abe and Tsuda (1987). Two loci, *Px-1* and *Px-2,* control the isozyme of beet PX and two alleles were recognized at the *Px-1* locus. The genotypes of each PX phenotype designated were phenotype 1: $Px\text{-}1^{2/2}$, 2: $Px\text{-}1^{1/1}$ and 3: $Px\text{-}1^{1/2}$, respectively.

In the primitive and wild *Beta* species, two phenotypes were identified. Many individuals of primitive *B. vulgaris* and *B. vulgaris* ssp. *asiatica* expressed phenotype 1. Phenotypes 1, 2 and 3 were detected in ssp. *maritima,* in which polymorphism was clearly observed. In contrast, ssp. *macrocarpa* showed phenotypes 3 and 4, and ssp. *adanensis* phenotype 5. Isozymes of PX in those beet forms were clearly different from the rest of the materials studied.

Isocitric dehydrogenase (IDH, EC 1.1.1.42). Two closely located zones were recognized. Since the bands of the second zone were faint, zymograms of the first zone furthest from the origin were analyzed. Two phenotypes expressing three bands, but with different band positions, and one with four bands could be detected (Figs. 1 and 2). The phenotype showing four bands was a composite of the three-band types. Van Geyt and Smed (1984) recognized three- and five-band phenotypes in IDH. They assumed IDH to be controlled by two dimeric genes, but more genetical analysis is needed to confirm this. The major phenotypes in cultivated beet were 2 and 3. Phenotype 1 was occasionally detected in the sugar beet gene pool.

Phenotype 2 occurred in the primitive and wild *Beta* species with the exception of ssp. *maritima* (B0334), which showed polymorphism with three phenotypes.

Glucose phosphate isomerase (GPI, EC 5.3.1.9). At least two zones of GPI were recognized. Two or more additional bands appeared under the second zone close to the origin, after staining overnight. Maximally three bands could be observed in the first zone, but since the stainability was unsatisfactory for analysis, only the zymograms of the second zone were examined. Three phenotypes, namely two single-band phenotypes and one three-band phenotype, were detected in the second zone in cultivated and wild beets (Fig. 1). The three-band phenotype was common in cultivated beets. Phenotypic polymorphism was rarely revealed in fodder beet, but in contrast, the two sugar beet gene pools showed high polymorphism.

The wild *Beta* forms possessed similar phenotypes to the cultivated. Little difference of phenotype could be revealed between different wild beets. Van Geyt and Smed (1984) reported that crosses between the two single-band types reveal a pattern of three bands, probably reflecting a heterodimer.

6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44). Distinctive zymograms consisting of a single thick band with three closely adjacent bands were revealed with one exception. Three phenotypes (1–3) could be distinguished by the migration distances of the three bands (Fig. 1). Three phenotypes were detected in the cultivated beets. The major phenotype in cultivated or wild beets was overwhelmingly number 3, and the magnitude of phenotypic polymorphism was small. Phenotype 1 was occasionally found within fodder beets.

Phenotype 4, which lacked the thick band of phenotypes 1–3, was found in the primitive *B. vulgaris* (Fig. 1).

Acid phosphatase (APH, EC 3.1.3.2). A total of 13 bands was detected in the anodal zone. Of these, the most anodal isozymes have already been analyzed genetically (Abe and Tsuda 1987). Corresponding genotypes to phenotypes have been designated as follows: phenotype $1\text{–}Aph\text{-}1^{3/3}$, $2\text{–}Aph\text{-}1^{2/2}$, $3\text{–}Aph\text{-}1^{2/3}$, $4\text{–}Aph\text{-}1^{1/1}$ and $5\text{–}Aph\text{-}1^{1/3}$ (Fig. 1). The cultivated beet gene pools showed high phenotypic polymorphism. Phenotypes 1, 2 and 3 were observed in cultivated beets. Phenotype 2 was predominant. Phenotype 1 could be found in fodder beet accessions, but was rarely detected in either sugar beet gene pool.

Primitive or wild *B. vulgaris*, ssp. *asiatica*, ssp. *maritima* and ssp. *adanensis* were similar in phenotype to cultivated beets. However, ssp. *macrocarpa* uniquely possessed phenotypes 4 and 5. Therefore, allele $Aph\text{-}1^1$ was only to be found in ssp. *macrocarpa*.

Glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1). Two zones of active GOT could be visualized. Since the first zone close to the anode showed faint stainability, only the second zone was analyzed. The phenotypes were designated as in Fig. 1, and corresponded to the genotypes of the *Got-2* locus determined by Abe and Tsuda (1987) as follows: phenotype $1\text{-}Got\text{-}2^{2/2}$, $2\text{-}Got\text{-}2^{1/1}$ and $3\text{-}Got\text{-}2^{1/2}$. Three phenotypes were found in the cultivated beets. GOT differed from the other enzymes examined in that phenotypic polymorphism was hardly detected. Phenotypes 2 and 3 were found only in the fodder beets, Jaune Ovoide Barres (B1011) and Orange Barres (B1029). The remaining fodder beet and all of the sugar beet accessions showed phenotype 1.

In contrast, the primitive and wild *Beta* were more highly polymorphic for GOT.

Glutamate dehydrogenase (GDH, EC 1.4.1.2). The bands controlled by three alleles at the *Gdh-2* locus designated by Abe and Tsuda (1987) were analyzed. Besides these bands, the zone of the *Gdh-1* locus was observed, but the bands were faint and analysis of this zone was excluded. Three phenotypes, 1, 2 and 3, which correspond to the genotypes $Gdh\text{-}2^{3/3}$, $Gdh\text{-}2^{2/2}$ and $Gdh\text{-}2^{2/3}$, respectively, were found in the cultivated beets (Figs. 1 and 2). A high level of phenotypic polymorphism was recognized in the three gene pools, with the predominant phenotypes being 1 and 3.

In primitive and wild *Beta* species, two additional phenotypes 4 and 5, corresponding to genotypes $Gdh\text{-}2^{1/1}$ and $Gdh\text{-}2^{1/2}$, respectively were observed (Figs. 1 and 2). *B. vulgaris* (primitive), *B. vulgaris* ssp. *asiatica* and ssp. *maritima* had similar phenotypes to those of cultivated beets, but in contrast, ssp. *macrocarpa* and ssp. *adanensis* uniquely displayed phenotypes 4 and 5.

Malic enzyme (ME, EC 1.1.1.40). Although a total of seven heavily stained and clear bands could be detected in both cultivated and wild beets, the number of bands and migration distances from the origin did not vary within and between accessions (Fig. 1). It is concluded that beet ME may therefore be a monomorphic enzyme in contradiction to the results of Van Geyt and Smed (1984), who reported that several alleles of ME could be detected using PAGE.

Superoxide dismutase (SOD, EC 1.15.1.1). A total of three bands (one thick and two thin) were identified (Fig. 1), but with no detectable differences in band number and migration within and between accessions.

Phenotypic polymorphism

Genetic interpretation of eight enzymes – MDH, SDH, PGM, IDH, GPI, 6PGD, ME and SOD – has not been attempted, but phenotypic variation of all 13 enzymes has been assessed. The magnitude of variation in isozyme phenotypes was expressed by the estimation of phenotypic polymorphism (Pj) of Kahler et al. (1980). Pjs of 13 enzymes for 33 accessions falling within the three cultivated beet gene pools are shown in Table 2. Table 3 shows the average Pjs for the 3 beet gene pools for the 13 enzymes. Among the three gene pools, GDH polymorphism was the highest. Pjs of PGM, IDH, APH and MDH were also high in comparison with that of GOT, SDH and 6PGD, which were low. ME and SOD were monomorphic. It can be seen, therefore, that the degree of polymorphism varied between different enzymes. The average PJs within the three beet gene pools were as follows: fodder beet – 0.180, multigerm sugar beet – 0.247 and monogerm sugar beet – 0.233, respectively. There was a significant difference between Pj of fodder beet and multigerm sugar beet, and also between fodder and monogerm sugar beet ($t=-3.44$, $df=21$; $t=-2.50$, $df=20$). On the other hand, no significant difference existed between the Pj of multigerm sugar beet and monogerm sugar beet ($t=0.61$, $df=19$). Consequently, the magnitude of genetic variability in both sugar beet gene pools with regard to enzyme loci was larger than that of fodder beet. In addition, the weighted phenotypic polymorphism ($\bar{P}$) indicated that the two sugar beet gene pools represented a higher isozyme variability in comparison with the fodder beet gene pool.

