парники укрываются рамами — первые один-два дня с проветриванием, а потом без проветривания и полива. В конце сентября—начале октября собираются семена и высаживаются гибриды в двухлетнем возрасте. В открытый грунт при вспашке летом вносится на 1 $м^2$: 8 *кг* навоза, 30 *г* сернокислого аммония, 100 *г* суперфосфата, весной при отрастании — по 30—40 *г* сернокислого аммония, суперфосфата и калийной соли. В июне—июле необходимы одна-две подкормки навозной жижей, разбавленной водой (1:4), с незначительным добавлением минеральных удобрений (10 *л* на 1 $м^2$), после чего — полив водой. Все подкормки вносятся в бороздки с засыпкой землей. Весной желательно внесение древесной золы (300 *г* на 1 $м^2$). Против серой плесени (*Botrytis*) в июне растения опрыскиваются бордосской жидкостью. Пересадки проводятся, как обычно, через 4—5 лет.

При плохом соблюдении агротехники выявленные различия сортов сглаживаются и они становятся более однородными.

Все вышеописанные гибриды относительно морозоустойчивы и аналогично регале (*L. regale* Wils.) хорошо зимуют под укрытием листьев.

Питомникам цветочно-декоративных растений следует особое внимание уделить вегетативному размножению бульбоносных лилий, что выгодно экономически и ускоряет получение однородного сортового материала.

Таллинский ботанический сад
Академии наук Эстонской ССР

Поступила в редакцию
30/III 1970

EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. 19. KÖIDE
BIOLOOGIA, 1970, Nr. 4

EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. 19. KÖIDE
BIOLOOGIA, 1970, Nr. 4

https://doi.org/10.3176/biol.1970.4.14

V. JAASKA

PRELIMINARY DATA ON THE ENZYME DIVERGENCE IN THE DIPLOIDS OF THE GENUS *AEGILOPS* L.

V. JAASKA. ESIALGSED ANDMED ENSÜÜMIDE DIVERGENTSI KOHTA PEREKOND *AEGILOPS* L. DIPLOIDIDEL

В. ЯАСКА. ПРЕДВАРИТЕЛЬНЫЕ ДАННЫЕ О ДИВЕРГЕНЦИИ ФЕРМЕНТОВ У ДИПЛОИДОВ РОДА *AEGILOPS* L.

In previous reports (Jaaska, 1969; Jaaska, Jaaska, 1970) we showed that electrophoretic patterns of acid phosphatase isoenzymes supported the view of *A. speltoides* Tausch as the donor of the genome *B* to the tetraploid wheats and about *A. squarrosa* L. as the contributor of the third genome *D* to the hexaploids. However, the data did not exclude the possibility that some other diploid species of *Aegilops* may have phosphatase isoenzymes electrophoretically similar to those found in *A. speltoides* or in *A. squarrosa* and, in this respect, may serve equally well as the donors of the *B*- and *D*-genomes, respectively.

In this short communication we present a preliminary account of the electrophoretic diversity of seedling acid phosphatase, esterase, peroxidase and glutamate dehydrogenase isoenzymes among the diploid species of the genus *Aegilops* L.

Material and methods. Most of the seed samples were received from the World Collection of the Vavilov Institute of Plant Industry (Leningrad) through the courtesy

of Dr. E. Migushova. Some samples were obtained from Prof. J. Grigoryev (Leningrad). Enzyme extracts from 4-day-old etiolated seedlings were subjected to polyacrylamide gel electrophoresis and stained histochemically as described previously (Jaaska, 1969), except for performing electrophoresis in the gel slabs as well as in the glass tubes.

