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NAD-dependent aromatic alcohol dehydrogenase in wheats (*Triticum* L.) and goatgrasses (*Aegilops* L.): evolutionary genetics

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Summary. Evolutionary electrophoretic variation of a NAD-specific aromatic alcohol dehydrogenase, AADH-E, in wheat and goatgrass species is described and discussed in comparison with a NAD-specific alcohol dehydrogenase (ADH-A) and a NADP-dependent AADH-B studied previously. Cultivated tetraploid emmer wheats (*T. turgidum* s. l.) and hexaploid bread wheats (*T. aestivum* s. l.) are all fixed for a heterozygous triplet, $E^{0.58}/E^{0.64}$. The slowest isoenzyme, $E^{0.58}$, is controlled by a homoeoallelic gene on the chromosome arm 6AL of *T. aestivum* cv. 'Chinese Spring' and is inherent in all diploid wheats, *T. monococcum* s. str., *T. boeoticum* s. l. and *T. urartu.* The fastest isoenzyme, $E^{0.64}$, is presumably controlled by the B- and D-genome homoeoalleles of the bread wheat and is the commonest alloenzyme of diploid goatgrasses, including *Ae. speltoides* and *Ae. tauschii.* The tetraploid *T. timopheevii* s. str. has a particular heterozygous triplet $E^{0.56}/E^{0.71}$, whereas the hexaploid *T. zhukovskyi* exhibited polymorphism with electromorphs characteristic of *T. timopheevii* and *T. monococcum.* Wild tetraploid wheats, *T. dicoccoides* and *T. araraticum,* showed partially homologous intraspecific variation of AADH-E with heterozygous triplets $E^{0.58}/E^{0.64}$ (the commonest), $E^{0.58}/E^{0.71}$, $E^{0.45}/E^{0.58}$, $E^{0.48}/E^{0.58}$ and $E^{0.56}/E^{0.58}$ recorded. Polyploid goatgrasses of the D-genome group, excepting *Ae. cylindrica,* are fixed for the common triplet $E^{0.58}/E^{0.64}$. *Ae. cylindrica* and polyploid goatgrasses of the C^u-genome group, excepting *Ae. kotschyi,* are homozygous for $E^{0.64}$. *Ae. kotschyi* is exceptional, showing fixed heterozygosity for both AADH-E and ADH-A with unique triplets $E^{0.56}/E^{0.64}$ and $A^{0.49}/A^{0.56}$.

Key words: Alcohol dehydrogenase – Isoenzymes – Evolutionary genetics – *Triticum* – *Aegilops*

Introduction

The problem of wheat phylogeny has been attacked by using various isoenzyme systems as genetic markers in comparative studies of contemporary wheats and their relatives (Bhatia 1968; Jaaska 1969, 1976, 1978, 1980, 1981, 1983; Nakai et al. 1969; Nakai 1973; Mitra and Bhatia 1971). The concept of monophyletic origin of a primitive hexaploid wheat and of a primitive tetraploid wheat with ancestral biotypes of a diploid wheat, *Aegilops speltoides* and *Aegilops tauschii* ssp. *strangulata* as the diploid progenitors and the genome donors has been favoured on the basis of isoenzyme studies (Jaaska 1980, 1981, 1982). However, many details of wheat phylogeny have remained unsolved and require further genetic studies.

Isoenzymes encoded by separate gene loci, recently proposed (Jaaska and Jaaska 1984) to specify shortly heterozymes (heterologous isoenzymes), exhibit each a specific variation pattern among the contemporary taxa of wheats and their relatives (Jaaska 1980, 1981, 1982). The data on the evolutionary allozymic variation of different heterozymes provides thus supplementary information on wheat phylogeny. There is a continued need for developing new isoenzyme systems for evolutionary studies.

We have published studies on the evolutionary genetics of NAD-dependent aliphatic ADHs (Jaaska 1976, Jaaska and Jaaska 1980) and of a NADP-dependent aromatic ADH (Jaaska 1978) in wheats and their relatives. Here we extend the study to the NAD-dependent aromatic ADH. Meanwhile, an independent study on the chromosomal control of isoenzymes of this enzyme in a hexaploid bread wheat has already been published (Schmidt and Seliger 1982). Isoenzymes of aromatic ADH in rye and triticale are described in a separate paper (Jaaska and Jaaska 1984).