Genotype and allele frequency

For five isozyme-coding loci, *Lap, Px-1, Aph-1, Got-2* and *Gdh-2*, genotypes and allele frequencies could be determined by the comparison of bands of each accession and of two reference controls, namely ssp. *macrocarpa* ($Lap^{1/1}$, $Px\text{-}1^{1/1}$, $Aph\text{-}1^{1/1}$, $Got\text{-}2^{1/1}$ and $Gdh\text{-}2^{1/1}$) and ssp. *adanensis* ($Lap^{3/3}$, $Px\text{-}1^{1/1}$, $Aph\text{-}1^{1/1}$, $Got\text{-}2^{1/1}$ and $Gdh\text{-}2^{1/1}$), the genotypes of which had already been designated by Abe and Tsuda (1987). Table 4 shows the alleles observed in the three cultivated beet gene pools and the primitive/wild material. Allele Lap^2 was predominant in the cultivated beets and frequencies of Lap^3 varied from 11% (fodder beet) to 35% (monogerm sugar beet). On the other hand, allele Lap^3 was not observed in wild beet, but was found in primitive *B. vulgaris*, and allele Lap^1 was entirely specific to ssp. *macrocarpa*.

Allele $Px\text{-}1^2$ was predominant in the cultivated beets. The primitive *B. vulgaris* had similar allele frequencies to those observed in cultivated beets. The allele frequency of

Table 2. Phenotypic polymorphism (Pj) for 13 enzymes in 33 accessions of three cultivated beet gene pools

No.	Acc. no.	MDH	SDH	LAP	PGM	PX	IDH	GPI	6PGD	APH	GOT	GDH	ME	SOD
1	B1006	0.00	0.32	0.00	0.46	0.54	0.18	0.46	0.54	0.42	0.00	0.50	0.00	0.00
2	B1011	0.00	0.18	0.00	0.56	0.00	0.18	0.18	0.18	0.18	0.66	0.18	0.00	0.00
3	B1029	0.00	0.00	0.00	0.42	0.00	0.48	0.00	0.42	0.00	0.48	0.62	0.00	0.00
4	B1030	0.32	0.00	0.42	0.66	0.48	0.00	0.00	0.00	0.62	0.00	0.32	0.00	0.00
5	B1032	0.18	0.00	0.54	0.34	0.18	0.18	0.00	0.00	0.00	0.00	0.18	0.00	0.00
6	B1035	0.42	0.46	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.66	0.00	0.00
7	B1037	0.32	0.00	0.18	0.42	0.00	0.32	0.00	0.00	0.64	0.00	0.42	0.00	0.00
8	B1038	0.00	0.00	0.00	0.62	0.00	0.18	0.00	0.00	0.48	0.00	0.62	0.00	0.00
9	B1040	0.00	0.00	0.42	0.00	0.18	0.58	0.18	0.48	0.64	0.00	0.34	0.00	0.00
10	B1055	0.42	0.00	0.00	0.18	0.18	0.18	0.32	0.42	0.64	0.00	0.54	0.00	0.00
11	B1058	0.18	0.32	0.00	0.00	0.00	0.50	0.00	0.64	0.18	0.00	0.58	0.00	0.00
12	B1064	0.00	0.00	0.00	0.32	0.00	0.18	0.00	0.00	0.48	0.00	0.32	0.00	0.00
13	B0079	0.00	0.00	0.48	0.58	0.46	0.66	0.42	0.00	0.48	0.00	0.48	0.00	0.00
14	B0081	0.50	0.32	0.18	0.18	0.32	0.48	0.32	0.42	0.18	0.00	0.58	0.00	0.00
15	B0112	0.62	0.18	0.58	0.62	0.54	0.18	0.50	0.00	0.32	0.00	0.32	0.00	0.00
16	B0113	0.56	0.18	0.00	0.58	0.18	0.42	0.64	0.00	0.00	0.00	0.46	0.00	0.00
17	B0115	0.50	0.48	0.50	0.50	0.18	0.58	0.46	0.00	0.42	0.00	0.56	0.00	0.00
18	B0116	0.00	0.00	0.42	0.34	0.18	0.18	0.32	0.18	0.32	0.00	0.18	0.00	0.00
19	B0117	0.48	0.00	0.00	0.50	0.32	0.00	0.34	0.00	0.32	0.00	0.34	0.00	0.00
20	B0788	0.00	0.00	0.46	0.62	0.56	0.00	0.32	0.00	0.58	0.00	0.50	0.00	0.00
21	B1043	0.46	0.18	0.00	0.48	0.58	0.54	0.48	0.00	0.00	0.00	0.66	0.00	0.00
22	B1044	0.62	0.32	0.18	0.50	0.32	0.42	0.32	0.00	0.00	0.00	0.50	0.00	0.00
23	B1075	0.58	0.00	0.00	0.66	0.18	0.56	0.54	0.32	0.32	0.00	0.00	0.00	0.00
24	B0076	0.00	0.18	0.00	0.42	0.18	0.50	0.48	0.00	0.42	0.00	0.00	0.00	0.00
25	B0078	0.54	0.18	0.00	0.42	0.46	0.62	0.18	0.00	0.00	0.00	0.66	0.00	0.00
26	B0378	0.58	0.48	0.54	0.48	0.00	0.50	0.42	0.32	0.46	0.00	0.58	0.00	0.00
27	B1259	0.50	0.18	0.46	0.18	0.64	0.18	0.18	0.00	0.50	0.00	0.18	0.00	0.00
28	B1260	0.32	0.32	0.32	0.46	0.18	0.46	0.18	0.00	0.00	0.00	0.62	0.00	0.00
29	B1261	0.48	0.00	0.48	0.54	0.00	0.50	0.48	0.48	0.42	0.00	0.66	0.00	0.00
30	B1262	0.00	0.00	0.42	0.00	0.00	0.56	0.32	0.32	0.46	0.00	0.48	0.00	0.00
31	B1263	0.32	0.00	0.18	0.34	0.42	0.34	0.34	0.00	0.00	0.00	0.48	0.00	0.00
32	B1264	0.50	0.00	0.34	0.48	0.46	0.42	0.18	0.00	0.42	0.00	0.50	0.00	0.00
33	B1265	0.18	0.00	0.48	0.42	0.18	0.18	0.34	0.00	0.32	0.00	0.46	0.00	0.00

