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ISOENZYME DIVERSITY WITHIN GENUS *MELICA* (POACEAE) – SYSTEMATIC IMPLICATIONS

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Polyacrylamide gel electrophoresis (PAGE) of six enzymes was employed to reveal the systematic position and relationships of four species within genus *Melica.* The variation of the isoenzymes anodal esterase, cathodal peroxidase, acid phosphatase, superoxide dismutase, amylase and glutamate dehydrogenase was analyzed. The aim of the present study was to examine the variation of a set of isoenzymes in attempt to evaluate the systematic position and relationships of four species of genus *Melica* L. Systematic relationships were evaluated by calculating coefficient of divergence D. The species *M. uniflora* was clearly differentiated from *M. ciliata* and *M. transsilvanica.* Moreover, the species *M. uniflora* possessed two species-specific isoforms. It was shown that the species *M. nutans* is closely related to *M. uniflora* but genetically is a well defined entity within genus *Melica.* The results of the present study correlated well with the main morphological features of the examined taxa of genus *Melica*.

Keywords: Melica, PAGE, isoenzymes, variation, systematic relationships

The species *Melica nutans* L. is shortly-rhizomatous, long-lived perennial grass. Spikelets are 6–8 mm long, eventually nodding, with 2–3 fertile florets, falling together when ripe. Diploid, 2n=18. While several European *Melica* species are very variable and subjected to different taxonomic treatments, *Melica nutans* is morphologically homogenous [1]. This species occupies shady and often rocky places. It is distributed mostly of Europe, but rarely in Mediterranean region and the islands [14].

Species *M. uniflora* Retz. is rhizomatous, perennial grass. Spikelets are 3–7 mm long, erect, with 1 fertile floret, diploid, 2n=18. It occurs mainly in shady places. Its range of distribution in Europe is northwards to Scotland, and eastwards to Moldova [14] and the neighboring south-western regions of Ukraine [5].

The complex *Melica ciliata – M. transsilvanica* consists of group of sub-Mediterranean-continental species. Intricate morphological variability and traits overlapping makes the *Melica ciliata – M. transsilvanica* complex a taxonomically problematic group [4]. The morphological variability concerns mainly indumentum of plants' vegetative parts and type of panicle branching. For the existence of continual variability, the number of taxa and its rank differ significantly in the taxonomic conception of different authors [11, 14, 18]. This circumstance causes confusion in synonymy as well disagreement concerning the distribution of some taxa. In particular, question about the eastern border of *Melica ciliata* s.str. is still disputable. According to Szczepaniak & Cieślak [13], *M. ciliata* is sub-Mediterranean species whose main continuous geographical range covers the area from the Atlantic and Mediterranean region, Central Europe, to southern

Ukraine and the Crimea. However, Ukrainian authors found that *M. ciliata* s.str. is very rare species in Podolian upland's south regions [5] and Transcarpathia.

The analysis of morphological characters proved to be insufficient for the proper taxa distinction within the complex *Melica ciliata-M. transsilvanica*. So, alternative diagnostic molecular markers were used. Study of genetic and morphological variation in natural populations of *Melica ciliata* and *M. transsilvanica* by DNA markers [12, 13] demonstrated effectiveness of AFLP analysis. Moreover, it was shown that earlier reported localities in Poland are wrong and this species does not occur in Poland. Despite of effectiveness of AFLP method for large-scale studies within Europe, it is expensive and rather laborious for smaller regional studies. Alternatively, the isoenzyme analysis is also widely employed in biosystematic studies. Large-scale geographic patterns in widespread Euroasian woodland grass *M. nutans* were studied by sets of isoenzyme systems [15, 16, 17]. Electrophoretic patterns of six isoenzymes in Bulgarian populations of *M. uniflora* were examined in order to reveal their isoenzyme variation [1].

The aim of the present study was to examine the variation of a set of isoenzymes in attempt to evaluate the systematic position and relationships of the above listed species. Within genus *Melica* L. The analysis of populations from the main parts of species' distribution, e.g. Bulgaria, will result in adequate comparative data.

