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SHORT COMMUNICATION

Characterisation of Oat Genetic Resources Using Electrophoresis of Avenins

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Abstract: The prolamin (avenin) patterns of oat (*Avena sativa* L.) cultivars released in the Czech Republic and in the former Czechoslovak Republic were analysed by acid polyacrylamide gel electrophoresis (A-PAGE). Forty-nine oat *(Avena sativa L.)* accessions of domestic origin, maintained in the Czech collection of oat genetic resources, were analysed. The evaluated set contained 18 modern and 31 old cultivars. Thirty accessions showed a homogeneous prolamin pattern. The other accessions were heterogeneous with two or three different patterns, present at different percentages. Heterogeneity was present in 48% of the old cultivars, but only in 22% of the modern cultivars. Identity indexes within the heterogeneous accessions were calculated. The index values ranged from 0.09 to 0.75. Only in two heterogeneous cultivars (Dětenický Bílý, Valečovský Bílý) the identity index between their components was higher than 0.6, indicating, that their components were most likely sister lines. All analysed cultivars could be unambiguously distinguished by their prolamin pattern. The obtained prolamin patterns will be used to complete descriptor data of the genetic resources and might be useful also in oat breeding and research.

Keywords: avenins; genotype distinguishing; line; oats; PAGE

Genetic variation is often present within genetic resources, which is important to know before the utilisation of a resource in breeding or research. Most of the grown cultivars of self-pollinated species are pure lines or mixtures of closely related lines. Old, nowadays not grown materials, such as landraces and obsolete cultivars of a low thoroughbreeding level, are often composed of several lines that can be donors of a number of different properties. Vyhnánek *et al.* (2003) described only two uniform accessions in the set of 20 accessions from the historical spring barley collection.

A lot of methods have been used to describe genetic diversity within and among cultivars, relying on data of different kind (Mohammadi & Prasanna 2003). Storage proteins have frequently been used to study genetic diversity in many species, since they are highly polymorphic and environmentally stable, e.g. in wheat (Bradová & Matějová 2008), barley (Echart-Almeida & Cavalli-Molina 2000) and oats (Gregová *et al.* 1996; Morikawa & Arase 1999; Dvořáček *et al.* 2003). Prolamins (avenins in oats) are a highly heterogeneous fraction of cereal storage proteins. They can be readily extracted with alcohol solution from dry mature grain and resolved by gel electrophoresis. The analysis of prolamins using PAGE was therefore internationally recommended by the

Table 1. List of analysed oat cultivars with the number of proved avenin patterns (A-PAGE)

Accession number*	Accession name	Number of patterns
03C0700001	Český Žlutý	1
03C0700002	Chlumecký Žlutý	1
03C0700004	Dětenický Bílý	2
03C0700005	Hořický	1
03C0700006	Irbit	1
03C0700007	Nalžovský	3
03C0700008	Rychlik	1
03C0700009	Studnický	1
03C0700010	Stupický Bílý	1
03C0700011	Táborský	2
03C0700012	Valečovský Bílý	3
03C0700014	Brněnský Zlaťák	1
03C0700015	Jindřichovský Bílý	3
03C0700016	Valečovský 4	1
03C0700017	Valečovský 2	1
03C0700018	Valečovský Nepoléhavý	3
03C0700019	Valečovsky Ligovo II	2
03C0700020	Valečovsky Vítěz	2
03C0700021	Valečovsky Hvězdový	1
03C0700022	Slapský Vítěz	2
03C0700023	Doupovský	2
03C0700024	Olešenský Žlutý	1
03C0700025	Selekční M 2	1
03C0700026	Selecty Horský	1
03C0700027	Terrasol Bílý Krajový	3
03C0700028	Selecty Vítěz	2
03C0700029	Klatovský Bílý	3
03C0700031	Slapský Poloraný	3
03C0700039	Šumavský	2
03C0700043	Nahý	1
03C0700052	Jesenický Žlutý	1
03C0700071	Diadem	1
03C0700075	Gratus	3
03C0700774	Ligowo III	1
03C0700809	Krajová ze Ždiaru (Budžak)	2
03C0701152	Saturn	2
03C0701318	Hermes	2
03C0701354	Veles	1
03C0701355	Pan	1
03C0701403	Orlik	1
03C0701588	Adam	1
03C0701625	David	1
03C0701660	Galantský Skorý	1
03C0701917	Radius	1
03C0701981	Cyril	1
03C0701982	Dalimil	1
03C0701986	Mojacar	1
03C0701993	Jakub	1
03C0702126	Radošinský	1

*identification number in the Czech Information System of Genetic Resources (EVIGEZ)

International Seed Testing Association (ISTA) for the verification of species and cultivars (ISTA 1999) and individual avenin electrophoregrams were genetically interpreted by Portyanko *et al.* (1998). Despite of a number of DNA-based methods for studying polymorphism, analyses of storage proteins provide a lot of advantages. They are, among others, less demanding for equipment, cost-effective, and thus more accessible. The objective of the present study was to examine the genetic variation of selected oat genotypes by means of electrophoretic separation of grain storage proteins (avenins).