Table 3. Phenotypic polymorphism (Pj) of 13 enzymes in three cultivated beet gene pools

Enzymes	MDH	SDH	LAP	PGM	PX	IDH	GPI	6PGD	APH	GOT	GDH	ME	SOD	Average Pj	Average P
Fodder	0.153	0.107	0.165	0.332	0.130	0.247	0.095	0.223	0.356	0.095	0.440	0.000	0.000	0.180	0.121
Old sugar	0.393	0.151	0.255	0.505	0.347	0.365	0.424	0.083	0.267	0.000	0.416	0.000	0.000	0.247	0.166
Modern sugar	0.342	0.134	0.322	0.374	0.252	0.426	0.310	0.112	0.300	0.000	0.462	0.000	0.000	0.233	0.160

Table 4. Allele frequencies of 13 alleles in five loci in three cultivated beet gene pools and wild or primitive *B. vulgaris* species

Enzyme loci		*Lap*			*Px-1*		*Aph-1*			*Got-2*		*Gdh-2*		
Alleles		1	2	3	1	2	1	2	3	1	2	1	2	3
Fodder	(12)[a]	0.00	0.89	0.11	0.06	0.94	0.00	0.69	0.31	0.07	0.93	0.00	0.36	0.64
Old sugar	(11)	0.00	0.84	0.16	0.15	0.85	0.00	0.85	0.15	0.00	1.0	0.00	0.23	0.77
Modern sugar	(10)	0.00	0.65	0.35	0.11	0.89	0.00	0.86	0.14	0.00	1.0	0.00	0.33	0.67
B. vulgaris	(3)	0.00	0.67	0.33	0.10	0.90	0.00	0.60	0.40	0.17	0.83	0.00	0.40	0.60
B. asiatica	(2)	0.00	0.53	0.47	0.00	1.0	0.00	0.58	0.42	0.00	1.0	0.00	0.40	0.60
B. maritima	(3)	0.00	1.0	0.00	0.33	0.67	0.00	0.88	0.12	0.17	0.83	0.00	0.13	0.87
B. macrocarpa	(2)	0.63	0.37	0.00	0.15[b]	0.15[b]	0.73	0.27	0.00	0.63	0.37	0.73	0.28	0.00
B. adanensis	(1)	0.00	1.0	0.00	0.00[b]	0.00[b]	0.00	1.0	0.00	1.0	0.00	1.0	0.00	0.00

[a] Number of accessions used [b] Other alleles were found

Px-1^1 in ssp. *macrocarpa* was comparatively higher than for cultivated beets and other wild beets. New isozymes close to *Px-1* bands were discovered in ssp. *macrocarpa* and ssp. *adanensis*.

