Study of genetic and systematic relationships among species of genus *Festuca* (*Poaceae*) by means of isoenzyme markers

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Abstract. Polyacrylamide gel electrophoresis (PAGE) was used to study the isoenzyme composition of five taxa of genus *Festuca* in an attempt to reveal their genetic and systematic relationships. The species *F. valesiaca*, *F. rupicola, F. galiciensis,* and *F. macutrensis* were closely related but different entities within genus *Festuca*. The species *F. pallens* occupied a more remote position in regard to species belonging to *F. valesiaca* agg. Thus, the division of thin-leaved fescues into aggregates based on taxonomy (morphology and anatomy) was supported by an independent biosystematic approach relying on molecular markers. Thus, the results generally support the recent systematic and taxonomic treatment of narrow-leaved fescues.

Key words: *Festuca,* isoenzymes, PAGE, systematic relationships

Introduction.

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

Studies of the genus in the Ukraine are rather scarce (Tzvelev 1976; Tveretinova 1977; Bednarska 2007, 2014). It was found that most narrow-leaved fescues demonstrate high variability of morphological and anatomical traits, including diagnostic ones. The species complex of *F. valesiaca* agg. is the most complicated. Thus, the morphometric parameters of *F. macutrensis* Zapał. (2n = 28) and *F. rupicola* Heuff. (2n = 42) in West Ukraine are practically overlapping. Along with this, in the different populations of *F. macutrensis* merely 20–45 % of the plants possess leaves with confluent sclerenchyma strands – a diagnostic trait of the species. Similarly, in the populations of *F. rupicola* there are plants (5–10 %) with fused sclerenchyma strands – a trait quite untypical for that species (Bednarska 2014). The existence of plants with intermediate traits, as well as of untypical plants for both species makes difficult the proper identification of the taxa.

It seems necessary to apply other approaches, using molecular markers, so as to reveal the systematic structure and phylogenetic relationships of the narrow-leaved fescues.

Several isoenzyme studies of fescues (Livesey & Norrington-Davis 1991; Aiken & al. 1993, 1994; Aiken & Lefkovitch 1995; Angelov 2002, 2003; Angelov & Ivanova 2012) have been conducted in an attempt to investigate species delimitation based on isoenzyme markers.

The present study includes five taxa of *Festuca* from the Ukraine: *F. pallens* Host, *F. valesiaca* Gaud.,

F. rupicola Heuff., *F. macutrensis* Zapał., and *F. galiciensis* Bednarska nom. prov. The last four taxa belong to *F. valesiaca* agg. (*F. valesiaca* group) sensu Arndt (2008). The species of this group are characterized with 3–5 separate leaf sclerenchyma strands (in some species they may be secondarily fused), 5–7 ribs and short trichomes on both sides of the leaf. The species *F. pallens* is a member of *F. glauca* agg. Characteristic traits of this species aggregate are a well-developed sclerenchyma ring, 9–13 ribs and long trichomes on ribs. All above mentioned species occur in the western regions of the Podolian Upland (West Ukraine). Together, they form complicated population complexes (sometimes 2–3 species within one and the same biotope) and make species delimitation very difficult.

The purposes of the present study are: 1) to assess the genetic and systematic relationships among the above-mentioned species of genus *Festuca* by means of isoenzyme markers; 2) to evaluate the systematic position of *F. pallens* as a representative of *F. glauca* agg.

Material and methods.

Plant samples (30–35 seedlings per each species) belonging to the natural Ukrainian populations of these taxa were individually studied. The enzymes superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase(G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH), glutamate-oxaloacetate transaminase (GOT), glutamate dehydrogenase (GDH), and isocitrate dehydrogenase (IDH) were examined on 8 cm long 7.5% polyacrylamide slab gels (Davis (1964). The following staining recipes were used: SOD (Baum & Scandalios 1982), G-6-PDH, 6-PGDH and GDH (Shaw & Prasad 1970), GOT (Przybylska & al. 1982), and IDH (Henderson 1965). Each isoform was designated by a number reflecting its migration (in mm) from the origin.

Systematic relationships among the above mentioned taxa of genus *Festuca* were assessed by coefficient of divergence D (Stuessy 1990), according to the following formula:

$$D = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x_{ij} - x_{ik}\right)^2}$$

where **N** is the number of isoforms for each enzyme, and $\mathbf{x_{ij}}$ and $\mathbf{x_{ik}}$ are the frequencies of $\mathbf{i}^{\text{-th}}$ isoform in taxa **j** and **k**. An index of group affinity (GA) was calculated for each taxon as a sum of its **D** values.

