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SYSTEMATIC RELATIONSHIPS AMONG SIX TAXA OF GENUS *ELYMUS* AS REVEALED BY ELECTROPHORESIS OF SEED PROTEINS

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Systematic affinities among *Elymus dahuricus*, *E. repens*, *E. hispidus*, their hybrid *E.* x *mucronatus*, *E. elongatus*, *E. pycnanthus* were studied by polyacryamide electrophoresis (PAGE) and sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins. Affinities among the taxa were assessed by coefficient of taxonomic similarity S. An index of group affinity (GA) was also calculated for each taxon as a sum of its S values. The results indicated that most of studied taxa are nearly equidistantly positioned within the examined group of genus *Elymus.* The species *E. pycnanthus* proved to be most distantly positioned within the group as its index GA (1.08) was the lowest one. The species *E. dahuricus* (GA=1.21) was also comparatively distant within the studied group of genus *Elymus.* It should be noted that the hybrid *E.* x *mucronatus* is more close positioned to *E. repens* than to the other parent *E. hispidus* as judged by the composition of its seed proteins. The results of the present study clearly showed that within mixed populations of *E. repens*, *E. hispidus* and *E. ×mucronatus well developed hybrid swarms can be observed.*

Keywords: Elymus, seed proteins, electrophoresis, systematic affinities

Introduction. *Elymus* (L.) L. is the widest distributed genus of Triticeae tribe and comprises some 150 species worldwide [6]. The main problem in delimitation of *Elymus* arises from the lack of clean-cut morphological traits at generic level and the presence of numerous intergeneric hybrids [9].

Six taxa of genus *Elymus*, namely, *Elymus dahuricus* Turcz. ex Griseb., *E. repens* (L.) Gould, *E. hispidus* (Opiz) Melderis, their hybrid *E.* x *mucronatus* (Opiz) Konert, *E. elongatus* (Host) Runemark, *E. pycnanthus* (Godron) Melderis were included in the present study. *Elymus repens* is a common, widespread perennial grass, native to Europe and Asia. This species is characterized by wide phenotypic plasticity allowing it to occupy different habitats. *Elymus hispidus* is distributed from Central Asia to C. France in Europe. It occurs in dry, usually sandy or stony habitats. *Elymus hispidus* and *E. repens* can easily crossbreed in nature giving rise to relatively common hybrid *E.* x *mucronatus* [14]. The genetic variation of *Elymus hispidus* and *E. repens* and its implications for the intraspecific taxonomy were studied by DNA markers [13].

Electrophoretic analysis of seed proteins is widely used for evaluation of systematic relationships, genetic variation of natural populations and cultivars of different plant taxa (10, 3, 12). SDS-PAGE technique is also widely used for seed protein characterization and genotype sample classification [1, 2, 15].

The purpose of the present study was to assess systematic and genetic relationships among the above listed *Elymus* taxa by electrophoresis of seed proteins.

Material and methods

Seeds from natural populations of *E. repens*, *E. hispidus* and their hybrid *E.* x *mucronatus* were collected in Southern Poland (Niecka Nidziańska Basin) by Dr. M. Szczepaniak. Seed accessions of *Elymus dahuricus, E. elongatus* and *E. pycnanthus* were provided by USDA, ARS, WRPIS, Washington State University, Regional Plant Introduction Station.

Proteins of seeds were extracted by 0.01M tris, 0.08M glycine, 20 % sucrose, pH 8.3, ratio seeds: buffer = 1:6. Anodal seeds proteins were electrophoretically resolved in vertical polyacryamide slab gels (7.5 % separating, 3 % stacking gels) using slightly modified tris-glycine discontinuous system [4]. Acidic vertical polyacryamide slab electrophoretic system [11] was employed to resolve the cathodal forms with spacer of 3 % and 7.5 separating gel. The length of separating gel was 7 cm (anodal forms), 5 cm (cathodal forms) while spacer was 1 cm long.

Gels were stained with Coomasie Briliant Blue R-250 (0.1 %) in 10 % acetic acid, 45 % methanol for 2 hours and destained in 10 % acetic acid, 10 % methanol for a night.

Discontinuous sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Gardiner et al. [5]. The stacking gel contained 5 % acrylamide and 12 % acrylamide separating gels were used. The length of stacking and separating gel was 2 cm and 9 cm, respectively.

