PHOSPHOGLUCOSE ISOMERASE (PGI) POLYMORPHISM IN *SORBUS TORMINALIS* (L.) CRANTZ

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ABSTRACT

Dormant buds of individual *Sorbus torminalis* trees collected from eight Polish populations were tested with horizontal gel electrophoresis according to one locus of PGI enzyme system. Three allozymes of different frequency were identified in each population. Genetic variability based on PGI locus A was high within seven populations (mean Ho=0.602 and mean Pg=0.491). Only one population with A_3A_3 genotypes was monomorphic, most probably due to intensive vegetative propagation or founder effect. The level of genetic differences among populations based on one variable locus reached GST=0.13.

KEY WORDS: *Sorbus torminalis*, electrophoresis, PGI, genetic diversity.

INTRODUCTION

The genus *Sorbus* L. (Rosaceae: Maloideae) comprises around 250 species, distributed mainly in north temperate regions (Phipps et al. 1990). In Poland, the following six species growing in the wild have been recognised: *S. aria*, *S. aucuparia*, *S. chamaemespilus*, *S. graeca*, *S. intermedia* and *S. torminalis* (Mirek et al. 1995).

Sorbus torminalis is native to Europe, Asia Minor, Caucasus and North-western Africa. The species originated in East Asia and reached Europe during the Miocene (Gabrielian 1978). In Europe, the most numerous and richest *S. torminalis* stands are situated in the southern parts of its range, most frequently growing in the lower highland biotopes (Browicz and Gostyńska-Jakuszewska 1966).

European species belonging to the genus *Sorbus* were intensively examined in the last decade including *S. torminalis* (Proctor et al. 1989; Proctor and Groenhof 1992; Prat and Daniel 1993; Aas et al. 1994; Raspé and Jacquemart 1998; Krzakowa and Bednorz 1999; Demesure et al. 2000; Raspe et al. 2000; Oddou-Muratorio et al. 2001). From eleven enzyme systems investigated in 73 *S. torminalis* populations (Demesure et al. 2000), none was examined according to phosphoglucose isomerase (PGI; EC 5.3.1.9). PGI, as one of the most important enzyme systems, catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. When it is active in chloroplasts, the enzyme reaction is one of a sequence of steps that converts 3-phosphoglycerate to starch in the light (Gottlieb 1983). The PGI enzyme system is a good marker for population variability description.

In Poland, the *S. torminalis* range reaches its north-eastern limits (Fig. 1) and the species grows mainly in lowlands. *S. torminalis* is one of the few woody species protected by

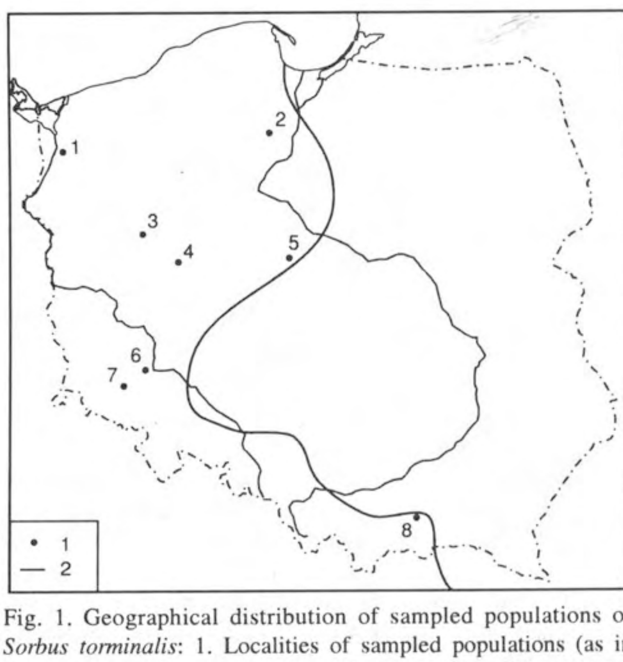

Fig. 1. Geographical distribution of sampled populations of *Sorbus torminalis*: 1. Localities of sampled populations (as in Table 1), 2. North-eastern range of *S. torminalis* in Poland (After Goetz 1928).

