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Genetic diversity of *Avena strigosa* Schreb. ecotypes on the basis of isoenzyme markers

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Abstract: Genetic diversity was analyzed in 19 ecotypes of the diploid oat *A. strigosa* originating from various geographical regions of the world. Six isoenzyme systems (AAT, ACP, EST, LAP, MDH, PX) were studied and 16 loci were identified. Only two loci (*Est4* and *Mdh2*) were polymorphic. Ecotypes were characterized by the percentage of polymorphic loci (P=3.3%), the mean number of alleles per locus (A=1.04) and intrapopulation diversity (H_S=0.013). Total genetic diversity (H_T=0.07) and interpopulation diversity (D_{ST}=0.057) were examined as well. The value of the coefficient of gene differentiation (G_{ST}=0.821) indicated that diversity among populations was an important contributor to total variability. Genetic similarity between *A. strigosa* populations was very high (I_N=0.94). Cluster analysis did not demonstrate strongly differentiated groups among the ecotypes examined.

Key words: *Avena strigosa*, isozyme, genetic diversity, genetic similarity, plant germplasm

1. Introduction

Bristle oat *Avena strigosa* Schreb. is an annual spring crop included into diploid oats (Baum 1977; Loskutov 2007). The centre of the primal origin and diversity of *A. strigosa* is claimed to be the northwestern part of the Pyrenees on the Iberian Peninsula (Frey 1991; Kropač 1981). Archeological data indicate that in Europe that species had been known to man as early as the Bronze Age (ca. 2 000 – 700 years B.C.) (Kropač 1981). Until the early 20th century, bristle oat was cultivated as a cereal or used for fodder purposes in a number of countries of western, northern and central Europe, mainly in rocky or mountain areas of England, Scotland and Wales (Kropač 1981). In Poland, it was grown until the 1940's in the Podhale region as an additive to crops of *A. sativa* (Miczyński 1949-1950; Frey 1989).

Nowadays the economic significance of bristle oat in Europe is negligible. In the area of its occurrence, covering 22 countries of the western, central and northern Europe (Kropač 1981; Frey 1991), bristle oat is known as a weed accompanying crops of spring cereals or – less frequently – as a ruderal plant (Frey 1989; Korniak 1997). A substantially greater significance is ascribed to *A. strigosa* in the economy of South America and Australia, where it is utilized as a fodder or cover crop (Bauer & Reeves 1999; Suttie & Reynolds 2004).

Bristle oat is a speirochoric species, spreading exclusively with sowing material (Kornaś 1987a). There are no reports on its occurrence in natural and semi-natural phytocenoses (Frey 1989). Thus, a tendency for its extinction was observed along with the abandoning of *A. strigosa* cultivation in Europe. In the 1980's it was claimed a species threatened with extinction (Kropač 1981; Kornaś 1987b; Warcholińska 1994; Weibull *et al.* 2002). Although numerous new stations and increasing population numbers of that species were observed in the 1990's in north-eastern and south-eastern Poland (Korniak 1997; Korniak & Frey 1999; Kieć 2003), it is still a sparsely occurring taxon. For this reason, the knowledge on the level of genetic diversity of genetic resources of that species protected *ex situ* in gene banks is of great significance.

The objective of the reported study was to evaluate genetic diversity of genetic resources of *A. strigosa* at the isoenzymatic level. Results presented are a part of a research project on morphological and molecular diversity of that species.

2. Material and methods

The study was performed on 19 ecotypes of *A. strigosa* originating from various geographical regions (Table 1). Seventeen accessions were germplasm collection preserved in a gene bank of the Plant Breeding and Acclimatization Institute (IHAR) in Radzików. The other two ecotypes (*A. strigosa* var. *glabrescens* and *A. strigosa* var. *subpilosa*) were collected as weeds from cultivated fields located in the northeastern Poland and taxonomically identified by Professor T. Korniak from the Department of Botany and Nature Protection, the University of Warmia and Mazury in Olsztyn (UWM). All seed samples of examined ecotypes were reproduced for 3 years in the experimental field of the Educational and Research Station, UWM, in Tomaszkowo.

