Systematic relationships among eight taxa of genus *Festuca* from the Ukraine, as revealed by seed proteins electrophoresis

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Abstract. Polyacrylamide gel electrophoresis (PAGE) was employed to analyze seed protein composition in natural populations of eight Ukrainian taxa of the genus *Festuca* in an attempt to reveal systematic relationships among them. The results of the study have shown that the species *F. polesica* is the most distantly positioned taxon within the studied group of genus *Festuca*. The species *F. pallens*, *F. psammophila* and *F. ovina* are well defined entities, with specific patterns of their seed proteins. These taxa are differentiated from each other and clearly distinct from the taxa of *F. valesiaca* group. The species of *F. valesiaca* agg., namely, *F. rupicola*, *F. arietina, F. macutrensis,* and *F. galiciensis* are closely related taxa demonstrating a high level of similarity, as judged by their seed proteins. Other biochemical markers are needed to clarify the systematic relationships among the species of *F. valesiaca* agg.

Key words: *Festuca,* PAGE, seed proteins, systematic relationships

Introduction

Genus *Festuca* L. is among the largest genera of *Poaceae* and encompasses about 300 species, but some authors suggest that even more taxa of *Festuca* exist. Recent taxonomic treatments of the European fescues follow mainly *Flora Europaea* (Markgraff-Dannenberg 1980).

Limits of the genus and taxonomic rank of the numerous taxa belonging to the thin- leaved fescues are still disputable. It is mainly due to lack of material for study from many regions of Europe, including the Ukraine. The studies of Alexeev (1975) and Tzvelev (1976; 2010), which encompass the territory of the former USSR, are among the main systematic treatments of genus *Festuca* in Eastern Europe. Until recently, the study of Tveretinova (1977) was the only systematic treatment of this genus in the Ukraine. The last revision of genus *Festuca* confirmed 17 species of the type subgenus *Festuca,* section *Festuca,* which occur in the flora of the Ukraine (Bednarska 2007; Tzvelev 2010). It includes all thin-leaved, densely caespitose species of the genus. These species may be divided into two major groups according to the anatomical structure of their leaves. The first group includes species with a sclerenchyma ring, the so-called "ovinoid" type structure: *F. ovina* agg. (*F. ovina* L., *F. filiformis* Pourr., *F. airoides* Lam.), *F. glauca* agg. [*F. pallens* Host, *F. psammophila* (Hack. ex Celak) Fritsch] and *F. beckeri* agg. [*F. beckeri* (Hack.) Trautv., and *F. polesica* Zapal.]. The second group comprises species with 3–5 sclerenchyma bundles, which in some species may be secondarily fused into a ring, the so-called "sulcata" type structure (from *F. sulcata* s.l.), which is characteristic of the species of *F. valesiaca* agg.

The species complexes of "ovinoid" taxa differ significantly from each other, but recent studies have demonstrated the complicated systematics of this group. For example, identification of *F. psammophila* populations occurring in the eastern regions of its range is problematic (Bednarska 2011). The question about distinctness of *F. polesica* Zapał. remains disputable, bearing in mind the existence of transitional forms to *F.beckeri* (Hack.) Trautv., as well as hybrids with *F. ovina* L. (Bednarska 2009). However, most problematic is the systematics of species belonging to *F. valesiaca* agg. Due to overlapping of the diagnostic traits and scarcity of small-scale studies, the systematic structure of this group is quite disputable (Pils 1984; Arndt 2008). A study of the Ukrainian taxa of *F. valesiaca* agg. has revealed a very complicated continuum of variability. Many of them deserve a species rank, as it was proved for *F. galiciensis* Bednarska nom. prov. (Bednarska 2014a).

Along with this, diversity of the naturally occurring populations is significantly higher than the number of formally described taxa. For example, in Western Ukraine (Rohatyn Opillia region), *F. rupicola* with green and glaucous leaves may frequently occur within one and the same biotope. Earlier, the latter form was wrongly identified as *F.pseudodalmatica* Krajina ex Domin (Bednarska 2007). However, the results of long-term observations have shown that both forms belong to *F. rupicola* and the glaucous one should be designated as *F. rupicola* f. *glaucescens* (Bednarska 2014a).

Summarizing most of the thin-leaved fescues has demonstrated high variability of morphological and anatomical traits, including diagnostic ones. So, there is a need to apply different new approaches, including biochemical ones, so as to reveal the systematic structure and phylogenetic relationships of the thin-leaved fescues.