TABLE 1. Geographical coordinates and numbers of sampled populations of *Sorbus torminalis*.

No. of population	Population (Locality)	No. of trees	Latitude	Longitude	Altitude (m)
1	'Puszcza Bukowa' near Szczecin	11	53°20' N	14°38' E	60-70
2	'Brzęki im. Zygmunta Czubińskiego' reserve	27	53°39' N	18°22' E	98-101
3	'Brzęki przy Starej Gajówce' reserve	21	52°28' N	16°28' E	100
4	Wielkopolski National Park	22	52°16' N	16°48' E	85-100
5	'Kawęczyńskie Brzęki' reserve	19	52°24' N	18°37' E	120-130
6	'Brekinia' reserve	21	51°17' N	16°21' E	110
7	Forest District Jawor	32	51°01' N	16°08' E	300-395
8	'Białowodzka Góra' reserve	26	49°41' N	20°38' E	500-550

law in Poland. Its localities are marginal, dispersed and scanty and the species is considered to be disseminated in Polish forests (Stecki 1949; Olaczek 1976; Pacyniak 1991).

It is well known that the amount and pattern of genetic variation determine the ability of forest tree species to adapt to the variability and changes of environmental conditions. It is particularly important for rare and declining species (such as *S. torminalis* in Poland), when in small populations there is a great threat of losing genetic diversity, which determines the population fitness and survival.

The purpose of this study is to quantify the genetic variation within and among populations of *S. torminalis* in Poland according to PGI allozyme markers, not examined in the species till now.

MATERIAL AND METHODS

Plant material

A total of 8 populations were sampled from the entire range of *S. torminalis* in Poland (Fig. 1, Table 1). From 11 to 32 individual trees were sampled per population. In March 2000, dormant buds were collected, just before emerging, from a total of 179 trees.

Electrophoresis procedures

Ten dormant buds, collected from individual trees, were homogenised in 0.12 M Tris/HCl pH 7.5 which was mixed with 4%w/v polyvinylpyrolidone (PVP 40.000), 0.07% Na-EDTA, 0.2% DTT and 0.13% bovine serum albumin. The extracts were absorbed on Whatman chromatography 3 MM paper wicks (size 4×4 mm). Electrophoresis was performed on horizontal, 11% starch gels (Sigma) in Ashton buffer system pH 8,1 and staining procedure was adopted from Vallejos (1983) with some minor modifications. Enzyme separation was carried out with a constant voltage of 150 V.

Data analysis

Calculations of genetic parameters such as observed heterozygosity (Ho), expected heterozygosity (He) and total genetic diversity (HT), mean diversity within populations (HS), fixation indices (F), polymorphism indices of genotypes (Pg), relative measure of genetic differentiation between populations (GST,DST) and genetic similarities among populations based on gene frequency (Nei 1972), genotypes frequencies (Hedrick 1974) and genetic distances (DN, DH) were constructed as described by Jain and Workman (1967), Nei (1987) and El-Kassaby (1991) using the GEN computer program (Nowak-Bzowy and Bzowy – unpublished).

RESULTS

Electrophoretically detected PGI band patterns are grouped in one zone of activity showing three allozymes. One-banded electrophoretic phenotypes are considered as homozygotes of their locus, whereas heterozygotes represent three-banded phenotypes indicating dimeric structure of the enzyme (Fig. 2). Such configuration of PGI band patterns is very similar to that described earlier in *Sorbus latifolia* (Aas et al. 1994).

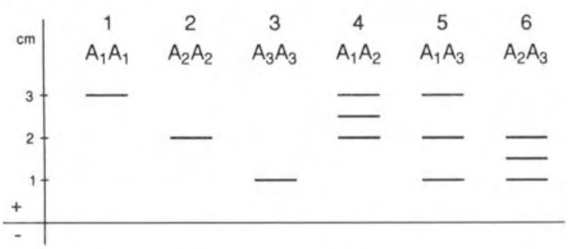

Fig. 2. PGI. Schematic allozyme phenotypes of *Sorbus torminalis*.