Results and discussion

Superoxide dismutase. A total of eleven isoforms of the enzyme were detected in the studied *Festuca* species (Table 1). Five isoforms, namely 18, 25, 29, 33, 36, and 47, were monomorphically fixed across the entire group. With the exception of *F. pallens*, isoform 18 was also fixed. Isoform 31 was rare in *F. galiciensis* and *F. macutrensis*. The values of coefficient D ranged from **0.08** (*F. macutrensis* vs. *F. pallens*) to **0.30**, when the latter species was contrasted to *F. valesiaca*.

Table 1. Isoform frequencies of superoxide dismutase in the studied species of genus *Festuca*.

	Isoform										
Species	**15**	**18**	**20**	**23**	**25**	**29**	**31**	**33**	**36**	**39**	**47**
F. valesiaca	1.00	1.00	0.24	0.70	1.00	1.00	0.31	1.00	1.00	0.85	1.00
F. rupicola	1.00	1.00	0.18	0.48	1.00	1.00	0.58	1.00	1.00	0.64	1.00
F galiciensis	1.00	1.00	0.32	0.36	1.00	1.00	0.08	1.00	1.00	0.32	1.00
F. macutrensis	1.00	1.00	0.28	0.65	1.00	1.00	0.10	1.00	1.00	0.48	1.00
F. pallens	1.00	0.25	0.14	0.14	1.00	1.00	0.16	1.00	1.00	0.45	1.00

Glucose-6-phosphate dehydrogenase. Five isoforms of G-6-PDH were electrophoretically resolved in the studied species group of *Festuca* (Table 2). Isoform 26 was common and invariant for all examined species. The remaining isoforms were shared by all studied taxa, with frequencies fluctuating from 0.16 to 0.60. The values of coefficient D for the pair-wise comparisons among the species varied within a wide range: from 0.08 (*F. macutrensis* vs. *F. galiciensis*) to 0.31, when the former species was compared to *F. pallens*.

Table 2. Isoform frequencies of glucose-6-phosphate dehydrogenase in the studied species of genus *Festuca*.

	Isoform				
Species	**4**	**10**	**12**	**18**	**26**
F. valesiaca	0.32	0.43	0.32	0.32	1.00
F. rupicola	0.37	0.37	0.37	0.17	1.00
F galiciensis	0.50	0.50	0.50	O.15	1.00
F. macutrensis	0.60	0.60	0.60	0.26	1.00
F. pallens	0.18	0.20	0.18	0.16	1.00

6-phosphogluconate dehydrogenase. Six isoforms altogether of the enzyme 6-PGDH were detected in the examined taxa of genus *Festuca* (Table 3). Two isoforms, namely 16 and 18, were monomorphic across the entire group. With the exception of *F. pallens* and *F. galiciensis,* isoform 20 was also invariant. Isoform 24 was rare in *F. pallens.* The values of coefficient D ranged within a wide interval: from 0.10 (*F. macutrensis* vs. *F. rupicila*) to 0.39, when the species *F. galiciensis* was contrasted to *F. pallens*.

Table 3. Isoform frequencies of 6-phosphogluconate dehydrogenase in the studied species of genus *Festuca*.

	Isoform					
Species	**16**	**18**	**20**	**22**	**24**	**26**
F. valesiaca	1.00	1.00	1.00	0.24	0.14	1.00
F. rupicola	1.00	1.00	1.00	0.32	0.28	0.75
F galiciensis	1.00	1.00	0.85	0.52	0.42	0.86
F. macutrensis	1.00	1.00	1.00	0.12	0.22	0.71
F. pallens	1.00	1.00	0.24	0.18	0.06	0.42

Glutamate-oxaloacetate transaminase. Seven isoforms of GOT were electrophoretically resolved in the studied species group of *Festuca* (Table 4). Isoform 25 was common and invariant for all examined species. Isoforms 20 and 29 were species-specific for *F. pallens*, while isoforms 51 and 55 were detected in *F. rupicola* only. The values of coefficient D for pair-wise comparisons within *F. valesiaca* agg. ranged within a narrow interval: from 0.11 (*F. macutrensis* vs. *F. galiciensis*) to 0.15, when the species *F. macutrensis* was contrasted to *F. valesiaca.*

Table 4. Isoform frequencies of glutamate-oxaloacetate transaminase in the studied species of genus *Festuca*.

	Isoform						
Species	**20**	**22**	**25**	**29**	**51**	**55**	**59**
F. valesiaca	0.00	0.00	1.00	0.00	0.00	0.00	0.00
F. rupicola	0.00	0.12	1.00	0.00	0.06	0.06	0.25
F galiciensis	0.00	0.00	1.00	0.00	0.00	0.00	0.00
F. macutrensis	0.00	0.00	1.00	0.00	0.00	0.00	0.00
F. pallens	0.09	0.09	1.00	0.09	0.00	0.00	0.09

Glutamate dehydrogenase. Three isoforms of the enzyme were found in *F. valesiaca:* isoform 10 was dominant, while isoform 8 and doublet 10/12 were rare. The species *F. rupicola, F. macutrensis* and *F. galiciensis* possessed isoform 10. The latter isoform was prevalent in *F. pallens* and isoform 12 was rare.