The present study includes eight taxa of *Festuca* from the Ukraine: *F. ovina* L., *F. psammophila* Hack. ex Celak., *F. pallens* Host, *F. polesica* Zapal., *F. rupicola* Heuff., *F. macutrensis* Zapal., *F. arietina* Klokov, and *F. galiciensis* Bednarska nom. prov. The last four taxa belong to *F. valesiaca* agg.

Electrophoretic studies of seed proteins are widely employed for estimation of systematic relationships, genetic variation of natural populations and cultivars of different plant taxa (Carreras & al. 1997; Yüzbaşioğlu &. al. 2008; Stoyanova & Boler 2010). Different electrophoretic techniques are also widely used for seed protein characterization and genotype sample classification (Aiken & al. 1998; Turi & al. 2010).

The aim of the present study was to analyze seed protein composition in an attempt to reveal systematic relationships among the above-mentioned taxa of genus *Festuca*.

Material and methods

Seeds from natural populations of the above-mentioned taxa were collected in the Ukraine by Dr. I. Bednarska (Table 1). Each population sample consisted of 20–30 plants which were identified first by their anatomical and morphological traits. Then seed population samples were collected. Vouchers were deposited at the Institute of Ecology of the Carpathians Herbarium of the Ukrainian National Academy of Sciences (LWKS).

Table 1. Locality list of the studied taxa of genus *Festuca* from the Ukraine.

Taxon	Number LWKS	Locality
F. ovina	1667	Kyiv region, Vyshgorod district, village Hotyanivka 06.07.2014 leg. I.Bednarska, Kostikov, 50°38'54.82" 30°33'17.26"
F. psammophilla		Lviv region, Yavoriv district, village Stradch 16.07.2014 leg. I.Bednarska, 49°53'45.09" 23°45'18.69"
F. pallens		Ternopil region, city Kremenets, tract Divochi Skeli 15.07.2014 leg. I.Bednarska, 50° 7'5.78" 25°43'38.35"
F. pallens	1677	Ivano-Frankivsk region, Galych district, village Podilla 19.07.2014 leg. I.Bednarska, 49°16'36.11" 24°44'28.97"
F. polesica	1666	Kyiv region, Vyshgorod district, village Hotyanivka 06.07.2014 leg. I.Bednarska, Kostikov, 50°37'3.91" 30°33'8.85"
F. polesica	1682	Volyn region, Lyubeshovsky district, village Lyubotin, river Tzir 2013 leg. Orlov, 51°50'46.66" 25°19'44.61"
F. polesica	1657	Kharkiv region, town Bogodukhov 18.06.2014 leg. I.Bednarska, 50° 8'44.38" 35°32'16.72"
F. rupicola	1673	Ivano-Frankivsk region., Rohatyn district, v. Kuropatnyky (green plants) 19.07.2014 leg. I.Bednarska, 49°17'1.98" 24°40'8.87"
F. rupicola	1679	Ivano-Frankivsk region., Rohatyn district, v. Fraga (green plants) 19.07.2014 leg. I.Bednarska ,49°28'2.47" 24°26'49.50"

Proteins of seeds were extracted by 0.01 M tris, 0.08 M glycine, 20 % sucrose, pH 8.3, and seeds : buffer ratio = 1:6. Anodal seed proteins were electrophoretically resolved in vertical polyacrylamide slab gels (7.5 % separating, 3 % stacking gels) using a slightly modified tris-glycine discontinuous system (Davis 1964). Acidic vertical polyacrylamide slab electrophoretic system (Reisfeld & al. 1962) was employed to resolve the cathodal forms with spacer of 3 % and 7.5 separating gel. The length of separating gel was 6 cm (anodal forms), 7 cm (cathodal forms), while the spacer was 1 cm long.

Gels were stained with Coomasie Briliant Blue R-250 (0.1 %) in 10 % acetic acid, 45 % methanol for two hours, and destained in 10 % acetic acid, 10 % methanol for a night.

