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Research Article

ISOENZYME VARIATIONS AND GENETIC IDENTITIES AMONG TAXA OF *FESTUCA* SERIES *PSAMMOPHILAE* M. PAWLUS (POACEAE)

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Summary: Isoenzyme variations and genetic identities among *F. vaginata, F. psammophila, F. pallens*, *F. polesica* and *F. ovina* were examined by means of polyacrylamide gel electrophoresis. The isoforms of the enzymes glutamate-oxaloacetate transaminase, malate dehydrogenase, glutamate dehydrogenase, isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase were examined. Based on mean allelic frequencies/locus/taxon, Nei' genetic identities I were calculated. The results showed that *F. vaginata*, *F. psammophila* and *F. polesica* were closely related. The species *F. pallens* was relatively distant from the above mentioned three species. The specific position of *F. pallens* is confirmed also by its ecological characteristics: it is the only species growing on carbonate rocks, while the rest taxa are typical psammophytes. The results showed distinct differences between the Ukrainian populations of *F. pallens* and *F. psammophila* and confirmed the hypothesis about the occurrence of the latter species in Ukrainian Roztocha. In conclusion, all studied species should be considered as a separate series of closely related taxa - series *Psammophilae*.

Keywords: Electrophoresis; *Festuca*; genetic identities; isoenzymes; species.

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INTRODUCTION

Festuca L. is one of the most complex genera in Poaceae. The species concept in genus *Festuca* has undergone drastic changes over the time. More than a century ago, relatively few, broadly defined taxa were recognised. Lately, the species concept became narrower and a large number of finely split taxa are recognised today.

Most of the *Festuca* species belong to the group of thin-leaved fescues. According to the type of leaf anatomy,

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thin-leaved fescues could be divided into three main groups: species with leaves as *F. rubra*, type *F. valesiaca* and type *F. ovina* (interrupted sclerenchyma ring). Two main treatments exist in Eastern Europe. Pawlus (1983) treated the species with interrupted sclerenchyma ring into two series: *Ovinae* M. Pawlus (= *F. ovina* agg.) and series *Psammophilae* M. Pawlus, which unites the rest species. According to the second one (Tzvelev, 1976; Bednarska, 2003), among the species with interrupted sclerenchyma ring there are several small species aggregates, namely *F. ovina* agg., *F. glauca* agg. (or *F. pallens* s.l.) and *F. beckeri* agg. The phylogenetic relationships among these species groups and their adequate taxonomic treatment still remain unresolved.

The present study included five taxa of *Festuca* from Ukraine and Bulgaria*: F. vaginata* Waldst. & Kit. ex Willd.*, F. psammophila* Hack. ex Čelak., *F. pallens* Host, *F. polesica* Zapał. and *F. ovina* L., which represent all above-mentioned species aggregates.

The above-mentioned five taxa of fescues exhibit high variability and overlapping of morphological and anatomical characters, including diagnostic ones, leading to identification difficulties/ missidentification and uncertainties in their taxonomy.

Isoenzymes are more reliable genetic markers than those previously used in plant biosystematics. The most significant advantage of isoenzymes is the simple genetic basis of their polymorphism. Being proteins, they can directly reflect alterations in the genome. Hence, changes in the electrophoretic mobility of enzymes provide an extremely useful method of evaluating genetic differences and systematic relationships within taxonomically complicated plant groups. Several isoenzyme analyses in fescues were conducted in an attempt to investigate species delimitation based on isoenzyme markers (Livesey and Norrington-Davis, 1991; Aiken et al., 1993; Aiken et al., 1994; Aiken and Lefkovitch, 1995; Guldahl et al., 2001).

The aim of the present work was to study the isoenzyme variations and genetic affinities among the above-listed species of genus *Festuca*.

MATERIALS AND METHODS

The isoforms of the enzymes glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), malate dehydrogenase (MDH, EC 1.1.1.37), glutamate dehydrogenase (GDH, EC 1.4.1.2), isocitrate dehydrogenase (IDH, EC 1.1.1.6) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) were examined in natural populations (25-28 plants/population) from Bulgaria (*F. vaginata*) and Ukraine. The isoforms were resolved on 7.5% separating gel (3% stacking gel) polyacrylamide slabs (Davis, 1964). Staining of gels followed procedures described by Shaw and Prasad (1970) for MDH and GDH, Przybylska et al. (1982) for GOT, Henderson (1965) for 6PGDH and Yeh and O´Malley (1980) for IDH.

Different genes (loci) coding for the same enzymes (isoenzymes) were designated according to the relative mobility of the enzymes they specify. That is, the gene encoding the most anodal isoforms was designated by (1), the next most anodal one by (2), etc. In each locus the allele encoding the fastest isoform

was designated by (a), the next fastest by (b), and so on. Based on mean allelic frequencies/locus/taxon (Table 2), genetic identities (I) were calculated (Nei, 1972). An index of group affinity (GA) was calculated for each taxon as a sum of its I values.

