EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. 20. KÖIDE
BIOLOOGIA. 1971, NR. 2

ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ТОМ 20
БИОЛОГИЯ. 1971, № 2

https://doi.org/10.3176/biol.1971.2.11

УДК 575.1/3+581.167 : 633.11

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BIOCHEMICAL STUDY OF PHYLOGENETIC RELATIONSHIPS IN THE *D*-GENOME GROUP OF THE GENUS *AEGILOPS* L.

The polyploid species of *Aegilops* L. can be arranged in two groups characterized by the presence of common genomes — *D* and C^u, respectively. The genome analysis by H. Kihara (1954) and karyomorphologic studies by M. S. Chennaveeraiah (1960) have revealed four polyploid species, *A. ventricosa* Tausch, *A. crassa* Boiss., *A. juvenalis* (Thell.) Eig and *A. cylindrica* Host, possessing the genome *D* derived from the diploid species *A. squarrosa* L. The other diploids suspected to be engaged in the origin of the above-mentioned polyploids are *A. caudata* L. (genome *C*), *A. comosa* Sibth. et Sm. (genome *M*), *A. uniaristata* Vis. (genome M^u) and *A. umbellulata* Zhuk. (genome C^u).

Our recent studies (Jaaska, 1970) have revealed distinct electrophoretic differences in phosphatase isoenzymes between most of the diploid species of *Aegilops* L. From these data it follows that acid phosphatase electrophoretic patterns can be used to identify the composite diploid genomes in the polyploid species of *Aegilops*, as it has been done in the case of the polyploid species of *Triticum* (Jaaska, 1969; Jaaska, Jaaska, 1970). The present communication deals with the variability of phosphatase and esterase isoenzymes in the *D*-genome group of the genus *Aegilops* L.

Materials and methods. Most of the seed samples were obtained from the World Collection of the Vavilov Institute of Plant Industry (Leningrad) through the courtesy of Dr. E. Migushova. Some collections were received from Prof. J. Grigoryev (Leningrad) and Dr. P. Tshopanov (Ashkhabad). Enzyme extracts from the etiolated seedlings were prepared and analyzed electrophoretically as described previously (Jaaska, 1969), except for performing electrophoresis in acrylamide gel slabs.

Results and discussion

Seven different strains of *Aegilops squarrosa* L. originated from Azerbaijan, Turkmenia and Uzbekistan all showed the presence of an electrophoretically constant doublet of major phosphatases and two faster-moving minor fractions, as seen in Fig. 1A. A slight shift in the mobility of the fastest minor band was revealed in one strain. In addition to these bands of general occurrence, a fraction of varying mobility and staining intensity, depending on the strain, can be seen.

Esterase enzymograms of *A. squarrosa* L. revealed a cluster of closely spaced fractions of intermediate mobility. These bands appear as fused together in the photograph in Fig. 1B. However, original enzymograms

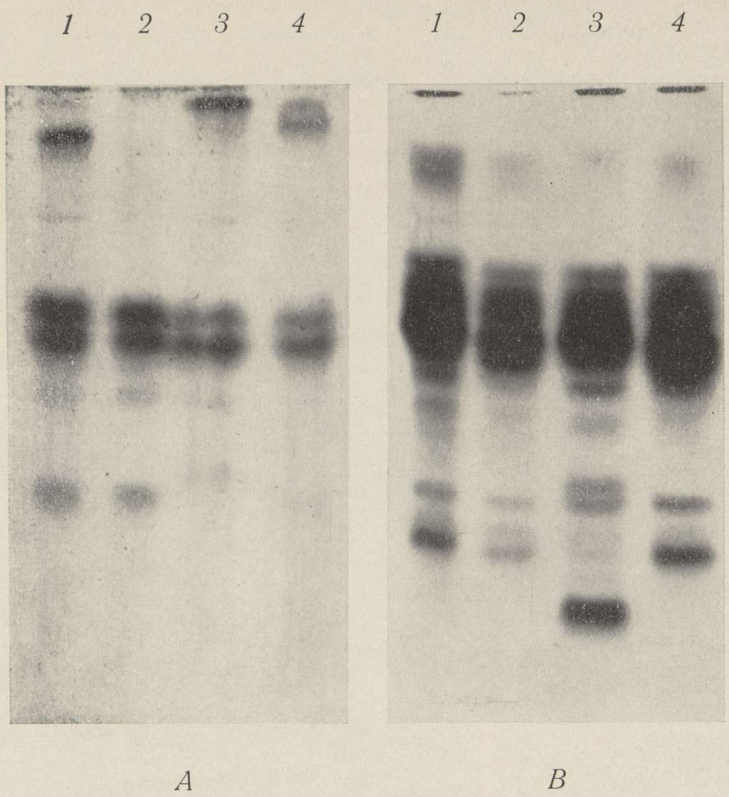

Fig. 1. Acid phosphatase (A) and esterase (B) electrophoretic patterns of *A. squarrosa* L. Enzymograms for the strains *G* (*1*), *K*-33 (*2*), *K*-160 (*3*) and *KD* (*4*).