Material and methods

Plant material

Brackets give the number of accessions studied.

1. Hexaploid wheats of the type section *Triticum* (= *T. aestivum* L. emend. Thell.): *T. spelta* L. (16), *T. macha* Dek.

et Men. (2), *T. compactum* Host (6), *T. sphaerococcum* Perc. (2), and *T. aestivum* L. s. str. (36), including a series of nullitetrasomic and ditelocentric chromosome lines of the cultivar 'Chinese Spring'.

2. Tetraploid wheats of the section *Dicoccoidea* Flaksb. (= *T. turgidum* L. emend. Thell.), including the cultivated *T. turgidum* L. s. str. (8), *T. durum* Desf. (4), *T. aethiopicum* Jakubz. (4), *T. polonicum* L. (2), *T. isphaghanicum* Heslot (1), *T. carthlicum* Nevski (2), *T. dicoccon* (Schrank) Schuebl. (24), *T. karamyschevii* Nevski (1), and the wild emmer *T. dicoccoides* (Koern.) Schweinf. (22).

3. Polyploid wheats of the section *Timopheevii* A. Filat. et Dorof., including the hexaploid *T. zykovskyi* Men. et Eriz. (2), the cultivated tetraploid *T. timopheevii* Zhuk. (5), and the wild tetraploid *T. araraticum* Jakubz. (32).

4. Diploid wheats of the section *Monococcon* Dum., including the cultivated *T. monococcum* L. s. str. (3) and two wild species, *T. boeoticum* Boiss. (10, involving the two-awned var. 'thaoudar') and *T. urartu* Thum. ex Gandil. (6).

5. Diploid goatgrasses: *Aegilops tauschii* Coss. (54), involving ssp. *tauschii* (12) and ssp. *strangulata* (42), *Ae. speltoides* Tausch s. l. (18), involving both the awned and awnless forms, *Ae. mutica* Boiss. (1), *Ae. bicornis* (Forsk.) Jaub. et Sp. (5), *Ae. longissima* Schweinf. et Muschl. s. str. (3), *Ae. sharonensis* Eig (6), *Ae. searsii* Feldm. et Kislev (1), *Ae. caudata* L. (9), *Ae. comosa* Sibth. et Sm. s. l. (3), including ssp. *heldreichii* Boiss. (2), *Ae. umbellulata* Zhuk. (6), and *Ae. uniaristata* Vis (3).

6. Polyploid goatgrasses of the D-genome group: the tetraploids *Ae. cylindrica* Host., (4), *Ae. ventricosa* Tausch (1), and *Ae. crassa* Boiss. ssp. *macrathera* (Boiss.) Zhuk. (6); the hexaploids *Ae. crassa* ssp. *crassa* (6) and *Ae. juvenalis* (Thell.) Eig (2).

7. Polyploid goatgrasses of the C^u-genome group: the tetraploids *Ae. triuncialis* L. (12), incl. ssp. *persica* (Boiss.) Zhuk. (4), *Ae. biuncialis* Vis. (9), *Ae. ovata* L. emend Roth (8, including *Ae. geniculata* Roth), *Ae. kotschyi* Boiss. (10), *Ae. variabilis* Eig (2), and *Ae. columnaris* Zhuk. (4) and the hexaploid *Ae. ovata* ssp. *recta* Zhuk. (6).

Biochemical methods

Enzyme extracts were prepared and subjected to electrophoresis in photopolymerized polyacrylamide gel slabs as described previously (Jaaska 1978) except for substituting 5 mM cysteine for 5 mM dithiothreitol in the extraction buffer.

After electrophoresis, the gels were stained for NAD-AADH in a histochemical reaction mixture made as follows: 25 ml of 0.1 M KH_2PO_4-NaOH buffer (pH about 7.4) or 0.1 M Tris-HCl buffer (pH about 8.8) saturated with cinnamyl alcohol (about 1.6 ml/l), 2 ml of NAD (5 mg/ml), 2 ml of tetranitroblue tetrazolium (2 mg/ml) and 0.2 ml PMS (2.5 mg/ml). In the reaction mixture for the aliphatic NAD-ADH, cinnamyl alcohol was omitted from the buffer, ethyl alcohol was substituted (1.0 ml/25 ml of buffer).