Affinities among the taxa within the studied group were assessed by coefficient of taxonomic similarity S [7]. Values of coefficient S for each pair-wise comparison among the taxa were calculated separately for each set of seed proteins (anodal, cathodal, SDS-PAGE). Then, mean values of coefficient S as an average on the three data sets were calculated in order to assess affinities among taxa within the studied group of *Elymus.* An index of group affinity (GA) was calculated for each taxon as a sum of its S values.

All calculations were done with the STATISTICA 7.0 package (Stat Soft Inc.). The data were analyzed by using Cluster Analysis (Ward's method and Euclidean distances) as well as some univariate statistical methods.

Results and Discussion

Anodal seed proteins. Totally eighteen migrating to the anode electrophoretic bands were detected in the studied taxa of genus *Elymus* (Table 1). Band 53 was shared by all taxa. Excepting *E. pycnanthus*, bands 29 and 32 were common for the studied group. Bands 44, 47 and 50 occurred in all but one taxon. Band 34 was observed in the studied populations of *E. pycnanthus* and *E. hispidus* only. Band 12 proved to be species specific for *E. pycnanthus* while electrophoretic band 45 was characteristic of *E. hispidus.* The values of coefficient of taxonomic similarity S varied from 0.13 (*E. x mucronatus* vs *E. hispidus*) to 0.63 in the comparison between *E. dahuricus* and *E. elongatus*. It should be noted that the hybrid *E. x mucronatus* was more close to *E. repens* (S=0.53) than to the other parent *E. hispidus* (S=0.38) as judged by the composition of their anodal seed proteins. The species *E. pycnanthus* was the most distant taxon within the whole studied group of genus *Elymus.*

Cathodal seed proteins. In total, seventeen cathodally migrating bands were electrophoretically resolved in the studied taxa of genus *Elymus* (Table 2). Bands 30 and 52 were common for all taxa. Bands 32 and 58 were shared by all taxa but one. Excepting *E. elongatus* and *E. pycnanthus*, electrophoretic band 26 was detected throughout the whole studied group of genus *Elymus.* Species pair *E. elongatus* and *E. pycnanthus* shared rare electrophoretic band 60. The species *E. elongatus* possessed unique bands 14 and 64, while electrophoretic bands 12 and 19 were found in the studied populations of *E. pycnanthus* only. Band 46 was characteristic of *E. dahuricus.* Most values of coefficient of taxonomic similarity S varied in wide range – from 0.16 (*E. repens* vs *E. pycnanthus*) to 0.58 in the comparison between *E. repens* and *E. dahuricus.* It could be noticed that the hybrid *E. x mucronatus* was more close to *E. repens* (S=0.73) than to

the other parent *E. hispidus* (S=0.17) as revealed by the profiles of their cathodal seed proteins. The values of coefficient of taxonomic similarity S as well the existence of species specific bands evidenced that the species *E. elongatus* and *E. pycnanthus* were most distantly positioned taxa within the whole studied group of genus *Elymus.*

SDS-PAGE seed proteins. Totally twenty three protein bands were resolved by SDS-PAGE in the examined group of genus *Elymus* (Table 3). Six electrophoretic bands, namely 29, 34, 39, 60, 71, 74 were observed in all studied taxa. Bands 47, 55 and 64 were shared by all but one taxon. Band 26 was rare as it was found in *E. dahuricus* and *E repens* only. Similarly, electrophoretic bands 36 and 58 were common for species pairs *E. x mucronatus – E. hispidus* and *E. repens – E. x mucronatus*, respectively. Bands 16 and 21 were species-specific for *E. elongatus* while electrophoretic band 66 was unique for *E. dahuricus.* The values of coefficient of taxonomic similarity S varied from 0.09 (*E. dahuricus* vs *E. repens*) to 0.75 in the comparison between *E. pycnanthus* and *E. elongatus*. It should be noted that the hybrid *E. x mucronatus* is more close positioned to *E. repens* (S=0.45) than to the other parent *E. hispidus* (S=0.32) as judged by the composition of their SDS-PAGE seed proteins.

