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Research Article

ISOENZYME VARIATION AND SYSTEMATIC RELATIONSHIPS AMONG FOUR XEROPHYTES OF GENUS *FESTUCA* FROM BULGARIA

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Summary: The isoenzyme variation of *Festuca hirtovaginata* (Acht.) Markgr. Dann., *F. thracica* (Acht.) Markgr. Dann., *F. hercegovinica* Markgr. Dann. and *F. oviniformis* Vett. was examined by means of four molecular markers. Systematic relationships among the above mentioned taxa were assessed by calculating coefficient of divergence D. The mean values of coefficient D averaged over the four enzymes varied in a rather narrow range from 0.21 to 0.31 for all pair-wise comparison among the studied species of genus *Festuca.*. It could be assumed that the studied taxa are well differentiated and closely related entities within genus *Festuca*. The species *F. oviniformis* and *F. hirtovaginata* proved to be most closely related for the set of isoenzyme markers employed. *Festuca thracica* was relatively more distantly positioned within the studied group. As a whole, it could be concluded that the four studied species are more or less closely related within genus *Festuca*. The acknowledgement of *F. hirtovaginata* and *F. thracica* as closely related but different species and the treatment of *F. hercegovinica* as a distinct species seemed justified.

Keywords: *Festuca*, xerophytes, isoenzyme variation, systematic relationships.

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INTRODUCTION

The present study included four xerophytes of genus *Festuca,* namely, *F. hirtovaginata, F. thracica F. hercegovinica* and *F. oviniformis*. The species *F. hercegovinica* is a newly described taxon (Markgraff-Dannenberg, 1980) that grows on silicates. The species *F. oviniformis* grows on serpentinites. *Festuca hirtovaginata* and *F. thracica* have varied taxonomic history. Earlier *F. thracica* was treated as variety/subspecies of *F. ovina* or both taxa were considered as forms of *F. duriuscula* (Achtarov, 1953). Later

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Markgraff-Dannenberg (1980) changed the taxonomical status ascribed by Achtarov and ranked *F. hirtovaginata* and *F. thracica* as species.

Isoenzymes can be used for evaluating genetic differences and systematic relationships within taxonomically complicated plant groups. In the last two decades several isoenzyme studies of fescues (Aiken et al., 1993; Aiken et al., 1994; Aiken and Lefkovitch, 1995; Guldahl et al., 2001) were carried out to investigate species delimitation.

The purpose of the present study was to reveal the isoenzyme variation and systematic relationships among the above-mentioned four species of genus *Festuca*.

MATERIALS AND METHODS

The enzymes anodal peroxidase (PER), EC 1.11.1.7, acid phosphatase (ACP), EC 3.1.3.2, superoxide dismutase (SOD), EC 1.15.1.1 and amylase (AMY), EC 3.2.1.1, were analyzed individually in natural populations of the above listed taxa. Anodally migrating isoforms were resolved on 7.5% polyacrilamide slab gels as described by Davis (1964). The length of gels was 11 cm for AMY and SOD, 9 cm for ACP and 6 cm for anodal PER. The following staining recipes were used: AMY (Reisfeld et al. 1962), PER (Przybylska et al., 1982), ACP (Shaw and Prasad, 1970), SOD (Baur and Schorr, 1969).

Systematic relationships among the above mentioned taxa of genus *Festuca* were assessed by calculating 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 mean frequencies of i-th isoform in taxa j and k and presented graphically as a dendrogram using the non-weighted pair-group method with arithmetic mean (UPGMA) (Statistica 7.0).

RESULTS AND DISCUSSION

Anodal peroxidase

Totally thirteen isoforms of the enzyme from the studied species were electrophoretically resolved (Table 1). Most of the isoforms were shared by all species. Except for *F. thracica,* isoform 19 was monomorphically fixed throughout the studied group. Similarly, isoform 48 was monomorphic in all taxa, but was absent in *F. hercegovinica.* Isoform 40 was invariant in *F. hirtovaginata* and *F. thracica* and it was absent in *F. hercegovinica* and *F. oviniformis.* The values of coefficient D varied in a wide range (0.18-0.50). *Festuca hirtovaginata* and *F. thracica* proved to be most closely positioned (D=0.18), while the species pair *hirtovaginata-hercegovinica* was most distant (D=0.50).