Results and discussion

Fig. 1 demonstrates the existing diversity in acid phosphatase electrophoretic patterns among the diploid species of *Aegilops*. The electrophoretic mobility of major isoenzymes varies considerably, *A. speltoides* having the most faster-moving band and *A. squarrosa* — the most slower-migrating doublet of bands. No other diploid species of *Aegilops* possess phosphatase isoenzymes, electrophoretically similar to those of *A. speltoides* which thus still seems to be the only suitable genome carrier to account for the fastest-moving phosphatase isoenzymes in the tetraploid and hexaploid wheats.

Several different forms of *A. speltoides* Tausch, some of them specified as *A. aucheri* Boiss., were studied and all showed generally similar phosphatase enzymograms, thus confirming the genetic closeness of the two taxa. Since there is no sufficient reproductive barrier between them, most of systematists (Chennaveeraiah, 1960; Zohary, Imber, 1963, etc.) consider *A. aucheri* to be an intra-specific type of *A. speltoides*.

A. mutica Boiss. is typically characterized by three closely spaced major isoenzymes (Fig. 2*A*, enzymograms *1—3*), although one of the three strains studied showed the presence of an additional faster-moving fraction. Although the fastest band in the triplet of *A. mutica* phosphatase almost reaches the electrophoretic mobility of the *speltoides* isoenzyme, by the general appearance of the phosphatase enzymogram, *A. mutica* appears to be unsuitable for the role of the *B*-genome donor for the tetraploid wheats, especially for the Timopheevi-group.

Three species, *A. longissima* Schweinf. et Muschl., *A. sharonensis* Eig, and *A. bicornis* Forsk., all grouped in the section *Sitopsis* of the genus on the morphological basis, possessed electrophoretically similar doublets of phosphatase isoenzymes. Three different strains of *A. longissima* and three additional strains of *A. sharonensis*, all showed electrophoretically closely similar doublets of major phosphatases (Fig. 2*A*, enzymograms *4—9*). Enzymological data thus confirm close genetic affinity of the two taxa, sharing major phosphatase isoenzymes. *A. longissima* and *A. sharonensis* form another pair of taxa in the section *Sitopsis* which are comparatively interfertile, morphologically differ in only minor characters and share karyomorphologically similar genomes (Chennaveeraiah, 1960; Roy, 1959, etc.). For these reasons, *A. sharonensis* is sometimes regarded (Chennaveeraiah, 1960) as an intra-specific form of *A. longissima*.

A. bicornis seems to be somewhat more divergent from *A. sharonensis* and *A. longissima*, being separated from them by a firm reproductive barrier (Roy, 1959). This is a clear example showing that, although in all other cases reproductively isolated diploid species of *Aegilops* possess electrophoretically different phosphatase isoenzymes, a single biochemical character — electrophoretic identity of phosphatases — alone cannot serve as a species criterion. However, it would be a good diagnostic character in distinguishing most diploid species of *Aegilops*.

Electrophoretic identity in phosphatase isoenzymes, indicating similar molecular structure of the genes controlling them, suggests phylogenetic closeness and probable common origin of the three *S*-genome carriers. This is evident from close morphological similarity of *A. sharonensis* and *A. bicornis*, as well as from biogeographical data. The two taxa seem to be vicarious, *A. sharonensis* being confined to the Mediterranean coast of Palestina, and *A. bicornis* replacing it in the Saharo-Sinaian territory (Eig, 1936).

The genome of *A. speltoides* which we propose to designate preferentially as B^S is clearly distinguishable from the *S*-genome of the other members in the section *Sitopsis* in the structure of genes controlling electrophoretically divergent phosphatase isoenzymes. This evidence argues against the involvement of the *S*-genome of *A. bicornis* in the origin of the tetraploid wheats as suggested by E. R. Sears (1956). However, the present data

