EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. 21. KÖIDE
BIOLOOGIA. 1972, NR. 3

ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ТОМ 21
БИОЛОГИЯ. 1972, № 3

https://doi.org/10.3176/biol.1972.3.02

УДК 575.1/3+582.542.1+581.19:577.15

VELLO JAASKA

ENZYME VARIABILITY AND PHYLOGENETIC RELATIONSHIPS IN THE GRASS GENERA *AGROPYRON* GAERTN. AND *ELYMUS* L. I. *AGROPYRON INTERMEDIUM* (HOST) BEAUV. AND *AGROPYRON ELONGATUM* (HOST) BEAUV.

In a series of recent publications (Jaaska, 1969, 1971, 1972, etc.) an attempt has been made to use electrophoretic isoenzyme patterns for elucidating phylogenetic relationships and the degree of genetic divergence in the grass genera *Triticum* L., *Aegilops* L., *Haynaldia* Schur. and *Secale* L., which comprise the subtribe *Triticinae* Benth. Although that work is to be continued, we now extend our studies to the closely related genera *Elymus* L. s. lat. and *Agropyron* Gaertn. s. lat. of the neighbouring subtribe *Elyminae* Benth. This paper involves isoenzyme studies in those wheatgrass species which are known to hybridize readily with wheats and thus are thought to be most closely related to them.

Starting from the pioneering work of N. Tzitzin (Цицин, 1933), a large number of hybridization studies between the *Triticum* and *Agropyron* species has been carried out (for reviews see Cauderon, 1958; Smith, 1943). These investigations have reached the conclusion that wheats are most readily and successfully crossed with the two wheatgrass species, *Agropyron intermedium* (Host) Beauv. (incl. *A. trichophorum*) and *A. elongatum* (Host) Beauv.

The cytogenetic studies of the F_1 hybrids from the *Triticum* × *Agropyron* crosses have lead investigators to contradicting views concerning the origin and genome composition of the hexaploid *A. intermedium* (=*A. glaucum*) and the decaploid *A. elongatum* (=*A. rigidum*). Several authors (Вакар, 1935, 1938; Хвостова et al., 1963; et al.) who observed a high degree of chromosome pairing in their F_1 hybrids attributed it to allosyndesis and concluded that two genomes of *A. intermedium* and three genomes of *A. elongatum* are homologous to the *AB* and *ABD* genomes of the polyploid wheats, respectively. Other authors (Armstrong, 1936, 1945; Matsumura, 1949; Peto, 1936, 1939) observed much less chromosome pairing in their F_1 hybrids and supposed only one genome to be in common to wheats and wheatgrasses.

More extensive hybridization and cytogenetic studies (Cauderon, 1958; Gaul, 1953; Matsumura, Muramatsu, 1956; Schulz-Schaeffer et al., 1971; Хижняк, 1938) have found the degree of chromosome pairing to vary considerably in the F_1 plants derived from crosses between different accessions of wheat and wheatgrass and even in different cells and plants

of the same cross combination, pointing to the existence of some kind of genic control of chromosome pairing. These and other authors (Ohlendorf; 1952; Stebbins, Pun, 1953) found the chromosome pairing observable in the F_1 hybrids to be mainly due to autosyndesis. From this evidence it has been inferred that wheats and wheatgrasses have no whole genomes in common, and only some chromosome segments may show partial homology. However, no final conclusion has been reached concerning which of the three wheat genomes shares much homology with the composite genomes of *A. intermedium*. Thus, B. Vakar (Вакар, 1948) and S. Matsumura (1949) have suggested that one genome of the hexaploid *A. intermedium* is closely related to the wheat *B*-genome, while several other investigators (Dvořák, 1971; Evans, 1964; Gaul, 1953; Larson, Atkinson, 1970) have found that the chromosomes of the wheat *D*-genome show highest affinity to the wheatgrass chromosomes and are able to interchange them in the hybrids.

In the current paper we present an additional evidence derived from electrophoretic isoenzyme studies in support of the view that none of the three wheat genomes is represented unmodified in genomes of *A. intermedium* and *A. elongatum*, which seem to have been arisen through auto-alloploidy from a common autotetraploid wheatgrass and an unknown diploid precursor.

Material and methods

Plant material. The following taxa and seed accessions were involved in the present study:

I. *Agropyron intermedium* (Host) Beauv., syn. *Agropyron glaucum* (Desf.) Roem. et Schult.: 1) Accession C 75, a reproduction of Canadian origin with 2n = 42, received from Dr. Y. Cauderon, 2) Accession C 506, a reproduction of French origin with 2n = 42, received from Dr. Y. Cauderon, 3) Accession P 318, a reproduction from seeds collected at Provalsk steppe, Ukrainian SSR, received from Dr. Y. Prokudin, 4) Accession P 378, a reproduction from seeds originating near Tiraspol, Moldavian SSR, received from Dr. Y. Prokudin, 5) Accession 1196, an original sample collected in Rumania, received from the Botanical Garden of University in Iasi (Rumania).

II. *Agropyron intermedium* var. *villosum* Schmahl., syn. *Agropyron trichophorum* (Link) Richt.: 1) Accession J 1/70, an original sample collected by the author in Tajik SSR, 2) Accession P I. 210990, a reproduction of seeds originating from Afganistan, received from the U.S. Department of Agriculture, Pullman, Washington (U.S.D.A.), 3) Accession P.I. 222961, a reproduction from Iran, received as *A. intermedium* from the U.S.D.A., 4) Accession P 319, a reproduction from seeds collected at Provalsk steppe, Ukrainian SSR, received from Dr. Y. Prokudin.