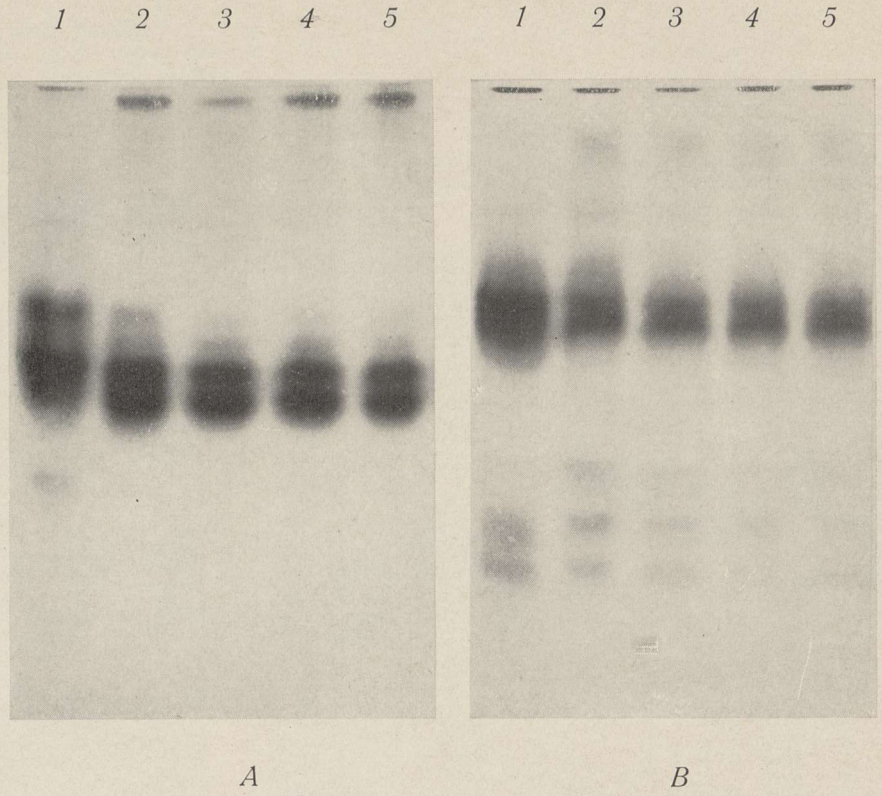

Fig. 2. Acid phosphatase (A) and esterase (B) electrophoretic patterns of *A. ventricosa* Tausch. Enzymograms for the strains *K*-245 (*1*), *K*-41 (*2*), *K*-196 (*3*), *K*-63 (*4*) and *K*-46 (*5*).