The isoenzyme bands are labelled by numerical superscripts reflecting their relative electrophoretic mobilities (united R_f values) in the electrophoretic system used. The AADH-heterozyme nomenclature as described and discussed by Jaaska and Jaaska (1984) is followed, the major NAD-dependent AADH studied here being designated AADH-E.

Results

Wheats (Triticum L.)

All diploid wheats of different morphology (various botanical varieties of *T. monococcum* s. str., *T. boeoticum* s. l. and *T. urartu*) and geographic origin (Armenia, Azerbaijan, Iran, Iraq, Turkey, etc.) showed an invariant one-banded electrophoretic phenotype of AADH-E with electromorph $E^{0.58}$ (1–2, Fig. 1). A symmetrical triplet with electromorphs $E^{0.58}$ and $E^{0.64}$ as the outer bands (3–5 and 8–10, Fig. 1) was characteristic of all accessions of hexaploid bread wheats (*T. aestivum* L. emend. Thell.) and of cultivated tetra-

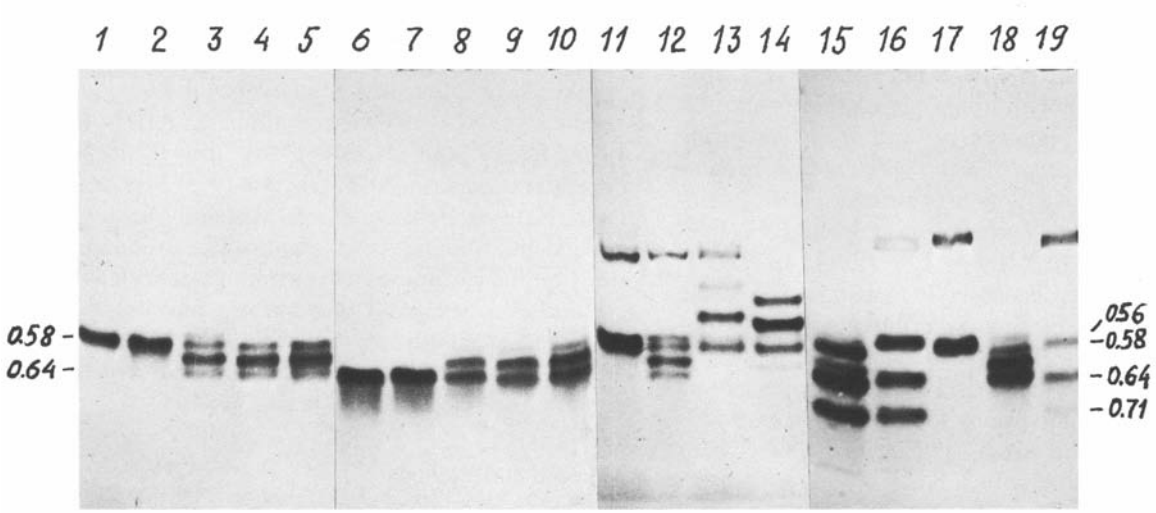

Fig. 1. Enzymograms of a NAD-dependent aromatic alcohol dehydrogenase in wheats; *1 – Triticum boeoticum; 2 – T. urartu; 3 – T. araraticum; 4 – T. dicoccoides; 5 – T. dicoccon; 6–8 – T. aestivum* cv. 'Chinese Spring': nulli 6A/tetra 6B (6), ditelo-6AS (7); cv. 'Chinese Spring' (8); *9 – T. sphaerococcum; 10 – T. spelta; 11–13 – T. araraticum; 14–15 – T. diccocoides; 16 – T. timopheevii; 17* and *19 – T. zhukovskyi; 18 – T. spelta*

ploid emmer wheats (*T. turgidum* L. emend. Thell.) of different morphology (botanical varieties of the linneons *spelta, macha, sphaerococcum, compactum, aestivum* s. str., *turgidum* s. str., *durum, polonicum, dicoccum,* etc.) and geographic origin (aborigenous accessions from Mediterranean, Transcaucasian and Asian countries).

The relative intensity of the bands in the triplet varied depending on the seedling age, accession and species studied. In hexaploid wheats, as a rule, the fastest band was more intense than in tetraploids.