III. *Agropyron elongatum* (Host) Beauv., syn. *Agropyron rigidum* (Schrad.) Roem. et Schult.: 1) Accessions C 746 and C 747, reproductions of presumably USSR origin with 2n = 70 for C 747, received from Dr. Y. Cauderon, supplied by Dr. N. V. Tzizin, 2) Accession C 721, a reproduction of Bulgarian origin with 2n = 70, received from Dr. Y. Cauderon, 3) Accession C 1, a reproduction of Canadian origin, received from Dr. Y. Cauderon, 4) Accession K 149, a reproduction of seeds originating from Stavropol Kraij, USSR, received from the Botanical Garden of Stavropol, 5) Accession 1316, a reproduction of seeds of presumably Rumanian origin, received from the Botanical Garden of Vacratot (Hungary), 6) Accession P.I. 172688, a reproduction of Turkish origin, received from USA under the name *A. intermedium*. Although indistinguishable from the hexaploid *A. intermedium* in spike characters, the plants of this accession proved to be non-rhizomatous, indicating that they should be taxonomically treated as belonging to *A. elongatum*, 7) Accession MSU 36-10, provided by Dr. J. R. Schaeffer of Montana State University, USA; cytologically a hexaploid with 2n = 42, originally received from the Saratov Agricultural Institute, USSR.

IV. *Agropyron* sp., Accession C 300 of French origin, received from Dr. Y. Cauderon as a diploid race of *A. elongatum* with 2n = 14.

V. *Triticum araraticum* Jakubz., Accession K-30216, a reproduction from Nakhitshevan ASSR, received from Vavilov Institute of Plant Industry (Leningrad).

VI. *Triticum aestivum* (L.) Thell. ssp. *macha* (Dek. et Men.) Mac Key, syn. *Triticum macha* Dek. et Men., Accession K-28165, a reproduction from the Georgian SSR, received from Vavilov Institute of Plant Industry (Leningrad).

Biochemical methods. Etiolated 5 to 7 day old seedlings were individually crushed each in 0.2 ml of cold homogenization buffer, containing 0.05 M tris-hydroxymethylaminomethane (Tris), 0.035 M ascorbic acid, 1 mM EDTA-Na_2Mg and 1 mM $Na_2S_2O_5$. After removal of cell debris about 20—40 mg of sucrose and 3—5 mg of Sephadex G-200 were added to the homogenates which were immediately subjected to electrophoresis in polyacrylamide gel slabs as described previously (Jaaska, 1972). After electrophoresis, the gels were stained for acid phosphatase, esterase, leucine aminopeptidase, glutamate dehydrogenase and sorbitol dehydrogenase activities, using common histochemical procedures (Jaaska, 1972).

The gels were photographed in a diffuse transmitting light for a permanent record. The enzymograms were photographically enlarged to a convenient length, using isoenzyme bands of a wheat control sample on the same slab as standard markers.

Results

Acid phosphatases. Fig. 1 presents examples of enzymograms for selected accessions of *Agropyron intermedium, Agropyron elongatum* and *Agropyron* sp. of different geographic origin. Enzymograms for *Triticum araraticum* and *Triticum aestivum* ssp. *macha* are also given for comparison. To facilitate description, the bands are designated by the distances of migration from the origin to the anode expressed in arbitrary units on the scale in the figure. Each enzymogram designated by an arabic number is obtained from tissue extract of a single individual (seedling).

The staining area remaining near the origin at the site of sample application is presumably due to structurally bound enzyme aggregates which fail to migrate in the gel matrix. This staining was found in almost all extracts, except in those from the diploid *Agropyron* sp. (enzymograms *33—36*).

The next staining area in the phosphatase enzymograms of polyploid wheatgrasses appears as a band at the migration distance of about 1.6—1.8. Some accession populations revealed this band in most individuals (enzymograms *1—5* for Accession P 378), while in other populations many individuals lacked it, as seen in Fig. 1. Its electrophoretic mobility was found to vary slightly, revealing intrapopulation polymorphism with either two closely spaced bands in some individuals (enzymogram 10) or only one of the two (or more) electrophoretic variants in others. This phosphatase band was revealed to occur with varying frequency in accessions of all the three taxa of polyploid wheatgrasses we tested, but was not found in the diploid wheatgrass (enzymograms *33—36*).

The most characteristic for the polyploid wheatgrass taxa are the four successive major phosphatase isoenzymes of intermediate electrophoretic mobility at migration distances from 2.2 to 3.0. The segregation observed among the wheatgrass individuals in accessions suggests that the four major isoenzymes behave as consisting of two doublets of independent genetic control. Individuals in several accessions of *A. intermedium* (enzymograms *1—5*) as well as of *A. elongatum* (enzymograms *18—21, 23—26*) revealed only a slower-moving doublet of isoenzymes designated as a doublet *S*. The fastest band of this doublet at the migration distance of about 2.5 is labelled as S_1, and the slowest at about 2.2—S_2. Some other accessions consisted mostly of individuals which lacked the doublet *S* and revealed instead of it a doublet of phosphatase bands of somewhat higher mobility. This faster doublet is designated by the letter *F*, and the two isoenzyme bands located at migration distances 3.0 and 2.7 are referred to as F_1 and F_2, respectively. Acid phosphatase phenotypes with only fast isoenzyme doublet *F* were encountered with high frequency in several accessions of rhizomatous *A. intermedium* (for example in Accessions

J $^{1}/_{70}$, P. I. 210990), and also in some accessions of non-rhizomatous *A. elongatum* (for example, Accession P. I. 172688, enzymograms *13—17* in Fig. 1).

In addition to the two "homozygous" acid phosphatase phenotypes with only one major isoenzyme doublet, many populations of both *A. intermedium* and *A. elongatum* involved individuals exhibiting apparently "heterozygous" phosphatase phenotypes with both the *S* and *F* isoenzyme doublets in enzymograms. The examples of such heterozygous phenotypes characterized by the four successive phosphatase isoenzymes (S_1, S_2, F_1, and F_2) are seen in enzymograms *7*, *10*, *11*, *16*, and *28* in Fig. 1.

