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# Isoenzyme variation and genetic affinity among *Dasypyrum villosum, Elytrigia repens* and *Elymus caninus*

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***Abstract:***

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Electrophoretic variation between and among populations of *D. villosum, Et. repen*s and *El. caninus* was studied in order to evaluate the phylogenetic position of *Dasypyrum* within tribe *Triticeae*. Several genetic (I) and phenetic (SI, S, D) indeces were calculated in order to assess more precisely genetic affinities among the species examined. Mean values of I (range 0.96 - 0.99) indicated high levels of genetic similarity among the populations within each species. The species *D. villosum* was clearly distant (I=0.44) from both *Et. repens* and *El. caninus*, while the latter two species exhibited relatively little divergence (I=0.81) at isoenzyme level. Genetic data were supported by phenetic analysis. Similarity indexes SI and S showed that *D. villosum* was substantially isolated from *Et. repens* and *El. caninus*. Index D indicated that the species *Et. repens* and *El. caninus* were equally distant (D=0.16 in both cases) from *D. villosum*, while the former two species demonstrated more close relationship as judged by isoenzymes examined. The results were discussed in the ligth of DNA sequence data about the position of *Dasypyrum*.

***Key words:*** *Dasypyrum villosum, Elytrigia repens, Elymus caninus*, isoenzyme variation, genetic affinity

## Introduction

The genus *Dasypyrum* (Cos. et Dur.) Durand belongs to subtribe Triticinae of tribe Triticeae (Tzvelev 1976). Two species of *Dasypyrum* are recognized in Europe - the widespread annual *D. villosum* (Cos. et Dur.) Cand. and North African and Greek perennial *D. hordeaceum* (Humphries, 1978). Initially *D. villosum* was described as *Secale villosum* L. Lately it was considered as member of genera *Triticum* and *Agropyrum* but in 1888 Durand (see Humphries, 1978) validated *Dasypirum* as a distinct genus within *Triticeae*. For its morphology and distribution ability to hybridize with some Triticum species (Depace et al. 1988, Cappocchi et al. 1991) *Dasypirum* is considered as closely related to *Triticum, Agropyrum* and *Secale*. However, some recent phylogenies of *Triticeae* based on DNA molecular data (Kellogg, 1992a, Kellogg, 1992b, Mason-Gamer, Kellogg, 1996) indicated close relationships among *Dasypirum*, *Elytrigia* and *Elymus*.

The aim of the study was to assess genetic affinities among *D. villosum, Et. repens* and *El. caninus* by means of isoenzymes in attempt to evaluate phylogenetic position of *Dasypyrum* within tribe *Triticeae*.

## Material and Methods

Enzymes peroxidase (PER), aspartate aminotransferase (AAT), glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH) and glutamate dehydrogenase (GDH) were examined in natural populations of *Et. repens, El. caninus* and *D. villosum* (**Table 1**). Vouchers are deposited at Herbarium of Institute of Botany (SOM).

**Table 1**. Taxa and populations examined

| Taxon | Population designation | Number of individuals | Locality | Voucher number |
|---|---|---|---|---|
| Et. repens | 1 | 31 | Chepan Mt., near Dragoman | Co-588 |
| | 2 | 33 | Vitosha Mt., near Boyana | Co-589 |
| | 3 | 28 | Sredna gora Mt., near the village of Anton | Co-590 |
| El. caninus | 4 | 35 | Rila Mt., the valley of Rilska river | Co-591 |
| | 5 | 37 | Rila Mt., the valley of Stara reka | Co-592 |
| D. villosum | 6 | 40 | Chepan Mt. around Dragoman | Co-225 |
| | 7 | 35 | Strouma valley region, Kozuh hills | Co-226 |
| | 8 | 34 | Sredna gora Mt., near the village of Mirkovo | Co-227 |
| | 9 | 41 | Thracian plain region, around village of Levka | Co-228 |

Fresh leaves were ground in 0.01 M Tris, 0.08 M. glycine, 0.005 M cysteine, 20% sucrose, pH 8.3. Ion-exchange resin Dowex 1 x 8 (0.4 g / 1 g tissue) was added to the extraction buffer. Crude extracts were centrifuged at 10000 rpm for 10 minutes. The supernatant was used as a source of enzymes.