Analysis of a series of nulli-tetrasomics and ditelocentrics of the 'Chinese Spring' wheat showed the triplet phenotype of AADH-E, except nullisomics for the chromosome 6A and the 6AS (α) ditelosomic line, which lacked the two slower bands of the triplet and had only the fastest isoenzyme $E^{0.64}$ (6–7, Fig. 1). This result implies that the slowest isoenzyme $E^{0.58}$ is controlled by a homoeoallelic gene on the longer arm of the chromosome 6A and that the band of intermediate mobility is evidently a hybrid heterodimer. Compensated nullisomics for chromosomes 6B and 6D revealed the ordinary triplet phenotype, except for reduced intensity of the fastest band. This suggests that homoeoallelic genes of AADH-E on the latter two chromosomes control the isoenzyme $E^{0.64}$ and show gene dosage effect.

The triplet $E^{0.58}/E^{0.64}$ was also most frequent among the accessions of the two wild-growing tetraploid wheats, *T. dicoccoides* and *T. araraticum,* accounting about 50 and 75% of the accessions studied, respectively. The two species revealed, however, remarkable intra-specific variation of AADH-E with three additional triplet phenotypes in common: $E^{0.58}/E^{0.71}$ (15, Fig. 1), found for seven accessions of *T. dicoccoides* from Israel (6) and Syria (1) and for one accession of *T. araraticum* from Turkey; $E^{0.45}/E^{0.58}$ (13, Fig. 1) was fixed in one accession of *T. dicoccoides* from Israel and in one accession of *T. araraticum* from Iraq; and $E^{0.48}/E^{0.58}$ (14, Fig. 1), characteristic of three accessions of *T. araraticum* from Iraq (2) and Azerbaijan (1) and of one accession of *T. dicoccoides* from Israel.

Two accessions of *T. araraticum,* one from Azerbaijan and one from Iraq, showed only the A-genome electromorph $E^{0.58}$. One accession of *T. araraticum* from Iraq, was fixed for a rare electromorph of slightly slower mobility, $E^{0.56}$. Several accessions of *T. araraticum* showed a broad band (11, Fig. 1) considered to be the heterozygous $E^{0.56}/E^{0.58}$ triplet.

A triplet phenotype combining the slower electromorph $E^{0.56}$ with the fastest electromorph $E^{0.71}$ (16, Fig. 1) was found fixed in five morphologically differing accessions of *T. timopheevii.* This species is a tetraploid component of the endemic Zhanduri-coenosis of Western Georgia, now extinct and the components maintained only in collections. The hexaploid component of the Zhanduri-coenosis, *T. zhukovskyi,* revealed variation among the individual seedlings of the available reproductional accession with phenotypes $E^{0.56}/E^{0.58}$ (a broader band, 17 in Fig. 1), $E^{0.58}/E^{0.71}$ (19, Fig. 1), and $E^{0.56}/E^{0.58}/E^{0.71}$ recorded. The observed polymorphism of AADH-E in *T. zhukovskyi* presumably reflects the genetic instability of this allohexaploid with various isozyme combinations of its parental species, *T. timopheevii* and *T. monococcum.*

Goatgrasses (Aegilops L.)

Figure 2 exemplifies electrophoretic variation of AADH-E among the goatgrass species.

The fastest homoeoallelic isoenzyme of hexaploid bread wheats, the homoeozyme $E^{0.64}$, shown to be controlled by the wheat 6B and 6D homoeoalleles, was the most frequent alloenzyme of diploid goatgrasses. It was characteristic of all accessions of *Ae. speltoides* (including its awned and awnless forms) and *Ae. tauschii* (including ssp. *tauschii* and ssp. *strangulata*) studied, the presumed donors of the wheat B and D genomes, respectively.

The wheat A-genome homoeozyme $E^{0.58}$ was a rare alloenzyme of diploid goatgrasses, found for one accession of *Ae. sharonensis,* one accession of *Ae. umbellulata* and one accession of *Ae. comosa* ssp. *heldreichii.* Two accessions of *Ae. umbellulata* proved homozygous for a fast electromorph, $E^{0.71}$. A slow allozyme $E^{0.48}$ was found in a heterozygous triplet with the common allozyme $E^{0.64}$ in rare individuals of a single accession of *Ae. speltoides* from Turkey.