Affinities among the taxa within the studied group were assessed by the coefficient of similarity SI=M/M+N, where M is the number of bands common for the compared taxa, and N is the sum of absent bands in each compared taxon. The values of coefficient SI for each pair-wise comparison among the taxa were calculated separately for each set of seed proteins (anodal, cathodal). Then, the mean values of SI coefficient as an average of the two data sets were calculated, in order to assess affinities among the taxa within the studied group of *Festuca.* An index of group affinity (GA) was calculated for each taxon as a sum of its SI values.

Table 1. Continuation.

Taxon	Number LWKS	Locality
F. rupicola f. glaucescens	1678	Ivano-Frankivsk region, Rohatyn district, v. Fraga (glaucous plants) 19.07.2014 leg. I.Bednarska, 49°28'2.47" 24°26'49.50"
F. rupicola	1675	Ivano-Frankivsk region, Galych district, village Podilla (green plants) 19.07.2014 leg. I.Bednarska, 49°16'30.88" 24°44'29.95"
F. rupicola f. glaucescens	1674	Ivano-Frankivsk region, Galych district, village Podilla (glaucous plants) 19.07.2014 leg. I.Bednarska, 49°16'33.46" 24°44'28.45"
F. macutrensis locus classicus	1622	Lviv region, Brody district, village Sukhovolya mountain Makitra 01.07.2013 leg. I.Bednarska, 50° 2'17.32" 25°14'53.75"
F. arietina	1668	Cherkasy region., Kaniv district, between village Keleberda and town Kaniv 07.07.2014 leg. I.Bednarska, Kostikov, 49°44'22.51" 31°33'53.09"
F. arietina	1445	Belarus. Homiel region., Vietka district, the village Odnopolie, river Sozh 17.06.2010 leg. I.Bednarska, 52°41'50.70" 30°58'30.66"
F. galiciensis sp. nova locus classicus	1672	Ivano-Frankivsk region., Rohatyn district, v. Kuropatnyky (glaucous plants) 19.07.2014 leg. I.Bednarska, 49°17'2.47" 24°40'8.10"

Results and discussion

Anodal seed proteins. A total of 23 migrating to the anode electrophoretic bands were detected in the studied taxa of genus *Festuca* (Table 2). Bands 10, 25 and 53 were shared by all taxa. Except for *F. polesica*, bands 31 and 38 were common for all taxa too. Electrophoretic bands 38, 39, 43, and 50 occurred in most of the studied taxa. Bands 16 and 20 were rare and shared by two taxa (*F. ovina, F. pallens*) only. Similarly, bands 27 and 36 were observed in the studied populations of *F. psammophila* and *F. pallens* only. Band 42 was found in *F. polesica* and *F. pallens*. Band 49 proved to be species-specific for *F. ovina*, while electrophoretic band 55 was characteristic of *F. polesica.* It should be emphasized that the typical *F. rupicola* and *F. rupicola*

Table 2. Banding profiles of anodal seed proteins in the studied taxa of *Festuca*.*

Taxon	Electrophoretic band																						
	10	16	18	20	25	27	29	31	33	36	37	38	39	42	43	44	46	47	48	49	50	53	55
1. *F. ovina*	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0
2. *F. psammophilla*	1	0	1	0	1	1	0	1	1	1	0	1	1	0	1	0	0	1	1	0	0	1	0
3. *F. pallens*	1	1	1	1	1	1	0	1	0	1	0	1	0	1	0	1	1	0	1	0	0	1	0
4. *F. polesica*	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	1	1
5. *F. rupicola*	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	1	0	0	1	1	0
6. *F. macutrensis*	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0
7. *F. arietina*	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0
8. *F. galiciensis*	1	0	0	0	1	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0	1	1	0

* 1 – band present, 0 – band absent. Each band was designated by a number reflecting its migration (in mm) from the origin.

f. *glaucescens* possessed identical patterns of their seed proteins. For this reason both forms are considered in Tables 2, 3 and 4 as *F. rupicola.*

The values of SI coefficient varied within a wide range, from 0.21 (*F. polesica* vs *F. macutrensis*) to 1.00, when *F. arietina* was contrasted to *F. macutrensis* (Table 3). Mention deserves the fact that the taxa of *F. valesiaca* group demonstrated close mutual affinity and all pair-wise comparisons among them resulted in very high values (0.89–1.00) of the similarity index. The species *F. polesica* was the most distantly positioned taxon within the whole studied group of genus *Festuca.* Its values of SI coefficient were the lowest ones and varied within a narrow range (0.21–0.36) for all pair-wise comparisons among the studied taxa.

