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NADP-dependent Aromatic Alcohol Dehydrogenase in Polyploid Wheats and their Diploid Relatives. On the Origin and Phylogeny of Polyploid Wheats

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Summary. The three major isoenzymes of the NADP-dependent aromatic alcohol dehydrogenase (ADH-B), distinguished in polyploid wheats by means of polyacrylamide gel electrophoresis, are shown to be coded by homoeoalleles of the locus *Adh-2* on short arms of chromosomes of the fifth homoeologous group. Essentially codominant expression of the *Adh-2* homoeolleles of composite genomes was observed in young seedlings of hexaploid wheats (*T. aestivum* s.l.) and tetraploid wheats of the emmer group (*T. turgidum* s.l.), whereas only the isoenzyme characteristic of the *A* genome is present in the seedlings of the timopheevii-group tetraploids (*T. timopheevii* s.str. and *T. araraticum*).

The slowest-moving B^3 isoenzyme of polyploid wheats, coded by the homoeoallele of the *B* genome, is characteristic of the diploid species *Aegilops speltoides* s.l., including both its awned and awnless forms, but was not encountered in *Ae. bicornis, Ae. sharonensis* and *Ae. longissima*. The last two diploids, as well as *Ae. tauschii, Ae. caudata, Triticum monococcum* s.str., *T. boeoticum* s.l. (incl. *T. thaoudar*) and *T. urartu* all shared a common isoenzyme coinciding electrophoretically with the band B^2 controlled by the *A* and *D* genome homoeoalleles in polyploid wheats. *Ae. bicornis* is characterized by the slowest isoenzyme, B^4, not found in wheats and in the other diploid *Aegilops* species studied.

Two electrophoretic variants of ADH-B, B^1 and B^2, considered to be alloenzymes of the A genome homoeoallele, were observed in *T. dicoccoides, T. dicoccon, T. turgidum* s.str. and *T. spelta*, whereas B^2 was characteristic of *T. timopheevii* s.l. and only B^1 was found in the remaining taxa of polyploid wheats. The isoenzyme B^1, not encountered among diploid species, is considered to be a mutational derivative which arose on the tetraploid level from its more ancestral form B^2 characteristic of diploid wheats.

The implication of the ADH-B isoenzyme data to the problems of wheat phylogeny and gene evolution is discussed.

Key words: Aromatic alcohol dehydrogenase – Isoenzymes – Wheat phylogeny – *Triticum* L. – *Aegilops* L.

Introduction

Electrophoretic analysis of enzymes has found increasing application in biosystematic and evolutionary studies. We have used electrophoretic analysis of alcohol dehydrogenase (Jaaska 1976a), aspartate aminotransferase (Jaaska 1976b), esterase and acid phosphatase (Jaaska 1969, 1971, 1974, 1976c. Jaaska and Jaaska 1970) isoenzymes to study the genome composition and origin of polyploid wheats. Each of the enzymes studied showed its own pattern of phylogenetic differentiation among polyploid wheats and their diploid relatives, thus providing supplementary data to the knowledge of wheat phylogeny.

The number of enzymes suitable to serve as genetic markers in the studies of wheat phylogeny is, at present, quite limited. Brewer et al. (1969) found that among twelve different enzymes studied, only one, phosphatase, revealed electrophoretically detectable differentiation between the three genomes of hexaploid wheat. There is, thus, an urgent need to search for new enzyme markers of wheat genomes and chromosomes.

Our recent work (Jaaska and Jaaska 1978) has demonstrated the presence, in wheat embryos and young seedlings, of three alcohol dehydrogenases of different substrate and coenzyme specificities, electrophoretic mobilities and developmental variation patterns during germination. One of these enzymes, the NAD-specific alcohol dehydrogenase of wheat embryos, has already been extensively studied in relation to wheat genetics and phylogeny (Hart 1969, 1970, 1973; Jaaska 1976a; Mitra and Bhatia 1971). The two other alcohol dehydrogenases of wheat seedlings utilize only aromatic alcohols, cinnamyl and coniferyl alcohols, as substrates. One of the two enzymes is active only with NADP as a coenzyme, whereas the second one is a coenzyme-unspecific dehydrogenase.

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In this paper, we report the data about the electrophoretic variation and genetic control of aromatic alcohol dehydrogenases in polyploid wheats and their diploid relatives.

Material and Methods

Plant Material

The seed accessions used are labeled by a code letter indicating their origin, followed by a collection number: A – the author's collection; B – received from a botanical garden; G – from the California State University, Riverside, USA (Dr. B.L. Johnson); K – from the Vavilov collection of the All-Union Institute of Plant Industry (VIR), Leningrad, USSR (Drs. A.A. Filatenko, E.F. Migushova and R.A. Udaczin); V – various other sources. The second letter L, if added, indicates that the seeds are collected in nature. The number of accessions studied is indicated in the brackets.

1. Hexaploid wheats (*T. aestivum* L. s.l.): *T. spelta* L. (25); *T. macha* Dek. et Men. (10); *T. aestivum* L. s.str. (98); *T. compactum* Host (5); *T. sphaerococcum* Perc. (8); and a series of nulli-tetrasomic and ditelosomic chromosome lines of *T. aestivum* cv. 'Chinese Spring' developed by Sears (1965, 1966) and kindly provided us by Dr. Olga Mitrofanova (VIR, Leningrad).

2. Tetraploid wheats of the emmer group comprising *T. turgidum* L. s.l.

2a. Cultivated emmers: *T. turgidum* L. s.str. (17); *T. durum* Desf. (60); *T. turanicum* Jakubz. (1); *T. aethiopicum* Jakubz. (3); *T. polonicum* L. (8); *T. jakubzinerii* Udacz. (1); *T. ispahanicum* Heslot (1); *T. carthlicum* Nevski (6); syn. *T. persicum* Vav. ex Zhuk. 1923; non Aitch. et Hemsl. 1888; *T. dicoccon* (Schrank) Schuebl. (36); *T. karamyschevii* Nevski (5); syn. *T. dicoccon* ssp. *georgicum* (Dek. et Men.) Flaksb. et *T. dicoccon* var. 'chwamlicum' Supat., = *T. palaeocolchicum* Menabde, nom. illeg.