The frequency of individuals with phenotypes heterozygous for phosphatase isoenzyme doublets varied widely among the different accessions studied. Many accessions revealed no or only few individuals with the heterozygous phosphatase phenotype, while some accessions (for example, the Accessions C 721, C 746 and C 747) involved a high percent of heterozygous individuals.

In most accession populations studied heterozygous phosphatase phenotypes appeared as consisting of four successive isoenzyme bands of equal or only slightly different staining intensity, indicating a roughly equal activity of individual isoenzymes. However, in some accessions more complex variation of isoenzymes in heterozygous individuals was observed. For example, in Accession C 721, some individuals lacked one of the four major isoenzymes (as in enzymogram *30*) or revealed decreased activity of several major isoenzymes (evidenced by comparatively fainter staining intensity, as in enzymograms *31* and *32*). Some accessions revealed individuals with slightly shifted electrophoretic variants of major isoenzymes. For example, the two major bands, S_1 and S_2, in enzymogram *29* (Fig. 1) each actually consists of two very closely spaced sub-bands which were readily distinguishable in original enzymograms, but appear fused together in the photograph. A small shift in the mobility of both isoenzymes of the doublet *S* is also seen in enzymograms *24—25*. In some other individuals only one of the two *S*-isoenzymes was electrophoretically shifted.

These examples indicate a more complex pattern of genetic control of individual isoenzymes in some genotypes of the polyploid wheatgrass which needs more detailed further study to disclose its nature. However, as a rule, the electrophoretic mobility of the four major phosphatase isoenzymes, F_1, F_2, S_1, and S_2, and its variation, proved to be similar for the most of the studied accessions of *A. intermedium* and *A. elongatum* which could not be distinguished on the basis of their acid phosphatase electrophoretic patterns. From this evidence it follows that the two polyploid wheatgrass species must involve closely related basic genomes which carry cistrons for the same major types of phosphatase isoenzymes.

Enzymograms *6* and *22* in Fig. 1 present acid phosphatase pattern of *Triticum araraticum* in comparison with those for *A. intermedium* and *A. elongatum,* respectively, obtained after electrophoresis on the same polyacrylamide gel slab. The most characteristic for the phosphatase pattern of *T. araraticum* is a fast-moving major band at the migration distance of about 3.5—3.9. This broad band, actually consisting of two closely-spaced, fusing sub-bands, was previously shown (Jaaska, 1969) to be under the genetic control of the genome *B* contributed by the diploid species *Aegilops speltoides*. Comparison of enzymograms in Fig. 1 shows that the two wheatgrass species possess only a comparatively weak band (or two bands) at the migration distance approximately (but not exactly) coinciding with that of the major phosphatase band controlled by the wheat *B*-genome. This could probably not yet be taken as a firm evidence

of the presence of the *B*-genome in wheatgrasses, since a similar weak band was also observed in many diploid *Aegilops* and *Secale* species.

A doublet of weakly stained minor phosphatase isoenzymes of unknown genome control seen in the enzymograms of *T. araraticum* approximately coincide with the major doublet *S* of wheatgrasses. This coincidence in the electrophoretic mobility seems to be fortuitous.

The acid phosphatase pattern of the hexaploid wheat (enzymograms *12* and *27* in Fig. 1) has been interpreted (Jaaska, 1969) as consisting of three doublets of major isoenzymes, each genetically controlled by one of the three composite genomes of the hexaploid wheat. Comparison of enzymograms in Fig. 1 clearly shows that the electrophoretic mobilities of major phosphatase isoenzymes of wheatgrasses are distinctly different from those of the hexaploid wheat. Thus the four successive bands in wheat enzymograms which were shown (Jaaska, 1969, 1970) to be controlled by the wheat genomes *A* and *B* exhibit higher electrophoretic mobility extending from 2.9 to 3.9 in Fig. 1 than the fastest-moving doublet *F* in wheatgrass enzymograms. The wheatgrass doublet *S* isoenzymes are shifted towards higher electrophoretic mobility with regard to the bands of the slowest wheat phosphatase doublet (at about 2.0 and 2.4 in Fig. 1) which was previously shown to be under the genetic control of the genome *D* derived from the diploid precursor *Aegilops tauschii* Coss.

When taking into account previous evidence (Jaaska, 1969) that the polyploid wheats have retained their major phosphatase isoenzymes electrophoretically invariant and similar to those found in the contemporary representatives of the diploid genome donors then the results obtained here provide evidence in favour of the view that two of the three wheat genomes, *A*, and *D*, have not been involved in the origin of the polyploid wheatgrasses. The polyploid wheatgrasses, *A. intermedium* and *A. elongatum*, and the polyploid wheats seem to have independent origin involving different diploid precursors, since their composite genomes carry structurally divergent cistrons for major phosphatase isoenzymes as evidenced by the observed differences in electrophoretic mobilities.

Enzymograms *33* to *36* in Fig. 1 present acid phosphatase electrophoretic patterns for a diploid wheatgrass of uncertain systematic position. This diploid wheatgrass species of French origin has been considered (Cauderon, 1958; Simonet, 1935) as a diploid race of *A. elongatum* due to its morphological similarity with the decaploid *A. elongatum*. However, comparison of enzymograms in Fig. 1 clearly shows that the three major phosphatase isoenzymes characteristic of the diploid wheatgrass are electrophoretically different from those found in the polyploid *A. elongatum*. This implies that the genome of the diploid wheatgrass growing on the sandy dunes of the Western Mediterranean is not involved in the polyploid *A. elongatum*. The diploid wheatgrass studied here proved to be monomorphic with respect to acid phosphatase isoenzymes.

Esterases. Photographs of polyacrylamide gel slabs stained histochemically after electrophoresis for an esterase activity with 1-naphthyl acetate as substrate are presented in Fig. 2. The esterase enzymograms are more complex than those of acid phosphatase, showing the presence of numerous isoenzyme bands of differing staining intensity (e.g., activity) and electrophoretic mobility. As previously in the case of wheat and *Aegilops* esterases (Jaaska, 1969, 1971), the wheatgrass isoenzyme bands can be subdivided in two groups having intermediate and fast electrophoretic mobilities.