Table 1. List of *A. strigosa* ecotypes

Ecotypes	Accession number[1]	Other name or number	Geographical origin
BRA	51730	SAIA 3	Brazil (Rio Grande de Sul)
ESP1	51582	PI 258730	Spain (Lugo)
ESP2	51733	CC7062, CAV 2838	Spain
ESP3	51742	AVE 1874, ESP-78 338a	Spain
FRA1	51105	Pied de Mouche	France
FRA2	51584	PI 258732	France
POLpd1	51520	E0941, POL10	Poland (Lublin Province, Łuków)
POLos3	51598		Poland (Masovia Province, Chrzęsne near Tłuszcz)
POLpr4	51750	E1489, POL1	Poland (Podkarpacie Province, Bircza)
POLrd5	51755		Poland (Masovia Province, Zwoleń)
POLneg6	-	var. *glabrescens*[2]	Poland (Warmian-Mazurian Province)
POLnes7	-	var. *subpilosa*[2]	Poland (Warmian-Mazurian Province)
RUS	51499	Kaukazus B	Russia
SLO1	51734	AVE 1714, CSK-77 210a	Slovakia (Hrabova Raztoka)
SLO2	51736	AVE 1438, CSK-74 152	Slovakia (Mociar, Kokava nad Rimavicou)
TFR	51741	AVE 1129	Spain (Tenerife, Los Rodeos)
UKR	51579	PI 258727	Ukraine (Chernivtsi)
URG	51578		Uruguay (Montevideo)
WAL	51731	S75	United Kingdom (Wales)

Explanations: [1]Germplasm collection of the gene bank at the Plant Breeding and Acclimatization Institute, Radzików; [2]Collected by T. Korniak

A horizontal starch gel was employed to separate electrophoretic variants of six enzyme systems: aspartate aminotransferase (AAT; EC 2.6.1.1), acid phosphatase (ACP; EC 3.1.3.2), esterases (EST; EC 3.1.1.1), leucyloaminopeptidase (LAP; EC 3.4.11.1), malate dehydrogenase (MDH; EC 1.1.1.37), and peroxidase (PX; EC 1.11.1.7). Analyses were carried out for 190 individuals (10 individuals per ecotype). One seed from each individual was germinated under greenhouse conditions, in pots filled with soil. Fresh leaves of 14-day-old individual seedlings were homogenized with 40 µl of 0.7% B-mercaptoethanol (for AAT, ACP, EST, LAP, MDH) or with 40 µl of double-distilled water (for PX). Extracts were absorbed into Whatman chromatography 3MM paper wicks and loaded onto 11% starch gel. Electrophoresis was performed in a 0.1 M lithium-borate buffer at pH 8.3 (Zieliński 1987). Gels were run at 300V and 60mA at 4°C for 5-6 h. The staining procedure was performed as described by Wendel & Weeden (1989) with modifications. Each locus was numbered respectively, beginning with the most anodal migrating locus (e.g. *Aat1*, *Aat2*, etc.). Alleles in each locus were labeled according to their distance in mm from the origin (e.g. *Est2-16*, *Est2-20*, etc.).

Genetic diversity of all ecotypes was estimated from allele frequencies (p), the percentage of polymorphic loci (P), the mean number of alleles per locus (A), total genetic diversity (H_T), intrapopulation diversity (H_S), interpopulation diversity (D_{ST}) and the proportion of total diversity found among the populations ($G_{ST}=D_{ST}/H_T$). Parameters of genetic diversity were calculated using POPGENE 1.32 computer software (Yeh & Yang 1999). A locus was considered polymorphic if the frequency of its most common allele did not exceed 0.95. Allele frequencies were used in the principal component analysis (PCA) and construction of a PC plot of ecotypes. Genetic similarities between the ecotypes calculated according to Nei's (1978) genetic identity (I_N), were summarized in a UPGMA phenogram (Sneath & Sokal 1973). The significance test (LSD), UPGMA phenogram and PCA were performed by means of STATISTICA 7.1 computer software.

3. Results

3.1. Genetic variability within and among ecotypes

The six enzyme systems assayed in this study were found to be encoded by 16 loci. Most of them were revealed by EST and PX (4 loci), and only 2 loci by AAT,