2b. Wild emmer, *T. dicoccoides* (Koern.) Aaronsohn (14).

3. Tetraploid wheats of the timopheevii group (*T. timopheevii* Zhuk. s.l.), including the wild-growing *T. araraticum* Jakubz. (22) and the cultivated *T. timopheevii* Zhuk. s.str. (3).

4. Diploid wheats: *T. monococcum* L. s.l., including the cultivated *T. monococcum* L. s.str. (25) and the wild-growing *T. boeoticum* Boiss. s.l. (26); involving the one-awned var. 'boeoticum' and the two-awned var. 'thaoudar'; *T. urartu* Thum. ex Gandilian (10).

5. Dilpoid goatgrasses: *Aegilops tauschii* Coss. (26); syn. *Ae. squarrosa* auct. non L.; *Ae. speltoides* Tausch (49); involving the awnless var. 'speltoides' (25); syn. *Ae. aucheri* sensu Zhuk. 1928, non Boiss. 1844, and the awned var. 'ligustica' Bornm. (24); syn. *Ae. ligustica* Cosson, = *Ae. speltoides* auct. non Tausch; *Ae. mutica* Boiss. (5); syn. *Amblyopyrum muticum* (Boiss.) Eig.; *Ae. bicornis* (Forsk.) Jaub. et Sp. (7); *Ae. longissima* Schweinf. et Muschl. s.str. (6); *Ae. sharonensis* Eig. (9), and *Ae. caudata* L. (5).

Tissue Extracts and Electrophoresis

The seeds were germinated in sterile glass dishes on sheets of filter paper saturated with 8 mM $Ca(NO_3)_2 . 4H_2O$ and 1 mM EDTA-Na_2Mg, in a thermostat at 26° C in the dark for 4-7 days. Individual shoots of etiolated seedlings were crushed in 0.1 or 0.2 ml of a cold buffer containing 0.05 M tris-hydroxymethylamino methane (tris), 0.01 M EDTA and 5 mM cysteine hydrochloride. After removal of cell debris, a suitable amount (20-50 mg) of a sucrose – Sephadex G-200 4:1 mixture was added to the enzyme extracts to increase their viscosity.

The tissue extracts were immediately subjected to electrophoresis in a polyacrylamide gel slab (60 × 45 × 3 mm) in a vertical Plexiglass-made cathode chamber by photopolymerizing between two fluorescent lamps of a freshly prepared mixture composed of 10 per cent acrylamide, 0.12 per cent N,N′–methylene-bisacrylamide, 0.25 M tris, 0.125 M HCl, 0.2 per cent triethanolamine and 0.5 mg per cent riboflavine. The upper cathode buffer contained 0.01 M tris and 0.08 M glycine, whereas the lower anode buffer was 0.1 M tris-acetate at a pH of about 8.9. Electrophoresis in the anodal direction was carried out in a refrigerated Plexiglass-made apparatus by maintaining the current at about 10-12 mA per slab for about 2.5 h until the marker dye, bromophenol blue, reached the lower end of the gel.

Enzyme Staining

After electrophoresis, the gels were stained in a histochemical reaction mixture made as follows: 26 ml of 0.012 M cinnamyl alcohol in 0.1 M tris-HCl buffer (pH 8.8), 2 ml of NADP (2.5 mg/ml), 2 ml of tetranitroblue tetrazolium (2 mg/ml) and 0.3 ml of phenazine methosulfate (2.5 mg/ml).

Results

Two groups of isoenzyme bands can be distinguished on the polyacrylamide gel electrophoretic enzymograms of aromatic alcohol dehydrogenase (Figs. 1-3). The slow-moving group of isoenzymes consisting of 2-3 bands has been shown (Jaaska and Jaaska 1978) to belong to a unspecific coenzyme, aromatic alcohol dehydrogenase, which is active with both NAD and NADP. This enzyme shows only a limited electrophoretic variation between

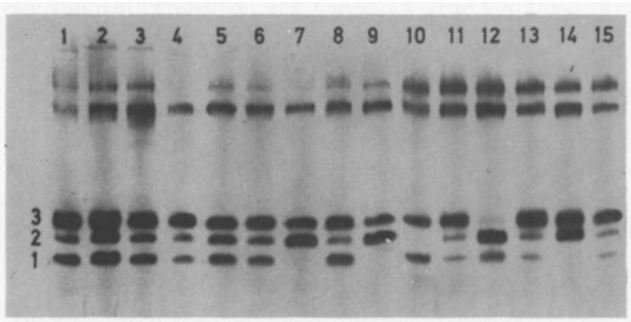

Fig. 1. Electrophoretic phenotypes of aromatic ADH of hexaploid wheats: 1 – *T. aestivum* var. 'erythrospermum', K-39662 from Turkey; 2 – *T. compactum* var. 'echinodes', K-12491 from Afghanistan; 3 – *T. sphaerococcum* var. 'globosum', K-23770 from Pakistan; 4 – *T. macha* var. 'letschumicum', K-28181 from Georgia; 5 – *T. spelta* var. 'vulpinum', K-45365 from Azerbaijan; 6 – *T. spelta* var. 'bactiaricum', K-45750 from Iran; 7 – *T. spelta* var. 'albispicatum', K-24696 from Switzerland; 8 – *T. spelta* var. 'bactiaricum', K-45814 from Iran; 9 – *T. spelta* var. 'arduini', K-20495 from Spain; 10-15 – ditelosomics and nulli-tetrasomics of *T. aestivum* cv. 'Chinese Spring': 10 – nulli-5D/tetra-5A, 11 – ditelo-5DL, 12 – nulli-5B/tetra-5D, 13 – ditelo-5BL, 14 – nulli-5A/tetra-5D, 15 – ditelo-5AL. The origin is at the top and the anode is at the bottom

Triticum and *Aegilops* species which is of no diagnostic value. Distinct intra- and interspecific variation was, however, observed in the anodally fast-moving group of isoenzymes representing NADP-specific aromatic alcohol dehydrogenase. Independent patterns of interspecific variation suggest that the two aromatic dehydrogenases, the NADP-specific and the coenzyme-unspecific enzymes, are controlled by separate gene loci. For convenience of further description, the NADP-specific aromatic alcohol dehydrogenase will be labelled ADH-B, leaving the designation ADH-A for the NAD-specific alcohol dehydrogenase of embryos studied previously (Jaaska 1976a).