RESULTS AND DISCUSSION

Genetic interpretation of enzyme banding patterns was based on two lines of evidence – the known subunit structure of enzymes and their segregation patterns within species. Three/two gene loci and dimeric subunit structure are supposed for the examined enzymes (Brown and Munday, 1982; Figueiras et al., 1984, Perez de la Vega and Allard, 1984). One gene locus has been reported for GDH (Gottlieb, 1982). The patterns of variation observed in the studied species of genus *Festuca* conform to the above-mentioned genetic models. The studied populations of each taxon were electrophoretically similar. Hence, the data for a taxon were pooled and mean frequencies were calculated. Mean allelic frequencies in the studied species are presented in Table 1. Totally, five enzymes, putatively encoded

Table 1. Mean allele frequencies in the studied species of genus *Festuca.*

Gene locus	Alelle	*F. vaginata*	*F. polesica*	*F. psammophila*	*F. pallens*	*F. ovina*
IDH 1	a	0.00	0.00	0.50	0.75	0.10
	b	1.00	1.00	0.50	0.25	0.90
IDH 2	a	1.00	1.00	1.00	0.50	0.00
	b	0.00	0.00	0.00	0.50	1.00
6PGDH 1	a	0.00	0.52	0.18	0.85	0.25
	b	0.27	0.38	0.34	0.15	0.75
	c	0.73	0.00	0.48	0.00	0.00
6PGDH 2	a	0.50	0.68	0.10	0.65	0.75
	b	0.50	0.32	0.90	0.35	0.25
GDH 1	a	0.25	053	0.35	0.00	0.00
	b	0.75	0.47	0.15	0.80	0.10
	c	0.00	0.00	0.50	0.20	0.90
GOT 1	a	1.00	1.00	1.00	1.00	0.50
	b	0.00	0.00	0.00	0.00	0.50
GOT 2	a	1.00	1.00	1.00	1.00	1.00
GOT 3	a	1.00	1.00	0.55	0.25	0.00
	b	0.00	0.00	0.45	0.75	1.00
	c	0.00	0.00	0.00	0.00	0.00
MDH 1	a	1.00	1.00	0.70	0.20	0.00
	b	0.00	1.00	0.30	0.80	1.00
MDH 2	a	0.50	0.90	1.00	0.70	0.10
	b	0.50	0.10	0.00	0.30	0.90

by ten gene loci, namely IDH 1, 2, GDH 1, 6-PGDH 1, 2, GOT 1, 2, 3, MDH 1, 2 IDH 1, 2 were scored. Most alleles were shared by all studied species, which is an indication for their close relationships. Except for *F. pallens,* the studied taxa were monomorphically fixed for allele a of GOT 1. All examined species were invariant for allele a of GOT 2.

Genetic identities values for all pair-wise comparisons among the studied species are presented in Table 2. The values for coefficient I varied from 0.93 (*F. vaginata* vs. *F. polesica*) to 0.44 when *F. ovina* was contrasted to *F. polesica.* The species *F. vaginata, F. polesica* and *F. psamophila* were genetically tightly related, while *F. pallens* was relatively isolated within the group. The latter species together with *F. ovina* form their own cluster (Fig 1).). The index of group affinity contributed further to revealing the relationships within the examined group of genus *Festuca.* Lower values of the index GA mean greater distance for a given taxon, and vice versa, higher values indicate closer affinity within the group. The values of the index GA for *F. vaginata* (3.07), *F. psamophila* (3.04) and *F. polesica* (2.91) indicated their close affinity within the studied group. The species *F. pallens* (GA=2.66) was relatively distant while *F. ovina* (GA=2.11) proved to be the most isolated species within the group. Therefore, the examined species could be arranged by their decreasing affinity and increasing genetic divergence as follows: *F. vaginata* , *F. psamophila*, *F. polesica, F. pallens, F. ovina.*

Morphologically the studied species demonstrated close affinity. As most species of genus *Festuca,* they can be differentiated mainly on the basis of subtle morphological and anatomical differences. Nevertheless, data from the present study showed some distinct differences in their isoenzyme structure. Similar observations were recorded also in previous studies. It was shown that other closely related taxa of genus *Festuca* were differentiated due to fixed or close to being fixed differences of some isoenzyme markers (Aiken et al., 1993; Aiken and Lefkovitch, 1995; Guldahl et al., 2001)

The specific position of *F. pallens* is confirmed also by its ecological characteristics – it is the only species growing on carbonate rocks, while the rest taxa are typical psamophytes which occur in pine forests sands, river terraces sands, open dunes, etc. Beside morphological differences, this fact is an additional argument to consider *F. psammophila* as a separate species (Tzvelev, 1976;

Table 2. Genetic identities (I) for all pair-wise comparisons among the studied species of genus *Festuca.*

	Species	Genetic identity (I)				
		1	2	3	4	5
1	*F. vaginata*	1.00				
2	*F. polesica*	0.93	1.00			
3	*F. psammophylla*	0.90	0.90	1.00		
4	*F. palens*	0.78	0.74	0.63	1.00	
5	*F. ovina*	0.46	0.44	0.61	0.60	1.00

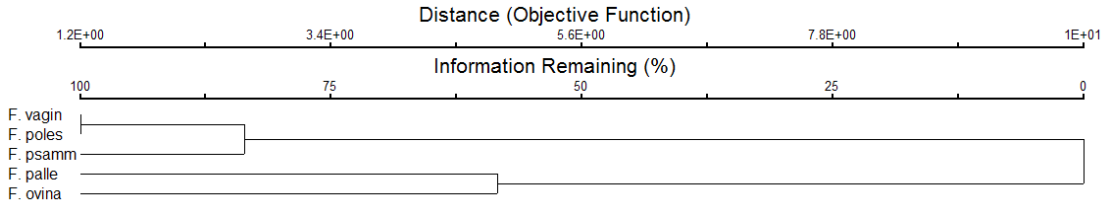

Figure 1. Cluster dendrogram (Distance measure: Euclidean (Pythagorean), Group linkage method: Ward's method) based on mean allelic frequencies (Table 1).

Tveretinova, 1977) and answers the question if it grows in Western Ukraine (Pawlus, 1983). The results from a previous study on seed proteins also showed distinct differences between Ukrainian populations of *F. pallens* and *F. psammophila* (unpublished) and confirmed the hypothesis about occurrence of the latter species in Ukrainian Roztocha (Bednarska, 2003). The results from the present study point out that all studied species should be considered as a separate series of closely related taxa – series *Psammophilae*.

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