Isoenzyme variation and genetic relationships between *Elymus repens*, *E. hispidus* and *E.* ×*mucronatus* (*Triticeae*, *Poaceae*)

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Abstract. Polyacrylamide gel electrophoresis (PAGE) was employed to examine the variation of ten isoenzyme markers in an attempt to assess the systematic and genetic relationships between *Elymus repens*, *E. hispidus* and their hybrid *E.* ×*mucronatus*. It was found that the hybrid shared most isoenzyme profiles and isoforms of its parents and high genetic affinity among the studied taxa was revealed. The isoenzyme structure of the hybrid resembled rather the structure of *E. repens*. Two alternative interpretations of systematic and genetic relationships among the hybrid *E.* ×*mucronatus* and its parents are discussed.

Key words: *Elymus,* PAGE, isoenzymes, hybridization, genetic relationships

Introduction.

Reticulate evolutionary processes, such as multiple independent origins and/or past hybridization events, have played a crucial role in the origin of contemporary species of genus *Elymus* (the wheat tribe *Triticeae*) (Mason-Gamer & al. 2010), namely they are closely related, share common genomes and have the ability for intergeneric or interspecies outcrossing. These findings support the view that the taxonomy of *Elymus* is complicated by continuous morphological variation and the taxonomic units based on the set of diagnostic characters are often difficult to distinguish (Baum 1983; Salomon & Lu 1992).

Elymus repens (L.) Gould [syn. *Elytrigia repens* (L.) Nevski], quackgrass, is a cosmopolitan species occurring almost worldwide (Hultén & Fries 1986). The species is native to entire Europe and Asia (Tsvelev 1976; Melderis 1980) and was introduced to North America and South America (Tsvelev 1976). *E. repens* is characterized by a very wide phytocoenotic scale and occurs in fairly different ecological conditions such as dry sandy sites, field margins, roadsides, and periodically flooded areas. It is very resistant to salinity and high concentration of heavy metals in the soil. It may also create new forms adapted to stress conditions in the environment (Brej 1998). *E. repens* is also a creeping, aggressive perennial grass that is a serious weed in the majority of northern temperature regions across the world (Holm & al. 1977).

Elymus repens exhibits remarkable morphological variation within its wide area of distribution, hence the different taxonomic treatments have been used by the various researchers (e.g. Szabó 1981; Prokudin 1982; Szczepaniak 2009a). Many subspecies, varieties and forms of *E. repens* were determined (Tsvelev 1976; Melderis 1980; Conert 1998), on the other hand infraspecific taxonomic units were not distinguished (Prokudin 1982; Kosina 1995).

Elymus repens is an allohexaploid species (2n= 6x=42) which has emerged in the result of a complex pattern of reticulate evolution by hybridization and

introgression not only among the taxa of *Triticeae*, but also from divergent sources beyond the tribe (Mason-Gamer 2008). It is a highly self-sterile species which needs cross-pollination for seed production. *E. repens* creates natural hybrids with the species of *Elymus* L., *Triticum* L., *Thinopyrum* Á. Löve and *Hordeum* L. genera (Melderis 1980; Mahelka & al. 2007; Szczepaniak & al. 2007).

Elymus hispidus (Opiz) Melderis [syn. *Elytrigia intermedia* (Host) Nevski, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey], an intermediate wheatgrass, is a species of the Mediterraneo-Irano-Turanian connective element and occurs starting from Central Asia, across the Central and South Europe, and reaching up to the Mediterranean part of France and Spain in the west (Melderis 1980). *E. hispidus* is comparatively rare across Europe, mainly due to its requirement of xerothermic or steppic habitats (Szczepaniak 2001; Mahelka & al. 2007). This thermophilous species grows in grasslands on dry and sun-exposed slopes, gypsum rock outcrops, in abandoned quarries, very often on stony calcareous or chalk soils derived from gypsum or loess, seldom in thermophilous scrubs and in midfield and roadside scarps (Szczepaniak 2001; Mahelka & al. 2007).

