Georgi Angelov

Genetic relationships between *Lophopyrum elongatum* and *Dasypyrum villosum* (*Triticeae* - *Poaceae*) as revealed by isoenzymes

Abstract

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Polyacrylamide gel electrophoresis was used to reveal genetic relationships between *Lophopyrum elongatum* and *Dasypyrum villosum*. Allozymes indicated that gene diversity occurrs primarily within than between populations of both taxa. Unbiased genetic identities (I) and distances (D) were calculated. Pairwise comparisons between populations of *L. elongatum* and *D. villosum* resulted in mean value of D equal to 0.889 - an indication that *Lophopyrum* and *Dasypyrum* are distantly related within the tribe *Triticeae*. The present isoenzyme study tended to support data derived from nuclear DNA sequences but it is incongruent with data from chloroplast DNA sequencing.

Introduction

The tribe *Triticeae* includes some of economically most important cultivated grass species - wheat, barley, rye. The high proportion of polyploids and intensive hybridization between the species makes their classification difficult. Thus, the number of recognized genera varied drastically - from one (Stebins 1956) to 38 genera accepted by Löve (1984).

Due to its importance, the tribe *Triticeae* has been subjected to numerous studies using morphology (Baum & al. 1987; Frederiksen & Seberg 1992; Seberg & Frederiksen 2001), cytogenetics (Hsiao & al. 1986; Wang 1989) and isoenzymes (McIntyre 1988; Jarvie and Barkworth 1990). In the last years, generic relationships within *Triticeae* have been studied by different molecular techniques (Monte & al. 1993, Hsiao & al. 1995, Kellogg & Appels 1995). Three comprehensive studies of chloroplast DNA in monogenomic genera within *Triticeae* (Mason-Gamer & Kellogg 1996b; Petersen & Seberg 1997; Mason-Gamer & Kellogg 2000) suggest a close affinity between *Lophopyrum* A. Löve and *Dasypyrum* (Cosson & Dur.) T. Durand.

The species *Lophopyrum elongatum* (Host) Á. Löve (*Elytrigia elongata* (Host) Nevski, *Elymus elongatus* (Host) Runemark) and *Dasypyrum villosum* (L.) P. Candargy (V genome) are both diploids. *Lophopyrum elongatum* is also treated as a member of genus *Thinopyrum* under the name *T. elongatum* (Host) D. R. Dewey. Genome designations and generic delimitation of *Lophopyrum* (E genome) and *Thinopyrum* (J genome) are still dis-

putable (Wang 1985; Jauhar 1988, 1990). However, delimitation of *Lophopyrum* and *Thinopyrum* as well the relationships between the respective genomes are beyond the scope of this study.

The present study aimed at assessing genetic relationships between *L. elongatum* and *D. villosum* by means of isoenzymes. In particular, the findings are compared with chloroplast DNA data sets suggesting close affinity between *Lophopyrum* and *Dasypyrum*.

Material and methods

Live plants were collected from natural populations of *L. elongatum* and *D. villosum* (Table 1). Voucher specimens were deposited at herbarium of Institute of Botany, Bulgarian Academy of Sciences (SOM).

Fresh leaves were crushed in grinding buffer consisting of 0.01 M Tris, 0.08 M glycine, 0.005 M cysteine, 20% sucrose, pH 8.3. Ion-exchange resin Dowex 1×8 (0.4 g / 1 g tisue) was added to the extraction medium. Homogenates were centrifuged and the supernatant was used as a source of enzymes. Aspartate aminotransferase (AAT), glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH) and glutamate dehydrogenase (GDH) were resolved on 7.5% polyacrylamide slabs with 3% stacking gel (Davis 1964). The lenght of separating and stacking gel was 5 cm and 1.5 cm, respectively. Electrophoresis was conducted until indicator dye bromphenol blue reached the end of the gel (1 front) for GDH and 1.5 fronts for the remaining enzymes. Enzyme staining was performed as described previously (Angelov 2000).

Table 1. Species and populations examined.

Species	Population number *	N	Locality	Voucher number
L. elongatum	1	30	Bulgaria, Black Sea coast, Pomorie	Co-604
	2	34	Bulgaria, Strouma valley region, Marikostinovo	Co-605
	3	35	Bulgaria, Black Sea coast, Nesebar	Co-400
D. villosum	4	29	Bulgaria, Thracian Lowland, Ognyanovo	Co-624
	5	31	Bulgaria, Pirin Mt., Goleshevo	Co-625
	6	32	Bulgaria, Strouma valley region, Marikostinovo	Co-600
	7	25	Bulgaria, Sredna gora Mt., Chavdar	Co-626

* Population number is followed in the succeeding tables. N - number of individual plants examined

Zones of activity on a gel, which varied independently of other such zones, were considered to be encoded by single gene loci. The fastest anodally migrating zone of activity was designated as the first locus encoding a particular enzyme, the next fastest as the second locus, and so on. Within a zone of activity, the most anodal band was designated the a allele, the next fastest the b allele, and so on.