Material and methods

The enzymes anodal esterase (EST), cathodal peroxidase (PER), acid phosphatase (ACP), superoxide dismutase (SOD), amylase (AMY) and glutamate dehydrogenase (GDH) were analyzed individually in two natural populations of each *Melica transsilvanica, M. ciliata*, *M. uniflora,* and *M. nutans* from Bulgaria*.* Leaves were grinded in 0.01 M Tris, 0.08 M glycine, 0.005 M cysteine, 20 % sucrose, pH 8.3. Anodally migrating isoforms were resolved on 7.5 % polyacrilamide slab gels as described [3]. The cathodal PER was run on 7.5 % polyacrylamide slab gels [7]. The length of gels was 10 cm for AMY, 7.5 cm for ACP and GDH, 7 cm for SOD, 6.25 cm for anodal EST and 6 cm for cathodal PER. The following staining recipes were used: AMY [7], PER [6], EST [8], GDH and ACP [9], SOD [2].

Systematic relationships among the above mentioned taxa of genus *Melica* were assessed by calculating coefficient of divergence D (15) according to the following formula:

$$D = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x_j - x_k\right)^2}$$

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.

Results and discussion

Anodal esterase. Totally eight isoforms of the enzyme were electrophoretically resolved in *Melica transsilvanica, M. ciliata, M. uniflora* and *M. nutans* (Table 1). Isoform 43 was shared by all species, being rare (frequency of 0.04) in *M. ciliata*. Excepting *Melica transsilvanica,* isoforms 16 and 32 were common for the rest of taxa. Similarly, isoform 19 was observed in all taxa but *M. uniflora.* The values of coefficient D indicated that the species *M. uniflora* and *M. nutans* were most closely related (D=0.11) while the former was most distantly positioned (D=0.25) from *Melica transsilvanica.*

Cathodal peroxidase*.* Nine isoforms of the enzyme were found in the populations of the studied species group (Table 2). Most of isoforms were shared by all species. Excepting *Melica transsilvanica,* isoform 28 was monomorphically fixed throughout the studied group. Isoform 34 was invariant in *M. uniflora* and *M. nutans.* Isoform 55 was species-specific for the species

M. uniflora. The values of coefficient D varied in a narrow range (0.14–0.18) and the studied taxa proved to be almost equidistantly positioned as judged by molecular marker cathodal peroxidase.

Table 1

Mean isoform frequencies of anodal esterase in the studied populations of *Melica uniflora, M. nutans, M. ciliata* and *M. transsilvanica*

Species	Isoforms							
	9	11	16	19	24	31	32	43
M. uniflora	0.00	0.79	0.74	0.00	0.00	0.09	0.23	0.31
M. nutans	0.12	0.48	0.64	0.12	0.42	0.22	0.65	0.84
M. ciliata	0.03	0.00	0.96	0.96	0.00	0.00	0.16	0.04
M. transsilvanica	0.00	0.00	0.00	0.12	0.75	0.12	0.00	0.12

Table 2

Mean isoform frequencies of cathodal peroxidase in the studied populations of *Melica uniflora, M. nutans, M. ciliata* and *M. transsilvanica*

Species	Isoforms								
	20	25	28	30	32	34	38	50	55
M. uniflora	0.58	1.00	1.00	1.00	0.00	1.00	0.42	0.39	0.13
M. nutans	0.14	0.82	1.00	0.14	0.72	1.00	0.92	0.78	0.00
M. ciliata	0.75	1.00	1.00	0.25	0.79	0.71	0.17	0.00	0.00
M. transsilvanica	0.62	0.88	0.62	0.62	0.00	0.75	0.50	0.00	0.00

Acid phosphatase. In total, nine isoforms of ACP were detected in the studied species of genus *Melica* (Table 3). Most of isoforms, namely 11, 14, 26, 30, 34, were shared by all examined species. Similarly, isoform 29 occurred in all studied taxa but *M. uniflora.* The values of coefficient D ranged from 0.09 (*M. nutans* vs *M. transsilvanica*) to 0.14 for the species pairs *M. transsilvanica/ M. uniflora* and *M. ciliata*/ *M. nutans*, respectively.