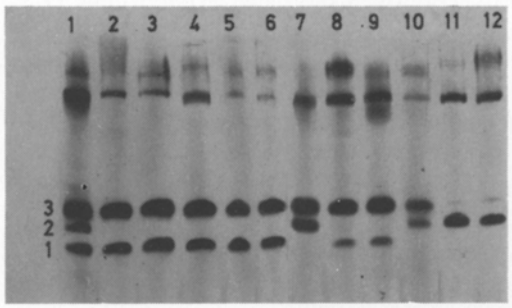

Fig. 2. Electrophoretic phenotypes of aromatic ADH in hexa- and tetraploid wheats: 1 – *T. spelta* var. 'arduini', K-47010 from Iran; 2 – *T. durum* var. 'apulicum', K-14377 from Turkey; 3 – *T. turgidum* var. 'speciosissimum', K-15125 from Azerbaijan; 4 – *T. carthlicum* var. 'fuliginosum', K-19759 from Georgia; 5 – *T. polonicum* var. 'levissimum', K-40162 from Germany; 6 – *T. aethiopicum* var. 'arraseita', K-5155 from Ethiopia; 7 – *T. dicoccon* var. 'rufum', K-81 from Germany; 8 – *T. dicoccon* var. 'farrum', K-6392 from Azerbaijan; 9 – *T. dicoccoides* ssp. *incertum*, K-41966 from Israel; 10 – *T. dicoccoides* ssp. *horanum* K-5198 from Israel; 11 – *T. timopheevii* var. 'timopheevii' (var. 'typicum auct.'), V-49/69 from Georgia; 12 – *T. araraticum* var. 'thumanjani', V-15/75 from Armenia. The origin is at the top and the anode is at the bottom

Hexaploid wheats reveal, as seen in Figure 1, two electrophoretic phenotypes of ADH-B by combining three isoenzyme bands labeled B^1, B^2 and B^3 in the decreasing order of their anodal mobility. One phenotype combines all the three isoenzymes with bands B^1 and B^3 stained intensely, whereas the band B^2 varies widely in its intensity, depending upon the seedling age and the tissue extraction efficiency. The second ADH-B phenotype lacks band 1 and combines bands 2 and 3.

The data about the distribution of ADH-B electrophoretic phenotypes among hexaploid wheats are given in Table 1. The phenotype combining all the three ADH-B isoenzymes was found in all the accessions of *T. aestivum* s.str., *T. macha*, *T. compactum* and *T. sphaerococcum* studied, whereas both phenotypes were encountered in *T. spelta*.

The chromosomal location of genes coding the ADH-B isoenzymes in hexaploid wheats was studied with the use of compensating nulli-tetrasomics and ditelosomics of *T. aestivum* cv. 'Chinese Sping' developed by Sears (1965, 1966). The variety 'Chinese Spring' and most of its nulli-

Table 1. Distribution of the ADH-B phenotypes in hexaploid wheats

Species	Number of accessions with the phenotypes	
	$B^1B^2B^3$	B^2B^3
T. aestivum	98	0
T. spelta ssp. *spelta*	6	12
T. spelta ssp. *kuckuckianum*	7	0
T. macha	10	0
T. compactum	5	0
T. sphaerococcum	8	0

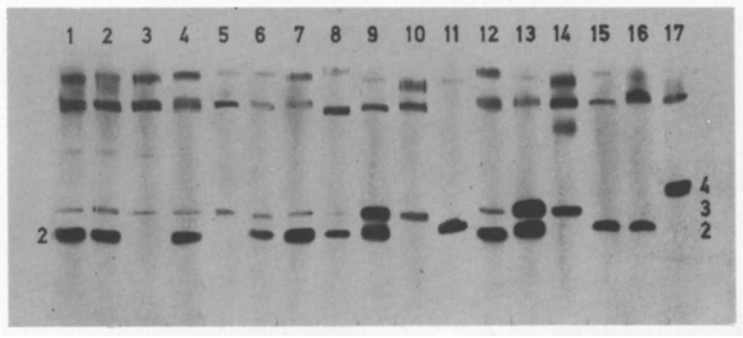

Fig. 3. Electrophoretic phenotypes of aromatic ADH in wild tetraploid wheats and their diploid relatives: 1 – *T. boeoticum* ssp *thaoudar*, K-40118 from Iraq; 2 – *T. monococcum* var. 'flavescens', K-35915 from Georgia; 3 – *T. monococcum* var. 'hornemanii', V-48/69 from the West-Georgian endemic population 'Zhanduri'; 4 – *T. boeoticum*, AL-42/71 from Azerbaijan; 5-6 – *T. urartu*, AL-47/75 from Armenia; 7 – *T. boeoticum*, AL-45/75 from Armenia, 8 – *T. araraticum*, AL-53/75 from Armenia; 9 – *T. dicoccoides*, K-5201 from Israel; 10 – *Ae. speltoides*, G-768 from Iraq; 11 – *Ae. tauschii*, AL-19/71 from Azerbaijan; 12 – *T. boeoticum*, AL-42/71 from Azerbaijan; 13 – *T. dicoccoides*, K-5198 from Isarael; 14 – *Ae. speltoides*, G-1591 from Turkey; 15 – *Ae. longissima*, K-209 from Israel; 16 – *Ae. sharonensis*, K-203 from Israel; 17 – *Ae. bicornis*, G-1424 from Egypt. The origin is at the top and the anode is at the bottom

tetrasomic combinations revealed typical three-banded ADH-B phenotypes. It varied only among the nulli-tetrasomic series of the fifth homoeologous chromosome group.