Table 1

Banding profiles of anodal seed proteins in the studied taxa of *Elymus*.
1 – band present, 0 – band absent. Each band was designated by a number reflecting its migration (in mm) from the origin

Taxon	Electrophoretic band																	
	5	11	12	17	20	22	24	26	29	32	34	35	40	44	45	47	50	53
E. dahuricus	1	1	0	1	0	0	0	1	1	1	0	0	1	1	0	1	1	1
E. repens	0	1	0	1	1	0	0	0	1	1	0	1	0	0	0	1	1	1
E. x *mucronatus*	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0	1	1	1
E. hispidus	1	1	0	1	0	0	0	0	1	1	1	1	0	1	1	1	0	1
E. elongatus	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1
E. pycnanthus	0	0	1	1	1	1	1	0	0	0	1	0	1	1	0	1	1	1

Table 2

Banding profiles of cathodal seed proteins in the studied taxa of *Elymus*.
1 – band present, 0 – band absent. Each band was designated by a number reflecting its migration (in mm) from the origin

Taxon	Electrophoretic band																
	12	14	19	26	30	32	35	40	42	44	46	48	52	55	58	60	64
E. dahuricus	0	0	0	1	1	0	1	1	0	0	1	0	1	0	1	0	0
E. repens	0	0	0	1	1	1	1	0	0	0	0	0	1	0	1	0	0
E. x *mucronatus*	0	0	0	1	1	1	0	1	0	0	0	0	1	0	1	0	0
E. hispidus	1	0	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0
E. elongatus	0	1	0	0	1	1	0	1	1	1	0	1	1	1	1	1	1
E. pycnanthus	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	0

Table 3

Banding profiles of SDS-PAGE seed proteins in the studied taxa of *Elymus*.
1 – band present, 0 – band absent. Each band was designated by a number reflecting its migration (in mm) from the origin

Taxon	Electrophoretic band																					
	16	21	24	26	29	32	34	36	39	41	43	45	47	49	51	55	58	60	64	66	71	74	80
E. dahuricus	0	0	1	1	1	1	1	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	0
E. repens	0	0	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	1
E. x *mucronatus*	0	0	1	0	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0	0	1	1	1
E. hispidus	0	0	1	0	1	0	1	1	1	1	0	0	1	1	1	1	0	1	1	0	1	1	1
E. elongatus	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0
E. pycnanthus	0	0	0	0	1	0	1	0	1	0	1	1	1	1	1	1	0	1	1	0	1	1	0

Table 4

Mean values of coefficient of taxonomic similarity S for each pair-wise comparison among the studied taxa of *Elymus*

Taxon	Coefficient of taxonomic similarity S					
	1	2	3	4	5	6
E. dahuricus	1.00					
E. repens	0.36	1.00				
E. x *mucronatus*	0.29	0.58	1.00			
E. hispidus	0.12	0.35	0.23	1.00		
E. elongatus	0.27	0.15	0.24	0.24	1.00	
E. pycnanthus	0.17	0.16	0.16	0.23	0.39	1.00

Mean values of coefficient S for all pair-wise comparisons among the studied taxa calculated as an average on the three S data sets (anodal, cathodal, SDS-PAGE seed proteins) are presented in Table 4. The dendrogram of Cluster analysis based on this coefficient is shown on Fig. 1.