The esterases of intermediate electrophoretic mobility ranging from about 1.0 to 3.0 involve exceedingly variable number of bands amounting to up to 10 or more in some individuals. Comparison of enzymograms in

Fig. 2 readily reveals qualitative and quantitative differences between individuals belonging to the same accession population. This individual variation becomes more extensive when enzymograms for different accessions are compared. As a rule, accessions of *A. intermedium* showed more complex pattern of intermediate esterases than accessions of *A. elongatum* which often revealed only a cluster of closely spaced bands at about 1.8—2.5 (enzymograms *24—27*), resembling a similar cluster found in wheats (enzymogram *12*). Many individuals of *A. intermedium* and a few of *A. elongatum* possess a closely spaced doublet of isoenzymes at migration distances 2.8 and 3.8 (enzymograms *4—5* and *7—10*) which electrophoretically coincides with a similar doublet characteristic of *T. araraticum* (enzymogram *6*).

The series of fast-moving esterases with the migration distances ranging between 4.0 and 5.0 also shows individual intrapopulational variation in the band number and staining intensity. One of the fast-moving esterase isoenzymes at about 4.5 frequently found in enzymograms of *A. intermedium* and *A. elongatum* electrophoretically coincides with a band in wheat esterase enzymograms (*6* and *12* in Fig. 2). This wheat isoenzyme has been shown (Jaaska, 1969) to be controlled by the genome *A*. However, the fastest-moving wheat esterase at the migration distance of about 5.0—5.2 shown (Jaaska, 1969) to be specified by the genome *B* was only occasionally observed in wheatgrasses.

Many wheatgrass individuals revealed a triplet of fast-moving esterases which is characteristic of heterozygosity, allelic or genomic. Considerable quantitative variation in the staining intensity of individual isoenzymes in the triplets makes it rather difficult to estimate precisely the degree of heterozygosity and its genetic nature. Slight variation in the mobilities of closely spaced individual bands adds more complexity.

The esterase electrophoretic pattern of the diploid wheatgrass (enzymograms *32—34* in Fig. 2) is characterized by the presence of two major bands of intermediate mobility and of one fast-moving band. The electrophoretic mobility of the fast band is similar to that of the wheat esterase controlled by the genome *A*. The diploid wheatgrass studied here revealed similar esterase electrophoretic patterns with no individual variation in isoenzymes.

Other enzymes. In contrast to phosphatase and esterase which showed significant intraspecific polymorphism in the two polyploid wheatgrasses, several other enzymes proved electrophoretically invariable and identical for both species. The examples of such phylogenetically conservative enzymes are glutamate dehydrogenase (GDH), sorbitol dehydrogenase (SDH), and leucine aminopeptidase (LAP). One GDH, one SDH, and two closely spaced LAP bands found in enzymograms proved to be in common not only for all the wheatgrass taxa studied but also for the wheats. Similarly, wheats and wheatgrasses share several major anodal peroxidase isoenzymes, although variation was observed in some other bands. This evidence supports the view that *Triticum* and *Agropyron* species possess homoeologous genomes which have retained many genes still unchanged.

Discussion

The finding of the same five major acid phosphatase isoenzymes segregating in both polyploid wheatgrass species, the non-rhizomatous *A. elongatum* and the rhizomatous *A. intermedium*, clearly suggests that the two species are phylogenetically very closely related. Since the