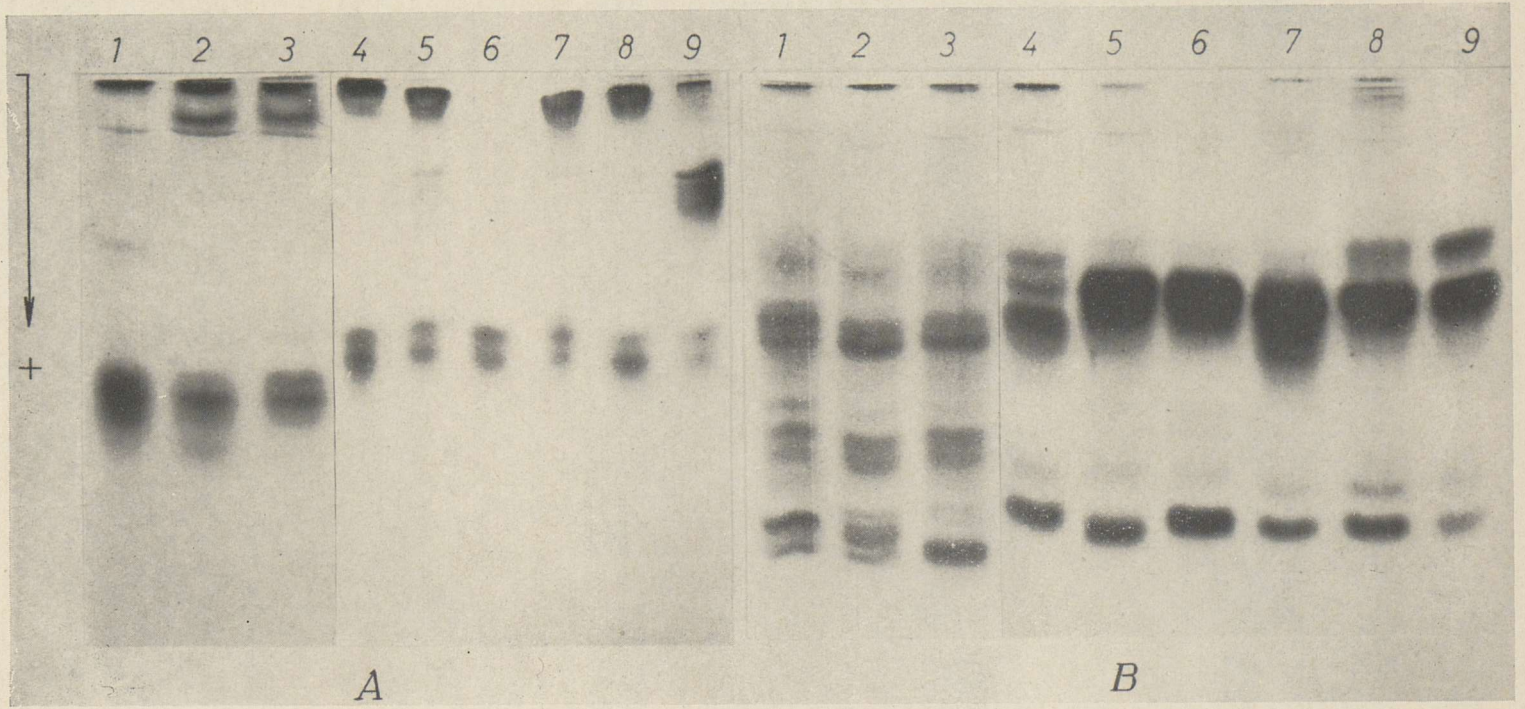

Fig. 2. Acid phosphatase (*A*) and esterase (*B*) enzymograms for different strains of *A. mutica* (*1—3*), *A. longissima* (*4—6*) and *A. sharonensis* (*7—9*).

of Dr. E. Migushova. Some samples were obtained from Prof. J. Grigoryev (Leningrad). Enzyme extracts from 4-day-old etiolated seedlings were subjected to polyacrylamide gel electrophoresis and stained histochemically as described previously (Jaaska, 1969), except for performing electrophoresis in the gel slabs as well as in the glass tubes.