Elymus hispidus exhibits a wide morphological variation, mainly in the degree of pubescence of different plant parts, such as glumes, lemmas and leaves (Szabó 1979; Assadi 1998; Szczepaniak 2009b). Therefore, infraspecific taxa have been determined at various taxonomic ranks as subspecies (Melderis 1980) or varietes (Assadi 1998; Szczepaniak & Cieślak 2003).

Elymus hispidus is an allohexaploid species (2n=6x=42) of heterogenous origin, with genome contributions from *Pseudoroegneria*, *Dasypyrum*, *Aegilops* and *Thinopyrum* genera (Mahelka & al. 2011).

Elymus repens and *E. hispidus* hybridize and backcross spontaneously and their hybrid *E. ×mucronatus* is relatively common in natural habitats, which was confirmed by the molecular and cytological studies (Mahelka & al. 2007; Szczepaniak & al. 2007), as well as by morphological (Mizianty & al. 2007) and anatomical evidence (Szczepaniak 2009b). Specimens of *E. ×mucronatus* are partially fertile and more vigorous than its parental species (Szczepaniak & al. 2007). Furthermore, unidirectional introgression towards *E. hispidus* was indicated (Mahelka & al. 2007; Szczepaniak & al. 2007).

Electrophoretic analysis of the isoenzymes has been also used to shed light on systematic relationships between the different taxa within *Triticeae* (Jaaska 1980, 1981; Jaaska & Jaaska 1982; McIntyre 1988; Sun & al. 1999).

The purpose of the present study was to assess the systematic and genetic relationships among *Elymus repens*, *E. hispidus* and their hybrid *E. ×mucronatus* by means of isoenzymes.

Material and methods

Ten enzymes were assayed, namely, esterase (EST), peroxidase (PER), acid phosphatase (ACP), superoxide dismutase (SOD), tetrazolium oxidase (TO), diaphorase (DIA), glutamate oxaloacetate transaminase (GOT), glutamate dehydrogenase (GDH), glucose-6-phosphate dehydrogenase (G-6-PDH), and amylase (AMY). Individuals of *Elymus* species from natural populations were studied (Table 1).

Table 1. Taxa and populations examined.

Taxon	Locality	Coordinates
E. repens	Poland, Niecka Nidziańska Basin, Janowiczki near Racławice	50°18'N 20°14'E
	Poland, Niecka Nidziańska Basin, Smoniowice near Racławice,	50°17'N 20°12'E
	Poland, between Zakopane and Kuźnice	49°16'N 19°58'E
	Bulgaria, Sredna gora Mt., Anton village	42°43'N 24°14'E
	Bulgaria, Rila Mt., Sapareva banja	42°17'N 23°15'E
	Former Soviet Union	PI 314197 *
E. ×mucronatus	Poland, Niecka Nidziańska Basin, Janowiczki near Racławice	50°18'N 20°14'E
	Poland, Niecka Nidziańska Basin, Smoniowice near Racławice	50°17'N 20°12'E
E. hispidus	Poland, Niecka Nidziańska Basin, Janowiczki near Racławice	50°18'N 20°14'E
	Poland, Niecka Nidziańska Basin, Smoniowice near Racławice	50°17'N 20°12'E
	Poland, Niecka Nidziańska Basin, Skorocice	50°24'N 20°38'E
	Bulgaria, Vrachnska Mt., Ledenika	43°12'N 23°29'E
	Bulgaria, Rila Mt., Palatovo village	42°15'N 23°02'E
	Russian Federation, Kursk	PI 273733 *

* number of seed collections provided by USDA, ARS, WRPIS, Washington State University, Regional Plant Introduction Station.

Leaves were ground in 0.01 M Tris, 0.08 M glycine and 0.005 M cysteine. Anodally migrating isoforms were resolved on 7.5% slab gels, as described by Davis (1964).

