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ELECTROPHORETIC STUDIES OF SEEDLING PHOSPHATASES, ESTERASES AND PEROXIDASES IN THE GENUS *TRITICUM* L.

Our previous studies (Jaaska and Jaaska 1968a,b) revealed clear-cut differences in polyacrylamide gel electrophoretic patterns of phosphohydrolases from wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) tissues. This finding showed the occurrence of species-specific diversity of the enzyme among the cereals. It also suggested that, during the phylogenetic differentiation of the grass family, considerable divergence of genetic systems determining the structure of phosphohydrolases had occurred.

The above considerations emphasized the desirability of studying the degree of enzyme diversity among the different taxonomic units of cereals. Furthermore, it was hoped that comparative electrophoretic studies of enzymes would provide additional evidence for elucidating the phylogenetic relationships between the taxa concerned.

In recent years, the use of proteins in evolutionary studies has received extensive treatment witnessed by numerous papers published as materials of several symposions (cf. Leone, 1964; Bryson, Vogel, 1965, etc.) or as separate theoretical works and reviews (Blagoveschensky, 1966; Ingram, 1963; Sibley, 1962; Vegotsky, Fox, 1962; Zuckerkandl, Pauling, 1965, etc.).

The applicability of protein characteristics in systematic and phylogenetic studies follows from one of the most fundamental concept of biochemical genetics, according to which the primary structure of proteins, i. e., their amino-acid sequence is determined by the nucleotide sequences of corresponding structural genes arranged along the DNA molecule (Anfinsen, 1959). The primary structure, in its course, determines other physico-chemical and biological properties of a protein.

Therefore, the knowledge of the primary structure provides the most direct and valuable characteristic of biological specificity and genetic individuality of protein. However, the determination of amino acid sequences is still a very time-consuming procedure, and at present it is

applied for only a limited number of proteins which can be comparatively easily separated in pure state. For this reason, other protein characteristics, such as their immunologic, enzymatic or molecular properties, which are more easily determined, have found much wider application in phylogenetic studies. Due to the development of new gel-electrophoretic methods for protein fractionation, electrophoretic properties of proteins have found especially wide application in various fields of population genetics and biosystematics (see for references Boulter et al., 1966; Leone, 1964; Shaw, 1965).

Protein electrophoresis has already found several successful applications in taxonomic and phylogenetic studies of cereals and grasses. Hall (1959) first applied agar gel electrophoresis to study the salt-soluble seed proteins from an artificial amphidiploid *Triticum aestivum* × *Secale cereale* and its parental species, showing the additiveness of parental proteins in the amphiploid pattern. Seed proteins of a spontaneous amphidiploid *Stipa viridula* × *Oryzopsis hymenoides* and its parental species were studied by Hall and Johnson (1963). Coulson and Sim (1964) compared seed proteins of various species of wheat and closely related genera by means of starch-gel electrophoresis. Recently, Johnson and Hall (1965), and Johnson (1967a,b,c) employed polyacrylamide gel electrophoresis of alcohol-soluble seed proteins to study genome homologies among various species of the genera *Aegilops* L. and *Triticum* L. and to determine possible genome donors for the polyploid species. Hunziker (1967) used protein electrophoresis combined with cytogenetic and geobotanic data to reveal phylogenetic affinities and differentiation of the *Agropyron scabriglume* complex in South America.

Theoretically, as it has already been pointed out by several authors (Hart, Bhatia, 1967; Boulter et al., 1966), electrophoretic studies of enzyme patterns in some cases may provide even better data for systematic and genetic studies than the study of proteins of an unknown nature. The present communication is primarily concerned with the study of polyacrylamide gel electrophoretic patterns of soluble acid phosphatases, esterases and peroxidases in the etiolated seedlings of the diploid, tetraploid and hexaploid wheats. In addition, the enzyme patterns for some diploid species of *Aegilops* L. which can be considered as possible genome donors to polyploid wheats were investigated. Studies of enzyme patterns for other diploid and polyploid *Aegilops* species are in progress at present, and the results are expected to be published in a separate paper.

Materials and Methods

Plant Material. The seed samples used in this study were received from the World Collection of Vavilov All-Union Institute of Plant Industry (Leningrad), except the sample of *Triticum thaoudar* Reut. obtained from the Botanical Garden of the Academy of Sciences of the Armenian SSR (Yerevan), and the strains of *T. aestivum* L. received from the Plant Breeding Station in Jõgeva, Estonia. The botanical names of the taxa studied, collection numbers of strains and places of origin are listed in the Table.

