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ISOENZYME VARIATION AND GENETIC RELATIONSHIPS AMONG FIVE SPECIES OF GENUS *FESTUCA* FROM UKRAINE

Angelov G. [1*] ***and I. Bednarska***[2]

[1]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 23, 1113 Sofia, Bulgaria*

[2]*Institute of Ecology of the Carpathians, NAS of Ukraine, Kozelnytska str., 4 Lviv 79026, Ukraine*

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Summary: Polyacrylamide gel electrophoresis (PAGE) was employed to analyze isoenzyme variation and genetic structure of *Festuca pallens*, *F. valesiaca*, *F. rupicola*, *F. macutrensis*, and *F. galiciensis* in an attempt to reveal genetic and systematic relationships among them. It was shown that the species *F. valesiaca*, *F. rupicola* and *F. macutrensis* were closely related but genetically different entities within genus *Festuca*. These three species were almost equidistantly and relatively far positioned from both *F. galiciensis* and *F. pallens.*

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INTRODUCTION

Festuca L. is one of the most complicated 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 recognized. Lately species concepts have become narrower and a large number of finely split taxa are recognized today.

Studies of the genus in Ukraine are rather scarce (Tzvelev, 1976; Tveretinova, 1977; Bednarska, 2007, 2014). It was found that most of the narrow-leaved fescues demonstrated high variability of morphological and anatomical traits, including diagnostic ones. So, there is a need to apply other approaches, using molecular markers, to reveal the systematic structure and phylogenetic relationships of the narrow-leaved fescues.

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

The present study included five taxa of *Festuca* from Ukraine: *F. pallens* Host, *F. valesiaca* Gaud., *F. rupicola* Heuff., *F. macutrensis* Zapał. and *F. galiciensis*

*Corresponding author: jorkata_1953@mail.bg

Bednarska nom. prov. The last four taxa belong to *F. valesiaca* agg.

The aim of the study was to assess isoenzyme variation, genetic and systematic relationships among the above-mentioned five species of genus *Festuca*.

MATERIAL AND METHODS

Seedlings (25-30/species, one-mon-old) deriving from natural populations of the above mentioned species were individually studied. The enzymes esterase (EST, *E.C.* 3.1.1.2.), NADP-malate dehydrogenase (NADP-MDH, *EC* 1.1.1.82) and amylase (AMY, *EC* 3.2.1.1) were examined. Anodal isoforms were resolved on 7.5 % polyacrilamide slab gels (Davis, 1964). Cathodal EST was run on 7.5 % polyacrylamide slab gels (Reisfeld et al., 1962). The length of gels was 8 cm for NADP-MDH and 6 cm for EST. Amylase was run anodally on 6 % polyacrylamide gels, containing 0.5% starch for 8 h at 150 V. The following staining recipes were used: EST (Schmidt-Stohn and Wehling, 1983), NADP-MDH (Shaw and Prasad, 1970), AMY (Przybylska et al., 1982). 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, x_{ij} and x_{ik} are the frequencies of i-th isoform in taxa j and k.

RESULTS AND DISCUSSION

Anodal esterase

Totally fifteen isoforms of the enzyme were detected in the studied species of *Festuca* (Table 1). Most of the isoforms were common for all species studied. Isoform 42 was invariant (frequency of 1.00) throughout the whole group. Except for *F. galiciensis*, isoform 38 was also fixed. Isoforms 13, 15, 27 and 45 were absent in *F. pallens* while isoforms 10, 23, 45 and 47 were not found in *F. galiciensis.* The values of coefficient D ranged from 0.23 to 0.37 and the studied taxa were almost equidistantly positioned in respect to anodal esterase.

Cathodal esterase

Totally five isoforms were resolved in the studied species (Table 2). Isoforms 11, 28 and 33 were shared by all species examined. Except for *F. galiciensis*, isoform 23 was common for the whole group studied. Isoform 37 was absent in *F. pallens* while it occurred with different frequencies in the populations of all species belonging to *F. valesiaca* group. The values of coefficient D for the pair-wise comparisons among the members of *F. valesiaca* agg. varied in a narrow range (0.23 – 0.29), an indication for close mutual affinities. The species *F. pallens* proved to be most distant in respect to cathodal esterase**.** The values of its comparisons with the species group *F. valesiaca, F. rupicola* and *F. macutrensis* were much higher (0.38 - 0.48) and indicated the more remote position of *F. pallens* within the studied group of genus *Festuca*. The value of comparison between *F. galiciensis*, and *F. pallens* was much lower (0.26) thus suggesting a closer relationship among them.

Table 1. Isoform frequencies of anodal EST in five species of genus *Festuca*.

Species	Isoform														
	10	13	15	17	19	21	23	27	30	33	35	38	42	45	47
F. valesiaca	0.15	0.43	0.18	0.46	1.00	0.48	0.32	0.65	1.00	1.00	1.00	1.00	1.00	0.54	0.45
F. rupicola	0.24	0.19	0.31	0.60	0.75	0.18	0.12	0.15	0.70	0.95	1.00	1.00	1.00	0.12	0.34
F galiciensis	0.00	0.23	0.26	0.50	0.32	0.28	0.00	0.32	0.55	1.00	0.45	0.50	1.00	0.00	0.00
F. macutrensis	0.42	0.12	0.60	0.14	1.00	0.10	0.50	0.42	1.00	1.00	1.00	1.00	1.00	0.25	0.15
F. palens	0.16	0.00	0.00	0.19	0.85	0.00	0.38	0.00	1.00	0.34	0.85	1.00	1.00	0.00	0.12

Each isoform is designated by a number reflecting its migration (in mm) from the origin.