All polyploid goatgrasses of the D-genome group, except the tetraploid *Ae. cylindrica,* revealed the $E^{0.58}/E^{0.64}$ triplet which is also characteristic of polyploid wheats. *Ae. cylindrica* and the polyploids of the C^u-genome group, except *Ae. kotschyi,* proved fixed (monomorphic) for the homozygous $E^{0.64}$, the commonest allozyme of diploid goatgrasses. *Ae. kotschyi* has a unique heterozygous triplet $E^{0.56}/E^{0.64}$.

AADH-E versus ADH-A

The band with the R_f value 0.56 on the AADH-E enzymograms of goatgrasses (Fig. 2), except *Ae. kotschyi,* reflects the activity of the major NAD-dependent ADH-A of aliphatic alcohols, as checked by staining of parallel gels in histochemical reaction mixtures with cinnamyl (aromatic) and ethyl (aliphatic) alcohols as substrates. The relative intensity of the ADH-A band on the enzymograms varied significantly, depending on the seedling age (decreases with age) and on the growth conditions (intensifies with partial anaerobiosis by the accumulation of CO_2 in the closed germination vessel), as described previously (Jaaska and Jaaska 1980).

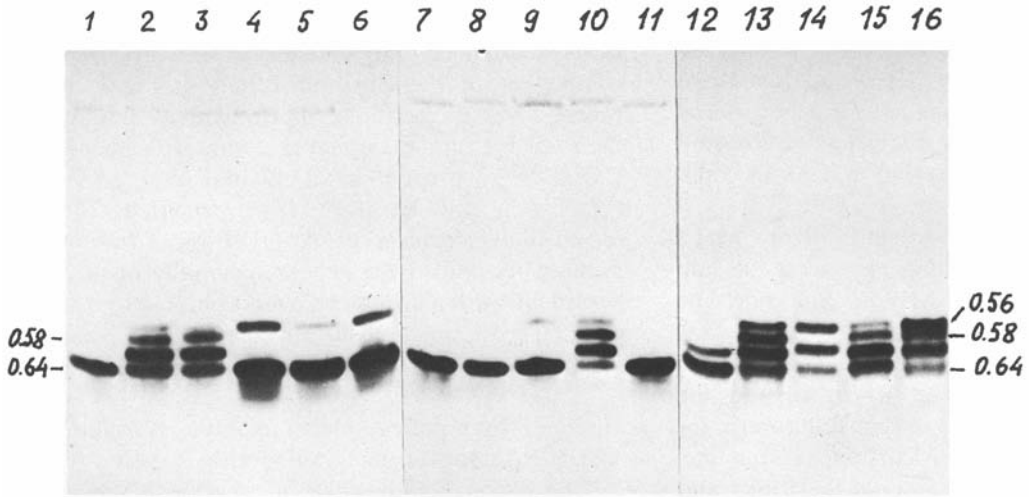

Fig. 2. Enzymograms of a NAD-dependent aromatic alcohol dehydrogenase in goatgrasses: *1 – Aegilops tauschii; 2 – Ae. juvenalis; 3 – Ae. ventricosa; 4 – Ae. columnaris; 5 – Ae. ovata* ssp. *recta; 6 – Ae. ovata; 7 – Ae. umbellulata; 8 – Ae. caudata; 9 – Ae. cylindrica; 10 – Ae. crassa* ssp. *crassa; 11 – Ae. triuncialis; 12 – Ae. tauschii* ssp. *tausschii; 13 – Ae. crassa* ssp. *macrathera; 14 – Ae. kotschyi; 15–16 – Ae. crassa* ssp. *crassa* (15) and ssp. *macrathera* (16)

Table 1. Electrophoretic mobilities of AADH-E and ADH-A in wheats and goatgrasses (expressed in unified R_f units)