Dehusked seeds were sterilized in a freshly prepared medium made by mixing of five volumes 96 per cent ethyl alcohol with one volume 30 per cent hydrogen peroxide. After 10 minutes the seeds were generously washed in several changes of distilled water and sown in Koch dishes on two sheets of filter paper saturated with 5 ml 2×10^{-4}M $CaSO_4$ solution. The seeds were germinated in the dark, in a thermostat at 25 °C for 4—5 days.

The List of Taxa, Collection Numbers and Places of Origin

Botanical names	Collection number	Place of origin
I. Diploid wheats		
T. boeoticum Boiss. ssp. *thaoudar balanssae*	K-40117	Iraq
T. thaoudar Reut.	—	Armenia
T monococcum L.	K-20748	—
II. Tetraploid wheats		
T. dicoccum Schrank var. *farrum*	K-7349	—
T. durum Desf. var. *melanopus*	K-41652	—
T. persicum Vav. var. *stramineum*	K-18013	Armenia
T. turgidum L. var. *specsiosissimum*	K-44548	Italy
T. polonicum L. var. *levissimum*	K-40162	—
T. timopheevi Zhuk. var. *typicum*	K-29548	West Georgia
III. Hexaploid wheats		
T. macha Dek. et Men. var. *letschumicum*	K-28168	West Georgia
T. spelta L. var. *coeruleum*	K-20538	Spain
T. vavilovii Jakubz. var. *vaneum*	K-29533	Armenia
T. sphaerococcum Perc. var. *globosum*	K-5498	India
T. aestivum L. (cultivars Varma, Diana, Universal, Murat, Pikker)	—	—
IV. Diploid species of *Aegilops* L.		
A. speltoides Tausch	K-21, K-23	Asia Minor
A. squarrosa L.	K-33	Turkmenistan

Tissue Extracts and Electrophoresis. A 400—500 mg sample of excised coleoptiles and roots was homogenized by grinding in a pre-chilled mortar with an addition of 2.0 ml of cold buffer mixture at a pH of about 7.6—7.8, consisting of 0.1 tris-hydroxymethylaminomethane (Tris), 0.06 *M* ascorbic acid and 0.005 *M* EDTA. The resulting homogenate was centrifuged at 18,000 g for 30 minutes. To the supernatant in a small vial, sucrose was added up to a final concentration of about 30 per cent, together with a small amount (about 10 mg/ml) of Sephadex G-200 as an inert protein carrier. The protein extracts were stored frozen at —10 °C.

A modified method of vertical polyacrylamide gel electrophoresis, using only photopolymerized small-pore gel layer without spacer and sample layers, has been adopted as described in detail by Jaaska and Jaaska (1968b). All the electrophoretic runs were made in duplicate with two parallel gels for every sample simultaneously in each run.

The maintaining of the extraction conditions constant proved to be essential for obtaining reproducible enzyme patterns. It has been found that changes in the composition of the extraction medium or freezing and thawing of the homogenates without added sucrose resulted in certain changes of electrophoretic patterns of acid phosphatases.

Enzyme Staining. Acid phosphatase and esterase activities on gels were localized by using an azo-dye coupling method of Barka (1960) with α-naphthyl phosphate or α-naphthyl acetate as substrates, respectively, and hexaazotized pararosanilin as a coupler, in a modification described in our previous paper (Jaaska and Jaaska, 1968b).

For studying peroxidase isoenzymes, the gels were first incubated for about 20 minutes in a mixture of eight volumes of 0.1 *M* acetate buffer at pH 4.8 and of two volumes of 0.005 *M* solution of a substrate (o-dianisidine, benzidine, etc.) in 96 per cent