Considering the index of group affinity (GA), it could be noticed that the species *F. psammophila* (GA=2.81), *F. pallens* (GA=3.42) and *F. ovina* (GA=3.78) were relatively distant within the studied group of genus *Festuca*. The values of coefficient GA were higher and varied from 4.79 to 5.51 for all taxa of *F. valesiaca agg*. The species *F. arietina* (GA=7.51) demonstrated the highest affinity within the studied group. On the contrary, *F. polesica* (GA=2.14) was most distant within the group of *Festuca* taxa, as judged by the anodal seed proteins.

Table 3. Coefficient of similarity SI values for pair-wise comparisons among the studied taxa of genus *Festuca* – anodal seed proteins.

Taxon	Coefficient of similarity SI							
	1	2	3	4	5	6	7	8
1. *F. ovina*	1.00							
2. *F. psammophilla*	0.44	1.00						
3. *F. pallens*	0.44	0.50	1.00					
4. *F. polesica*	0.29	0.31	0.28	1.00				
5. *F. rupicola*	0.50	0.48	0.31	0.31	1.00			
6. *F. makutrensis*	0.50	0.50	0.36	0.21	0.95	1.00		
7. *F. arietina*	0.53	0.53	0.33	0.23	0.95	1.00	1.00	
8. *F. galiciensis*	0.54	0.50	0.29	0.29	0.95	0.89	1.00	1.00

Cathodal seed proteins. A total of nine cathodally migrating bands were electrophoretically resolved within the studied taxa of genus *Festuca* (Table 4). Band 10 was detected throughout the entire studied group. Band 49 was shared by all taxa but *F. polesica*. Electrophoretic band 60 was shared by all taxa of *F. valesiaca agg*. The species pair *F. polesica – F. ovina* shared electrophoretic band 46. The species *F. ovina* possessed a unique band 51, while electrophoretic band 54 was found in the studied populations of *F. psammophila* only.

Table 4. Banding profiles of cathodal seed proteins in the studied taxa of *Festuca*.*

Taxon	Electrophoretic band								
	10	46	49	51	54	56	58	60	63
1. *F. ovina*	1	1	1	1	0	0	1	0	1
2. *F. psammophilla*	1	0	1	0	1	0	1	0	1
3. *F. pallens*	1	0	1	0	0	0	1	0	1
4. *F. polesica*	1	1	0	0	0	0	1	0	0
5. *F. rupicola*	1	0	1	0	0	0	0	1	0
6. *F. macutrensis*	1	0	1	0	0	0	0	1	0
7. *F. arietina*	1	0	1	0	0	1	1	1	0
8. *F. galiciensis*	1	0	1	0	0	0	0	1	0

* 1 – band present, 0 – band absent. Each band was designated by a number reflecting its migration (in mm) from the origin.

Considering the SI coefficient values, it was found out that *F. polesica* occupied a remote position to all taxa of *F. valesiaca agg,* as in most cases its SI coefficient equaled 0.20 (Table 5). The former species was closer to *F. ovina* (SI=0.43) and *F. pallens* (SI=0.40), as revealed by the profiles of their cathodal seed proteins. Pair-wise comparisons among *F. ovina, F. pallens* and *F. psammophila* resulted in SI coefficient values similarity within the range of 0.57–0.80, which indicate tighter affinities among them. The values of SI coefficient within *F. valesiaca* group varied from 0.60 to 1.00 and suggested a close affinity among the respective taxa.

Table 5. Coefficient of similarity SI values for pair-wise comparisons among the studied taxa of genus *Festuca* – cathodal seed proteins.

Taxon	Coefficient of similarity SI							
	1	2	3	4	5	6	7	8
1. *F. ovina*	1.00							
2. *F. psammophilla*	0.57	1.00						
3. *F. pallens*	0.66	0.80	1.00					
4. *F. polesica*	0.43	0.33	0.40	1.00				
5. *F. rupicola*	0.43	0.43	0.50	0.25	1.00			
6. *F. makutrensis*	0.28	0.33	0.40	0.20	0.60	1.00		
7. *F. arietina*	0.38	0.43	0.50	0.20	1.00	0.60	1.00	
8. *F. galiciensis*	0.28	0.33	0.40	0.20	0.60	1.00	0.60	1.00

The species *F. polesica* possessed the lowest index value of group affinity (GA=2.21) and occupied the remotest position within the entire studied group of genus *Festuca.* The species *F. ovina* (GA=3.31) and *F. pallens* (GA=3.62) were also distantly positioned in respect to the remaining taxa. The values of GA index for the taxa of *F. valesiaca* group were higher and

varied from 4.41 to 4.69 (*F. arietina*), which indicates close affinity among them.