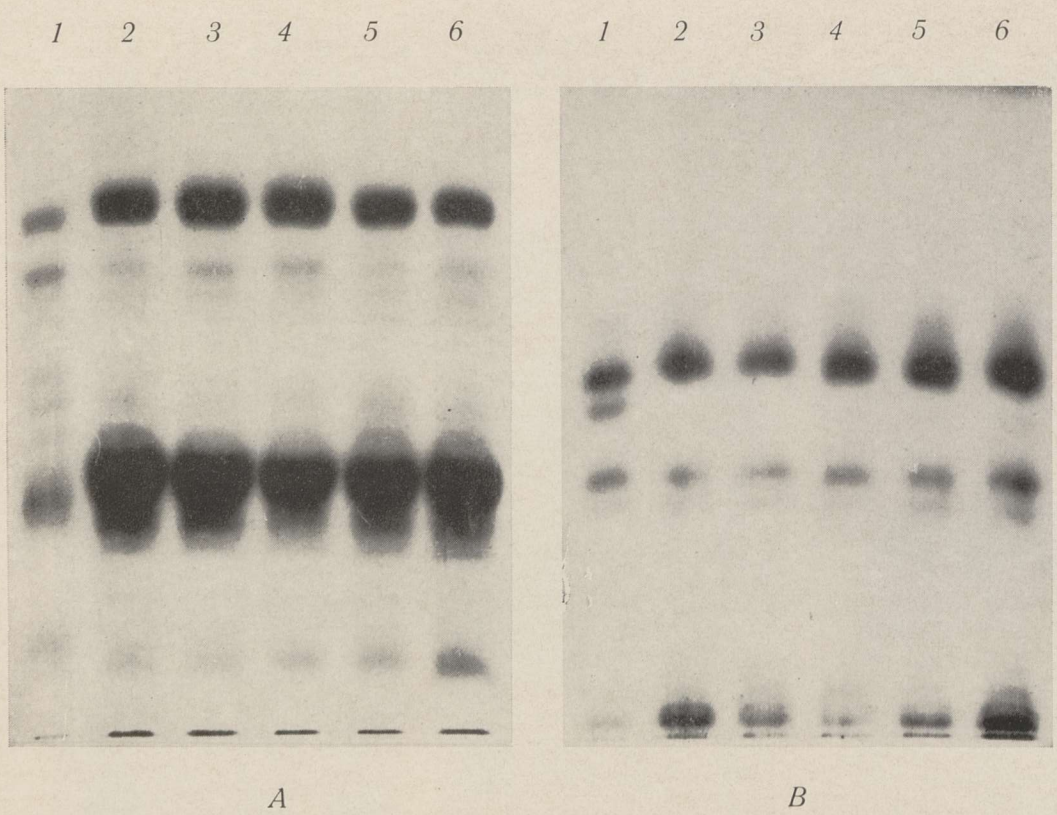

Fig. 3. Acid phosphatase (A) and esterase (B) electrophoretic patterns of *A. cylindrica* Host (*1—5*) and *A. crassa* Boiss (*6*). The origin of the strains: *1* — *G*, from Uzbekistan, *2* — from the Crimea, *3* — *K*-26, from Uzbekistan, *4* — *KD*, from Turkmenia, *5* — *K*-227, from Armenia, *6* — *K*-256, from Uzbekistan.

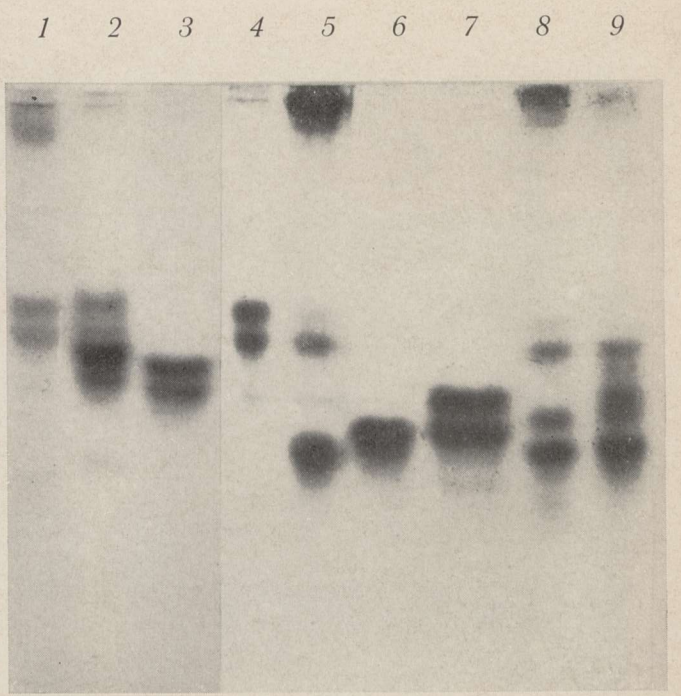

Fig. 4. Acid phosphatase electrophoretic patterns of the polyploid species and of their presumable diploid progenitors: *1 — A. squarrosa, 2 — A. ventricosa, 3 — A. uniaristata, 4 — A. squarrosa, 5 — A. cylindrica, 6 — A. caudata, 7 — A. comosa, 8 — A. crassa, 9 — A. juvenalis.*

enabled us to distinguish up to 6 fractions in this cluster. No qualitative variation among the seven strains of *A. squarrosa* studied was observed.

In addition, a series of fast-moving esterases showing a distinct intra-specific variation was present. The number of bands in this group varied from three to six, depending on the strain. Four characteristic esterase phenotypes found among the seven strains studied are presented in Fig. 1B.