Forty-nine oat (*Avena sativa* L.) accessions were analysed that were bred and/or grown in the territory of the Czech Republic and also in the former Czechoslovak Republic and are maintained in the collection of oat genetic resources. The list of analysed cultivars together with results is given in Table 1. Both modern (released after the year 1950; 18 accessions) and old cultivars (landraces and obsolete cultivars; 31 accessions) were involved. The electrophoretic analysis was carried out by vertical polyacrylamide electrophoresis, avenins were separated by acid polyacrylamide gel electrophoresis (A-PAGE) using a method described in the Czech standard ČSN 46 1085-2 (1998) with certain specific modifications for oats (Polišenská *et al.* 2010). One hundred seeds of each accession were analysed, two seeds of the cultivar Abel were added to each gel in order to produce reference bands. The number of identical protein patterns was counted, the different protein patterns within one accession were given letters from A through C depending on their frequency in the analysed sample. Graphical illustration of avenin patterns for each protein band was created using relative electrophoretic mobility (REM) and relative colour intensity (RCI) according to Vyhnánek and Bednář (2003) using a macro in the Excel program. The identity index (ii) was calculated for heterogeneous patterns of avenins based on calculations described by Hadačová *et al.* (1980) and for cereal proteins used e.g. by Šašek *et al.* (1982).

All genotypes of analysed cultivars were unambiguously distinguished based on their protein patterns. From one to three protein patterns per accession were detected. Thirty homogeneous, 11 dimorphic and 8 trimorphic accessions were found. Among 18 modern cultivars, 14 of them were homogeneous (78%), 3 were dimorphic (17%) and 1 (5%) trimorphic. Among 31 old accessions, 16 of them were homogeneous (51%), 8 were dimorphic (26%) and 7 (23%) trimorphic. The list of analysed cultivars together with the numbers of obtained patterns is given in Table 1. The illustration of avenin patterns created on the basis of REM and RCI values for uniform accessions is shown in Figure 1. From the group of dimorphic accessions only protein patterns from Dětenický Bílý had a high identity index (0.75), indicating them as sister lines. In the other accessions the identity index was lower, ranging from 0.20 to

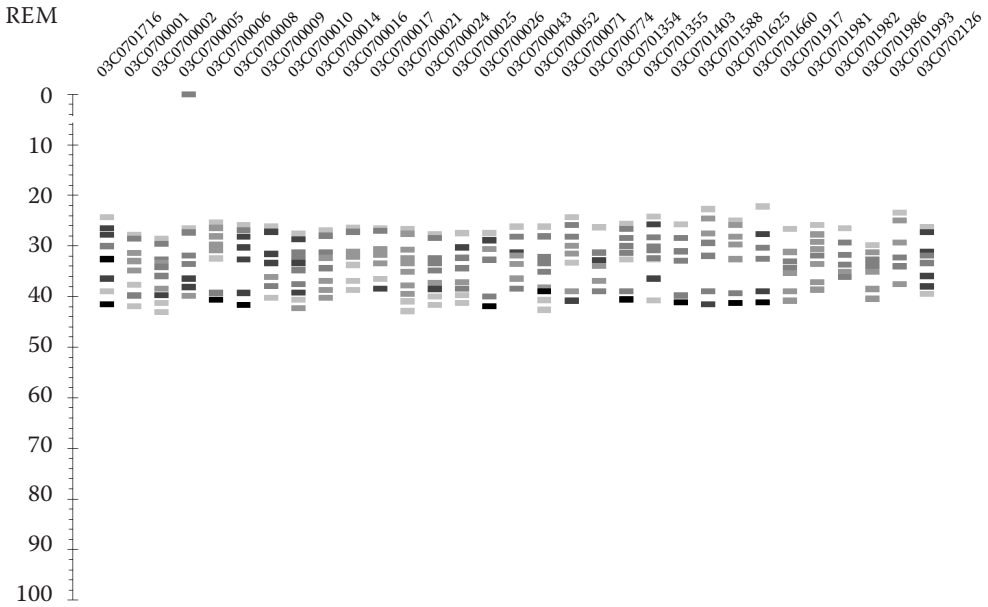

Figure 1. Avenin patterns from A-PAGE for 30 homogeneous oat accessions; the first accession is cultivar Abel, used as reference cultivar