Species, subspecies	AADH-E	ADH-A
T. aestivum s.l.	0.58/0.64	0.56/0.62
T. turgidum s.l.	0.58/0.64	0.56/0.62[a]
T. dicoccoides	0.58/0.64[a]	0.56/0.62
T. zhukovskyi	0.56/0.58/0.71[a]	0.56
T. timopheevii s. str.	0.56/0.71	0.56
T. araraticum	0.58/0.64[a]	0.56
T. monococcum s. str.	0.58	0.56
T. boeoticum s.l.	0.58	0.56
T. urartu	0.58	0.56
Ae. speltoides s.l.	0.64[a]; 0.48	0.56; 0.40
Ae. bicornis	0.64	0.56
Ae. longissima	0.64	0.56
Ae. sharonensis	0.64[a]; 0.58	0.56
Ae. searsii	0.64	0.56
Ae. caudata	0.64	0.56
Ae. comosa s.l.	0.64[a]; 0.58	0.56
Ae. uniaristata	0.64	0.56
Ae. umbellulata	0.64[a]; 0.58; 0.71	0.56
Ae. tauschii		
ssp. *tauschii*	0.64	0.61
ssp. *strangulata*	0.64	0.56
Ae. mutica	0.64	0.56
Ae. cylindrica	0.58/0.64	0.56/0.61
Ae. ventricosa	0.58/0.64	0.56
Ae. crassa		
ssp. *macrathera*, 4×	0.58/0.64	0.56
ssp. *crassa*, 6×	0.58/0.64	0.56/0.61
Ae. juvenalis	0.58/0.64	0.56
Ae. biuncialis	0.64	0.56
Ae. triuncialis	0.64	0.56
Ae. kotschyi	0.56/0.64	0.49/0.56
Ae. variabilis	0.64	0.56
Ae. columnaris	0.64	0.56
Ae. ovata		
ssp. *ovata*, 4×	0.64	0.56
ssp. *recta*, 6×	0.64	0.56

[a] The most frequent phenotype (allozyme, homoeozyme)

The table presented summarizes and compares the electrophoretic mobility data of the two major NAD-dependent alcohol dehydrogenases, AADH-E and ADH-A, in wheat and goatgrass species. The table also includes the data of our earlier papers (Jaaska 1976, 1981; Jaaska and Jaaska 1980) on the evolutionary variation of ADH-A in unified R_f values for the electrophoresis system used here.

It is seen from the table that AADH-E and ADH-A exhibit independent and distinctly different variation patterns among the wheat and goatgrass species, although in some species the R_f values are very close and even overlapping.

Discussion

A recent investigation of the major NAD-dependent aromatic alcohol dehydrogenase in 6A-nullisomic plants of the wheat cultivar 'Carola' by Schmidt and Seliger (1982) has discovered that this enzyme, which we have labelled AADH-E, is distinct from the major NAD-dependent aliphatic alcohol dehydrogenase, ADH-A. This result is now confirmed by the analysis of nulli-tetrasomics of the 'Chinese Spring' wheat, whereas the analysis of its ditelosomics enabled the localization of one of the homoeoalleles on the chromosome arm 6AL (β). ADH-A is known (Hart 1970) to be controlled by homoeoalleles on the chromosomes of the wheat fourth homoeologous group.

Both enzymes showed fixation of heterozygosity in the hexaploid bread wheats and in the tetraploid emmer wheats with triplet electrophoretic phenotypes, as characteristic of dimeric enzymes. The electrophoretic mobilities of homoeoallelic isozymes (homoeo-

zymes) of the two enzymes are very close in wheats (Table 1). There are, however, characteristic differences in the intra- and interspecific variation pattern of the two ADHs in wheats.

The most impressive, perhaps, is extensive intraspecific, partially homologous variation of AADH-E in the two wild tetraploid wheats. *T. araraticum* and *T. dicoccoides,* in contrast with a diagnostic divergence and essential monomorphism of ADH-A in the two species, as shwon previously (Jaaska 1976; Jaaska and Jaaska 1980). A novel A-genome-controlled homoeozyme, ADH-$A^{0.62}$, has become fixed in a heterozygous condition in the Emmer wheats, whereas the ancestral allozyme, ADH-$A^{0.56}$, shared by diploid wheats and goatgrasses, has remained homozygous in the Timopheevii wheats (Jaaska 1976).

Since no intra- or interspecific variation of the two ADHs has been discovered in diploid wheats, it may be assumed that the variation observed has been induced at the tetraploid level. The differentiation process of the two contemporary wild tetraploid wheats from an initial allotetraploid precursor has evidently had different effects on the loci controlling AADH-E and ADH-A.