Five strains of *A. ventricosa* Tausch of unknown origin deposited in the Vavilov Collection (Leningrad) from different sources (countries) all showed qualitatively identical acid phosphatase and esterase electrophoretic patterns (Fig. 2A and Fig. 2B, respectively), revealing no intra-specific isoenzyme variability. Phosphatase pattern was characterized by the presence of four closely successive bands of intermediate electrophoretic mobility. Esterase pattern consisted of two closely spaced major bands of intermediate mobility and of three fast-moving minor fractions.

Five different strains of *A. cylindrica* Host derived from Uzbekistan, Turkmenia, Armenia and Crimea all showed electrophoretically identical acid phosphatase patterns (Fig. 3A) consisting of three distinct bands of intermediate mobility and a diffuse staining area near the origin. Esterase enzymograms presented in Fig. 3B were also exactly similar for all the strains studied, revealing no intra-specific variation.

Acid phosphatase pattern of *A. crassa* Boiss. (three strains from Uzbekistan and Tajikistan) consisted of two doublets of major isoenzymes (enzymogram *6* in Fig. 3A). Phosphatase enzymogram for *A. juvenalis* (Thell.) Eig was very similar to that of *A. crassa*, showing the presence of the same four major bands, and, in addition, three weak, scarcely distinguishable bands closely spaced to the others. Esterase patterns of *A. crassa* (enzymogram *6* in Fig. 3B) and of *A. juvenalis* were almost identical, consisting of up to ten electrophoretically distinct isoenzyme bands.

Origin of the polyploids. *A. ventricosa* is considered (Kihara, 1954) to be an allotetraploid involving the *D*-genome of *A. squarrosa* and the *M*-genome of *A. uniaristata*. The synthetic amphidiploid of *A. squarrosa* × *A. uniaristata* proved to be (Matsumoto, Kondo, 1942; Matsumoto et al., 1957) morphologically very similar to *A. ventricosa*, and the hybrid between them showed good fertility and chromosome pairing. Acid phosphatase pattern in Fig. 4 is consistent with this view; the electrophoretic mobilities of four successive phosphatase isoenzymes in *A. ventricosa* (enzymogram 2) closely correspond to the major phosphatase isoenzymes in *A. squarrosa* (enzymogram 1) and *A. uniaristata* (enzymogram 3). However, it should be emphasized that in this case the enzymological data alone are not conclusive and sufficient to prove the origin of *A. ventricosa*, since the diploids of the *S*-genome group *A. longissima*, *A. sharonensis* and *A. bicornis*, as it is shown in a separate communication (Jaaska, 1970), possess phosphatase isoenzymes which are electrophoretically almost similar to those of *A. uniaristata*.

The origin of *A. ventricosa* as an amphidiploid between *A. squarrosa* and *A. uniaristata* is somewhat unexpected in view of disjunct contemporary areas of the three species (Eig, 1936). *A. ventricosa* is restricted to the western part of the Mediterranean Basin, occurring in Spain, Morocco, Algeria, and extending eastwards to Italy. *A. uniaristata* is only sporadically found in Italy, being more common in the Adriatic zone of Jugoslavia and at the shore of the Sea of Marmara in Greece. *A. squarrosa* has a wide distribution area ranging from Iran, Iraq and Transcaucasia through Turkmenia eastwards up to South-East Kazakhstan. Thus, the contem-

porary areas of *A. squarrosa* and *A. uniaristata* do not overlap. To explain the above amphidiploid origin of *A. ventricosa*, one must assume that *A. squarrosa* and *A. uniaristata* (or its precursor form) had overlapping areas in ancient times. The present distribution pattern of the three species seems to be the result of the eastward migration of *A. squarrosa* and the westward distribution of *A. uniaristata* and *A. ventricosa*, the latter being a more succesful colonizer.

According to Kihara (1954) and Chennaveeraiah (1960), *A. crassa* contains the genome *D* of *A. squarrosa* and the genome M^{cr} of unknown origin, suspected to be derived from *A. comosa*. Both the tetraploid and hexaploid forms of *A. crassa* are known, the hexaploid form being suggested (Kihara et al., 1959; Chennaveeraiah, 1960) to contain two *D*-genomes and a *M*-genome.

Similarly, *A. cylindrica* has been suggested (Kihara, 1954; Chennaveeraiah, 1960) to be an amphidiploid between *A. caudata* and *A. squarrosa*. Both the genome *C* of *A. caudata* and the genome *D* of *A. squarrosa* were shown (Kihara, Matsumura, 1941; McFadden, Sears, 1946) to be present in *A. cylindrica*. Recently, this conclusion has found a biochemical confirmation derived from the electrophoretic study (Johnson, 1967) of alcohol-soluble seed proteins and showing the *cylindrica* proteinogram to be the sum of the fractions found in *A. squarrosa* and *A. caudata*.