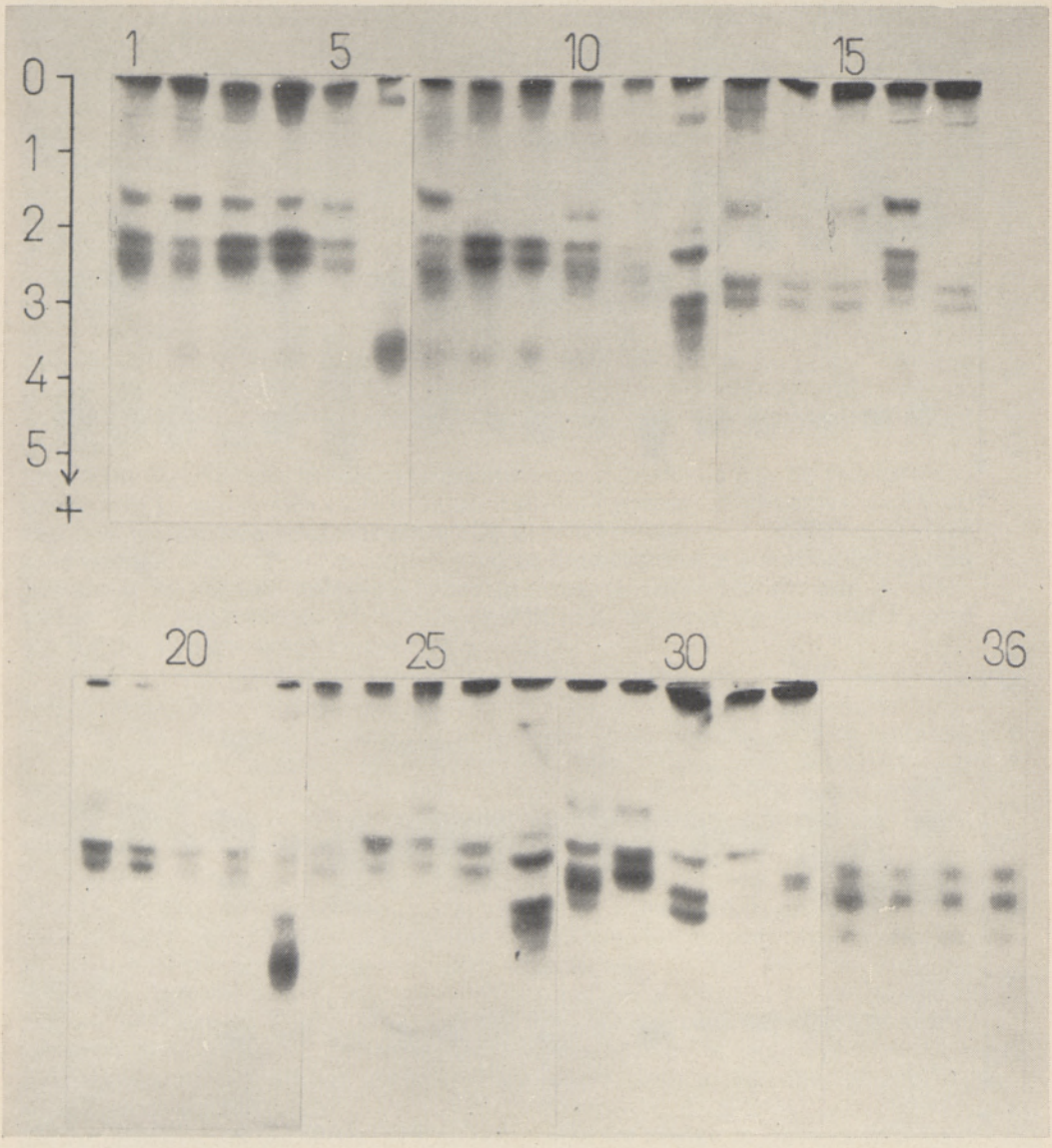

Fig. 1. Polyacrylamide gel electrophoretic patterns of acid phosphatase from etiolated seedlings of *Agropyron intermedium* (enzymograms *1—5* for the Accession P 378 and *7—11* for 1196), *Agropyron elongatum* (enzymograms *13—17* for the Accession P. I. 172688, *18—21* for 1316, *23—26* for K 149, and *28—32* for C 721), *Agropyron* sp. with 2n=14 (enzymograms *33—36* for the Accession C 300), *Triticum araraticum* K-30216 (enzymograms *6* and *22*), and *Triticum aestivum* ssp. *macha*, K-28165 (enzymograms *12* and *27*).

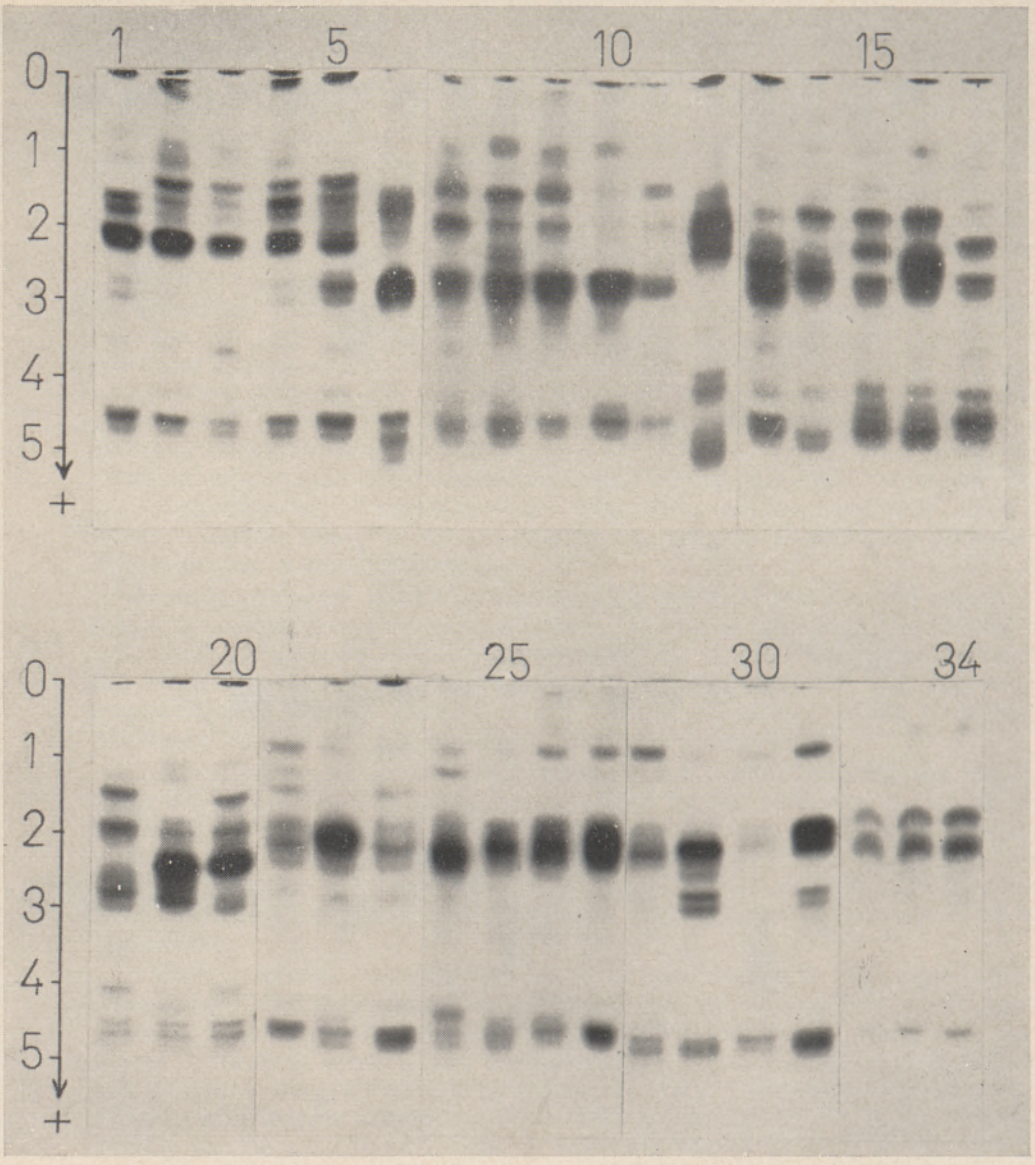

Fig. 2. Polyacrylamide gel electrophoretic patterns of esterase from etiolated seedlings of *Agropyron intermedium* (enzymograms *1—5* for the Accession P 318, *7—11* for 1196, *13—17* for P 378, and *18—20* for C 76). *Agropyron elongatum* (enzymograms *21—23* for Cl, *24—25* for K 140, and *28—31* for C 721). *Agropyron* sp. with 2n=14 (enzymograms *32—34* for C 300), *Triticum araraticum* K-30216 (enzymogram *6*), and *Triticum aestivum* ssp. *macha* K-28165 (enzymogram *12*).

structure of isoenzymes is strictly genetically controlled, our data suggest that the two species share closely related basic genomes, carrying similar cistrons for the observed major acid phosphatase isoenzyme types.