Table 3

Mean isoform frequencies of acid phosphatase in the studied populations of *Melica uniflora, M. nutans, M. ciliata* and *M. transsilvanica*

Species	Isoforms							
	11	14	18	22	26	29	30	34
M. uniflora	0.32	0.32	0.22	0.46	1.00	0.00	0.59	0.59
M. nutans	0.52	0.64	0.00	0.00	0.64	0.08	0.32	0.32
M. ciliata	0.04	0.79	0.00	0.63	0.46	0.21	0.79	0.12
M. transsilvanica	0.63	0.89	0.12	0.00	0.50	0.25	0.77	0.12

Superoxide dismutase. Nine isoforms of the enzyme marker SOD were detected in the studied species of genus *Melica* (Table 4). Monomorphically-fixed isoform 60 was common for the whole species group. Isoforms 30, 40, 44 and 48 were also detected with different frequencies in all examined taxa. Isoform 57 was species-specific for *M. uniflora.* The values of coefficient D fluctuated from 0.06 (*M. nutans* vs *M. transsilvanica*) to 0.18 when *M. ciliata* and *M. transsilvanica* were contrasted.

Table 4

Mean isoform frequencies of superoxide dismutase in the studied populations of *Melica uniflora, M. nutans, M. ciliata* and *M. transsilvanica*

Species	Isoforms								
	25	30	40	44	48	50	54	57	60
M. uniflora	0.58	0.42	0.58	0.23	0.13	0.97	0.39	0.33	1.00
M. nutans	0.23	0.16	0.32	0.00	0.54	0.45	0.18	0.00	1.00
M. ciliata	0.00	0.78	1.00	0.21	0.04	0.00	0.00	0.00	1.00
M. transsilvanica	0.00	0.12	0.75	0.12	0.75	0.75	0.00	0.00	1.00

Amylase. Totally eight isoforms of the enzyme were electrophoretically resolved in *Melica transsilvanica, M. ciliata, M. uniflora* and *M. nutans* (Table 5). Isoform 10 was shared by all studied species. Excepting *M. uniflora*, isoform5 was also common for the whole group. Isoforms 40 and 44 were observed in *M. uniflora* and *M. nutans* only. In similar, isoform 8 was specific for species pair *Melica transsilvanica, M. ciliata.* The values of coefficient D varied in a wide range – from 0.11 (*M. uniflora* and *M. nutans*) to 0.30 when *M. ciliata* and *M. transsilvanica* were compared.

Table 5

Mean isoform frequencies of amylase in the studied populations of *Melica uniflora, M. nutans, M. ciliata* and *M. transsilvanica*

Species	Isoforms							
	5	8	10	17	20	26	40	44
M. uniflora	0.00	0.00	0.22	1.00	0.00	0.00	0.88	0.44
M. nutans	0.42	0.00	0.48	1.00	0.34	0.16	0.48	0.24
M. ciliata	0.79	0.26	0.53	0.00	1.00	1.00	0.00	0.00
M. transsilvanica	0.25	0.25	0.12	0.88	0.00	0.00	0.00	0.00