Acid phosphatase

In total, twelve isoforms of ACP were detected in the examined species of genus *Festuca* (Table 2). Most isoforms were common for all examined species. Similarly, isoforms 7, 32, 39 occurred in all studied taxa, but were absent in *F. oviniformis*. The values of coefficient

Table 1. Mean isoform frequencies of anodal peroxidase in the studied populations of *F. hercegovinica, F. ovinoformis, F. thracica* and *F. hirtovaginata*.

Species	Isoform												
	5	10	11	15	17	19	22	23	25	40	43	48	52
F. hercegovinica	0.00	0.00	0.43	0.00	0.00	1.00	0.40	0.62	0.00	0.00	0.76	0.81	0.95
F. oviniformis	0.20	0.06	0.13	0.13	0.13	1.00	0.33	0.20	0.20	0.00	0.93	1.00	1.00
F. thracica	0.13	0.07	0.14	0.00	0.13	0.67	0.20	0.20	0.13	1.00	1.00	1.00	0.00
F. hirtovaginata	0.00	0.00	0.20	0.47	0.05	1.00	0.05	0.00	0.00	1.00	1.00	1.00	0.00

Table 2. Mean isoform frequencies of acid phosphatase in the studied populations of *F. hercegovinica, F. ovinoformis, F. thracica* and *F. hirtovaginata*.

Species	Isoform											
	7	9	13	17	19	23	32	35	37	39	42	47
F. hercegovinica	0.10	0.47	0.95	0.24	0.52	0.86	0.14	0.81	0.05	0.15	0.57	0.35
F. oviniformis	0.00	0.14	0.21	0.75	0.43	0.77	0.00	0.37	0.34	0.00	0.13	0.87
F. thracica	0.43	0.77	0.77	0.14	0.14	0.28	0.72	0.61	0.15	0.68	0.45	0.22
F. hirtovaginata	0.72	0.55	0.32	0.38	0.45	0.48	0.58	0.34	0.09	0.35	0.65	0.18

D ranged from 0.25 (*F. hirtovaginata* vs *F. thracica*) to 0.50 when the species pairs *F. oviniformis / F. thracica* were compared.

Superoxide dismutase

Seven isoforms of SOD were observed in the studied species of genus *Festuca* (Table 3). Monomorphically-fixed isoforms 9 and 57 were common for the whole species group. Except for *F. thracica,* isoform 32 was monomorphic throughout the studied group. Similarly, isoform 74 was invariant in all taxa, but was absent in *F. oviniformis*. The values of coefficient D fluctuated from 0.07 (*F. hercegovinica* vs *F. thracica*) to 0. 31 when the species *F. oviniformis* and *F. hirtovaginata* were contrasted.

Amylase

Totally nine isoforms of the enzyme were electrophoretically resolved in the examined species (Table 4). Isoforms 19, 25 and 34 were shared by all studied species. Except for *F. oviniformis*, isoforms 3, 23 and 44 were also common for the whole group. Similarly, isoforms 12 and 27 were observed in all studied taxa, but were absent in *F. thracica*. The values of coefficient D varied in a comparatively wide range - from 0.16 (*F. hercegovinica* vs *F. thracica*) to 0.29 when *F. hercegovinica* and *F. oviniformis* were compared.

The mean values of coefficient D averaged over the four enzymes surveyed are shown in Table 5. The values varied in a rather narrow range - from 0.21 to 0.31 for all pair-wise comparisons. It could be assumed that the studied taxa are well differentiated and closely related entities within genus *Festuca*.

Systematic relationships among the above mentioned taxa are presented graphically as a dendrogram (Fig.1). The species *F. oviniformis* and *F. hirtovaginata* proved to be most closely related. *Festuca thracica* was relatively more distantly positioned within the studied group. As a whole, the four studied species are more or less closely related within genus *Festuca*. Morphologically and ecologically the studied species demonstrated close affinity. Nevertheless, the presented data showed some quantitative differences in their isoenzyme structure. Other closely related taxa of genus *Festuca* were also differentiated due to fixed or close to

Table 3. Mean isoform frequencies of superoxide dismutase in the studied populations of *F. hercegovinica, F. ovinoformis, F. thracica* and *F. hirtovaginata.*