Isocitrate dehydrogenase. A doublet consisting of isoforms 26 and 28 was observed in the studied species of genus *Festuca*.

When averaged for the enzymes SOD, G-6-PDH, 6-PGDH, and GOT, the mean values of coefficient D for the comparisons of *F. pallens* with species of *F. valesiaca* group produced the highest values (0.20–0.23) and indicated the remote position of the former species within the studied group (Fig. 1; Table 5). Values of coefficient D for pair-wise comparisons within *F. valesiaca* agg. ranged within a narrow interval: from 0.11 (*F. macutrensis* vs. *F. galiciensis*) to 0.15, when the species *F. macutrensis* was contrasted to *F. rupicola.* Generally, the mean values of D for the comparisons within *F. valesiaca* agg. were twice lower than the values for comparisons of *F. pallens* and suggested close mutual affinities among the members of *F. valesiaca* group, as well a remote position of *F. pallens*.

Table 5. Mean values of coefficient of divergence D for each pair-wise comparison among the studied species of *Festuca*.

	Coefficient D				
Species	**1**	**2**	**3**	**4**	**5**
1. *F. valesiaca*	0.00				
2. *F. rupicola*	0.12	0.00			
3. *F galiciensis*	0.14	0.13	0.00		
4. *F. macutrensis*	0.13	0.15	0.11	0.00	
5. *F. pallens*	0.23	0.20	0.23	0.21	0.00

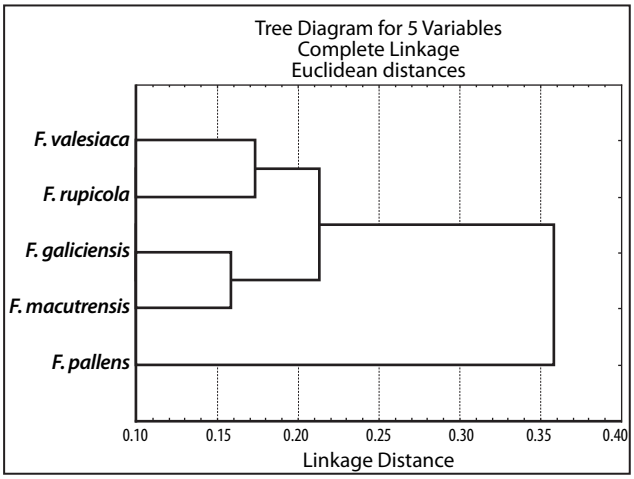

Fig. 1. Dendrogram of Cluster analysis for the studied *Festuca* species based on mean values of coefficient D (Table 5).

Analysis of isoenzyme data demonstrated that the studied taxa could be distinguished by isoenzymes. Several monomorphically-fixed isoform differenc-

es in the isoenzyme structure of the studied species were detected. For example, isoform 18 of SOD was invariant and common for the members of *F. valesiaca* agg. and clearly differentiated this group from *F. pallens,* which is a representative of *F. glauca* agg. A similarly contrasting pattern delimited *F. pallens* in respect to isoform 20 of the enzyme 6-phosphogluconate dehydrogenase. This isoform was monomorphically fixed (*F. valesiaca, F. rupicola, F. macutrensis*), or nearly fixed (*F. galiciensis*), while it was seldom found in *F. pallens*. Thus, clear-cut differences of the isoenzyme markers SOD and 6-PGDH enabled the distinguishing of *F. pallens* (*F. glauca* agg.) from the studied species of *F. valesiaca* agg. On the other hand, some isoforms were species-specific for some of the examined *Festuca* taxa. For example, isoforms 20 and 29 of GOT were species-specific for *F. pallens.* Similarly, isoforms 51 and 55 of GOT were detected in *F. rupicola* only. These distinct differences should be regarded as diagnostic for the respective species and discriminated them properly within the studied group. Similar patterns of isoenzyme variation have been found in other studies of fescues. In an earlier study of Bulgarian populations, it was demonstrated that the species *F. valesiaca* and *F. rupicola*, separated mainly on the basis of subtle morphological differences, were isoenzymatically well characterized as distinct genetic entities (Angelov and Ivanova, 2012). Isoenzyme markers were also used to assess the species boundaries in *F. ovina* complex (Aiken & al. 1993, 1994; Aiken & Lefkovitch 1995; Angelov 2003).

Summarizing the results, it was clear that the species *F. pallens*, as a representative of other species aggregates, occupied a more remote position in regard to the species belonging to *F. valesiaca* agg. Thus, the division of thin-leaved fescues into aggregates based on taxonomy (morphology and anatomy) was supported by an independent biosystematic approach relying on molecular markers. It could be concluded that the present results generally support the recent systematic and taxonomic treatment of narrow-leaved fescues.

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