The isoenzyme B^2 is totally lacking in the nulli-5D/tetra-5A (enzymogram 10, Fig. 1) and nulli-5D/tetra-5B strains but it was present in the ditelo-5DL (11, Fig. 1). This result suggests that the isoenzyme B^2 is controlled by a gene located on the short arm of the chromosome 5D. The nulli-5B/tetra-5D (12, Fig. 1) and nulli-5B/tetra-5A strains reveal a sharp reduction in the staining intensity of band 3, instead it two closely spaced and weakly stained bands appear on the enzymograms. At the same time, the ditelo-5BL strain shows the ordinary threebanded ADH-B pattern. Such a result implies that the major isoenzyme B^3 of band 3 is controlled by a gene on the short arm of the chromosome 5B, whereas the two minor isoenzymes, also located on the enzymograms at the site of the band 3, are under independent genetic control. And, at last, that band 1 was found to be lacking on the enzymograms of the nulli-5A/tetra-5D (14, Fig. 1) and nulli-5A/tetra-5B strains. It was, however, present in the ditelo-5AL (15, Fig. 1). This implies that band 1 consists of a single isoenzyme B^1 which is under the control of a locus on the short arm of the chromosome 5A.

A significant increase in the intensity of band 2 on the enzymograms (12 and 14, Fig. 1) of the 5D tetrasomics reflects the dosage effect of the gene controlling the isoenzyme B^2 in hexaploid wheats and located on the chromosome 5D.

In conclusion, from the results of a study of nulli-tetrasomics and ditelosomics of 'Chinese Spring' it follows that homologous genes of the locus labelled *Adh-2*, controlling the structure of the three major ADH-B isoenzymes in hexaploid wheats, are located on short arms of chromosomes of the fifth homoeologous group. The three homologous genes will further be named, following the general nomenclature as proposed by Washington (1971), as homoeoalleles of the locus *Adh-2*.

Tetraploid wheats of the emmer group, comprising *T. turgidum* s.l., reveal, as seen in Figure 2, two two-banded electrophoretic phenotypes of ADH-B by combining the same three isoenzyme bands 1, 2 and 3 which were found in hexaploid wheats. The isoenzyme B^3 is common for both phenotypes, which differ with respect to the presence of either B^1 or B^2 as variants. Isoenzyme B^3 was shown above to be under the control of the *Adh-2* homoeoallele of the chromosome 5B; the isoenzymes B^1 and B^2 of emmer wheats should be considered as alloenzymes of the 5A chromosome homoeoallele.

The observed distribution of the two ADH-B phenotypes, B^1B^3 and B^2B^3, among the emmer tetraploids is given in Table 2. Both phenotypes were encountered in *T. dicoccoides, T. dicoccon*, and *T. turgidum* s.str., whereas the remainder taxa of emmers were monomorphic with respect to the phenotype B^1B^3.

Tetraploid wheats of the timopheevii group, i.e. the cultivated *T. timopheevii* s.str. and its wild relative *T. araraticum*, showed invariant ADH-B phenotypes (11-12, Fig. 2) with B^2 as a major isoenzyme in all accessions of different geographic origin (Georgia, Armenia, Azerbaijan, Iraq, Syria).

Diploid – All diploid wheats, the cultivated *T. monococcum* s.str. and its wild relatives *T. boeoticum*, (including ssp. *thaoudar*) and *T. urartu* shared a common major isoenzyme together with a minor fraction of lower electrophoretic mobility. The major isoenzyme band of diploid wheats corresponds on the enzymograms (1-3, Fig. 3) to that of the isoenzyme B^2 of emmer wheats, whereas the minor band coincides with the second major isoenzyme, band 3, of emmers. As seen in Figure 3, the basic ADH-B electrophoretic phenotype of diploid wheats is identical with that of *T. araraticum* (and of *T. timopheevii* s.str. as well), whereas *T. dicoccoides* and other emmers are characterized by the presence of two major ADH-B isoenzymes.

Intraspecific differentiation with two electrophoretic phenotypes was observed among the accessions of the cultivated einkorn *T. monococcum* s.str. As seen from the data in Table 3, 14 accessions of *T. monococcum* s.str. out of the 25 accessions studied had the null-phenotype of ADH-B – B^0, lacking the major isoenzyme band 2 (3, Fig. 3) and showing the presence of the minor component only. There was, however, no clear-cut correlation between the presence or absence of the major isoenzyme B^2 and the geographic origin or variety of *T. monococcum* s.str.

Table 2. Distribution of the ADH-B phenotypes in tetraploid wheats

Species	Number of accessions with the phenotypes		
	B^1B^3	B^2B^3	B^2
T. dicoccoides	3	11	0
T. dicoccon from West-Europe	5	11	0
T. dicoccon from Transcaucasia	20	0	0
T. dicoccon ssp. *georgicum*	0	5	0
T. turgidum	14	3	0
T. durum	60	0	0
T. turanicum	1	0	0
T. polonicum	8	0	0
T. ispahanicum	1	0	0
T. aethiopicum	3	0	0
T. carthlicum	6	0	0
T. araraticum	0	0	22
T. timopheevii	0	0	3

Table 3. Distribution of the ADH-B isoenzyme phenotypes among the diploid wheats and *Aegilops* species

Species	Number of accessions with the phenotypes				
	B^0	B^1	B^2	B^3	B^4
T. monococcum s. str.	14	0	11	0	0
T. boeoticum	0	0	26	0	0
T. urartu	1	0	10	0	0
Ae. speltoides var. *speltoides*	9	0	0	20	0
Ae. speltoides var. *ligustica*	7	0	0	18	0
Ae. mutica	5	0	0	0	0
Ae. bicornis	0	0	0	0	7
Ae. longissima s. str.	0	0	6	0	0
Ae. sharonensis	0	0	9	0	0
Ae. tauschii	0	0	26	0	0
Ae. caudata	0	0	5	0	0

The null-phenotype B^0 was very rare in wild diploid wheats and was, in our sample, encountered only in an accession of *T. urartu* collected in nature in Armenia.

The observed independent pattern of intraspecific variation of the major and the minor component of the NADP-dependent aromatic alcohol dehydrogenase in diploid wheats gives further support to their separate genetic control by different gene loci, as shown above by the analyses of nulli-5B chromosomal lines of the hexaploid wheat.

The isoenzyme B^1 of emmer wheats, thought to be an allelic variant of the A genome homoeoallele, as well as other electrophoretic variants of ADH-B were not encountered in the sample of diploid wheats analyzed.