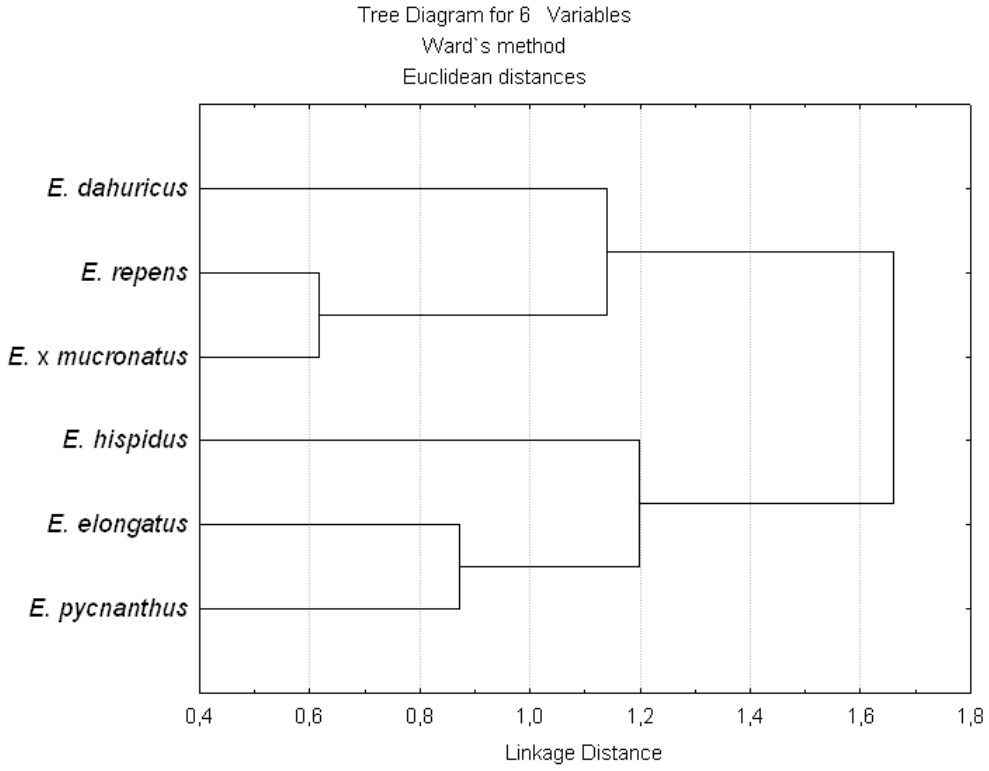

Fig. 1. The dendrogram of Cluster analysis for six *Elymus* species on the base S coefficient

As it could be seen, values ranged from 0.13 in the comparison between *E. pycnanthus* and *E. x mucronatus* to 0.58 when the latter taxon was contrasted to *E. repens*. Most of coefficient S values were within the range of 0.23 – 0.39 which is an indication for nearly equidistant position for the most of studied *Elymus* taxa. Index of group similarity contributed to revealing systematic relationships within the examined group of genus *Elymus.* Lower values of index GA mean greater distance for given taxon, and vice versa, higher values are indicate a closer affinity within the group. The species *E. pycnanthus* proved to be most distantly positioned within the group as its index GA (1.08) was the lowest one. The species *E. dahuricus* (GA=1.21) was also comparatively

distant within the studied group of genus *Elymus* (Fig. 1.). Values of GA for the rest of taxa were varying from 1.25 (*E. elongatus*) to 1.60 for *E. repens*. As a whole, index GA also indicated that most of studied taxa are nearly equidistantly positioned within the examined group of genus *Elymus*. It should be emphasized that the hybrid *E. x mucronatus* is more close positioned to *E. repens* (Fig. 1.) than to the other parent *E. hispidus* as judged by the composition of its seed proteins. Analysis of polyphenol compounds and isoenzymes led to similar conclusions (unpubl. res.). These inferences are different from previous cpDNA [8] and AFLP [14] studies that suggested more close affinity of *E. ×mucronats* to *E. hispidus* and unidirectional introgression. However, it cannot be excluded that F1 or later-generation hybrids may also backcross with E. repens, which suggests possibility of bidirectional introgression towards both parental species. The previous and present studies clearly showed that within mixed populations of *E. repens*, *E. hispidus* and *E. ×mucronatus well developed hybrid swarms can be observed.*