Enzymes were resolved on 7.5% polyacrylamide slabs as separating gel with 3% stacking gel (Davis, 1964). Cathodal isoforms of PER were run on 7.5% separating and 3% stacking gel (Reisfeld et al., 1961). The length of the separating gel for cathodal PER was 6 cm. The rest of enzymes were resolved on 5 cm long gels. Electrophoresis in cathodal direction was carried out until the indicator dye, pyronin G, reached the gel end (1 front) for the enzyme cathodal PER.The duration of electrophoresis in anodal direction was 1.5 fronts of indicator bromphenol blue for all of enzymes surveyed. Staining protocols were described previously (Angelov, 2000).

For enzymes AAT, G-6PDH, 6-PGDH and GDH gene number was assigned on the basis of the known subunit structure of the enzyme and patterns of segregation within populations. Different gene loci coding for the same enzyme (isoenzymes) were designatad according to relative mobilities of respective isoenzymes (Crawford, Smith, 1982, 1984). Thus, the gene locus specifying the most anodal isoenzyme was designated 1, the next 2 etc. At each locus, alelle coding the fastest isoform was assigned a, the next b etc. Allelic frequencies were determined and genetic identities were calculated (Nei, 1972). Mean genetic identities were calculated for all pair-wise comparisons within and among species.

The enzyme cathodal PER was not included in genetic identity analysis. In this case isoform frequencies were determined. Each isoform was assigned a number which reflects its gel migration in mm from the origin (Perez de la Vega, Allard, 1984).

Three measures of phenetic affinity (SI, S and D) were calculated according to the following formulas:

$$SI = \frac{M}{M+N}$$

where **M** is the number of isoforms common for both taxa and **N** is the sum of species specific isoforms,

$$S = \frac{a+d}{a+b+c+d}$$

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

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

where **N** is the number of isoforms for each enzyme, $\mathbf{x_{ij}}$ and $\mathbf{x_{ik}}$ - the frequency of **i**-th isoform in taxa **j** and **k**.

## Results and Discussion

Six gene loci - three for GOT, and one locus for G6PDH, 6PGDH and GDH were included in genetic identity analysis. Alellic frequencies in populations examined are presented in Table 2. The species Et. repens was monomorphic at gene loci GOT-1 and GOT-2 and nearly invariant at GOT-3. El. caninus was nearly fixed at GOT-1 whereas four and three alleles of loci GOT-2 and GOT-3 were found, respectively. Five alleles of GOT-3, two of them rare, were observed in the latter species. Totally four alleles of GDH were detected. Some of them were rare - allele GDH-1a in *Et. repens* and *El. caninus* while alleles GDH-1c and GDH-1d were absent in the species El. caninus and D. villosum, respectively.

**Table 2.** Allelic frequencies of gene loci in Et. repens, El. caninus and D. villosum

| Gene | Alelle | Et. repens | | | El. caninus | | D. villosum | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| GOT-1 | a | 1.00 | 1.00 | 1.00 | 1.00 | 0.94 | 0.50 | 0.50 | 0.50 | 0.50 |
| | b | | | | | 0.06 | | | | |
| | c | | | | | | 0.50 | 0.50 | 0.50 | 0.50 |
| GOT-2 | a | | | | 0.04 | | 0.14 | 0.22 | 0.17 | 0.27 |
| | b | | | | 0.09 | 0.10 | 0.86 | 0.78 | 0.83 | 0.73 |
| | c | | | | 0.75 | 0.80 | | | | |
| | d | 1.00 | 1.00 | 1.00 | 0.12 | 0.10 | | | | |
| GOT-3 | a | 1.00 | 1.00 | 0.88 | 0.86 | 0.90 | 0.24 | 0.28 | 0.21 | 0.30 |
| | b | | | 0.12 | | | 0.47 | 0.39 | 0.49 | 0.37 |
| | c | | | | 0.14 | 0.10 | 0.06 | 0.09 | 0.05 | 0.10 |
| | d | | | | | | 0.02 | 0.06 | 0.10 | 0.00 |
| | e | | | | | | 0.21 | 0.18 | 0.15 | 0.23 |
| G6PDH | a | | | | | | 1.00 | 1.00 | 1.00 | 1.00 |
| | b | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| 6PGDH | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| GDH | a | 0.02 | 0.00 | 0.10 | | | 0.05 | 0.15 | 0.20 | 0.08 |
| | b | 0.05 | 0.14 | 0.18 | 0.08 | 0.24 | 0.72 | 0.60 | 0.65 | 0.75 |
| | c | 0.68 | 0.43 | 0.45 | | | 0.23 | 0.25 | 0.15 | 0.17 |
| | d | 0.27 | 0.41 | 0.37 | 0.82 | 0.76 | | | | |