Acid phosphatase electrophoretic patterns presented in Fig. 4 are in general agreement with the involvement of the genome *D* in *A. cylindrica* and *A. crassa*, showing the presence of a characteristic doublet of *squarrosa*-phosphatases in both polyploids. The comparison of enzymograms *5*, *6*, *7* and *8* in Fig. 4 suggests that faster-moving isoenzymes in *A. crassa* and *A. cylindrica* can be derived from the diploids *A. comosa* and *A. caudata*, respectively. It should be noted, however, that the faster-moving phosphatase bands in the polyploid patterns, compared with those in the diploids, showed a small but distinct shift toward higher electrophoretic mobility. The reason for this shift is not clear, but it is possible that there is some intra-specific variation in the electrophoretic mobility of acid phosphatase isoenzymes within the diploids *A. caudata* and *A. comosa*, since so far we have been able to study only two strains of unknown origin for both species, deposited in the Vavilov Collection from Cambridge. We have observed small shifts in the electrophoretic mobilities of major phosphatase isoenzyme doublets among the diploid wheats and *A. umbellulata* (unpublished data). However, since no other diploid *Aegilops* species possess electrophoretically suitable phosphatase isoenzymes to be suspected as the donors of the second genome in the two polyploids (see Jaaska, 1970), *A. comosa* and *A. caudata* can be considered as the most probable genome donors.

The presence, in the hexaploid *A. juvenalis*, of all the major phosphatase and esterase isoenzymes found in *A. crassa*, highly suggests that the genome DM^{cr} *of A. crassa* is present in *A. juvenalis* and that the tetraploid form of *A. crassa* can be considered as a probable precursor species. The origin of the third genome remains finally unsolved in the present study, since our preliminary enzymological data do not provide conclusive evidence. Phosphatase enzymograms in Fig. 5 seem to allow the involvement of the genome C^u of *A. umbellulata* in the origin of *A. juvenalis*, as it has been suggested by Kihara et al. (1959) on the basis of similarities in the glume characters and later supported by Chennaveeraiah (1960) on karyomorphological grounds. However, several esterase isoenzymes (Fig. 5B), highly active in *A. umbellulata*, are absent in *A. juvenalis*.

Our data clearly demonstrate that acid phosphatase patterns of the polyploid *Aegilops* species generally comprise a sum of isoenzymes found in the contemporary polyploid species which have contributed their genomes to the polyploids. Only small shifts in the electrophoretic mobility of faster-moving isoenzymes were observed. In the case of esterases, however, the disappearance from the polyploid of some isoenzymes found in the diploids was observed. The comparison of enzymograms in Fig. 1B, 2B ja 3B shows that several esterase isoenzymes characteristic of the diploid *A. squarrosa* are absent or appear faint in the polyploids. At present, we have no experimental evidence to explain the observed non-additiveness of esterase isoenzymes in the polyploids.

The observed constancy of the isoenzyme electrophoretic patterns in the polyploid species (*A. cylindrica*, *A. ventricosa* and *A. crassa*) is unexpected in several respects. Theoretically, genetic variability must be increased in the polyploids, since the duplicated loci can accumulate even deficient mutations without any or sufficient harmful phenotypic effect due to the compensating action of the second set of loci. The absence of intraspecific variability in esterase patterns of the polyploids is especially interesting in view of the obvious variation observed in the diploid *A. squarrosa*. From the data it seems to follow that only few strains, if not a single one, of *A. squarrosa* have given rise to initial constant amphidiploid forms of a polyploid. The constancy of phosphatase and esterase isoenzyme patterns of *A. cylindrica* throughout its large distribution area from the Crimea through Transcaucasia to Central Asia seems to suggest the origin of the contemporary tetraploid species by the colonization of an initial amphidiploid without further mutational divergence in esterase or phosphatase loci. This evidence presumably also suggests a comparatively recent origin of the polyploids, which have not yet had time to accumulate mutations in the esterase loci, as has done their more ancient diploid progenitor, *A. squarrosa*.