Our previous electrophoretic studies of acid phosphatase isoenzymes in the wheat group have shown (Jaaska, 1969; Jaaska, 1970) that the presence of a closely spaced doublet of phosphatase isoenzymes is characteristic of the diploid species. Of nine diploid *Aegilops* species eight were found (Jaaska, 1970) to possess two electrophoretically closely migrating major acid phosphatase isoenzymes. The diploid wheatgrass species studied here possessed even three electrophoretically distinct major phosphatase isoenzymes.

It was also found (Jaaska, 1969) that of the six major phosphatase isoenzymes in the hexaploid wheats one isoenzyme doublet has been contributed by the genome *A* of *Triticum monococcum* L., the second — by the genome *B* of *Aegilops speltoides* Tausch, and the third doublet — by the genome *D* of *Aegilops tauschii* Coss. This suggests generous additiveness, in the polyploids, of the isoenzymes controlled by the composite diploid genomes.

In the light of the above considerations, a hypothesis can be put forward according to which two closely related diploid precursor genomes, carrying cistrons for the two electrophoretically distinct doublets of acid phosphatase isoenzymes, *S* and *F*, have contributed to the origin of a common tetraploid precursor of the two polyploid wheatgrasses, *A. intermedium* and *A. elongatum*. For convenience of further description, we propose formula E_S and E_F for the two genomes respectively.

Two alternative possibilities concerning taxonomic treatment of the hypothetical diploid precursor should be considered. The first possibility is that the two genomes, E_S and E_F, have come from two genetically isolated, although closely related, diploid species. The second alternative is that only one diploid precursor species existed which, however, was polymorphous with respect to genes controlling two electrophoretically different doublets of major acid phosphatase isoenzymes.

Previously we have found (Jaaska, 1970) that among the ten diploid species of *Aegilops* seven are characterized by a particular, electrophoretically distinct phosphatase isoenzyme phenotype. No intraspecific variation in major acid phosphatase isoenzymes has been found in the diploids *Aegilops tauschii* and *T. monococcum*, except for a form of the wild wheat known as *T. urartu* Thum. (Jaaska, 1971a, b). Our recent unpublished study, however, has revealed intraspecific polymorphism of acid phosphatase isoenzymes in the diploid *Aegilops speltoides*, indicating the possibility of the second alternative.

In either cases the initial tetraploid precursor seems to have been arisen through autoploidy, since many accessions of the contemporary hexaploid *A. intermedium* and decaploid *A. elongatum* proved to be monomorphous or almost so, possessing in most individuals only one of the two phosphatase isoenzyme doublets, either *S* or *F*, and, consequently, only one of the two genomes.

The heterozygous genotypes characterized by the presence, in the seedlings, of both doublets of phosphatase isoenzymes, *S* and *F*, should then involve two different composite genomes, E_S and E_F, in the polyploid genome. They may have originated in two different ways. First, through introgressive hybridization in the ancient population, involving the initial autotetraploid, possessing one of the two genomes, with the diploid, carrying the second genome. The second possibility is hybridization at the tetraploid level, between two independently (either polytopically or diphyl-

etically) arisen autotetraploid forms, carrying different genomes, E_S and E_F.

No sufficient evidence is available at present to choose between the discussed alternatives, and the whole hypothesis about the common autotetraploid precursor to *A. intermedium* and *A. elongatum* needs further confirmation, since direct evidence for the genetic control of the two phosphatase isoenzyme doublets observed in the polyploid wheatgrasses by separate composite genomes, although highly plausible, is nevertheless lacking.

Most of the cytogenetic studies carried out with *A. intermedium* and *A. elongatum* have suggested in favour of their autoalloploid nature. Chromosome pairing studies in a polyhaploid plant of *A. intermedium* have lead D. R. Dewey (1962) to the preferential acception of segmental autoallohexaploid origin of this species with the proposed genome formula A_1A_2B. Similarly, Y. Cauderon (1958) has suggested the autoalloploid genome formula E_1E_2N for this species. Although rare trivalent and hexavalent chromosome associations were observed, both investigators considered strict autohexaploidy improbable and have suggested the involvement, in addition to two closely related diploid genomes, of a third more divergent genome in the origin of the hexaploid *A. intermedium*.

Our phosphatase isoenzyme data do not exclude the presence of the third more divergent genome in the polyploid wheatgrasses. It is quite possible that either the isoenzyme observed at the migration distance 1.6—1.8 or a weak band (or bands) at about 3.5—3.8, or both, are controlled by a separate genome.

The results concerning acid phosphatase and esterase electrophoretic patterns seem to suggest that none of the three wheat genomes is represented in unmodified form in the genomes of intermediate and elongate wheatgrasses. The involvement of the genomes *A* and *D* can be excluded due to distinctness of the phosphatase isoenzymes they control from those found in the polyploid wheatgrasses, while the evidence concerning the presence of genome *B* in wheatgrasses is less convincing. Possible suppression of some genes in the polyploid wheatgrass genome similar to that found (Jaaska, 1971) for the *A*-genome phosphatase loci in the tetraploid wheat *T. araraticum* Jakubz. should be considered and can well explain the observed reduced activity of wheatgrass phosphatase isoenzymes at 3.5—3.8 which are electrophoretically similar to those of *Ae. speltoides*. Our data, thus, dot not exclude the possibility of involvement of some form either belonging or closely related to the polymorphous diploid species *Aegilops speltoides*, as the donor of the third genome in the hexaploid intermediate wheatgrass. The available isoenzyme data are clearly insufficient to disclose with certainty the nature of the third genome donor to the hexaploid wheatgrass and a further more thorough study is required.