The isoenzyme B^2 was also found to be characteristic of several diploid *Aegilops* species – *Ae. tauschii* (11, Fig. 3), *Ae. longissima* (15, Fig. 3), *Ae. sharonensis* (16, Fig. 3), and *Ae. caudata*. As seen from the data in Table 3, the isoenzyme B^2 was found in all the analyzed accessions of these diploids and no variation was observed. *Ae. bicornis* is characterized by the presence of a unique electrophoretic variant B^4 (17, Fig. 3) not found in polyploid wheats and in other diploids studied here.

Intraspecific and intrapopulational polymorphism with two ADH-B phenotypes, B^3 and B^0, was observed in the diploid *Ae. speltoides* s.l. The null-phenotype, B^0, in *Ae. speltoides* revealed the presence, on enzymograms, of a weakly stained minor component, coinciding electrophoretically with the major isoenzyme B^3 and with the minor component of diploid wheats. The distribution of the two ADH-B phenotypes among the accessions of *Ae. speltoides* s.l. showed no correlation, as seen in Table 3, with the two morphological varieties of this species.

Aegilops mutica proved unique among the diploids studied with respect to its ADH-B. No seedling with a major ADH-B isoenzyme was encountered among the five accessions of this diploid studied and only the minor component was observed on the enzymograms.

Discussion

1 On the Origin and Phylogenetic Differentiation of Tetraploid Wheats

The results of the present study show that the two genetic groups of tetraploid wheats, *T. turgidum* s.l. and *T. timopheevii* s.l., are clearly differentiated with respect to their ADH-B isoenzyme composition. The cultivated *T. timopheevii* s.str. and its wild-growing relative, *T. araraticum*, share a common ADH-B phenotype with only one major isoenzyme, B^2, present. The same phenotype is also inherent to diploid wheats, whereas the isoenzyme B^3 is characteristic of *Ae. speltoides* – a recognized donor of the second genome to *T. timopheevii* (Jaaska 1974, 1976; Konarev et al. 1976; Kimber 1973, 1974). This result indicates that only the A genome homoeoallele of ADH-B is expressed in the seedling tissues of *T. timopheevii* s.l., whereas the homoeoallele of the second genome B^t is silent.

At the same time, codominant expression of the ADH-B homoeoalleles of both composite genomes, A and B^e, in the seedlings of emmer wheats is evident from the presence of two major ADH-B isoenzymes. The isoenzyme B^3, invariantly found to be present in all accessions and taxa of emmer and dinkel wheats and shown above to be under the genetic control of the B genome homoeoallele, was characteristic of only one diploid species of the wheat group – *Ae. speltoides* s.l. It was not encountered in any accession of *T. urartu*, *Ae. longissima* s.str., *Ae. sharonensis* and *Ae. bicornis*. This result is considered as further support to the conclusion of our previous isoenzyme studies (Jaaska 1974, 1976b, c) that, among the known contemporary diploids of the wheat group, *Ae. speltoides* s.l. is genetically most closely related to the ancestral diploid which has contributed its genome to the origin of both *T. araraticum* and *T. dicoccoides*.

The involvement of *Ae. speltoides* in the origin of emmer wheats has recently been strongly questioned by Kimber and coworkers (Gill and Kimber 1974; Kimber 1973, 1974; Kimber and Athwal 1972; Kimber and Larsen 1973; Waines and Kimber 1973) on the grounds of karyomorphologic and cytogenetic data, as well as by several other authors (Bedetti et al. 1974; Bozzini et al. 1973; Dass 1972; Johnson 1972, 1975; Konarev et al. 1976; Vitozzi and Silano 1976) on the basis of biochemical data.

The origin of the second genome of emmer and dinkel

wheats has been ascribed (Konarev et al. 1976) to *Ae. longissima* on the basis of immunochemical data showing that an antigenic component of a seed protein fraction characteristic of the two groups of polyploid wheats was also present in seeds of *Ae. longissima* s.l. (incl. *Ae. sharonensis*), but was not detected in seeds of *Ae. speltoides* s.l. (incl. *Ae. aucheri* sensu Zhuk.) and *Ae. bicornis*. A possible involvement of *Ae. longissima* in the speciation of emmer wheats has also been considered (Vitozzi and Silano 1976) from the similarity of the Sephadex gel-elution profiles of the seed α-amylase isoinhibitors. *Ae. bicornis* was favoured as the wheat B genome donor on the basis of cytogenetic and morphologic (Sears 1956), disease resistance (Ryan 1966), and flavonoid chromatographic (Dass 1972) data.

Our previous studies have shown (Jaaska 1974, 1976b, c) that acid phosphatase, esterase A, aspartate aminotransferase B and D isoenzymes in emmer wheats shown (Hart and Langston 1977) to be controlled by genes located on the chromosomes 4B, 3B, 6B, and 3B, respectively, are electrophoretically different from those found in *Ae. longissima* s.l. and *Ae. bicornis*. All these isoenzyme markers, as well as the present data argue against *Ae. longissima* s.l. and *Ae. bicornis* as the wheat B^e genome donors and, instead, reveal its more close genetic similarity with the genome of *Ae. speltoides* s.l. The isoenzyme data, however, do not exclude a possible introgression of some chromosomal material from *Ae. longissima* to the origin of the emmers' genome B^e which should seriously be considered in the light of the studies of the authors cited above. It may be speculated that *T. dicoccoides* and *T. araraticum* represent cytogenetically stabilized and evolutionary-wise very successful recombinational segregants of the hybrids between two or more different initial amphiploids, involving ancestral forms or precursors of the contemporary diploids *T. boeoticum, Ae. speltoides* and *Ae. longissima*.