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REFERENCES

1. *Aiken S., Gardiner E., Basset K.* et al. Implications from SDS-PAGE analyses in the classification of taxa of *Festuca* and *Lolium* (Poaceae) // Biochem. Syst. Ecol. 1998. Vol. 26. P. 51–533.
2. *Aiken S., Gardiner E., Forde M.* Taxonomic implications of SDS-PAGE analyses of seed proteins in North American taxa of *Festuca* subgenus *Festuca* (Poaceae) // Biochem. Syst. Ecol. 1992. Vol. 20. P. 615–629.
3. *Carreras M., Fuentes E., Merina M.* Seed proteins patterns of nine species of *Cactaceae* // Biochem. Syst. Ecol. 1997. Vol. 25. P. 43–49.
4. *Davis B.* Disc electrophoresis. I. Method and application to human serum proteins // Ann. New York Acad. Sci. 1964. Vol. 12. P. 404–427.
5. *Gardiner S., Forde M., Slack C.* Grass cultivar identification by sodium dodecylsulphate polyacrylamide electrophoresis // New Zeland J. Agricult. Res. 1986. Vol. 29. P. 193–206.
6. *Jensen K., Chen S.* Systematic relationships of *Elymus* and *Roegneria* (Poaceae) // Hereditas. 1992. Vol. 116. P. 127–132.
7. *Krzakova M., Melosik I.* Taxonomic value of electrophoretically detected peroxidase patterns in four *Sphagnum* species // Plant Peroxidase Newsletters. 2000. Vol. 14. P. 21–27.
8. *Mahelka V., Fehrer J., Krahulec F., Jarolímová V.* Recent natural hybridization between two allopolyploid wheatgrasses (*Elytrigia*, Poaceae): ecological and evolutionary implications // Ann. Bot. 2007. Vol. 100. P. 249–260.
9. *Melderis A. J.* Taxonomic notes on the tribe Triticeae, with special referenceto the genera *Elymus* L. sensu lato, and *Agropyron* Gaertner sensu lato // Bot. J. Linn. Soc. 1978. Vol. 76. P. 316–320.
10. *Raymond J., Inquello V., Azanza I.* The seed proteins of sunflower: comparative study of cultivars // Phytochem. 1991. Vol. 30. P. 2849–2856.
11. *Reisfeld R., Lewis U., Williams D.* Disc electrophoresis of basic proteins and peptides on polyacrylamide gels // Nature. 1962. Vol. 195. P. 281–283.
12. *Savoy C.* Peanut (*Arachis hypogea*) seed protein characterization and genotype sample classification using PAGE // Biochem. Biophys. Res. Commun. 1976. Vol. 68. P. 886–895.

13. *Szczepaniak M., Cieślak E.* Assessment of genetic variation in *Elymus repens* and *E. hispidus* using AFLP markers and its implications for intraspecific taxonomy: in Frey L. (ed.), "Problems of grass biology", W. Szafer Institute of Botany, Kraków, 2003. P. 271–286.
14. *Szczepaniak M., Cieślak E., Bednarek P.* Natural hybridization between *Elymus repens* and *E. hispidus* assessed by AFLP analysis // Acta Soc. Bot. Pol. 2007. Vol. 76. N 3. P. 225–234.
15. *Turi N., Farhatullah M., Rabani A.* et al. Study of total seed storage protein in indigenous brassica species based on SDS-PAGE electrophoresis // Afr. J. Biotechnol. 2010. Vol. 9. P. 7595–7602.

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СИСТЕМАТИЧНІ ЗВ'ЯЗКИ МІЖ ШІСТЬОМА ТАКСОНАМИ РОДУ *ELYMUS* ЗА ДАНИМИ ЕЛЕКТРОФОРЕЗУ БІЛКІВ НАСІННЯ

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Систематична спорідненість між *Elymus dahuricus*, *E. repens*, *E. hispidus*, їхнім гібридом *E.* x *mucronatus*, *E. elongatus* та *E. pycnanthus* була вивчена за допомогою поліакриламідного електрофорезу (PAGE) та електрофорезу в додецилсульфатному поліакриламідному гелі (SDS-PAGE) білків насіння. Ступінь спорідненості між таксонами оцінювали за коефіцієнтом таксономічної подібності S. Індекс групової спорідненості (GA) також був розрахований для кожного таксона як сума його значень S. Результати показали, що більшість досліджених таксонів є майже рівнодистанційно віддалені один від одного в межах дослідженої групи роду *Elymus*. З-поміж усіх видів *E. pycnanthus* виявився найбільш відмежованим, оскільки його індекс GA (1.08) був найменшим. Інший вид – *E. dahuricus* (GA = 1.21) також показав істотні відмінності у досліджуваній групі роду *Elymus*. Поряд із цим, виходячи зі складу білків насіння, гібридний нототаксон *E.* x *mucronatus* перебуває ближче до *E. repens*, ніж до іншого батьківського виду, яким є *E. hepisus*. Результати проведеного дослідження чітко показали, що у змішаних популяціях *E. repens*, *E. hepisus* та *E.* × *mucronatus* можна спостерігати добре розвинені численні скупчення гібридів.

Ключові слова: *Elymus,* білки насіння, електрофорез, систематична спорідненість