Results and discussion

Fig. 1 demonstrates the existing diversity in acid phosphatase electrophoretic patterns among the diploid species of *Aegilops*. The electrophoretic mobility of major isoenzymes varies considerably, *A. speltoides* having the most faster-moving band and *A. squarrosa* — the most slower-migrating doublet of bands. No other diploid species of *Aegilops* possess phosphatase isoenzymes, electrophoretically similar to those of *A. speltoides* which thus still seems to be the only suitable genome carrier to account for the fastest-moving phosphatase isoenzymes in the tetraploid and hexaploid wheats.

Several different forms of *A. speltoides* Tausch, some of them specified as *A. aucheri* Boiss., were studied and all showed generally similar phosphatase enzymograms, thus confirming the genetic closeness of the two taxa. Since there is no sufficient reproductive barrier between them, most of systematists (Chennaveeraiah, 1960; Zohary, Imber, 1963, etc.) consider *A. aucheri* to be an intra-specific type of *A. speltoides*.

A. mutica Boiss. is typically characterized by three closely spaced major isoenzymes (Fig. 2*A*, enzymograms *1—3*), although one of the three strains studied showed the presence of an additional faster-moving fraction. Although the fastest band in the triplet of *A. mutica* phosphatase almost reaches the electrophoretic mobility of the *speltoides* isoenzyme, by the general appearance of the phosphatase enzymogram, *A. mutica* appears to be unsuitable for the role of the *B*-genome donor for the tetraploid wheats, especially for the Timopheevi-group.

Three species, *A. longissima* Schweinf. et Muschl., *A. sharonensis* Eig, and *A. bicornis* Forsk., all grouped in the section *Sitopsis* of the genus on the morphological basis, possessed electrophoretically similar doublets of phosphatase isoenzymes. Three different strains of *A. longissima* and three additional strains of *A. sharonensis*, all showed electrophoretically closely similar doublets of major phosphatases (Fig. 2*A*, enzymograms *4—9*). Enzymological data thus confirm close genetic affinity of the two taxa, sharing major phosphatase isoenzymes. *A. longissima* and *A. sharonensis* form another pair of taxa in the section *Sitopsis* which are comparatively interfertile, morphologically differ in only minor characters and share karyomorphologically similar genomes (Chennaveeraiah, 1960; Roy, 1959, etc.). For these reasons, *A. sharonensis* is sometimes regarded (Chennaveeraiah, 1960) as an intra-specific form of *A. longissima*.

A. bicornis seems to be somewhat more divergent from *A. sharonensis* and *A. longissima*, being separated from them by a firm reproductive barrier (Roy, 1959). This is a clear example showing that, although in all other cases reproductively isolated diploid species of *Aegilops* possess electrophoretically different phosphatase isoenzymes, a single biochemical character — electrophoretic identity of phosphatases — alone cannot serve as a species criterion. However, it would be a good diagnostic character in distinguishing most diploid species of *Aegilops*.

Electrophoretic identity in phosphatase isoenzymes, indicating similar molecular structure of the genes controlling them, suggests phylogenetic closeness and probable common origin of the three *S*-genome carriers. This is evident from close morphological similarity of *A. sharonensis* and *A. bicornis*, as well as from biogeographical data. The two taxa seem to be vicarious, *A. sharonensis* being confined to the Mediterranean coast of Palestina, and *A. bicornis* replacing it in the Saharo-Sinaian territory (Eig, 1936).

The genome of *A. speltoides* which we propose to designate preferentially as B^S is clearly distinguishable from the *S*-genome of the other members in the section *Sitopsis* in the structure of genes controlling electrophoretically divergent phosphatase isoenzymes. This evidence argues against the involvement of the *S*-genome of *A. bicornis* in the origin of the tetraploid wheats as suggested by E. R. Sears (1956). However, the present data

should be considered as preliminary only since we have been able to study only one strain of *A. bicornis* available from Cambridge through the courtesy of Dr. Chr. Lehman (Gatersleben, DDR).