Based on allelic frequencies unbiased genetic identities (I) and distances (D) were calculated using the method of Nei (1978). Gene diversity statistics were calculated utilizing the procedure of Nei (1973). Total gene diversity (H_T), intrapopulational gene diversity (H_S), interpopulational gene diversity (H_{ST}), and the coefficient of gene differentiation (G_{ST}) are related by the equations $H_T=H_S+D_{ST}$ and $G_{ST}=D_{ST}/H_T$.

Results and discussion

All four enzyme systems displayed activity and more or less legible bands. The interpretation of the genetic basis of the enzyme phenotypes (electrophoretic patterns) was made given the known subunit structure of enzymes and their patterns of variation within the studied populations. The species *L. elongatum* and *D. villosum* are diploids and it could

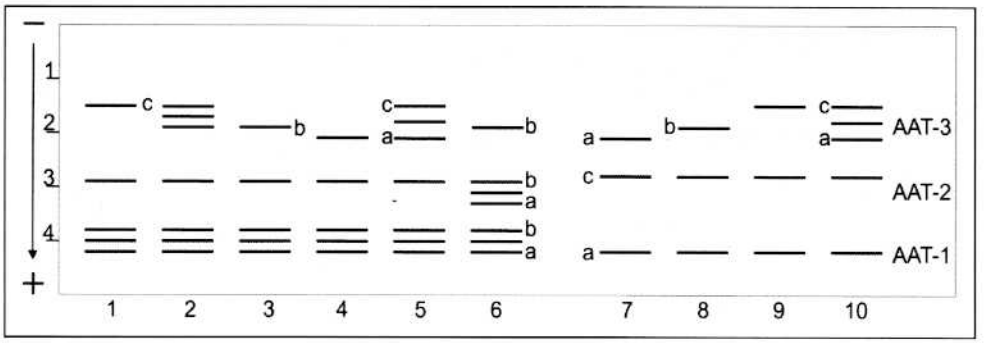

Fig. 1. Isoenzymes genotypes of aspartate aminotransferase in *D. villosum* (1-6) and *L. elongatum* (7-10). Origin at top, anode at bottom.

be assumed that enzyme phenotypes reflect the respective genotypes.

Aspartate aminotransferase is a dimeric enzyme (Huang & al. 1986; Scandalios & Sorensen 1975). Three zones of activity, exhibiting independent variation pattern, appeared on the gels (Fig. 1). These zones were interpreted as three gene loci coding isozymes of AAT in *D. villosum* and *L. elongatum*. Two bands occurred in most anodal zone, labeled AAT-1. Allele **a** was shared by *D. villosum* and *L. elongatum*, whereas allele **b** was observed in *D. villosum* only. Two alleles, **a** and **b**, at locus AAT-2 were found in *D. villosum*. Some plants displayed heterozygous genotype 6, formed by **aa** and **bb** homodimers and intermediate **ab** heterodimer. All examined plants of *L. elongatum* exhibited allele **c** at locus AAT-2. Alleles **a**, **b**, **c** at locus AAT-3 were shared by *D. villosum* and *L. elongatum*. Beside homozygous genotypes **aa**, **bb**,**cc** - genotypes 4, 6, 1, respectively, they

combined in heterozygous triplets **bc** (genotype 2) in *D. villosum* and **ac** (genotypes 5 and 10) in both species. Plants possess three gene loci encoding AAT, and in some instances four loci have been observed (Gottlieb 1981, 1982). Three gene loci and dimeric structure of AAT have been found in other grasses - maize (Doebley & al. 1984; Doebley & al. 1985), barley (Brown & al. 1978), wheats, *Aegilops* (Jaaska 1976, 1981), *Sorghum* (Morden & al. 1990).

Data about quartenary structure of GDH are rather controversial. The enzyme is considered to have monomeric (McLeod & al. 1983), tetrameric (Bayer 1988) and hexameric (Cammerts & Jacobs 1983) subunit composition. The pattern of variation observed in *D. villosum* and *L. elongatum* (Fig. 2) conforms to the first genetic model. *Dasypyrum villosum* displayed three alleles, namely **a**, **b**, **c**, in homozygous **aa**, **bb** (genotypes 1, 2) and heterozygous **bc** (genotype 3) combinations. Allele **c** (genotype 4) was shared by *D. villosum* and *L. elongatum* whereas allele **d** was observed only in the latter species. Monogenic control of GDH with up to five alleles have been observed in *Compositae* (Crawford & Smith 1984; Bayer & Crawford 1986; Bayer 1988), grasses (Morden & al. 1989; Morden & al. 1990; Jaaska 1994) and *Capsicum* (McLeod & al. 1983).