Table 2. Allele frequencies (p) at polymorphic loci of *A. strigosa* ecotypes

Ecotype	Alleles					
	Mdh2			*Est4*		
	27	*29*	*33*	*16*	*20*	*24*
BRA	0.3	0.7	0.0	0.3	0.0	0.7
ESP1	0.6	0.4	0.0	1.0	0.0	0.0
ESP2	0.0	1.0	0.0	0.0	0.0	1.0
ESP3	1.0	0.0	0.0	0.4	0.0	0.7
FRA1	0.0	1.0	0.0	1.0	0.0	0.0
FRA2	0.0	1.0	0.0	1.0	0.0	0.0
POLpd1	1.0	0.0	0.0	1.0	0.0	0.0
POLos3	1.0	0.0	0.0	1.0	0.0	0.0
POLpr4	1.0	0.0	0.0	0.0	0.0	1.0
POLrd5	1.0	0.0	0.0	0.0	0.0	1.0
POLneg6	1.0	0.0	0.0	0.8	0.2	0.0
POLnes7	1.0	0.0	0.0	1.0	0.0	0.0
RUS	0.0	0.1	0.9	1.0	0.0	0.0
SLO1	0.0	1.0	0.0	1.0	0.0	0.0
SLO2	0.0	1.0	0.0	1.0	0.0	0.0
TFR	0.0	0.3	0.7	0.1	0.4	0.5
UKR	0.9	0.1	0.0	1.0	0.0	0.0
URG	0.0	0.6	0.4	1.0	0.0	0.0
WAL	0.0	0.0	1.0	0.0	0.0	1.0
Mean (p)	0.46^{c}	0.38^{c}	$0.16^{a\,b}$	0.66^{d}	0.03^{a}	0.31^{b}

Explanations: $^{a, b, c, d}$ – different letters mean significant differences between values (LSD test, p=0.05)

ACP, LAP and MDH. Amongst the 16 loci analyzed, one esterase locus *Est4* and one *Mdh2* locus showed polymorphism, whereas the others were monomorphic in all the plants examined (Table 2). No heterozygous individuals were observed at polymorphic loci. The mean percentage of polymorphic loci per population was P=3.3% (Table 3). Both polymorphic loci (*Mdh2* and *Est4*) were observed only in the ecotypes from Brazil (BRA) and Tenerife (TFR). The ecotypes from Spain (ESP1), Russia (RUS), Ukraine (UKR) and Uruguay (URG) were polymorphic only in the *Mdh2* locus, whereas the *glabrescens* variety (POLneg6) from northeastern Poland and the ecotypes from Spain (ESP3) – only in the *Est4* locus. In the other ecotypes examined (58%), a lack of differences was revealed in each of the 16 loci observed.

The total number of alleles was 20, and the mean number of alleles per locus was 1.04, with a range of 1.00 to 1.25 (Table 3). In polymorphic loci the least frequent were *Est4-20* and *Mdh2-33* alleles (with allele frequencies p=0.03 and p=0.16, respectively) (Table 2).

Table 3. Mean number of alleles per locus (A), percentage of polymorphic loci (P), intrapopulation diversity (H_S) for *A. strigosa* ecotypes

Ecotype	A	P	H_S
BRA	1.13^{ab}	13	0.055^{bc}
ESP1	1.06^{a}	6	0.032^{abc}
ESP2	1.00^{a}	0	0.000^{a}
ESP3	1.06^{a}	6	0.032^{abc}
FRA1	1.00^{a}	0	0.000^{a}
FRA2	1.00^{a}	0	0.000^{a}
POLpd1	1.00^{a}	0	0.000^{a}
POLos3	1.00^{a}	0	0.000^{a}
POLpr4	1.00^{a}	0	0.000^{a}
POLrd5	1.00^{a}	0	0.000^{a}
POLneg6	1.06^{a}	6	0.021^{abc}
POLnes7	$1,00^{a}$	0	0.000^{a}
RUS	$1,06^{a}$	6	0.012^{ab}
SLO1	$1,00^{a}$	0	0.000^{a}
SLO2	1.00^{a}	0	0.000^{a}
TFR	1.25^{b}	13	0.068^{c}
UKR	1.06^{a}	6	0.012^{ab}
URG	1.06^{a}	6	0.032^{abc}
WAL	1.00^{a}	0	0.000^{a}
Mean	1.038	3.3	0.013

Explanations: $^{a, b, c}$ – different letters mean significant differences between values (LSD test, p=0.05)

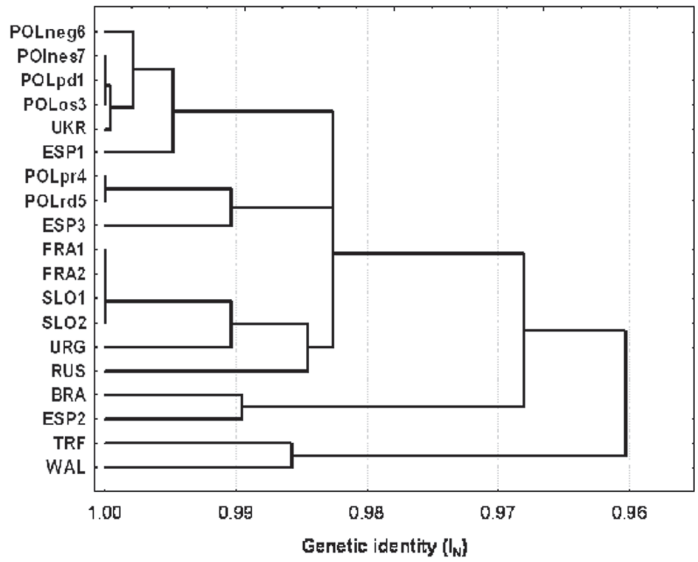

Fig. 1. UPGMA phenogram of 19 *A. strigosa* ecotypes based on Nei's (1978) genetic identity