The emmer wheats show genetic differentiation with two alloenzymes B^1 and B^2, evidently controlled by two alleles of the A genome homoeoallele of ADH-B. The alloenzyme B^2, coinciding electrophoretically with the basic ADH-B isoenzyme of diploid wheats and of several diploid *Aegilops* species, should be considered to be ancestral to the alloenzyme B^1 which, not encountered in diploids, has evidently arisen mutationally on the tetraploid level in a primitive emmer. The ancestral alloenzyme B^2 was found in only three species of emmers. It is most frequent in the wild emmer *T. dicoccoides*, frequent in the most primitive cultivated emmer *T. dicoccon* and rare in *T. turgidum* s.str. B^2 was not found in *T. durum, T. carthlicum, T. polonicum* and in the remaining species taxa of cultivated emmers which all were characterized by the presence of B^1 and, thus, should be considered as phylogenetically younger taxa of emmers in comparison with *T. dicoccon*.

2 *On the Origin of Hexaploid Wheats*

The three-banded electrophoretic phenotype of ADH-B, most common in hexaploid wheats and comprising all three major isoenzymes, B^1, B^2, and B^3, reflects codominant expression of the *Adh-2* homoeoalleles of the three composite genomes. Hexaploid wheats with such ADH-B phenotypes may be considered as amphidiploid derivatives of hybrids between cultivated emmer wheat with the phenotype B^1B^3 and the diploid *Ae tauschii* – a carrier of the isoenzyme B^2. The wild emmer, *T. dicoccoides*, seems to be a less suitable candidate for the role of a tetraploid precursor, since most of its accessions carry the phenotype B^2B^3. The occurrence of the alloenzyme B^1 in some accessions of *T. dicoccoides* may be explained by assuming either a mutational event from the ancestral alloenzyme B^2 or later introgression from the cultivated emmer *T. dicoccon* through occasional hybridization in nature.

Of special interest is the result that all the examined accessions (10) of the West-Georgian endemic hexaploid *T. macha*, including its compact-eared vartiety *palaeoimereticum*, reveal the presence of the alloenzyme B^1, whereas all the five accessions of its tetraploid companion in the cultural coenosis 'makha', *T. dicoccon* ssp. *georgicum*, is characterized by the alloenzyme B^2. This result implies that *T. dicoccon* ssp. *georgicum* (= *T. palaeocolchicum*) should not be considered as the tetraploid precursor of *T. macha*. The overall morphological similarity between *T. dicoccon* ssp. *georgicum* and *T. macha* var. 'palaeoimereticum' thus seems to be of convergent nature arisen through the artificial selection in a common cultural coenosis and it does not reflect immediate phylogenetic links between the two taxa as suspected by several authors (Kandelaki 1967; Swaminathan 1966 a.o.). There is also no evidence of introgression of the chromosomal material with the genetic information for the alloenzyme B^2 from *T. dicoccon* ssp. *georgicum* to *T. macha*. The presence of the ancestral alloenzyme B^2 in *T. dicoccon* ssp. *georgicum*, however, indicates its phylogenetic antiquity. All the remaining 20 accessions of *T. dicoccon* of Transcaucasian origin, belonging to the botanical varieties *farrum* and *rufum*, shared the alloenzyme B^1.

The observed intraspecific genetic differentiation of *T. spelta* with respect to the ADH-B alloenzymes B^1 and B^2 requires a special discussion in view of its obvious correlation with the geographic distribution. Thus, all the seven Transcaucasian and Asian accessions of *T. spelta* examined, including those from the Azerbaijan SSR (2), Nakhitshevan ASSR (1), Tadjik SSR (1) and Iran (3), carried the phylogenetically younger alloenzyme B^1. Most of the West-European spelts (12 out of 18 analyzed), including all 5 accessions from Spain, 3 from Switzerland, 2 from Germany, 1 from Jugoslavia and 1 from Denmark,

revealed the presence of the ancestral alloenzyme B^2. The alloenzyme B^1 was found in 5 accessions of *T. spelta* from Germany (out of 7 analyzed) and in one accession from Switzerland (out of 4 analyzed).

There are two possible ways of origin of the Asian spelts with the alloenzyme B^1: firstly, as a primary amphiploid, combining the genomes of a cultivated emmer with the alloenzyme B^1 and *Ae. tauschii*; secondly, as a secondary mutational or hybridizational derivative of the free-threshing *T. aestivum* s.str., which is monomorphic with respect to the alloenzyme B^1. At present, most triticologists (Dorofeev, 1971; Gandilian 1972, a.o.) are inclined to recognize both the primary as well as the secondary origin of present-day Asian spelts. The ADH-B isoenzyme data fail to contribute additional evidence in this respect.

Two analogous hypotheses have been put forward to explain the origin of West-European spelts. Since the diploid donor of the wheat D genome, *Ae. tauschii*, is of the East-Mediterranean distribution and does not extend to West-Europe, then most triticologists (Dorofeev 1971; Flaksberger 1930; Mac Key 1954; Schiemann 1951, a.o.) recognize the secondary mutational or hybridizational origin of the European *T. spelta* from *T. aestivum* s.str. or *T. compactum*. In view of this hypothesis, the observed wide distribution of the alloenzyme B^2 among the West-European spelts may be taken as an evidence in favour of its introgression from a cultivated emmer *T. dicoccon*. The introgressional origin of the alloenzyme B^2 in the West-European spelts is supported by the data (Table 2) about its high frequency among West-European accessions of *T. dicoccon* (in 11 accessions out of 16 examined).

If the West-European spelts possessing the alloenzyme B^1 may be considered as mutational derivatives of *T. aestivum*, the same cannot be asserted with respect to the spelts carrying the alloenzyme B^2, since the reversed mutational change from B^1 to B^2 was never observed among the numerous primitive accessions of *T. aestivum* s.str. analyzed.

The theory of the Asian origin of West-European spelts favoured by McFadden and Sears (1946) has received further support (Kuckuck 1959, 1964) after the discovery of *T. spelta* in Iran (Kuckuck and Schiemann 1957). Some triticologists (Menabde 1948, a.o.) consider the West-Georgian endemic spelt *T. macha* as the most primitive hexaploid wheat preserved to our days.

Although the theory of introgressional and mutational origin of the West-European spelts looks to be quite convincing and well-grounded, there is still no firm belief to reject the alternative migrational theory of their allochtonic Asian origin from a primitive emmer carrying the ancestral alloenzyme B^2. The ADH-B data, however, favour the concept of polyphyletic origin of West-European spelts. No reason remains to consider the West-European spelts with the alloenzyme B^2 as simple migrational derivatives of Asian spelts, including *T. macha*, which are all monomorphic with respect to the phylogenetically younger alloenzyme B^1.