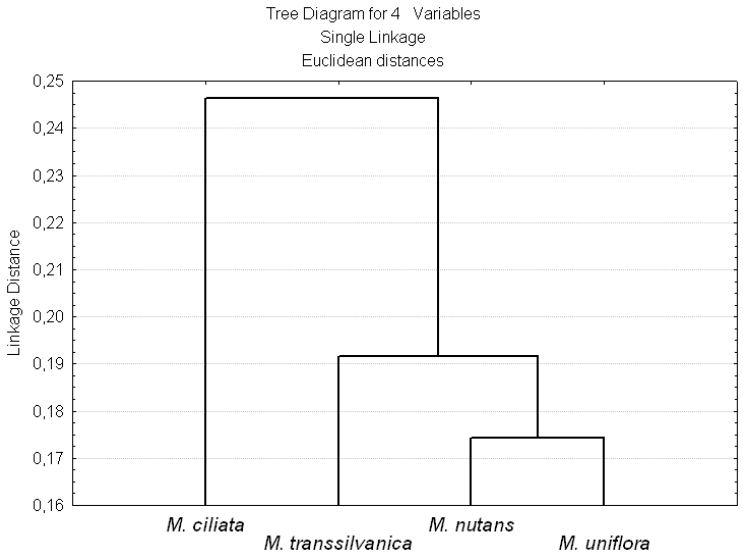
Glutamate dehydrogenase. In total, five isoforms of the enzyme were detected in the studied species of genus *Melica*. Isoforms 13 and 15 were common for the whole studied group. The species *M. nutans* shared the electrophoretic patterns of *M. uniflora.* Thus, the latter two taxa possessed identical isoenzyme structure in respect to the enzyme marker glutamate dehydrogenase.

The mean values of coefficient D averaged over the five enzymes surveyed, namely anodal EST, catodal PER, ACP, SOD and AMY, are shown in Table 6 and graphically in Figure.

Table 6

Mean values of coefficient D for pair-wise comparisons among the studied taxa of genus *Melica*

Species	Mean values of coefficient D				
	Number	1	2	3	4
M. uniflora	1	0.00			
M. nutans	2	0.12	0.00		
M. ciliata	3	0.17	0.17	0.00	
M. transsilvanica	4	0.17	0.13	0.19	0.00



The dendrogram of Cluster analysis for four *Melica* species on the base D coefficient

Pair-wise comparisons of *M. uniflora* with species *M. ciliata* and *M. transsilvanica* resulted in high values of coefficient D equal to 0.17 in both cases. Thus, it was clearly differentiated from species pair *M. ciliata* and *M. transsilvanica.* It should be noted that the mean value of coefficient D for the comparison between *M. nutans* and *M. uniflora* was the lowest one (0.12) – an indication for high similarity of isoenzyme structure of both taxa. In addition, the species *M. uniflora* possessed two species-specific isoforms. This result demonstrated that the species *M. nutans* and *M. uniflora* are closely related taxa within genus *Melica.* It should be pointed out that both *M. nutans* and *M. uniflora* are rhizomatous plants while the species *M. ciliata* and *M. transsilvanica* are caespitose ones. Moreover, *M. nutans* and *M. uniflora* are among the minority of species within the genus which are characterized with obtuse and glabrous lemma, while the rest of species have pointed (acute) lemma with long hairs. Thus, they were clearly differentiated from both *M. ciliata* and *M. transsilvanica.* Thus, the results of the present study correlated with the main morphological features of the examined taxa of genus *Melica*.

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РІЗНОМАНІТНІСТЬ ІЗОЕНЗИМІВ У МЕЖАХ РОДУ *MELICA* (POACEAE) – РЕЗУЛЬТАТИ ВИКОРИСТАННЯ В СИСТЕМАТИЦІ

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З метою оцінки систематичного положення та взаємовідносин між чотирма видами роду *Melica* було використано електрофорез шести ферментів у поліакриламідному гелі (PAGE). Проведено аналіз мінливості ізоферментів анодної естерази, катодної пероксидази, кислої фосфатази, супероксиддисмутази, амілази і глутаматдегідрогенази. Систематичні відносини оцінювали шляхом розрахунку коефіцієнта дивергенції. Встановлено, що за набором зазначених молекулярних маркерів *Melica uniflora* чітко відрізняється від *M. ciliata* та *M. transsilvanica*. Крім того, *M. uniflora* містить дві видоспецифічні ізоформи. Також було показано, що *M. nutans* тісно пов'язана з *M. uniflora*, проте генетично вид досить добре вирізняється в межах роду *Melica*. Представлені результати виявилися добре скорельовані з діагностично значущими ознаками морфологічної будови досліджених таксонів роду *Melica*.

Ключові слова: Melica, PAGE, ізоензими, мінливість, систематичні взаємовідносини