Cathodal isoforms were run on 7.5 % gels (Reisfeld & al. 1962). Amylase was fractionated on 6 % gels, 0.5 % starch for 8 hours at 150 V. The gel length for anodal EST, ACP, DIA and GOT was 9 cm, and cathodal PER was 7 cm and 6 cm for the other enzymes. The following staining recipes were used: PER, GOT, AMY (Przybylska & al. 1982.), ACP, G-6-PDH, GDH (Shaw & Prasad 1970), EST (Schmidt-Stohn & Wehling 1983), SOD (Jaaska & Jaaska 1982), DIA (Wendel & Weeden 1989), and TO (Baur & Schorr 1969). Each isoform was assigned a number reflecting its gel migration (in mm) from the origin.

The studied taxa are polyploids and produce complex banding patterns difficult for genetic interpretation. For this reason, phenotypic analysis (isoform presence/absence, isoform frequency) was used. Thus, the mean values of phenetic coefficients of similarity (S) and distance D (Stuessy 1990) were calculated for the six most polymorphic enzymes EST, PER, ACP, SOD, DIA, and TO, according to the following formulas (Stuessy 1990):

S = (a + d)/a + b + c + d),

where **a** is the number of isoforms common for compared taxa, **b** and **c** are the specific isoforms, **d** is number of isoforms absent from both taxa; and

$$D = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x_{ij} - x_{ik}\right)^2}$$

where N is the total isoform number for each enzyme, and x_{ij} and x_{ik} the mean frequencies of i^{th} isoform in taxa j and k.

Results

Anodal esterase. A total of fourteen isoforms of the enzyme were detected (Table 2). Most of them were shared by all studied taxa. Isoform 52 was species-specific for *E. hispidus*.

Cathodal peroxidase. The isoenzyme variation of cathodal PER is shown in Table 3. Four isoforms, namely, 13, 32, 38, and 44 were monomorphically fixed across the entire studied species group. Isoform 17 was also invariant or nearly fixed in most populations and taxa. The monomorphic isoform 28 was characteristic of *E. hispidus,* while isoform 48 was found in the studied populations of *E.* ×*mucronatus* only.

Table 3. Mean isoform frequencies of cathodal PER in the studied populations of *E. repens*, *E. hispidus* and *E.* ×*mucronatus*.

Taxon	Isoforms									
	13	17	28	32	36	38	44	48	54	58
E. repens	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.00	0.50	0.05
E. hispidus	1.00	0.92	1.00	1.00	0.25	1.00	1.00	0.00	1.00	0.50
E. ×*mucronatus*	1.00	1.00	0.00	1.00	0.00	1.00	1.00	0.45	0.00	0.10

Acid phosphatase. The mean isoform frequencies of ACP are presented in Table 4. Most of them were detected in all studied taxa. Isoforms 19 and 43 were invariant throughout the examined group of species. Isoforms 17 and 22 proved to be unique for *E. hispidus*.

Table 4. Mean isoform frequencies of ACP in the studied populations of *E. repens*, *E. hispidus* and *E.* ×*mucronatus*.

Taxon	Isoforms									
	13	17	19	22	24	26	29	36	43	50
E. repens	0.16	0.00	1.00	0.00	0.42	1.00	0.38	0.37	1.00	0.54
E. hispidus	0.34	0.45	1.00	0.42	0.21	0.64	0.74	0.24	1.00	0.32
E. ×*mucronatus*	0.22	0.00	1.00	0.00	0.32	0.48	0.12	0.32	1.00	0.47

Superoxide dismutase. Six isoforms of SOD were electrophoretically resolved in *E. repens*, *E. hispidus* and *E.* ×*mucronatus* (Table 5). Isoforms 30 and 49 were monomorphically-fixed across the whole group, while isoform 22 was nearly fixed in most populations and taxa. Isoform 44 was observed in the studied populations of *E.* ×*mucronatus* only.