Unlike the NAD-dependent AADH-E studied here, the NADP-dependent AADH-B studied previously (Jaaska 1978) is a monomeric enzyme controlled, in bread wheats, by homoeoallelic genes on the chromosomes of the fifth homoeologous group. This enzyme has its own pattern of evolutionary isoenzyme variation in the wheat group (Jaaska 1978).

A recent study (Nevo et al. 1982) has discovered considerable intraspecific variation at many enzyme loci with one or two, mainly rare, allozymic variants in the Israel populations of *T. dicoccoides.* AADH-E with a distinct and extensive allozymic variation is a new useful gene marker to study the genetic resources of wild tetraploid wheats in relation to their evolutionary history. An item of interest in this respect is that the electrophoretic phenotype of AADH-E characteristic of cultivated *T. timopheevii* s. str., $E^{0.56}/E^{0.71}$, has not been encountered in the analyzed sample of its wild relative *T. araraticum.* Electrophoretic screening of a representative geographic sample of wild tetraploid wheats for AADH-E would thus be desirable to understand phytogeographic aspects of their origin.

The variation pattern of AADH-E among the goatgrass species has its own peculiarities in comparison with that in wheats and of ADH-A.

Thus, diploid wheats and goatgrasses are essentially differentiated for different allozymes of AADH-E, $E^{0.58}$ and $E^{0.64}$, respectively, but share the same allozyme of ADH-A, $A^{0.56}$ (Table 1).

The carrier of the D-genome, *Ae. tauschii,* proved monomorphic for AADH-$E^{0.64}$, whereas intraspecific allozymic differentiation correlated with subspecific differentiation was earlier discovered for ADH-A in this species (Jaaska 1981).

The D-genome polyploids showed fixed heterozygosity for the triplet $E^{0.58}/E^{0.64}$. From this it may be assumed that the homeozyme $E^{0.58}$ of the polyploids has been derived from the contributors of their second genomes. $E^{0.58}$, essentially characteristic of diploid wheats, is indeed a rare allozyme of diploid goatgrasses also, although encountered only in three accessions of the sample analyzed. Electrophoretic analysis of a more representative sample of diploid goatgrasses for AADH-E is needed in order to evaluate the significance of the AADH-E isoenzyme data in identifying the second genome of the D-genome polyploid goatgrasses.

In contrast with the D-genome polyploids, fixation of heterozygosity at the AADH-E locus was not distributed in the C^u-genome polyploids, *Ae. kotschyi* being the sole exception. The latter species is morphologically quite similar to *Ae. triuncialis* and has even been treated under this species as *Ae. triuncialis* γ *kotschyi* (Boiss.) Boiss. and *Ae. triuncialis* ssp. *kotschyi* (Boiss.) Zhuk. *Ae. kotschyi* is, however, clearly distinct from *Ae. triuncialis* by having unique heterozygous triplets of both AADH-E and ADH-A (Table 1), It may be assumed that the heterozygosity at the two ADH loci has been introduced to *Ae. kotschyi* through allopolyploidy from a diploid precursor with divergent allozymes.

The cytogenetic studies of Kihara (1954) have suggested that *Ae. kotschyi* has a common genome composition, C^uS^v, with *Ae. variabilis.* The involvement of a *Sitopsis*-diploid with the genome S in the origin of *Ae. kotschyi* and *Ae. variabilis* was supported by later cytogenetic investigations (Tanaka 1955; Rubenstein and Sallee 1979), but questioned by Chennaveeraiah (1960) on the basis of karyomorphologic data.

The AADH-E and ADH-A isoenzyme data distinguish *Ae. kotschyi* from *Ae. variabilis,* although the analyzed sample of the latter species, two accessions, is too small for generalizations. The ADH isoenzyme data, however, failed to identify the donor of the second genome of *Ae. kotschyi.* The diagnostic homoeozymes of *Ae. kotschyi,* AADH-$E^{0.56}$ and ADH-$A^{0.49}$, were not encountered among the accessions of diploid goatgrasses analyzed, including the *Sitopsis*-diploids. Although the involvement of an unknown or extinct diploid in the origin of *Ae. kotschyi* may be asserted, further ADH isoenzyme analyses among the contemporary diploids are needed before final conclusions may be made.

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Note added in proof

Three *T. araraticum* and one *T. dicoccoides* from Turkey had the additional $E^{0.64}/E^{0.71}$-triplet.