Table 2. Frequency and relationship of avenin patterns for dimorphic oat accessions

Accession number*	Accession name	Percentage of pattern		Identity index
		A	B	
03C0700004	Dětenický Bílý	57	43	0.75
03C0700011	Táborský	58	42	0.25
03C0700019	Valečovský Ligovo II	82	18	0.27
03C0700020	Valečovský Vítěz	63	37	0.25
03C0700022	Slapský Vítěz	52	48	0.42
03C0700023	Doupovský	87	13	0.33
03C0700028	Selecty Vítěz	92	8	0.44
03C0700039	Šumavský	76	24	0.50
03C0700809	Krajová ze Ždiaru (Budžak)	94	6	0.20
03C0701152	Saturn	92	8	0.45
03C0701318	Hermes	88	12	0.27

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0.50. The frequency and relationship of avenin patterns within dimorphic oat accessions are given in Table 2, avenin patterns are shown in Figure 2. Černý and Šašek (1998) indicated the value of the identity index 0.6 as the lowest acceptable value for sister lines. Extremely low values could suggest that these lines should be considered also as admixtures. However, old landraces were developed by mass selection and if the materials do not exhibit any significant morphological distinctions, it is possible that these lines might be present in populations. Therefore based on information on the variety origin and lines ratio, in selected cases it would be possible to consider the accessions as materials containing non-sister lines.

In the group of accessions with three avenin patterns, only patterns A and B from Valečovský Bílý had a high identity index (0.70). The situation can be described similarly like for the group of dimorphic accessions. The frequency and relationship of avenin patterns within trimorphic oat accessions are given in Table 3, avenin patterns are shown in Figure 3. Some of the lines B and C were closer to

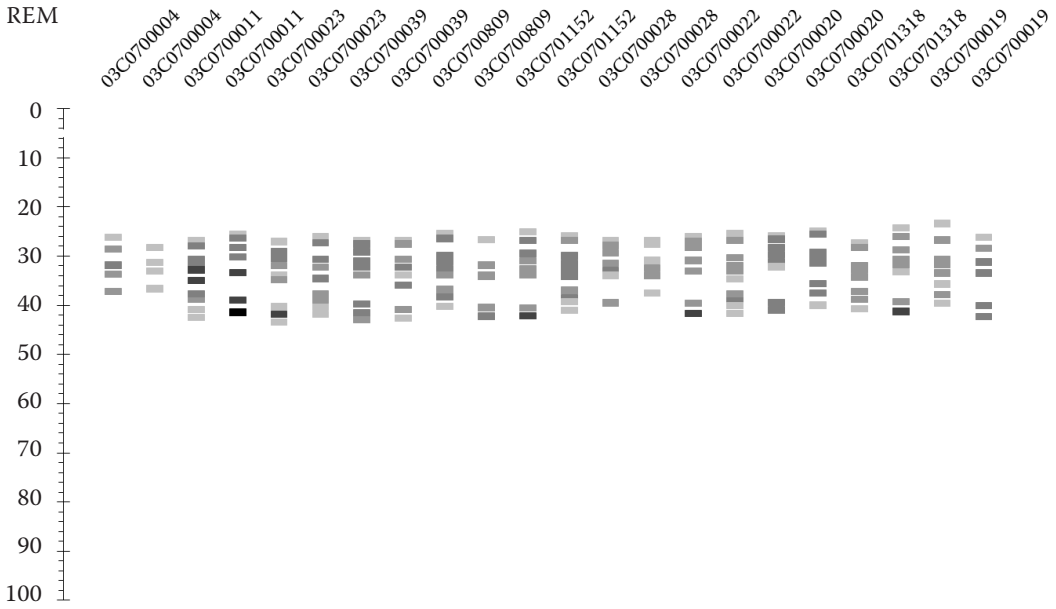

Figure 2. Avenin patterns from A-PAGE for 11 dimorphic oat accessions

Table 3. Frequency and relationship of avenin patterns for trimorphic oat accessions