Table 5. Mean isoform frequencies of SOD in the studied populations of *E. repens*, *E. hispidus* and *E.*×*mucronatus*.

Taxon	Isoforms					
	22	26	30	42	44	49
E. repens	1.00	0.70	1.00	0.25	0.00	1.00
E. hispidus	0.84	0.17	1.00	0.00	0.00	1.00
E. ×*mucronatus*	0.30	0.70	1.00	0.30	0.30	1.00

Table 2. Mean isoform frequencies of anodal EST in the studied populations of *E. repens*, *E. hispidus* and *E.* ×*mucronatus*.

Taxon	Isoforms													
	19	22	25	28	31	34	36	40	42	44	49	52	54	58
E. repens	0.05	0.21	0.58	0.83	0.36	0.42	0.51	0.25	0.00	0.47	0.27	0.00	0.58	0.42
E. hispidus	0.15	0.10	0.48	0.65	0.19	0.28	0.43	0.05	0.40	0.15	0.52	0.37	1.00	1.00
E. ×*mucronatus*	0.19	0.32	0.52	0.78	0.28	0.32	0.65	0.42	0.10	0.34	0.38	0.00	0.22	0.18

Diaphorase. Totally six isoforms of the enzyme were detected in the studied species group (Table 6). Two of them, 30 and 32 were monomorphic in two or all three taxa. Isoform 50 was characteristic of *E. repens*.

Table 6. Mean isoform frequencies of DIA in the studied populations of *E. repens*, *E. hispidus* and *E. ×mucronatus*.

Taxon	Isoforms					
	30	32	36	40	44	50
E. repens	1.00	1.00	0.86	0.88	0.10	0.13
E. hispidus	1.00	0.93	0.72	0.75	1.00	0.00
E. ×mucronatus	1.00	1.00	0.94	0.62	0.00	0.00

Tetrazolium oxidase. A set of three isoforms (35, 39, 54) was monomorphically-fixed across the whole group. Another three isoforms (11, 16, 22) were invariant or nearly fixed in *E. repens* and *E. ×mucronatus.*

Glutamate dehydrogenase. Isoform 16 was shared by all studied taxa. The species *E. hispidus* possessed also the rare isoform 18 in its populations.

Glucose-6-phosphate dehydrogenase. Triplet 21/24/27 was detected in *E. hispidus*, while another triplet 27/31/35 was observed in *E. repens*. A triplet with intermediate mobility (21/29/31) was found in the examined populations of *E. ×mucronatus*. Different types of triplets found in the studied *Elymus* taxa reflect dimeric structure of G-6-PDH generally observed in plants (Wendel & Weeden 1989).

Glutamate oxaloacetate transaminase. Numbered from the anode, three zones of independent isoenzyme variation were detected on the gels. The studied taxa shared the triplet 36/39/42 in zone I. The hybrid possessed additionally isoform 34 in zone II. Isoform 24 in zone III was invariant throughout the studied group of taxa. The occurrence of triplets indicates dimeric structure of GOT as it has been usually found in plant species (Wendel & Weeden 1989).

Amylase. Two zones of independent variation were detected in *E. repens*, *E. hispidus* and *E. ×mucronatus.* All examined taxa were monomorphic for isoform 19 in the anodally faster migrating zone. *E. repens* and the hybrid shared isoform 6 in the slower migrating zone. *E. hispidus* possessed the dublet 6/7 in the same zone.

The mean values of coefficient S ranged from 0.80 in the comparison between *E. repens* and *E. ×mucronatus* to 0.87 when the latter taxon was contrasted to *E. hispidus.* An intermediate value of 0.83 was obtained when *E. repens* and *E. hispidus* were contrasted. Considering distance coefficient D, similar tendencies were observed. The isoenzyme structure of *E. ×mucronatus* resembled more that one of *E. repens*, while it is more distant from *E. hispidus* as coefficient D is equal to 0.31 in this case.