Of the diploid species of *Aegilops* studied previously (Jaaska, 1970), only those of the *S*-genome group, i. e. *Ae. sharonensis* Eig and *Ae. bicornis* (Forsk.) Jaub. et Sp., possessed a doublet of major acid phosphatase isoenzymes which proved to be electrophoretically closely similar to the doublet *F* of the wheatgrasses. However, the fast-moving esterase isoenzymes of the two *Aegilops* diploids were electrophoretically distinct (enzymograms not presented) from those found in wheatgrasses.

Thus, available isoenzyme data seem to indicate that the two basic genomes of the intermediate and elongate wheatgrasses, E_S and E_F, are genetically distinct from any genome of the contemporary diploid *Aegilops* species. At the same time, the electrophoretic studies of some other enzymes, dehydrogenases, peroxidase, leucine aminopeptidase, as well as coinciding

electrophoretic mobilities of several isoenzymes clearly suggest the existence of homologous loci preserved unchanged or similar in the genomes of wheats and wheatgrasses. This is in one line with the cytogenetic data which indicate a partial homology (or homoeology) and overall karyotypic similarity between the *Triticum* and *Agropyron* chromosomes.

On the basis of these considerations, it seems reasonable to imply that the *Triticum-Aegilops* group and the *Agropyron-Elymus* group have become divergent at the diploid genome level before the origin of polyploid species. The existence of several contemporary diploid and autotetraploid wheatgrass species supports such an approach.

Our present isoenzyme studies exclude the diploid wheatgrass species growing on sandy dunes of the Western Mediterranean and frequently misidentified as the diploid race of *A. elongatum* as the genome donor and precursor to the two polyploid wheatgrass species. The diploid wheatgrass should be treated as a separate species which is distinct from and phylogenetically not closely related to the polyploid *A. elongatum*. The taxonomic position of the diploid wheatgrass remains to be clarified.

Preliminary isoenzyme studies (unpublished) also argue against some other known diploid or autotetraploid wheatgrasses, e. g., *A. cristatum* (L.) Gaertn. and *A. caespitosum* C. Koch, to be involved in the origin of the intermediate and elongate wheatgrasses.

Although the precursor species remain undiscovered in the present study, we are tentatively inclined to accept the hypothesis according to which an autotetraploid wheatgrass and an unknown diploid species have given rise to a common hexaploid precursor to both *A. intermedium* and *A. elongatum*. Our isoenzyme data suggest phylogenetic closeness and common composite genomes for the two polyploid wheatgrasses. This is in one line with the recent cytogenetic evidence of V. Lyubimova (Любимова, 1970) who has reported good pollen fertility and high degree of meiotic chromosome conjugation in the interspecific hybrids between the hexaploid *A. intermedium* and the decaploid *A. elongatum*.

Plants of various ploidy level extending from hexaploid to decaploid forms have been found (Schulz-Schaeffer, Jura, 1967) in some populations of *A. elongatum*. Acid phosphatase and esterase isoenzyme electrophoretic patterns of a hexaploid form of *A. elongatum* studied here were found to be qualitatively similar to those of the decaploid accessions. This evidence implies that the two additional genomes in the decaploid form contribute no new genetic information to the hexaploid form but only duplicate the genetic information already available in the hexaploid. If we, following the proposal of G. L. Stebbins and F. T. Pun (1953) for *A. intermedium*, designate the genome composition of the hexaploid *A. elongatum* as E_1E_2N, then the genome formula for the decaploid form should be designated as $E_1E_2E_3E_4N$.

It is not excluded that the species delimitation in the *intermedium-elongatum* group of wheatgrasses mainly on the basis of absence or presence of rhizomes should be revised. The hexaploid plants may prove belonging to the same polymorphic biological species which is segregating for genes controlling the formation of rhizomes (in *A. intermedium*). In our nursery we have observed plants of intermediate wheatgrass with widely varying degree of rhizome formation and spikelet pubescence. The need for more thorough biosystematic studies in the *intermedium-elongatum* species complex is evident.

Summary

Acid phosphatase, esterase, glutamate dehydrogenase, sorbitol dehydrogenase, leucine aminopeptidase and peroxidase isoenzyme patterns in the two wheatgrass species, *Agropyron intermedium* (Host) Beauv. and *A. elongatum* (Host) Beauv., were studied by the use of polyacrylamide gel electrophoresis and were compared with those in wheats and some other related species.

The two polyploid wheatgrasses exhibited significant intraspecific genetic polymorphism in acid phosphatase and esterase isoenzymes. The same five major acid phosphatase isoenzyme types were found segregating in both wheatgrass species, suggesting their close phylogenetic relatedness and the presence of common basic composite genomes. The intermediate wheatgrass revealed more extensive individual variation in esterase isoenzymes than the elongate wheatgrass. The hexaploid and decaploid plants of *A. elongatum* were found to possess qualitatively similar acid phosphatase and esterase isoenzyme patterns, indicating that the two additional genomes in the decaploid add no new genetic information for isoenzymes but only repeat that already present in the hexaploid genome.

Both wheatgrasses, *A. intermedium* and *A. elongatum,* have suggested to possess a common autoallohexaploid precursor which has arisen from an autotetraploid wheatgrass and an unknown diploid species as progenitors. The genome formula for the hexaploid is designated as E_1E_2N and that for the decaploid *A. elongatum* — $E_1E_2E_3E_4N$.