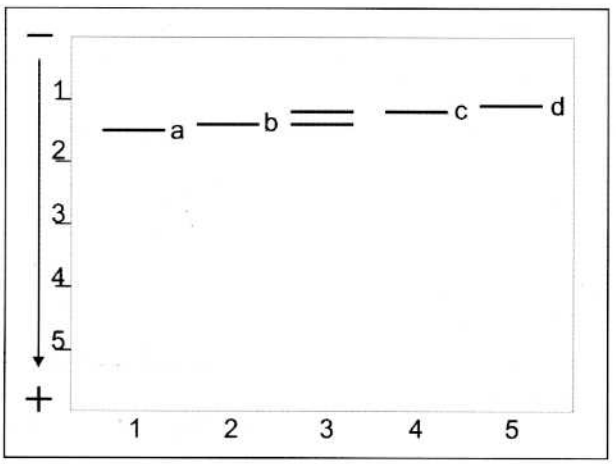

Fig. 2. Isoenzymes genotypes of glutamate dehydrogenase in *D. villosum* and *L. elongatum*. Origin at top, anode at bottom.

Glucose-6-phosphate dehydrogenase is considered to be encoded by one locus (Weber & Stetter 1981; Guries & Ledig 1982; Wheeler & Guries 1982) or by two gene loci (Millar 1983). Occurrence of dublets (Weber & Stetter 1981) and triplets (Jaaska 1994) implies monomeric or/and dimeric subunit structure. McIntyre (1988) reported two invariant loci in *D. villosum*, and one locus in *Agropyron (Lophopyrum) elongatum*. These discrepancies among different data sets as well the lack of inter- and intraspecific variation make difficult the interpretation of G6PDH pattern in *D. villosum* and *L. elongatum*. It could be better explained assuming monogenic control by a common allele for both taxa (Table 2).

The dimeric enzyme 6PGDH is commonly encoded by two gene loci (Stuber &

Table 2. Allelic frequencies at six gene loci in the studied populations of *L. elongatum* and *D. villosum*.

Gene	Allele	*L. elongatum*			*D. villosum*			
		1	2	3	4	5	6	7
AAT-1	a	1,00	1,00	1,00	0,50	0,50	0,50	0,50
	b				0,50	0,50	0,50	0,50
AAT-2	a					0,30		
	b				1,00	0,70	1,00	1,00
	c	1,00	1,00	1,00				
AAT-3	a	0,75	0,62	0,70	0,25	0,40	0,30	0,45
	b	0,25			0,60	0,50	0,70	0,55
	c		0,38	0,30	0,15	0,10		
G6PDH	a	1,00	1,00	1,00	1,00	1,00	1,00	1,00
6PGDH	a				1,00	1,00	1,00	1,00
	b	1,00	1,00	1,00				
GDH	a					0,12	0,20	
	b				0,70	0,60	0,45	0,90
	c	0,62	0,40	0,45	0,30	0,28	0,35	0,10
	d	0,38	0,60	0,55				

Goodman 1980; Figueiras & al. 1984). However, in some studies (McIntyre 1988; Jaaska 1994; Guldahl & al. 2001) only one locus of otherwise digenically controled enzymes have been detected. Variation pattern observed in *D. villosum* and *L. elongatum* could be interpreted supposing one gene locus with two alternative and fixed alleles (Table 2).

As it was stated above, the four enzyme systems were interpreted as encoded by six putative loci, namely, three for AAT and one locus each for G6PDH, 6PGDH and GDH. The number of loci for AAT and GDH is the same as normally found in diploid plants (Gottlieb 1982). Allelic frequencies at six gene loci in studied populations of *D. villosum* and *L. elongatum* are presented in Table 2. Fifteen alleles were encountered across the loci surveyed. Allele **a** of G6PDH was fixed in both taxa. Two alleles of 6PGDH, namely, **a** and **b**, were invariant and diagnostic for *L. elongatum* and *D. villosum*, respectively. Alleles **a** and **b** were characteristic of *D. villosum*, whereas allele **d** was specific for *L. elongatum*. The rest of alleles were shared by both species examined. The proportion of polymorphic

Incongruence between different data sets in *Triticeae* have been thoroughly examined and discussed by Mason-Gamer & Kellogg (1996b) and Kellogg & al. (1996). It was concluded that different portions of the genome of diploid *Triticeae* have distinct histories. Discrepancies observed most probably reflect the separate evolution of nuclear and chloroplast genomes. Evolutionary history of the tribe *Triticeae* could be best presented as a net than a tree. In this sense, at present it is difficult to resolve consistently relationships among particular genera within the tribe *Triticeae*.

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