Summarizing the results of this study, it was observed that *E. ×mucronatus* is closer to *E. repens* as judged by the set of examined enzyme markers.

Discussion

In the present study of *E. repens*, *E. hispidus* and *E. ×mucronatus* it was found that the hybrid shared most of isoenzyme profiles and isoforms of its parents, but no evidence for strict summation was observed. Nevertheless, a high genetic affinity among the studied taxa was revealed. It could be expected, bearing in mind that parental species share common genomes. Assuming the genomic constitutions of *E. repens* and *E. hispidus* to be SSH and SJJ, respectively, the genomic formula of the hexaploid hybrid should be SSSJJH (Assadi & Runemark 1995). It has been demonstrated that the genome S is next to the closely related J-E genomic complex (Jarvie & Barkworth 1990). It has been also shown that the S genome has almost always dominance on the morphology of the taxa of which it is a component (Assadi 1998; Assadi & Runemark 1995). Allopolyploids between E/J and S genome species shown isoenzyme profiles resembling those of the S genome taxa, thus reflecting patterns of variation based on morphological and anatomical data (Jarvie & Barkworth 1990). Isoenzyme analysis of polyploid taxa provided also evidence of gene silencing and intergenomic supression of redundant genes in polyploids (Wilson & al. 1983; Galili & Feldman 1984; Wendel 2000).

The results of the present study could be interpreted in the light of the above-mentioned evidence about differential gene expression and silencing in polyploids. It could be assumed that S genome dominates over J genome and some isoenzyme gene loci/alleles coded by the latter are suppressed or silenced in the hybrid. The derivative allopolyploid species in *Tragopogon* is of very recent origin and it could be expected that the genomes of its parents have not changed substantially after the hybridization event. Electrophoretic studies also demonstrated that both copies of the duplicated gene usually retain expres-

sion in young polyploids (Gottlieb 1982), while in older polyploids loss of duplicated gene expression is common (Wendel 2000). In the case of *E.* ×*mucronatus,* where several different genomes interact, it could hardly be expected that isoenzyme profiles would reflect exactly its genome composition. Furthermore, some isoforms found in the hybrid were not observed in its parents. It could be assumed that other species carrying more or less different genomes may be involved in its parentage as it was suggested for some species of genus *Festuca* (Aiken & al. 1993). Alternatively, the observed non-additivity could be explained by the assumption that the analyzed specimens of *E.* ×*mucronatus* are not strict allopolyploids, but segmental ones originating from a backcross by *E. repens* pollen to more or less sterile F_1 hybrid. In this case, one would expect a greater contribution of *E. repens* parent to the genome of *E.* ×*mucronatus.* A study of the exact genome composition of *E.* ×*mucronatus* would permit deciding which of the alternative interpretations of isoenzyme data is more plausible. These inferences are different from the earlier cpDNA (Mahelka & al. 2007) and AFLP (Szczepaniak & al. 2007) studies that suggested greater 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 means a possibility of bidirectional introgression towards both parental species. The earlier and present studies have clearly shown that within mixed populations of *E. repens*, *E. hispidus* and *E.* ×*mucronatus* some well developed hybrid swarms can be observed.

Different hybridization events depend on the frequency of parental species in the common habitats and the level of hybrids fertility. *E.* ×*mucronatus* is partially fertile (Szczepaniak & al. 2007) and has capability for intercrossing and backcrossing with the parental species, producing a conspicuous genetic and phenotypic variation for natural selection (Mahelka & al. 2007). In many populations, hybrids exceed both parental species in the more invasive characters, such as the stronger and more creeping rhizomes and robust culms (own field observations). Apparently, natural selection acting on these fit hybrids, creating a more invasive population, may be responsible for accelerating rates of the hybrid spread and the eventual local extinction of parental species (Mahelka & al. 2007; Szczepaniak & al. 2007).

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