The mean values of SI coefficient for all pair-wise comparisons among the studied taxa calculated as an average of the two SI data sets (anodal, cathodal seed proteins) are presented in Table 6. The mean values of SI coefficient for the pair-wise comparisons of *F. polesica* with the taxa of *F. valesiaca* group were low and fluctuated within a narrow range (0.21–0.28) suggesting lack of substantial affinity. The former species was closer to *F. ovina, F. pallens* and *F. psammophila,* as revealed by their seed proteins profiles. It could be noticed that the taxa belonging to *F. valesiaca* group were very close to each other as the SI values were high and varied from 0.75 to 0.95 for all pair-wise comparisons. The values of SI coefficient for *F. ovina, F. pallens* and *F. psammophila* were intermediate. These values indicated that the three above-mentioned taxa are almost equidistantly positioned to each other and well differentiated from the members of *F. valesiaca* group.

The index of group similarity has contributed to revealing the systematic relationships within the examined group of genus *Festuca.* The lower values of GA index meant a greater distance for a given taxon and vice versa, the higher values indicated closer affinity within the group. The species *F. polesica* proved to be most distantly positioned within the group, as its GA index (2.21) was the lowest one. The species *F. ovina* (GA=3.51), *F. pallens* (GA=3.54) and *F. psammophila* (GA=3.69) were also comparatively distant within the studied group of genus *Festuca.* The GA index values for the taxa of *F. valesiaca* group were high and varied from 4.81 (*F. rupicola*) to 5.03 for *F. galiciensis* – an indication of high biochemical resemblance and close relationships within the group.

Summarizing the results of the present electrophoretic study, it was evident that the species *F. polesica* was the most distantly positioned taxon within the studied group of genus *Festuca*. This species belongs to *F. beckeri agg*. The taxa of this taxonomic group are characterized by stout, rigid green leaves, with 3–7 ribs, and thickened sclerenchyma ring. The species *F. pallens* and *F. psammophila* are representatives of *F. glauca agg.* Their characteristic morphological traits are glaucous, pruinose, stout leaves, with 1–3 ribs, and a well-developed uniform sclerenchyma ring. The species *F. ovina* is type representative of *F. ovina agg.* – a group of taxa with thin, filiform leaves, 1 distinct rib, and a thin uniform sclerenchyma ring. The three above-mentioned species aggregates are characterized by the ovinoid type of their morphological and anatomical structures and all taxa are diploids (2n=14). According to the presented data, they are well defined entities, with specific patterns of their seed proteins. These taxa are differentiated from each other and clearly distinct from the taxa of *F. valesiaca* group.

The most stable and reliable diagnostic traits are characteristic for diploid *F. valesiaca* only. All other taxa form a complicated polyploid series, with a spectrum of transitional forms among them. The species *F. rupicola* (2n=42) has stable 3(-5) sclerenchyma bundles. Among all taxa of the group, *F. rupicola* has the widest range of distribution which is reflected in certain variability of its morphological traits. The remaining of taxa – *F. arietina, F. macutrensis* and *F.galiciensis* – are tetraploids (2n=28) with more restricted distribution, but the variability of their leaf anatomical traits is among the highest within the group (Bednarska 2000, 2014a, b; Bednarska & Orlov 2011). In these three taxa the sclerenchyma may be developed as isolated bundles, or fused to a different extent into a sclerenchyma ring. According to some authors (Tzvelev 1976; Bednarska 2014a), these taxa are of hybridogeneous origin, which explains their high polymorphism. This group is among the evolutionary youngest ones and the process of species differentiation is not completed yet. In fact, these taxa form a very complex continuum. It is very difficult to divide it into discrete taxa solely on the basis of morphological traits. Just like their morphological, anatomical and ecological peculiarities, the results of the electrophoretic analysis demonstrated that they are closely related taxa and the distinction among them based on seed proteins is problematic. Other biochemical markers are needed to clarify the systematic relationships among the species of *F. valesiaca* agg.

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