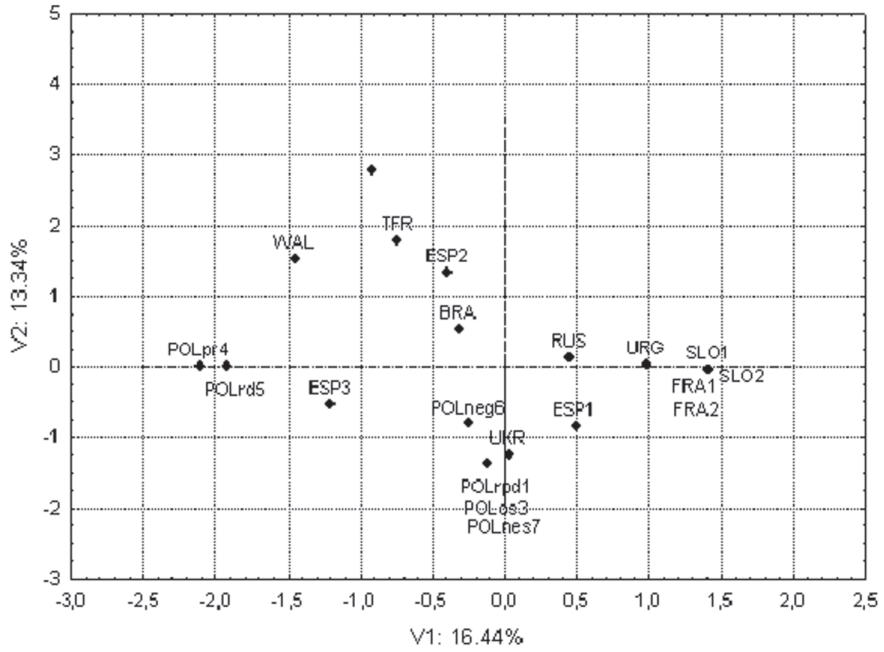

Fig. 2. Scatterplot of 19 *A. strigosa* ecotypes on principal components based on allele frequencies

Allele *Est4-20* was present only in two ecotypes (POLneg6, TRF). Allele *Mdh2-33* was detected in the ecotypes from Russia (RUS), Tenerife (TFR), Uruguay (URG) and Wales (WAL). The highest frequency was recorded for the *Est4-16* allele (p=0.66), which appeared in 79% of the ecotypes examined.

Mean total genetic diversity (H_T) was 0.07. Mean intrapopulation diversity (H_S) was 0.013. In 11 out of the 19 examined ecotypes of *A. strigosa*, the value of the intrapopulation diversity (H_S=0) indicated a lack of differences at the enzymatic level (Table 3). In the other ecotypes, the level of intrapopulation diversity ranged from 0.012 to 0.068. The ecotypes from Tenerife (TFR) (H_S= 0.068) and Brazil (BRA) (H_S= 0.055) turned out to be the most diverse.

The mean value of D_{ST} was estimated as 0.057, which shows a low degree of diversity within the examined ecotypes of *A. strigosa*. The mean value of G_{ST} for all loci was 0.821. It indicates that more than 80% of the total variation was due to differences between the populations.

3.2. Genetic similarity between ecotypes

The mean value of Nei's (1978) genetic identity (I_N) between 19 ecotypes of *A. strigosa* was 0.94 and ranged from 0.875 to 1.00. On the basis of I_N the greatest differences were observed between the ecotype from Wales (WAL) and ecotypes from France (FRA1, FRA2), Poland (POLpd1, POLos3, POLnes7) and Slovakia (SLO1, SLO2) (I_N=0.875). Ecotypes grouping by UPGMA clustering (Fig. 1) and PCA (Fig. 2) showed that most of the examined ecotypes formed one group. A slightly distinguishing group was formed by the ecotypes from Wales (WAL), Tenerife (TFR), Spain (ESP2) and Brazil (BRA).