The acid phosphatase and esterase isoenzyme electrophoretic patterns of the diploid wheatgrass species growing on sandy dunes of the Western Mediterranean proved to be distinctly different from those of the decaploid *A. elongatum,* suggesting that the two species are not phylogenetically closely related and should taxonomically be treated as separate species.

Acid phosphatase isoenzymes of the polyploid wheats controlled by the genomes *A* and *D* were found to be electrophoretically distinct from those characteristic of *A. intermedium* and *A. elongatum.* However, several enzymes and isoenzymes were found to be electrophoretically similar in both wheats and wheatgrasses, indicating the residual homology, i. e. homoeology, of their genomes which have retained many genes unchanged and in common. A possibility is considered that the donor of the *N*-genome in the wheatgrasses may prove to be phylogenetically related to the wheat *B*-genome donor *Aegilops speltoides* Tausch.

Acknowledgements — Thanks are due to Dr. Yvonne Cauderon (Versailles, France), Dr. Douglas R. Dewey (Logan, USA), Dr. Jurgen R. Schaeffer (Bozeman, USA), and Dr. Yuri Prokudin (Kharkov, USSR) for seeds and to Dr. Nikolai Tzvelev (Leningrad, USSR) for a consultation about wheatgrass nomenclature. Skillful technical aid in performing electrophoresis of Miss Kai Luik and Miss Marje Raava is gratefully acknowledged.

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

Received
Jan. 13, 1972

VELLO JAASKA

ENSÜÜMIDE VARIEERUVUS JA FÜLOGENEETILISED SEOSED KÕRRELISTE PEREKONDADES *AGROPYRON* GAERTN. JA *ELYMUS* L.

I. *Agropyron intermedium* (Host) Beauv. ja *Agropyron elongatum* (Host) Beauv.

Resümee

Orasheintel *A. intermedium* ja *A. elongatum* tehti polüakrüülamiidgeelelektroforeesi meetodi abil kindlaks happelise fosfataasi ja esteraasi isoensüümide liigisisene geneetiline polümorfism. Kõik viis happelise fosfataasi põhiisoensüümi osutusid ühiseiks mõlemale orasheinaliigile, viidates nende lähedasele fülogeneetilisele sugulusele ja genoomsele struktuurile. Esteraasi isoensüümide varieeruvus oli *A. intermedium*'il suurem kui *A. elongatum*'il. Viimase heksaploidsete ja dekaploidsete vormide happelise fosfataasi ja esteraasi ensümogrammid olid kvalitatiivselt sarnased. Seega ei anna kaks dekaploidi lisagenoomi heksaploidile enam uut geneetilist informatsiooni, vaid ainult kordavad olemasolevat.

Oletatakse, et mõlemal orasheinaliigil oli ühine autotetraploidsest orasheinast ja tundmatust diploidsest liigist tekkinud, autoalloheksaploidne, genoomse koostisega E_1E_2N eellane. Dekaploidse *A. elongatum*'i genoomset koostist tähistatakse valemiga $E_1E_2E_3E_4N$.

Läänepoolsete Vahemeremaade liivaluidetel kasvava diploidse orasheina happelise fosfataasi ja esteraasi isoensüümid osutusid dekaploidse *A. elongatum*'i omadest elektroforeetiliselt erinevaiks, mis viitab lähedase fülogeneetilise suguluse puudumisele nende kahe liigi vahel.

Nisugenoomide *A* ja *D* poolt determineeritud happelise fosfataasi isoensüümid olid elektroforeetiliselt orasheinte omadest erinevad. Selgus, et mitmed ensüümid ja isoensüümid olid orasheintele ja nisudele siiski ühised. See viitab genoomide osalisele homoloogiale, s. o. homoeoloogiale, mitmete muutumatuna ja ühistena säilinud geenide näol.

Eesti NSV Teaduste Akadeemia
Zooloogia ja Botaanika Instituut

Toimetusse saabunud
13. I 1972

БЕЛЛО ЯАСКА

ИЗМЕНЧИВОСТЬ ФЕРМЕНТОВ И ФИЛОГЕНЕТИЧЕСКИЕ СВЯЗИ В РОДАХ ЗЛАКОВ *AGROPYRON* GAERTN. И *ELYMUS* L.

I. *Agropyron intermedium* (Host) Beauv. и *Agropyron elongatum* (Host) Beauv.

Резюме

У пыреев *Agropyron intermedium* и *A. elongatum* методом электрофореза в полиакриламидном геле обнаружен внутривидовой генетический полиморфизм изоферментов кислой фосфатазы и эстеразы. Пять основных изоферментов кислой фосфатазы оказались общими для обоих видов пырея, указывая тем самым на их тесное филогенетическое родство и общность составных геномов. Пырей средний выявил более значительную изменчивость изоферментов эстеразы по сравнению с пыреем удлиненным. Гексаплоидные и декаплоидные растения *A. elongatum* имели качественно одинаковые электрограммы кислой фосфатазы и эстеразы.

Высказывается гипотеза, что *A. intermedium* и *A. elongatum* имели общий аутоаллогексаплоидный предшественник с геномным составом E_1E_2N, который возник от аутотетраплоидного пырея и неизвестного диплоида. Для декаплоидного *A. elongatum* предлагается геномный состав $E_1E_2E_3E_4N$.

Диплоидный пырей, растущий на песчаных дюнах Западного Средиземноморья, четко отличается от декаплоидного *A. elongatum* по изоферментам кислой фосфатазы и эстеразы и не является его предшественником.

Изоферменты кислой фосфатазы полиплоидной пшеницы, контролируемые геномами *A* и *D*, оказались электрофоретически отличными от изоферментов, найденных у пыреев. Некоторые другие изученные ферменты и изоферменты, однако, оказались электрофоретически одинаковыми для пшениц и пыреев, что указывает на частичную гомологию их геномов, которые сохранили ряд генов неизмененными и общими.

Институт зоологии и ботаники
Академии наук Эстонской ССР

Поступила в редакцию
13/I 1972