At the *Aph-1* locus, allele *Aph-1*^{2} was predominant and no allele *Aph-1*^{1} was observed. The frequency of allele *Aph-1*^{3} in fodder beet was higher than in both sugar beet gene pools. Primitive *B. vulgaris* and wild ssp. *asiatica*, ssp. *maritima* and ssp. *adanensis* had similar allele frequencies to those of cultivated beets; however, ssp. *macrocarpa* possessed a unique allele, *Aph-1*^{1}, which appears suitable for characterizing this subspecies.

Allele *Got-2*^{2} was predominant in the cultivated beets, although there was a low frequency of allele *Got-2*^{1} in fodder beets. The *Got-2* locus of sugar beets was fixed with respect to allele *Got-2*^{2}. Primitive *B. vulgaris*, wild ssp. *asiatica* and ssp. *maritima* had high frequencies of *Got-2*^{2} as with cultivated beets, but *Got-2*^{1} was of high frequency in ssp. *macrocarpa* and ssp. *adanensis*.

Only alleles *Gdh-2*^{2} and *Gdh-2*^{3} were found in cultivated beets, with no significant difference in frequency. Only *Gdh-2*^{1} and *Gdh-2*^{2} were found in ssp. *macrocarpa*, while *Gdh-2*^{1} appeared to be fixed in ssp. *adanensis*.

Heterozygosity in three cultivated gene pools

Table 5 shows the average heterozygosities (He) within the three beet gene pools. Heterozygosity for the *Got-2* locus was the lowest for the five loci analyzed, and was zero for both sugar beet gene pools. The heterozygosities of *Px-1* and *Lap* loci in fodder beet were lower than those in both sugar beets, but the He of *Aph-1* and *Gdh-2* loci in fodder beet were higher than those in both sugar beet gene pools. The average heterozygosities for five loci showed no significant difference between the three gene pools, a result which contrasted with the phenotypic polymorphism of the 13 enzymes.

Isozyme polymorphism in F_1 hybrid accessions

F_1 hybrid varieties derived from crosses between pure lines should show consistent isozyme structures among individuals in the varieties. With the exception of ME and SOD, which were monomorphic and GOT, which revealed low polymorphism only in fodder beets, ten enzymes showed isozyme polymorphism in the F_1 hybrid triploid varieties, namely the monogerm sugar beet. Particularly high phenotypic polymorphism was observed in GDH, IDH, PGM, MDH, LAP and GPI (Table 2), indicating that varieties of sugar beet consist of a range of heterogeneous genotypes showing isozyme variation. This is ascribed either to the low homozygosities of isozyme-coding loci in one or both parents of F_1 hybrids, or to the imperfect expression of cytoplasmic male sterility giving rise to uncontrolled cross-pollination. Other, more detailed observations could also be made. For instance, in terms of LAP, GOT and GDH, the variety Hilleshog Monotri (B0076) has been produced from parental genotypes which had identical allelic constitutions. The same applied to PX for varieties Hilma (B1261) and Monoire (B1262). In contrast, for LAP, Bush Mono G (B1260) must have resulted from parents possessing different alleles at this locus, resulting predominantly in heterozygous F_1 individuals.

Table 5. Average heterozygosities of five enzyme-coding loci in three cultivated beet gene pools

Enzyme loci	*Lap*	*Px-1*	*Aph-1*	*Got-2*	*Gdh-2*
Fodder beet	0.135	0.088	0.284	0.076	0.388
Old sugar beet	0.205	0.230	0.199	0.000	0.318
Modern sugar beet	0.297	0.166	0.222	0.000	0.348