3 On the Evolutionary Variation of ADH-B in Wheats

An intriguing feature in the results obtained is the conspicuous evolutionary stability of ADH-B in polyploid wheats. Indeed, only two mutation changes at the locus *Adh-2* have become fixed during the whole evolutionary history of polyploid wheats. One of them involves the A genome homoeoallele in emmer wheats, which has given rise to the alloenzyme ADH-B^1 from ADH-B^2. The second mutational change has resulted in a full inactivation of the B genome homoeoallele or its phenotypic product – the isoenzyme ADH-B^3 in a phylogenetic line of tetraploid wheats *T. araraticum – T. timopheevii*.

The absence of other electrophoretic variants of ADH-B in polyploid wheats, as well as of silent homoeoalleles or inactive ADH-B isoenzymes in the emmer and *aestivum* wheats, is striking and difficult to explain. On the grounds of the neo-darwinian selectogenetic concept of microevolution, one would expect to observe increased intraspecific genetic variation in polyploid species. The duplication of the whole genome through the polyploidization should essentially free one set of homologous genes from the control by natural selection. In polyploids, one set of homoeoalleles can accumulate even such mutations which will cause full inactivation of a gene or its product and would be harmful or lethal in diploids, owing to the compensatory action of other sets of homoeoalleles which continue to produce normal proteins and enzymes essential to the development of the organism.

Contrary to theoretical expectations, a silent allele with the ADH-B isoenzyme absent or inactive in seedling tissues was geographically widely distributed among the accessions of the cultivated diploid wheat, but was not encountered among the tetraploid emmer wheats and the hexaploid *aestivum* wheats. At the same time, polyploid wheats and especially *T. aestivum* s.str. possess much richer genetic resources with a wide amplitude of intraspecific variation in morphological and eco-physiological characters in comparison with the diploid wheat. It is noteworthy that the most frequent types of morphological mutations in hexaploid wheats, speltoids, square-heads and compactoids, are caused by genetic changes of the chromosome 5A (Ichikawa and Nishiyama 1967; Muramatsu 1963) where one of the *Adh-2* homoeoalleles is also located. The genes controlling such variable characters as growth habit (Tsunewaki and Jenkins 1961) and awnedness (Sears 1953, a.o.) are also located on the chromosomes of the fifth homoeologous group of hexaploid wheat.

The lack of the expected correlation between the ploidy level and the extent of intraspecific genetic variation has also been noted in our recent studies with respect to the acid phosphatase isoenzymes in wheat (Jaaska 1974) and goatgrass (Jaaska 1978) species. The results of the present study show that even in such genes as *Adh-2* loss mutations of which do not have any harmful effect even in a diploid organism, may be of very restricted evolutionary variation.

To explain these observations it seems reasonable to assume that the gene mutability is not a random process but its extent, specificity and direction are largely controlled by the peculiarities of the molecular structure of the genome DNA in each species. Natural selection acts only within the limits of those evolutionary potentialities which are predetermined by the genome molecular structure and directs the evolution towards the internal and external adaptation of organisms.

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Literature

Bedetti, C.; Bozzini, A.; Silano, V.; Vittozzi, L.: Amylase protein inhibitors and the role of *Aegilops* species in polyploid wheat speciation. Biochim. Biophys. Acta **362**, 299-307 (1974)

Bozzini, A.; Cubbadda, R.; Quatrucci, E.: Esterases in *Triticum* and some related species. Proc. 4th Int. Wheat Genet. Symp., Columbia, Missouri 783-789 (1973)

Brewer, G.J.; Sing, C.F.; Sears, E.R.: Studies of isoenzyme patterns in nullisomic-tetrasomic combinations of hexaploid wheat. Proc. Natl. Acad. Sci. (USA) **64**, 1224-1229 (1969)

Dass, H.C.: Phylogenetic affinities in *Triticinae* studied by thin-layer chromatography. Can. J. Genet. Cytol. **14**, 703-712 (1972)

Dorofeev, V.F.: The botanical composition and breeding value of Transcaucasian spelts. Bull. Agric. Sci. (USSR) 7, 44-56 (1971), in Russian

Flaksberger, K.: Ursprungszentrum und geographishe Verbreitung des Spelzes (*Triticum spelta* L.) Angew. Bot. **12**, 86-99 (1930)

Gandilian, P.A.: Spontaneous hybridization, mutations and problems of phylogeny of wheat. Genetica (Moscow) **8**, 5-19 (1972), in Russian

Gill, B.L.; Kimber, G.: Giemsa C-banding and the evolution of wheat. Proc. Natl. Acad. Sci. USA **71**, 4086-4090 (1974)

Hart, G.: Genetic control of alcohol dehydrogenase isozymes in *Triticum dicoccum*. Biochem. Genet. **3**, 617-625 (1969)

Hart, G.: Evidence of triplicate genes for alcohol dehydrogenase in hexaploid wheat. Proc. Natl. Acad. Sci. (USA) **66**, 1136-1140 (1970)

Hart, G.: Homoeologous gene evolution in hexaploid wheat. Proc. 4th Int. Wheat Genet. Symp., Columbia, Missouri, 805-810 (1973)

Hart, G.E.; Langston, P.J.: Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. Heredity **39**, 263-277 (1977)

Ichikawa, S.; Nishiyama, J.: Gamma-ray induced mutants in a Japanese variety of common wheat. Jap. J. Genet. **42**, 175-187 (1967)

Jaaska, V.: Electrophoretic studies of seedling phosphatases, esterases and peroxidases in the genus *Triticum* L. Eesti NSV TA Toimetised, Bioloogia **18**, 170-183 (1969)

Jaaska, V.: Phylogenetic differentiation of tetraploid wheats. Eesti NSV TA Toimetised, Bioloogia **20**, 202-205 (1971)

Jaaska, V.: The origin of tetraploid wheats on the basis of electrophoretic studies of enzymes. Eesti NSV TA Toimetised, Bioloogia **23**, 201-220 (1974), in Russian, English summary

