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VARIATION OF ESTERASE, ACID PHOSPHATASE, GLUTAMATE-OXALOACETATE TRANSAMINASE, MALATE DEHYDROGENASE AND GLUTAMATE DEHYDROGENASE IN BULGARIAN POPULATIONS OF *MELICA UNIFLORA* AND *M. CILIATA* (POACEAE)

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Abstract

Polyacrylamide gel electrophoresis (PAGE) was used to examine the variation of six enzymes in *M. uniflora* in order to assess the systematic relationships between both species. It was found that the populations of *Melica uniflora* and *M. ciliata* contain a large amount of isoenzyme variation. Each of the studied species possessed a substantial portion (ca 25%) of unique isoforms. The presence of species-specific isoforms enables to discriminate definitively *M. uniflora* from *M. ciliata*.

Key words: *Melica uniflora*, *M. ciliata*, PAGE, isoenzyme variation, systematic relationships

Introduction. Genus *Melica* encompasses six species in Bulgaria. This study includes two species – *Melica uniflora* and *M. ciliata*. Studies on isoenzyme variation in *Melica* are rather scarce. Geographic patterns of genetic variation in *M. nutans* were studied [1,2], electrophoretic spectra of six isoenzymes in *Melica uniflora* were examined [3], allozyme variation in populations of *M. ciliata*/*M. transilvanica* species complex was studied [4–6].

The aim of the study was to reveal the variation of esterase, acid phosphatase, glutamate-oxaloacetate transaminase, malate dehydtogenase and glutamate dehydrogenase in *M. uniflora* and *M. ciliata* in order to assess the systematic relationships between both species.

Material and methods. Natural populations of *M. uniflora*: Vitosha Mt. (Knyazhevo; Belata voda); Lyulin Mt. (Bankya, Klisura) and *M. ciliata*: Lozenska Mt. (Lozen), Sredna gora Mt. (Petrich, Hisar) were studied. On average, 30 plants per population of each species were examined. The isoforms of glutamate-oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), acid phosphatase (ACP), anodal and cathodal esterase (EST) and glutamate dehydrogenase (GDH) were resolved by polyacrylamide gel electrophoresis. Leaves were ground in 0.01 M Tris, 0.08 M glycine, 0.005 M cysteine and 20% sucrose at pH 8.3. Homogenates were centrifuged at 10 000 rpm for 10 min. The supernatant was used as a source of enzymes. The enzymes were resolved on 7.5% separating gel (3% stacking gel) polyacrylamide slabs using basic system [7]. Cathodal isoforms of EST were run on 7.5% separating acidic gel and 3% stacking gel [8]. The length of the separating gel was 7.5 cm for GDH and 6 cm for the rest of enzymes. Staining of GOT [9], MDH and GDH [10], ACP [11], EST [12] followed standard procedures. Each isoform was assigned a number reflecting its gel migration in mm from the origin [13]. Affinity between the species was assessed by the similarity index (SI), according to [14]: $SI = M/(M + N)$, where M is the number of common isoforms, N is the sum of specific isoforms for each species.

Results and discussion. In total, fourteen isoforms of anodal esterase were electrophoretically resolved in *M. uniflora* and *M. ciliata*. Isoform presence/absence data are presented on Table 1. Isoforms 9, 16, 32, 43 and 47 were shared by both species. Isoforms 11, 34 and 50 were found in *M. uniflora* only while six isoforms (19, 22, 27, 30, 37 and 40) were specific for *M. ciliata*. The value of the coefficient SI was 0.38.

Eight isoforms of the cathodal EST occurred in *Melica uniflora* and *M. ciliata* (Table 2). Isoforms 16, 22, 27 and 33 were common for both species. Isoforms 24, 31 and 48 were observed in *M. ciliata* only, whereas isoform 20 was characteristic of *M. uniflora*. The value of the coefficient SI was 0.50 and showed a relatively high resemblance between *M. uniflora* and *M. ciliata*.

Totally nine isoforms of the ACP enzyme were electrophoretically detected in *M. uniflora* and *M. ciliata* (Table 3). Most of the isoforms namely, 11, 14, 22, 26, 30 and 34, were recorded in both species. Each of the examined species possessed a unique isoform – isoforms 18 and 29 for *M. uniflora* and *M. ciliata*

Table 1

Isoform presence/absence data for anodal EST in the studied populations of *M. uniflora* and *M. ciliata*

Species	Isoforms													
	9	11	16	19	22	27	30	32	34	37	40	43	47	50
M. uniflora	+	+	+	–	–	–	–	+	+	–	–	+	+	+
M. ciliata	+	–	+	+	+	+	+	+	–	+	+	+	+	–

Table 2

Isoform presence/absence data for cathodal EST in the studied populations of *M. uniflora* and *M. ciliata*

Species	Isoforms							
	16	20	22	24	27	31	33	48
M. uniflora	+	+	+	–	+	–	+	–
M. ciliata	+	–	+	+	+	+	+	+

Table 3

Isoform presence/absence data for ACP in the studied populations of *M. uniflora* and *M. ciliata*

Species	Isoforms								
	11	14	18	22	25	26	29	30	34
M. uniflora	+	+	+	+	–	+	–	+	+
M. ciliata	+	+	–	+	+	+	+	+	+

respectively. The value of the coefficient SI was high (0.67) that is an indication for a close affinity between both taxa.

Seven isoforms of GOT were electrophoretically resolved in the studied populations of *M. uniflora* and *M. ciliata* (Table 4). Isoform 40 was common for both examined taxa. Isoforms 15, 18 and 21 were recorded in *M. uniflora* only, whereas isoforms 25 and 44 were species-specific for *M. ciliata*. The value of the coefficient SI was very low (0.14) and suggested for quite a different isoenzyme structure of both taxa.

In total, six isoforms of the enzyme MDH were detected in the examined populations of *M. uniflora* and *M. ciliata*. Three of them, namely isoforms 31, 34 and 41, were shared by both studied species, while isoforms 22, 25 and 29 were characteristic of *M. uniflora*. The value of the coefficient SI was equal to 0.50 thus indicating substantial similarity between the isoenzyme structures of *M. uniflora* and *M. ciliata*.

Three isoforms of the enzyme GDH (13, 14 and 15) were recorded in the examined populations of *M. uniflora* and *M. ciliata*. Two of them, namely isoforms 13 and 15, were common for both studied species. Isoform 14 was characteristic

Table 4

Isoform presence/absence data for GOT in the studied populations of *M. uniflora* and *M. ciliata*

Species	Isoforms						
	15	18	21	25	35	40	44
M. uniflora	+	+	+	–	+	+	–
M. ciliata	–	–	–	+	–	+	+

of *M. uniflora*. The value of SI was high (0.67) that is an indication for close affinity between both taxa.

Summarizing the result of the present study, it is evident that the populations of *M. uniflora* and *M. ciliata* contain a large amount of isoenzyme variation. Totally 47 isoforms of the six examined enzyme markers were observed. It could be noted that each of the studied species possessed a substantial portion (ca 25%) of unique isoforms. The presence of species-specific isoforms enables us to discriminate definitively *M. uniflora* from *M. ciliata*. The values of the coefficient SI ranged from 0.14 for GOT to 0.67 for ACP and GDH. The mean value of SI over the six examined enzymes was 0.48. The respective values for different taxa of genus *Festuca* were higher varying in the range of 0.63–0.84 [15,16]. It could be concluded that the species *M. uniflora* and *M. ciliat* are clearly defined taxa within genus *Melica*.

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