Discussion

Sugar beet as well as other cultivated beets such as Swiss chard, garden beet and fodder beet, falls within the section *Beta* of the genus *Beta* L. and constitutes a single species, *B. vulgaris* L., (Ford-Lloyd and Williams 1975; Ford-Lloyd 1986). Since sugar beet is known to be derived, in part, from a fodder beet population (Bosemark 1979; De Bock 1986), the significance of fodder beet as a genetic resource and the genetic relationship between sugar beet and fodder beet may be of importance to the sugar beet breeder. Root shape, fruit shape and leaf characters have been employed for conventional evaluation, leaving aside other economically important and highly selected characteristics. The limited number of reliable genetic markers available [for instance pigmentation gene (R) and non-restorer genes] causes certain difficulties when trying to understand relationships between species and when analyzing the genotype constitution of germplasm within species. We have analyzed isozymes to overcome such obstacles. All modern sugar beet varieties share a genetic base which, according to some, is not particularly narrow from the viewpoint of nuclear genes (Bosemark 1979), even though they have common ancestry at least in terms of the monogerm character and the S-cytoplasm used to create F_1 hybrids. We have, therefore, analyzed genetic diversity in this modern sugar beet gene pool and compared it with two other gene pools, namely that composed of old multigerm sugar beet varieties grouped together for comparative purposes because they do not possess the monogerm characteristic and are generally not F_1 hybrids containing the S-cytoplasm, and secondly the putatively ancestral fodder beets as a third gene pool.

Full genetic interpretation of isozymes has only been completed for five loci; otherwise, phenotypic variation of isozymes has been assessed. Two of the 13 enzymes

analyzed appeared to be monomorphic. Eleven enzymes showed variation of isozyme phenotypes. Of these, both high and low values of phenotypic polymorphism were found. No significant difference in polymorphism between multigerm and monogerm sugar beets was recognized for any enzyme. However, differences were found between fodder beet and both groups of sugar beets for three enzymes. The average polymorphism for 13 enzymes in both sugar beet groups was significantly higher than that in fodder beet. Small differences in allele frequencies of 13 alleles at five enzyme-coding loci were observed between the three gene pools.

Abe and Tsuda (1987) found a similar allele composition in fodder beet and sugar beet using the same five loci, and only a slight distinction of allele composition at the *Px-1* locus between them. Our results for allele composition in fodder beet and sugar beets were similar except that new alleles $Px\text{-}1^1$ and $Got\text{-}2^1$ were discovered, having low frequencies in fodder beet. Heterozygosity at the *Px-1* and *Lap* loci in fodder beet was smaller than that in both sugar beet groups. Consequently, in terms of both allele frequency and heterozygosity at the five loci, no significant difference of isozyme diversity was demonstrated between the three cultivated beet gene pools. In contrast, in terms of phenotypic polymorphism of the 13 enzymes, a highly significant difference in isozyme diversity was found between fodder beet and the two sugar beet groups, the sugar beet being more diverse.

As fodder beet is considered to have been one of the ancestors of sugar beet, greater isozyme diversity might have been encountered in the fodder beet gene pool. Our findings contradict this, and could be explained by the fact that hybridization between two gene pools of fodder beet and either wild beet or leaf beet gave rise to the first breeding material of sugar beet (Bosemark 1979). Even the small numbers of wild forms of beet used in our study showed a different pattern of allele diversity from that of cultivated forms, and may indicate further the contribution made to the diversity currently found amongst sugar beets. Existence of comparatively high levels of polymorphism within sugar beet supports to some extent Bosemark's view that genetic variation within present diploid and triploid hybrids is sufficiently large for breeding purposes (Bosemark 1979). It is still conceivable, however, that some unique and important genetic variation may be confined at present to fodder beet germplasm, as is exemplified by the exclusive occurrence of some alleles of four enzymes in our study. Even more significant is the fact that for eight enzymes, MDH, SDH, LAP, PGM, 6PGD, APH, GOT and GDH, several unique isozymes are to be found in primitive and wild beets, especially in ssp. *macrocarpa* and ssp. *adanensis*. This serves as an indication that truly novel genetic variation is primarily available well outside the normal breeding gene pools of sugar beet.

Finally, as regards the use of isozymes as genetic and taxonomic markers, the existence of alleles or isozymes which are confined solely to particular gene pools or taxa is significant. They may be used to monitor gene flow or introgression between such gene pools or taxa in either artificial or natural situations. They may be useful for instance to determine the degree to which hybridization may contribute to the "weed beet" problem within Europe (Evans and Weir 1981; Hornsey and Arnold 1979).

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