Genetic identiy values for all pair-wise comparisons among the populations of *Et. repens, El. caninus* and *D. villosum* are presented in Table 3. The populations of *Et. repens* were indistinguishable (I=O.99) for the set of enzymes surveyed. Comparisons within *El. caninus* resulted in identity value of 0.98. The corresponding values for populations of *D. villosum* ranged from 0.96 to 0.99 and mean value was 0.98. Mean value for comparisons among populations of *D. villosum* and *Et. repens* was equal to 0.44. The same value was obtained when comparisons among populations of *D. villosum* and *El. caninus* were made. Genetic identity for comparison between populations of *Et. repens* and *El. caninus* has mean value of 0.81.

Isoform frequencies of cathodal PER are presented in Table 4. Most of isoforms were monomorphic (frequency of 1.00) and were shared by all species examined. Isoforms 5 and 10 were diagnostic for *D. villosum*. Isoform 52 was observed in *El. caninus* only. Values of similarity index SI for comparison between *D. villosum* and species pair *Et. repens* and *E. caninus* were 0.42 and 0.54 respectively. Values of SI and S for comparison between the latter two species were 0.73 in both cases. Nearly the same values of coefficient D (0.17 and 0.15) were obtained when *D. villosum* was compared to *Et. repens* and *El. caninus,* respectively. Twice lower value (D=0.073) was obtained in the comparison between the latter two species.

Genetic identity statistics allowed some conclusions about interspecific and intraspecific genetic affinities to be drawn. The very high mean genetic identity values for pair-wise comparisons of populations within each species indicated insignificant interpopulational differentiation within species examined. The values obtained

**Table 3.** Genetic identity values for pair-wise comparisons among populations of Et. repens, El. caninus and D. villosum

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | x | | | | | | | | |
| 2 | 0.99 | x | | | | | | | |
| 3 | 0.99 | 0.99 | x | | | | | | |
| 4 | 0.83 | 0.81 | 0.82 | x | | | | | |
| 5 | 0.79 | 0.82 | 0.80 | 0.97 | x | | | | |
| 6 | 0.43 | 0.42 | 0.42 | 0.44 | 0.43 | x | | | |
| 7 | 0.42 | 0.46 | 0.46 | 0.45 | 0.45 | 0.98 | x | | |
| 8 | 0.49 | 0.42 | 0.44 | 0.43 | 0.41 | 0.99 | 0.96 | x | |
| 9 | 0.44 | 0.46 | 0.42 | 0.44 | 0.45 | 0.98 | 0.96 | 0.99 | x |

**Table 4.** Isoform frequencies of cathodal PER in Et. repens, El. caninus and D. villosum

| Taxon | Isoforms | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 14 | 22 | 24 | 28 | 30 | 32 | 36 | 40 | 48 | 50 | 52 |
| Et. repens | 0.00 | 0.00 | 1.00 | 0.64 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 0.50 | 0.00 | 0.00 |
| El. caninus | 0.00 | 0.00 | 1.00 | 0.72 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.58 | 0.64 | 0.42 | 0.34 |
| D. villosum | 0.33 | 1.00 | 0.67 | 0.00 | 0.33 | 1.00 | 1.00 | 1.00 | 1.00 | 0.83 | 0.00 | 0.79 | 0.00 |

correspond to the grand mean of 0.95 found for majority of species examined electrophoretically (Gottlieb, 1977, 1981) . Our data conform to the generalization that populations belonging to the same taxon are very similar isoenzymically (Crawford, 1989).