4. Discussion

Results of the conducted analyses demonstrated a low level of genetic diversity of 19 ecotypes of *A. strigosa*. A total of 87% of the revealed enzymatic loci were monomorphic. Over half the analyzed ecotypes (58%) in each of the 16 loci observed exhibited no differences. The value of the H_S parameter (0.013) points to low intrapopulation diversity of *A. strigosa* (Table 3), which is over 5-fold lower than the total gene diversity of the species (H_T=0.07). The genetic structure of bristle oat (P=3%, A=1.04, H_S=0.013) is not typical to other species of the genus *Avena* – a diploid *A. canariensis* (P=48%, A=1.65, H_S=0.247) (Morikawa & Leggett 1990) or a tetraploid *A. barbata* (P=36%, A=1.6, H_S=0.04) (Guma *et al.* 2006). The above parameters are also considerably lower as compared to the values determined for vascular plants by Hamrick & Godt (1989). According to those authors, the average proportion of polymorphic loci (P) in plants is 34% (range 29.0-57.7%), the mean number of alleles per locus (A) is 1.53 (range 1.44-1.93) and the mean intrapopulation diversity (H_S) is 0.113 (0.096-0.16), respectively.

Studies on isoenzyme variation in plants have revealed that several factors may influence the amount of genetic polymorphism and how this polymorphism is distributed within and among populations (Hamrick & Godt 1989; Hamrick 1989). A significant impact is ascribed in this respect to the reproduction system, mechanisms of pollination, the method of seed sowing, the stage of plant succession as well as the range and dynamics of a population (Hamrick 1989).

The low genetic variability of *A. strigosa* ecotypes demonstrated in the study obviously results from the

self-pollinating method of reproduction of that species. A relationship between autogamy and low levels of isoenzyme variation has been observed in self-pollinating *Lolium persicum* and *L. remotum* (Charmet & Balfourier 1994) or *Elymus alaskanus* (Diaz *et al*. 1999). In self-pollinating species, a high level of inbreeding not only leads to homozygosity and suppresses genetic variability within a population, but also increases genetic diversity between groups.

In case of *A. strigosa,* high homozygosity, manifested in a lack of heterozygotes and a low value of intrapopulation diversity (H_S= 0.013), does not lead to considerable differences between ecotypes on the isoenzymatic level (D_{ST}=0.057). The mean value of genetic similarity between the ecotypes analyzed in the study was also high (I_N =0.94) and ranged from 0.875 to 1.00. The grouping of the investigated ecotypes of bristle oat (Figs. 1-2) based on genetic similarity and allele frequency demonstrated that most of them constituted a relatively homogenous group. It suggests negligible diversity of *A. strigosa* ecotypes as affected by environmental factors linked with various geographical origins. A lack of such a dependency was shown by Oja (1999), who determined, based on isoenzymatic markers, a high similarity between populations of *Bromus tectorum* and *B. sterilis* representing gene reserves of various areas of Eurasia. Out of the *A. strigosa* ecotypes examined, slightly distinctive were two ecotypes originating from the area acknowledged as the centre of origin of that species (ESP2) and from geographically isolated areas (TFR). A similar individuality of populations from Spain and Tenerife was observed, based on isoenzymatic markers, by Charmet & Balfourier (1994) in *Lolium rigidum*. Cultivated ecotypes – the Welsh one (WAL) known as cv. 'S75' and the Brazilian one (BRA) known under the name 'Saia' were also shown to be slightly distinctive. Therefore the process of domestication and cultivation as well as the participation of man in the spreading of *A. strigosa* are of great importance. When comparing data on genetic diversity of crop cultivars and their wild ancestors in a number of plant species, Doebley (1990) demonstrated in the cultivable forms a decrease in the percentage of polymorphic loci (by 19%), in the number of alleles per locus (by 11%), and in their total heterozygosity (by 26%).

A lack of the effect of environmental factors resulting from the geographical origin may be linked with its specific soil requirements. Bristle oat occurs most often on sour soils included into poorer and the poorest soil complexes (Korniak & Kuszewska 1999). Such preferences in respect of the habitat have undoubtedly limited the cultivation of that species in the past, whereas contemporarily they restrict its natural occurrence to areas with barren soil as well as sandy and stone regions (Miczyński 1949-1950; Kropač 1981; Korniak 1997). Ample investigations demonstrate that rare species and those restricted to the habitat with specific conditions are characterized by a considerably lesser genetic diversity as compared to the wide-spread and frequently occurring ones (Hamrick 1989; Maki 2003; Gitzendanner & Soltis 2000).

The low level of isoenzymatic diversity of genetic resources of *A. strigosa* found in this study results from the self-pollinating method of reproduction, specific habitat preferences and the contribution of man to its propagation. However, in order to obtain a broader picture of genetic diversity of this species it would be necessary to use DNA markers and morphology.

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