Species	Isoform						
	9	25	32	38	48	57	74
F. hercegovinica	1.00	0.00	1.00	0.66	0.33	1.00	1.00
F. oviniformis	1.00	1.00	1.00	0.40	0.60	1.00	0.80
F. thracica	1.00	0.80	0.80	0.65	0.25	1.00	1.00
F. hirtovaginata	1.00	0.63	1.00	0.95	0.15	1.00	1.00

Table 4. Mean isoform frequencies of amylase in the studied populations of *F. hercegovinica, F. ovinoformis, F. thracica* and *F. hirtovaginata.*

Species	Isoform								
	3	10	12	19	23	25	27	34	44
F. hercegovinica	0.12	0.00	0.00	0.32	0.48	0.22	0.08	0.05	0.42
F. oviniformis	0.00	0,26	0.35	0.06	0.00	0.08	0.24	0.18	0.00
F. thracica	0.18	0.08	0.00	0.42	0.10	0.34	0.00	0.15	0.27
F. hirtovaginata	0.08	0.14	0.07	0.52	0.14	0.26	0.10	0.33	0.38

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

	Species	Coefficient D			
		1	2	3	4
1	*F. hercegovinica*	x			
2	*F. oviniformis*	0.26	x		
3	*F. thracica*	0.25	0.29	x	
4	*F. hirtovaginata*	0.30	0.31	0.21	x

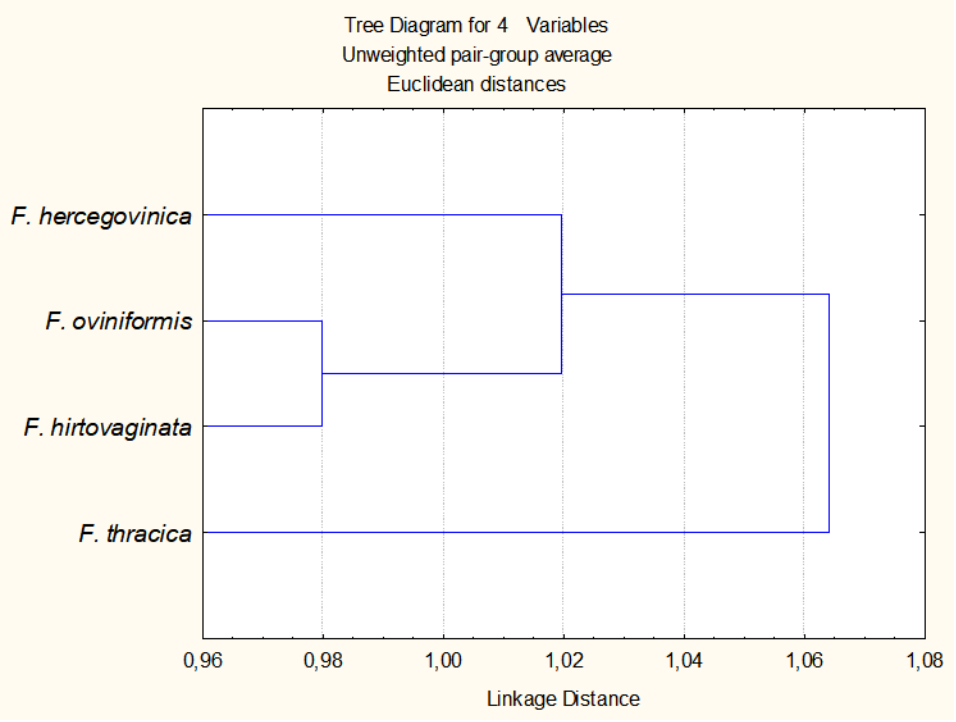

Figure 1. Dendrogram of Cluster analysis for the four studied *Festuca* species based on D coefficient.

being fixed differences (Aiken et al., 1993; Aiken and Lefkovitch, 1995; Guldahl et al., 2001). In this sense, the acknowledgement by Markgraff-Dannenberg **(**1980) of *F. hirtovaginata* and *F. thracica* as closely related but different species and the treatment of *F. hercegovinica* as a distinct species (Markgraff-Dannenberg, 1980) seems justified.

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