The populations show differences in heterozygosity level (Ho). The most heterozygotic was population No. 4 from Wielkopolski National Park (Table 3), while population No. 6 was monomorphic (see also Table 2). The most polymorphic were populations Nos. 7, 5 and 2 respectively. The fixation indices in populations nos. 1 and 5 are close to

TABLE 2. Allele frequencies in populations of *Sorbus torminalis* for PGI locus A.

Allele	Population							
	1	2	3	4	5	6	7	8
1	0.05	0.02	0.00	0.41	0.29	0.00	0.20	0.31
2	0.05	0.24	0.43	0.05	0.11	0.00	0.17	0.00
3	0.91	0.74	0.57	0.55	0.61	1.00	0.63	0.69

TABLE 3. Genetic diversity estimates in populations of *Sorbus torminalis* based on one PGI locus A. He – expected heterozygosity, Ho – heterosygosity observed, F – inbreeding coefficient, Pg – polymorphism index.

Population	He	Ho	F	Pg
1	0.1694	0.1818	-0.0732	0.3140
2	0.3930	0.4444	-0.1309	0.5624
3	0.4898	0.7619	-0.5556	0.3810
4	0.5331	0.9091	-0.7054	0.3140
5	0.5388	0.5789	-0.0746	0.7091
7	0.5386	0.7188	-0.3345	0.6816
8	0.4260	0.6154	-0.4444	0.4734
6	0.0000	0.0000		0.0000

zero indicating that the populations are in Hardy-Weinberg equilibrium with the exception of monomorphic population No. 6 and remaining populations of higher frequency of heterozygotes.

The dendrogram (Fig. 3A) shows the genetic similarities between populations based on allele frequencies. The populations form two main clusters (Fig. 3A) not connected with their geographic distribution. Also the dendrite (Fig. 3B) constructed on the basis of genotype frequencies shows similar connections between populations.

Most of enzyme systems investigated earlier are considered in *S. torminalis* as low polymorphic (6-PDH, PRX, SKDH, AAP, IDH, GOT) and some of them are even completely monomorphic (ACP, MR) in the populations studied. The most polymorphic enzyme systems were ME, ADH and PGM (Demesure et al. 2000). In the study presented here, PGI is a highly polymorphic enzyme system and although it is the most expensive to work with, it is worth to be recommended for broader investigations.