Table 2. Isoform frequencies of cathodal EST in five species of genus *Festuca*.

Species	Isoform				
	11	23	28	33	37
F. valesiaca	0.55	0.32	1.00	1.00	0.43
F. rupicola	0.22	0.57	0.84	1.00	0.72
F galiciensis	0.31	0.00	0.34	1.00	0.15
F. macutrensis	0.10	0.14	0.72	1.00	0.62
F. palens	0.00	0.38	0.22	0.82	0.00

Each isoform is designated by a number reflecting its migration (in mm) from the origin.

NADP-malate dehydrogenase

In total six isoforms of the enzyme were detected in the studied populations of *Festuca* taxa (Table 3). Isoform 20 was monomorphically fixed throughout the whole group. Isoform 24 was not observed in *F. pallens,* while isoform 27 was not found in *F. galiciensis.* Except for *F. galiciensis*, the values of coefficient D for the pair-wise comparisons among the species of *F. valesiaca* agg. were within the range from 0.12 (*F. valesiaca* vs. *F. macutrensis*) to 0.19 when *F. rupicola* was contrasted to the latter species. These values evidenced for close affinities among the above-mentioned three taxa. In contrary, pair-wise comparisons between species pair *F. galiciensis*, *F. pallens* and the species group *F. valesiaca, F. rupicola* and *F. macutrensis* resulted in much higher values of coefficient D (0.49 – 0.52) and indicated the more distant position of both *F. galiciensis* and *F. pallens* regarding NADP-malate dehydrogenase.

Amylase

Four isoforms were found in the studied taxa. Isoforms 11 and 12 were shared by all species. Isoform 13 was not detected in *F. macutrensis.* Isoform 21 was invariant (frequency of 1.00) in *F. valesiaca, F. rupicola*, *F. macutrensis* but absent in *F. galiciensis.* The values of coefficient D for the comparisons between

Table 3. Isoform frequencies of NADP-MDH in five species of genus *Festuca*.

Species	Isoform					
	13	15	18	20	24	27
F. valesiaca	0.35	0.25	0.30	1.00	1.00	1.00
F. rupicola	0.62	0.44	0.54	1.00	0.40	1.00
F galiciensis	0.16	0.10	0.12	1.00	0.25	0.00
F. macutrensis	0.44	0.22	0.40	1.00	0.75	1.00
F. palens	0.48	0.62	0.15	1.00	0.00	0.25

Each isoform is designated by a number reflecting its migration (in mm) from the origin.

F. valesiaca, F. rupicola, *F. macutrensis* and species pair *F. galiciensis*, *F. pallens* were high (0.37 – 0.55) and evidenced for an isolated position of the latter two species within the group.

Averaged over the four examined enzymes, the mean values of coefficient D showed that the species *F. galiciensis* was equidistantly and far positioned from *F. valesiaca* (D 0.44), *F. rupicola* (D 0.43) and *F. macutrensis* (D 0.42) while the last three taxa were much closer to each other (Table 4). The species *F. pallens* occupied a remote position to *F. valesiaca* (D 0.46) and *F. macutrensis* (D 0.44) and was more close (D 0.31) to *F. galiciensis*.

In a previous study of Bulgarian populations it was shown that 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).

Festuca macutrensis is among the poorly studied species. Information about it is often wrong, mainly due to its big overall resemblance to *F. rupicola* (Bednarska, 2000; Bednarska and Orlov, 2011). Both species are practically identical for their morphology, pubescence and leaf diameter. Main differences are found in sclerenchyma structure and ploidy level - 3 sclerenchyma strands and 2n=42 in *F. rupicola,* confluent strands and 2n=28 in *F. macutrensis*. The present isoenzyme data confirm their phylogenetic relationship revealed by morphology and anatomy.

Festuca galiciensis is a unique endemic taxon found in one locality (Western Ukraine) only (Bednarska, 2014). Like *F. macutrensis,* this species is among the members of *F. valesiaca* group with an entire sclerenchyma ring. However, in contrast to *F. macutrensis*, 5-year recordings showed reliable trait differences in respect to all other species of the group. Isoenzyme data for *F. galiciensis* confirmed its uniqueness and remoteness within *F. valesiaca* agg. In our opinion, *F. galiciensis* is of hybridogeneous origin and one of its parents is probably *F. pallens*. The data presented here are in good concordance with such a hypothesis.

The analysis of isoenzyme data showed that the examined taxa could be discriminated by isoenzymes. Several monomorphically-fixed isoform differences in the isoenzyme structure of the studied species were detected. On

Table 4. Mean values of coefficient of divergence D for each pair-wise comparison among the studied five species of genus *Festuca*.

Species	Coefficient D				
	1	2	3	4	5
1. *F. valesiaca*	0.00				
2. *F. rupicola*	0.22	0.00			
3. *F galiciensis*	0.44	0.43	0.00		
4. *F. macutrensis*	0.17	0.20	0.42	0.00	
5. *F. palens*	0.46	0.34	0.31	0.44	0.00

the other hand, a portion of isoforms were not found in some of the examined *Festuca* species. These specific isoform combinations form distinct isoenzyme patterns which distinguish the respective species within the group. Similar patterns of isoenzyme variation have been found in other studies of fescues. Isoenzyme markers were used to assess species boundaries in *F. ovina* complex (Aiken et al., 1993, 1994; Aiken and Lefkovitch, 1995; Angelov, 2003).

Summarizing the results, it could be concluded that the species *F. valesiaca*, *F. rupicola* and *F. macutrensis* were closely related but genetically different entities within genus *Festuca*. These species were almost equidistantly and relatively far positioned from both *F. galiciensis* and *F. pallens*.

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