Acid phosphatase isoenzymes of *A. uniaristata* Vis. (enzymogram *6*) are electrophoretically closely similar to those characteristic of the *S*-genome (enzymograms *3*, *4*, *5*). The systematic position of *A. uniaristata* in the genus has been the subject of discussion, and, most recently, M. S. Chennaveeraiah (1960) created a separate section *Uniaristatopyron* specially for this species. The present enzymological evidence on acid phosphatase isoenzymes seems to indicate possible phylogenetic connections of *A. uniaristata* with the *S*-genome group of the section *Sitopsis*.

A. comosa Sibth. et Sm. and *A. caudata* L., the two species clearly distinguishable morphologically and cytogenetically, possess electrophoretically distinctly different phosphatase patterns as well (Fig. 1, enzymograms *7* and *8*). Two strains were studied for both species and showed non-variant enzymograms. *A. umbellulata* Zhuk. was characterized by the presence of a closely spaced doublet of isoenzymes (enzymogram *9*), whereas *A. squarrosa* L. showed a doublet of electrophoretically distinct isoenzymes, in addition to a minor faster-moving component and a staining area near the origin (enzymogram *10*).

In total, seven different phosphatase isoenzyme phenotypes can be distinguished among the 12 diploid taxa (10 species) of *Aegilops*, when leaving non-specific fractions out of consideration.

Esterase enzymograms consisted of a complex series of isoenzymes and showed a clear variation among the diploids. However, contrary to acid phosphatases which proved to be genome-specific in major isoenzymes, esterases in many cases revealed distinct intra-specific variation in the isoenzyme composition. Fig. *2B* presents some examples of this variation. For example, up to 14 isoenzyme fractions can be distinguished in enzymograms for *A. mutica*, a group of the fastest isoenzymes showing distinct variation. Of particular interest is the finding of an intra-specific variation in esterase patterns among the strains of *A. longissima* and *A. sharonensis*, since the same strains shared electrophoretically similar doublets of phosphatase isoenzymes.

The remaining two enzymes studied, glutamate dehydrogenase (GDH) and peroxidase, proved to be even more conservative than phosphatases. Only one major GDH fraction of identical electrophoretic mobility was observed for all the diploid taxa. Similarly, three major electrophoretically distinct anodal peroxidase isoenzymes proved to be in common to all the diploids. The diploid wheat, *T. boeoticum*, showed GDH and peroxidase patterns identical with those of the diploid species of *Aegilops*. This evidence supports the view that all diploid species of the wheat group have evolved from a common ancestral form and possess homoeologous genomes sharing many loci controlling the protein structure still unchanged. From these data it also follows that separate genes as well as the proteins they control change and evolve at different rates.

REFERENCES

Chennaveeraiah M. S., 1960. Karyomorphologic and cytotaxonomic studies in *Aegilops*. Acta horti Gotoburg. **23** : 85—178.

Eig A., 1936. *Aegilops*. In: Die Pflanzenareale. Ed. by E. Hannig and H. Winkler. Fischer, Jena, Reihe **4** (4) : 43—50.

Jaaska V., 1969. Electrophoretic studies of seedlings phosphatases, esterases and peroxidases in the genus *Triticum* L. Eesti NSV TA Toimet., Biol. **18** (2) : 170—183.

Jaaska V., Jaaska Vilve, 1970. Biochemical data on the origin of Transcaucasian endemic wheats. Eesti NSV TA Toimet., Biol. **19** (4) : 344—354.

Roy R. P., 1959. Genome analysis of *Aegilops sharonensis*. Genetica **29** : 331—357.

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

Zohary D., Imber D., 1963. Genetic dimorphism in fruit type in *Aegilops speltoides*. Heredity **18** : 223—231.

Academy of Sciences of the Estonian SSR,
Institute of Zoology and Botany

Received
March 5, 1970