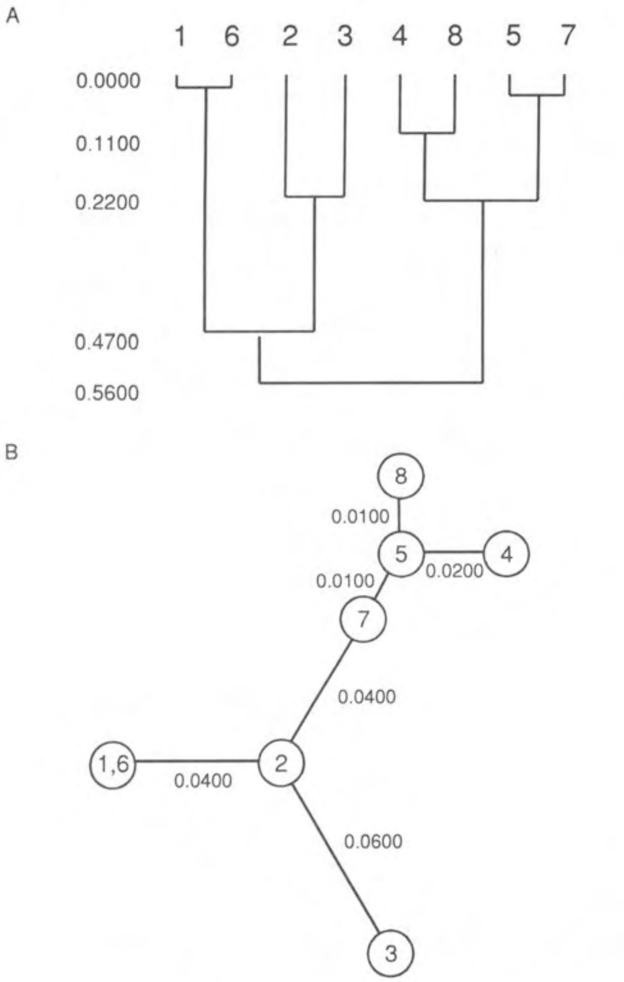

Fig. 3. Dendrogram (A) and dendrite (B) illustrate the genetic similarities of *Sorbus torminalis* populations based on gene and genotype frequencies respectively.

DISCUSSION

Adaptive strategies of species in marginal populations differ from species to species. *S. torminalis* populations in Poland are dispersed, so gene exchange between them must be limited and even may not occur at all. Existence of a monomorphic population with evident domination of one homozygotic genotype (population No. 6 with A_3A_3 PGI genotypes) or fixed heterozygosity (PGI-A_1A_3 genotype in population No. 4) may suggest strong influence of vegetative reproduction. Besides, long distances between populations (over 100 km) disturb genes exchange and may cause a certain amount of genetic differentiation through founder effect.

Extensive examinations of genetic diversity in *S. torminalis* based on five enzyme systems were carried out in 26 French populations (Prat and Daniel 1993) and later on eleven enzyme systems in 73 populations (Demesure et al. 2000). Not all investigated enzyme systems proved to be polymorphic, and the level of heterozygosity was low (Ho ranged from 0.086 to 0.274). The level of heterozygosity (Ho) in populations studied by us ranged from 0.182 to 0.909. The level of genetic differentiation within Polish populations HS=0.41 is higher than among populations GST=0.13 (total genetic diversity HT=0.47). Since the results of our study are based on the variability of one enzyme locus and the fact that PGI enzyme system was not investigated in *S. torminalis* so far, it is impossible to compare our results with those regarding to populations from other geographical regions.

In the woody plant species, the values of their mating system, seed dispersal, mechanism and evolutionary history of the species may have a major influence on genetic variation within and among populations (Hamrick et al. 1992). The broad investigations on genetic structure and gene flow in *S. torminalis* have been carried out recently (Oddou-Muratorio et al. 2001). Chloroplast DNA variation was investigated in French populations of the species at the same scale as the previous survey of isozyme diversity (Demesure et al. 2000) to confront patterns of variation for the two markers and to assess pollen/seed mediated gene flow. The coefficient of differentiation among populations (cytoplasmic GST_c=0.34) although higher than nuclear (GST_n=0.11) was one of the lowest found in forest trees so far, and the mean within-population diversity was relatively high.

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POLIMORFIZM FOSFOGLUKOIZOMERAZY (PGI) U *SORBUS TORMINALIS* (L.) CRANTZ

STRESZCZENIE

Osiem polskich populacji brekinii (*Sorbus torminalis*) zostało scharakteryzowane pod względem częstości elektroforetycznych fenotypów fosfoglukoizomerazy (PGI) określonych w pączkach śpiących pojedynczych drzew. Trzy allozymy jednego locus występowały w populacjach z różną częstością. Populacje okazały się wysoce polimorficzne (średnie wartości Ho=0,602 i Pg=0,491), z wyjątkiem jednej, która okazała się monomorficzna pod względem genotypu A3A3. Należy przypuszczać, że populacja ta utrzymuje się na skutek intensywnej wegetatywnej propagacji jednego genotypu. Poziom genetycznego zróżnicowania badanych populacji opartych na zmienności jednego locus osiągnął wartość GST=0,13.

SŁOWA KLUCZOWE: *Sorbus torminalis*, elektroforeza, PGI, struktura genetyczna.