Jaaska, V.: Alcohol dehydrogenase in polyploid wheats and in their diploid relatives. On the origin of tetraploid wheats. Genetika (USSR) **12**, 22-28 (1976a)

Jaaska, V.: Aspartate aminotransferase isoenzymes in the polyploid wheats and their diploid relatives. On the origin of tetraploid wheats. Biochem. Physiol. Pfl. **170**, 159-171 (1976b)

Jaaska, V.: Genome- and tissue-specific regulation of esterase and acid phosphatase isoenzymes in tetraploid wheats. Eesti NSV TA Toimetised, Bioloogia **25**, 132-145 (1976c), in Russian, English summary

Jaaska, V.: Electrophoretic study of acid phosphatase isoenzymes in the grass genus *Aegilops* L. Biochem. Physiol. Pfl. **172**, 133-153 (1978)

Jaaska, V.; Jaaska, Vilve: Biochemical data on the origin of Transcaucacian endemic wheats. Eesti NSV TA Toimetised. Bioloogia, **19**, 344-354 (1970)

Jaaska, V.H.; Jaaska, V.E.: Electrophoretic analysis of substrate specificity of the wheat alcohol dehydrogenase. Biokhimiya (1978), In Russian (in press)

Johnson, B.L.: Protein electrophoretic profiles and the origin of the *B* genome of wheat. Proc. Natl. Acad. Sci. (USA) **69**, 1398-1402 (1972)

Johnson, B.L.: Identification of the apparent *B*-genome donor of wheat. Can. J. Genet. Cytol. **17**, 21-39 (1975)

Kandelaki, G.V.: On the origin of *Triticum macha* Dek. et Men. Genetika (Moscow) 34-46 (1967)

Kimber, G.: The relationships of the *S*-genome diploids to polyploid wheats. Proc. 4th Int. Wheat Genet. Symp., Columbia, Missouri 81-85 (1973)

Kimber, G.: A reassessment of the origin of the polyploid wheats. Genetics 78, 487-492 (1974)

Kimber, G.; Athwal, R.S.: A reassessment of the course of evolution of wheat. Proc. Natl. Acad. Sci. (USA) **69**, 912-915 (1972)

Kimber, G.; Larsen, J.: The *B*-genome of polyploid wheat. Cereal Res. Comm. **1**, 17-26 (1973)

Konarev, V.G.; Gavriljuk, I.P.; Peneva, T.I.; Konarev, A.V.; Hakhimova, A.G.; Migushova, E.F.: On the nature and origin of wheat genomes on the basis of biochemistry and immunochemistry of the grain proteins. Selskokhoz. biologija (USSR) **11**, 656-665 (1976) In Russian

Kuckuck, H.: Neuere Arbeiten zur Entstehung der hexaploiden Kulturweizen. Z. Pflanzenzücht. **41**, 205-226 (1959)

Kuckuck, H.: Experimentelle Untersuchungen zu Entstehung der Kulturweizen. I. Die Variation des iranischen Spelzweizens und seine genetischen Beziehungen zu *Triticum aestivum* ssp. *vulgare* (Vill., Host) Mac Key, ssp. *spelta* (L.) Thell. und ssp. *macha* (Dek. et Men.) Mac Key, mit einem Beitrag zur Genetik des Spelta-Komplexes. Z. Pflanzenzücht. **51**, 97-140 (1964)

Kuckuck, H.; Schiemann, E.: Über das Vorkommen von Spelz und Emmer (*T. spelta* und *T. dicoccum*) im Iran. Z. Pflanzenzücht. **38**, 383-396 (1957)

MacKey, J.: Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas **40**, 65-180 (1954)

McFadden, E.S.; Sears, E.R.: The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J. Hered. **37**, 81-89, 106-116 (1946)

Menabde, V.: Wheats of Georgia. Tbilisi 1948, in Russian

Mitra, R.; Bhatia, C.R.: Isoenzymes and polyploidy. I. Qualitative and quantitative isoenzyme studies in the *Triticinae*. Genet. Res. **18**, 57-69 (1971)

Muramatsu, M.: Dosage effect of the spelta gene *q* of hexaploid wheat. Genetics **48**, 469-482 (1963)

Ryan, G.S.: Another feasible approach to study the phylogeny of cultivated hexaploid wheat. Wheat Inf. Serv. **22**, 14-17 (1966)

Schiemann, E.: New results on the history of cultivated cereals. Heredity **5**, 305-320 (1951)

Sears, E.R.: Nullisomic analysis in wheat. Amer. Nat. **87**, 245-252 (1953)

Sears, E.R.: The *B* genome of *Triticum*. Wheat Inf. Serv. **4**, 8-10 (1956)

Sears, E.R.: Nullisomic-tetrasomic combinations in hexaploid wheat. In: Chromosome Manipulations and Plant Genetics (ed. Riley, R.; and Lewis, K.R.) Suppl. to Heredity **20**, 29-45 Edinburgh and London; Oliver and Boyd. (1965)

Sears, E.R.: Chromosome mapping with the aid of telocentrics. Proc. 2nd Int. Wheat Genet. Symp. 1963, Suppl. to Hereditas **2**, 370-381 (1966)

Swaminathan, M.S.: Mutational analysis of the hexaploid *Triticum* complex. Proc. 2nd Intr. Wheat Genet. Symp. 1963, Suppl. to Hereditas **2**, 418-438 (1966)

Tsunewaki, K.; Jenkins, B.C.: Monosomic and conventional gene analyses in common wheat. II. Growth habit and awnedness. Jap. J. Genet. **36**, 428-443 (1961)

Vittozzi, L.; Silano, V.: The phylogenesis of protein α-amylase inhibitors from wheat seed and the speciation of polyploid wheats. Theor. Appl. Genet. **48**, 279-284 (1976)

Waines, J.G.; Kimber, G.: Satellite number and size in *Triticum monococcum* L. and the evolution of the polyploid wheats. Can. J. Genet. Cytol. **15**, 117-122 (1973)

Washington, W.J.: Homoeoallelism in *Triticum aestivum*. Can. J. Genet. Cytol. **13**, 169-172 (1971)

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