The species *D. villosum* was almost equally and distantly related to both *Et. repens* and *El. caninus* as jujded by low identity values. By contrast, the species *Et. repens* and *El. caninus* exhibited much closer genetic affinity. The mean value of their comparison was substantially higher than the grand mean of 0.67 calculated for congeneric species ((Gottlieb, 1977, 1981).

The phenetic data for enzyme cathodal PER revealed similar patterns of genetic relationships among the species. Considering coefficient D, the species *D. villosum* proved to be almost equally distant from the species pair *Et. repens* and *El. caninus*. This value of D indicates that a substantial genetic differentiation exists between *D. villosum* and the latter two species. Nearly twice lower value of coefficient D for comparison between *Et. repens* and *El. caninus* is an indication for their stronger genetic relationship. Similarity indeces SI and S also supported the observation that a close genetic affinity exists between the later two species whereas *D. villosum* is most distantly positioned within the studied group. Considering together genetic and phenetic data, it could be concluded that *Et. repens* and *Elymus caninus* are genetically more closely related than either is to *D. vilosum*.

Studies of chloroplast DNA (cpDNA) were employed to discern genetic relationships within tribe *Triticeae* (Kellogg, 1992b). The most distinctive marker was a unique deletion found in *D. villosum* and *Et. repens* (Kellogg, 1992a). When polyploids of *Elymus* were included in cpDNA analysis it was found that *Elymus* and *Elytrigia* formed a clade with *Dasypyrum* (Mason-Gamer, Kellogg, 1996a). It is known that *Elytrigia* and *Elymus* contain S genome (Asadi, Runemark, 1995). Thus, the deletion may be a useful marker for S genome but it will not distinguish S genome from V genome of *D. villosum*. In a set of papers (McIntyre, 1988, McIntyre et. al., 1988a, 1988b) available information on genome relationships in the tribe *Triticeae* has been summarized. The position of V genome varied but in general, it has tended to cluster with S, E, J genomes (McIntyre, 1988). These results conform, at least partially, with before-mentioned studies of cpDNA.

## Conclusion

The results of our isoenzyme study tend not to support cpDNA analysis as they indicate that *D. vilosum* is genetically distinct from both *Et. repens* and *El. caninus* at isoenzyme level. The position of *Dasypyrum* seems to be unstable and its phylogenetic relationships within *Triticeae* are rather unresolved. There exists a discrepancy between nuclear and chloroplast DNA data. The differences probably reflect separate evolutionary history of nuclear and chloroplast genomes (Kellogg et.al., 1996, Mason-Gamer, Kellogg, 1996b). Further research is needed to clarify phylogenetic position of *Dasypyrum* within tribe *Triticeae*.

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***Резюме***

**Изоензимна изменчивост и генетично родство между *Dasypyrum villosum, Elytrigia repens* and *Elymus caninus***

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Изследвана бе изоензимната изменчивост в естествени популации на *Dasypyrum villosum, Elytrigia repens* and *Elymus caninus* с цел да се установи филогенетичната позиция на *Dasypyrum* в триб *Triticeae.* Средните стойности на индекса за генетична идентичност показаха, че *D. villosum* е ясно разграничен (I=0.44) от *Et. repens* и *El. caninus*, докато последните два вида имат сравнително малки различия I=0.81) на изоензимно ниво. Фенетичните индекси за сходство SI и S са също индикатор за значителната изолираност на *D. villosum* от *Et. repens* и *El. caninus.* Индексът за генетична диференциация D показва, че видовете *Et. repens* и *El. caninus* са на значителна и еднаква дистанция от *D. villosum* . Резултатите от настоящето изоензимно изследване не подкрепят данните от анализа на cpDNA в триб *Triticeae,* ксито свидетелстват за тясно родство между ***Dasypyrum, Elytrigia* и *Elymus,*** Различията вероятно отразяват различната еволюционна история на ядрената и хлоропластната ДНК в триб *Triticeae.*