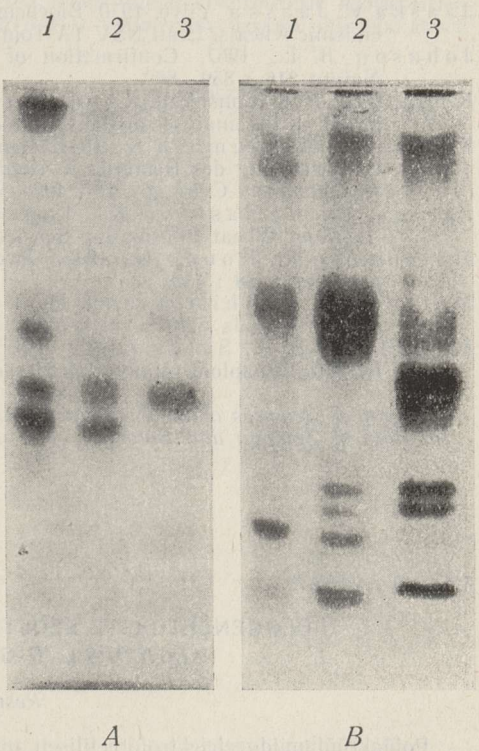

Fig. 5. Acid phosphatase (A) and esterase (B) electrophoretic patterns of *A. crassa* Boiss. (*1*), *A. juvenalis* (Thell.) Eig (*2*) and *A. umbellulata* Zhuk. (*3*).

Summary

Acid phosphatase isoenzyme composition of the polyploids presented a sum of isoenzymes found in the diploids presumed, on the basis of cytogenetic and karyomorphologic data, to be the genome donors and parental species, thus confirming the amphidiploid origin of the polyploids. Several

esterase isoenzymes characteristic of the diploids were absent or appeared faint on the enzymograms of the polyploids.

No intra-specific variation in phosphatase or esterase isoenzyme composition was observed in the polyploids, whereas a distinct polymorphism of esterases was found in the diploid species, *A. squarrosa* L.

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Received
March 5, 1970

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FÜLOGENEETILISTE SEOSTE BIOKEEMIAST PEREKOND *AEGILOPS* L. *D*-GENOOMI RÜHMAS

Resümee

Polüakrüülamiidgeelelektroforeetiliselt uuriti polüploidide *Aegilops ventricosa* Tausch, *A. crassa* Boiss., *A. juvenalis* (Thell.) Eig ja *A. cylindrica* Host ning nende diploidsete eellaste *A. squarrosa* L., *A. caudata* L., *A. comosa* Sibth. et Sm. ja *A. umbellulata* Zhuk. happelise fosfataasi ja esteraasi isoensüümset koostist.

Polüploidide happelise fosfataasi ensümogrammid kujutavad endast diploidsetele eellastele omaste isofermentide summat ning kinnitavad tsütogeneetilisi ja karüomorfoloogilisi andmeid polüploidide alloploidse päritolu kohta. Mõned diploidsetele eellastele iseloomulikud esteraaside isoensüümid polüploididel aga puuduvad või on nõrgenenud.

Diploidil *A. squarrosa* L. täheldati esteraaside koostises selgepiirilist liigisisest polümorfismi, mida ei esinenud polüploidsetel liikidel.

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Toimetusse saabunud
5. III 1970

ВЕЛЛО ЯАСКА

БИОХИМИЧЕСКОЕ ИЗУЧЕНИЕ ФИЛОГЕНЕТИЧЕСКИХ СВЯЗЕЙ В ГРУППЕ *D*-ГЕНОМА РОДА *AEGILOPS* L.

Резюме

Методом электрофореза в полиакриламидном геле изучался изоферментный состав кислой фосфатазы и эстеразы полиплоидных видов *Aegilops ventricosa* Tausch, *A. crassa* Boiss., *A. juvenalis* (Thell.) Eig и *A. cylindrica* Host, а также их диплоидных предшественников *A. squarrosa* L., *A. caudata* L., *A. comosa* Sibth. et Sm. и *A. umbellulata* Zhuk.

Изученные виды эгилопсов четко различаются по энзимограммам кислой фосфатазы. Изоферментный состав кислой фосфатазы полиплоидов, который является суммой изоферментов диплоидных предшественников, подтверждает их аллополиплоидную природу, выявленную цитогенетическими и кариоморфологическими исследованиями. У полиплоидов некоторые изоферменты эстеразы, характерные для современных диплоидов — доноров геномов, отсутствуют или выражены слабо.

Образцы полиплоидов различного происхождения имели константные изоферментные составы, тогда как у диплоида *A. squarrosa* L. обнаружен четкий внутривидовой полиморфизм в составе эстераз.

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Поступила в редакцию
5/III 1970