Accession number*	Accession name	Percentage of pattern			Identity index AB/AC/BC
		A	B	C	
03C0700007	Nalžovský	56	38	6	0.09/0.09/0.50
03C0700012	Valečovský Bílý	66	30	4	0.70/0.13/0.15
03C0700015	Jindřichovský Bílý	58	13	29	0.13/0.20/0.10
03C0700018	Valečovský Nepoléhavý	52	43	5	0.44/0.18/0.27
03C0700027	Terrasol Bílý Krajový	72	12	16	0.30/0.18/0.27
03C0700029	Klatovský Bílý	66	19	15	0.31/0.17/0.50
03C0700031	Slapský Poloraný	56	39	5	0.55/0.45/0.33
03C0700075	Gratus	71	16	13	0.15/0.35/0.44

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each other than to the prevailing line A. This was the case with the accessions Nalžovský, Klatovský Bílý and Gratus, where the identity index of line C to B was 0.50 (0.44 respectively) compared with much lower values to line A (0.09 in both cases with Nalžovský, 0.31/0.17 with Klatovský Bílý and 0.15/0.35 with Gratus).

The frequency and thus the probability of detection of various lines can be affected by environmental conditions, since natural selection might prefer some lines. The detection of line numbers could also be influenced by the number of analysed seeds. Maintenance breeding can also change the frequency and number of lines. Šašek *et al.* (1998) described a case where a dimorphic variety changed to a uniform one. The frequency of sister lines in an accession can influence its agronomic performance, since their interactions with environment might be different. Echart-Alemeid and Cavalli-Molina (2000), analysing 14 barley genotypes (landraces and cultivars), found intravarietal polymorphism in 12 cultivars. Černý and Šašek (1998), analysing gliadins and HMW-glutenins in wheat landraces, revealed polymorphism in 40% of the examined accessions, i.e. two to four protein lines. Pomortsev (2001), analysing hordeins in 147 Ethiopian barleys by starch gel electrophoresis, found from one to six different hordein patterns per accession. Por-

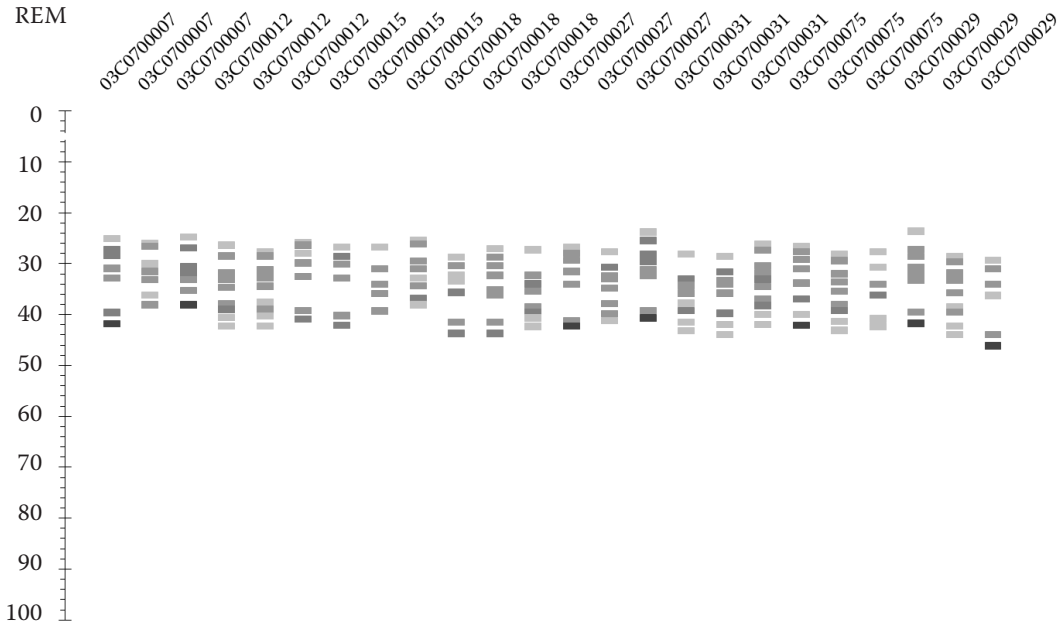

Figure 3. Avenin patterns from A-PAGE for 8 trimorphic oat accessions

TYANKO *et al.* (1998) determined about 8% of the 252 oat accessions (varieties, landraces, breeding lines) as heterogeneous, comprising two or three different avenin profiles.

An efficient exploitation of polymorphic genetic resources requires the separation and evaluation of lines with different protein patterns. A possible approach consists in the analysis of avenins in the endosperm of individual seeds, selection of eligible protein patterns and regeneration of plants from the corresponding embryos cultivated *in vitro*.

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