Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 66, No 6, 2013

BIOLOGIE

Botanique

GENETIC STRUCTURE AND AFFINITY AMONG XEROPHYTE TAXA OF *FESTUCA PSEUDOVINA* GROUP (POACEAE)

Georgi Angelov

(*Submitted by Academician V. Golemansky on February 26, 2013*)

Abstract

Polyacrylamide gel electrophoresis (PAGE) was employed to reveal genetic structure and affinity among four xerophyte taxa belonging to *Festuca pseudovina* group. Four enzymes (GOT, MDH, GDH and 6PGDH) putatively coded by eight gene loci were scored. Totally twenty-two alleles were found at the eight polymorphic loci in the studied *Festuca* taxa. Based on mean allelic frequencies, genetic identities were calculated. Analysis of the data demonstrated that the studied taxa were closely related but distinct genetic entities. It could be concluded that the present study is generally in support of the contemporary narrow species concept which is accepted for genus *Festuca.*

Key words: *Festuca*, xerophytes, PAGE, genetic structure, affinity

Introduction. Species *Festuca oviniformis* Vet., *F. thracica* (Acht.) Markgr.-Dnnbg., *F. hirtovaginata* (Acht.) Markgr.-Dnnbg., and *F. hercegovinica* Markgr.-Dnnbg. are Balkan endemics restricted to the southern parts of the Balkan Peninsula. They are xerophytes and belong to the group of *Festuca pseudovina* Hack. ex Wiesb. More than a century ago, relatively few, broadly defined *Festuca* taxa were recognized. Now the definitions of that species have narrowed, and large numbers of finely split taxa are circumscribed. Thus *Festuca thracica* and *F. hirtovaginata* have varied and contradictory taxonomical history. In older taxonomic treatments, they have been considered as forms of *F. duriuscula* [1] and varieties/subspecies of *F. ovina* [2, 3]. These taxa were later critically revised by Markgraff-Dannenberg [4, 5], who gave them species rank. The

species *F. hercegovinica* is a recently circumscribed taxon [4]. The species *F. oviniformis* is reported as endemic for Greece [4] and Bulgaria.

Isoenzymes are reliable genetic markers as the specificity in their electrophoretic patterns provides a useful tool for evaluating genetic differences and systematic relationships within taxonomically complicated plant groups. In the last two decades, several isoenzyme studies of sub/arctic [6, 7] and temperate zone fescues [8, 9] were conducted in attempt to investigate species delimitation in genus *Festuca* by means of isoenzyme markers.

The aim of the present study was to use PAGE to reveal the genetic structure and affinity among the above-mentioned four Balkan endemics of genus *Festuca*, thus contributing to their more precise delimitation.

Material and methods. On average, 25–30 plants per population belonging to eight natural Bulgarian populations, namely *F. oviniformis* (East Rhodope Mts – Zhalti chal, Kamenyane and Kazak villages); *F. thracica* (Central Rhodope Mts – Kurudere, Martsiganitsa); *F. hercegovinica* (Kresna Gorge, Rila Mt., near Bistritsa village); *F. hirtovaginata* (Rila Mt., Samokovishteto, along Bistritsa River), were examined.

The isoforms of enzymes glutamate-oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH) and 6-phosphogluconate dehydrogenase (6PGDH) were resolved by polyacrylamide gel electrophoresis. Leaf samples were extracted with 0.01M Tris, 0.08 M glycine buffer, containing 0.005M cysteine and 20% sucrose) at pH 8.3. Ion-exchange resin Dowex 1×8 (0.4 g/1 g fresh tissue) was added to the extraction buffer to eliminate polyphenols. The supernatant was used in the analysis. The enzymes were resolved on 7.5% separating gel (3% stacking gel) polyacrylamide slabs using the electrophoretic system [10]. The length of the separating gel was 7 cm and stacking gel was 2 cm long. Electrophoresis was conducted at 200V/25 mA. MDH and GDH isoenzymes were stained after [11], GOT – after [12], and 6 PGDH – after [13]. Different genes (loci) coding these enzymes were designated according to the relative mobility of the isoenzymes they specify. The gene coding the fastest isoforms was designated by 1, the next gene by 2, etc. In each locus the allele coding the fastest isoform was designated by a, the next by b, and so on [14]. Based on mean allelic frequencies, genetic identities (I) were calculated [15].

Results and discussion. Genetic interpretation of enzyme banding patterns was inferred from two lines of evidence – the known subunit structure of enzymes and their segregation patterns within species. Three gene loci and dimeric subunit structure are supposed for GOT in grasses [16, 17]. Similarly, the enzymes MDH and 6PGDH are considered as dimers coded by three genes [16, 17]. One gene locus has been reported for GDH and monomeric subunit structure has been supposed [18].

Banding patterns observed in the examined *Festuca* species conform to the above-mentioned studies. Due to weak banding intensity and unsatisfactory res-

olution, the slowest anodally migrating zones of MDH and 6PGDH were omitted. Thus four enzymes putatively coded by eight gene loci, namely GOT-1 (3 alleles), GOT-2 (2 alleles), GOT-3 (4 alleles); GDH-1 (5 alleles), MDH 1, 2 (2 alleles/each locus), and 6PGDH 1, 2 (2 alleles/each locus) were scored. Totally twenty-two alleles were found at eight polymorphic loci in the studied *Festuca* group. Allelic richness (2.75 alleles per locus) indicated that the studied taxa contained substantial genetic diversity in their populations.

Mean allelic frequencies in the studied species are presented on Table 1. Some of the alleles (MDH-1a, MDH-2a, 6PGDH-2a) were monomorphically fixed/nearly

T a b l e 1

Mean allele frequencies in the studied species of genus *Festuca*

Gene loci	Allele	*F. hirtovaginata*	*F. oviniformis*	*F. thracica*	*F. hercegovinica*
MDH-1	a	1.00	0.82	1.00	1.00
	b	0.00	0.18	0.00	0.00
MDH-2	a	1.00	1.00	1.00	0.92
	b	0.00	0.00	0.00	0.08
GOT-1	a	0.16	0.00	0.05	0.00
	b	0.84	1.00	0.90	1.00
	c	0.00	0.00	0.05	0.00
GOT-2	a	1.00	0.97	0.85	0.94
	b	0.00	0.03	0.15	0.06
GOT-3	a	0.05	0.00	0.20	0.10
	b	0.90	0.97	0.70	0.90
	c	0.00	0.03	0.10	0.00
	d	0.05	0.00	0.00	0.00
6PGDH-1	a	0.92	1.00	0.84	1.00
	b	0.08	0.00	0.16	0.00
6PGDH-2	a	0.94	1.00	1.00	1.00
	b	0.06	0.00	0.00	0.00
GDH-1	a	0.00	0.15	0.18	0.06
	b	0.11	0.05	0.18	0.19
	c	0.05	0.00	0.00	0.00
	d	0.24	0.09	0.10	0.13
	e	0.60	0.71	0.54	0.62

fixed and shared by most species. Two species-specific alleles, namely GDH 1c and GOT 3d, were detected in the studied populations of *F. hirtovaginata.* A unique allele GOT 1c was observed in *F. thracica.*

Genetic identity values for all pair-wise comparisons among the studied species are presented on Table 2. It is evident that *F. hirtovaginata* is equidistantly positioned from species pair *F. hercegovinica* – *F. thracica* as well the latter species from *F. oviniformis*. The species *F. oviniformis* proved to be genetically very closely related to both *F. hirtovaginata* and *F. hercegovinica* as judged by the examined set of enzymes. However, it should be pointed out that the analysis of another set of isoenzyme markers (anodal peroxidase, esterase and amylase) showed significant differences in the isoenzyme structure of *F. hercegovinica* and *F. oviniformis* [19]. Taking into account all available data, it could be stated that the species *F. hercegovinica* and *F. oviniformis* are closely related but distinct genetic entities. The value of coefficient I for comparison between *F. thracica* and *F. hercegovinica* was equal to 0.92 – an indication for close genetic affinity. However, electrophoretic survey of peroxidase, esterase and amylase demonstrated distinctive differences between them [20]. Additionally, the presence of species-specific alleles allows the species *F. hirtovaginata* (two alleles) and *F. thracica* (one allele) to be clearly distinguished within the studied *Festuca* group. The latter two species are morphologically and ecologically closely related taxa. As most species of genus *Festuca*, they differentiate mainly on the basis of subtle morphological differences. There are no firmly set criteria for sub/species ranking in genus *Festuca* [9]. Morphological differences between *F. thracica* and *F. hercegovinica* are comparable with those found in other species of the genus and correspond to narrow species concept accepted by modern researchers of genus *Festuca*.

The four examined fescues are representatives of the xeromorphic evolutionary line in genus *Festuca*. They are characterized with morphological and anatomical features for adaptation to extreme xeric conditions. These fescues exhibit subtle morphological differences. They differ mainly in leaf cross-section

T a b l e 2

Genetic identities (I) for all pair-wise comparisons among the studied *Festuca* species

Species		1	2	3	4
1	*F. hercegovinica*	×			
2	*F. thracica*	0.92	×		
3	*F. oviniformis*	0.99	0.78	×	
4	*F. hirtovaginata*	0.79	0.79	0.99	×

anatomy but their identification is difficult. Isoenzyme data presented here and data of other related studies [19, 20] evidenced that the four examined taxa are genetically well-defined entities. Despite their close morphological resemblance, these species proved to be discrete entities as revealed by different sets of enzyme markers. It could be concluded that the present study is generally in support of the contemporary narrow species concept which is accepted for genus *Festuca*.

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Institute of Biodiversity and Ecosystem Research
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., Bl. 23
1113 Sofia, Bulgaria
e-mail: gbangv@bio.bas.bg