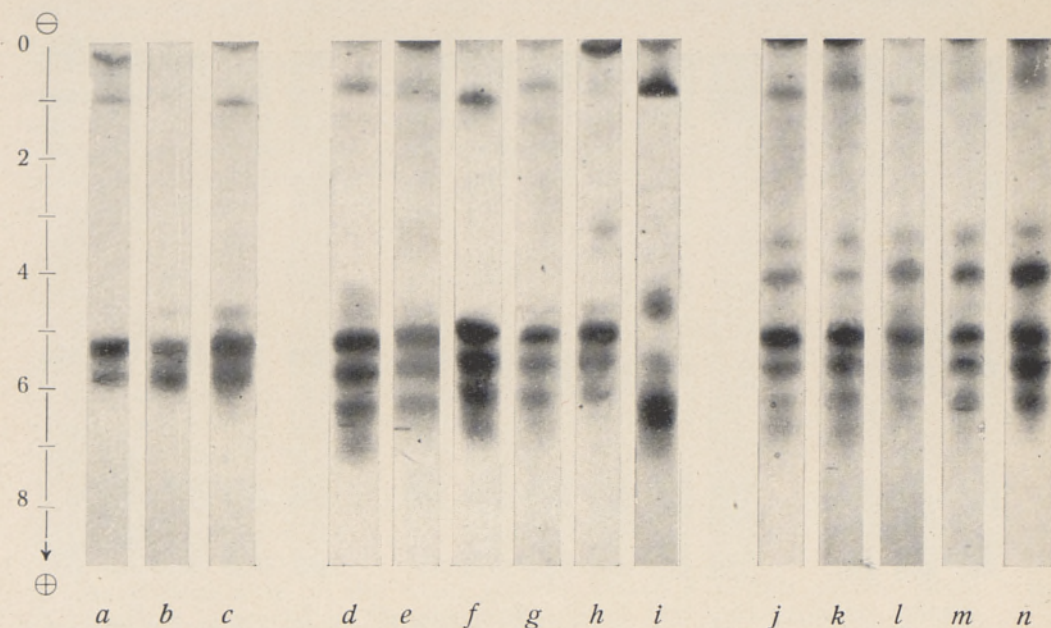

Fig. 1. Polyacrylamide gel electrophoretic patterns of seedling acid phosphatases for various wheat taxa. Enzymograms: *a* — *T. monococcum* L., *b* — *T. boeoticum* Boiss., *c* — *T. thaoudar* Reut., *d.* — *T. dicoccum* Schrank, *e* — *T. durum* Desf., *f* — *T. persicum* Vav., *g* — *T. turgidum* L., *h* — *T. polonicum* L., *i* — *T. timopheevi* Zhuk., *j* — *T. macha* Dek. et Men., *k* — *T. spelta* L., *l* — *T. vavilovii* Jakubz., *m* — *T. sphaerococcum* Perc., *n* — *T. aestivum* L.

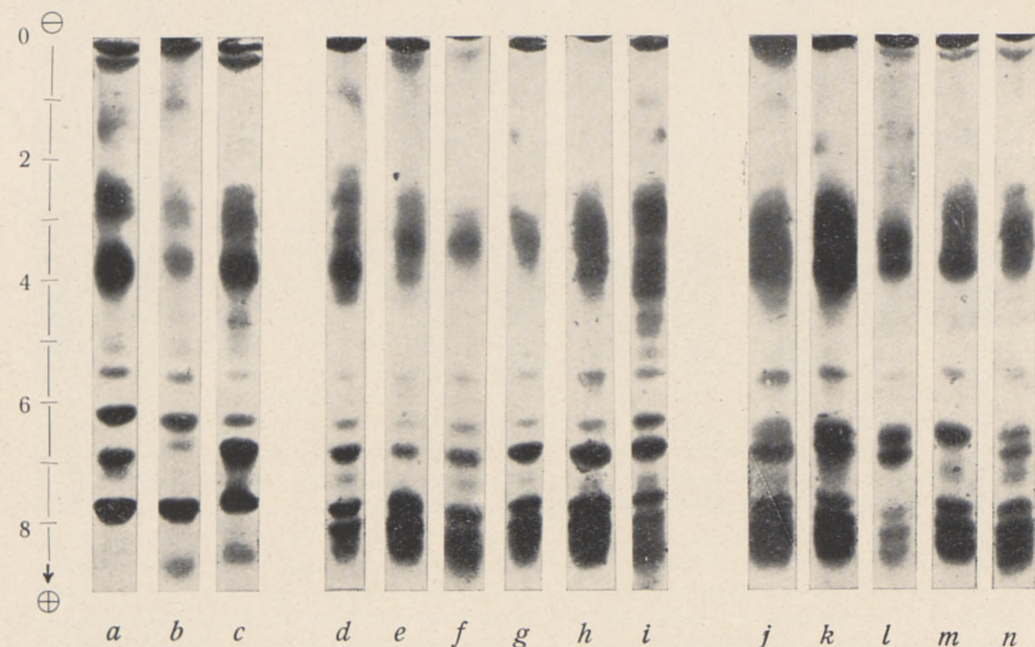

Fig. 2. Polyacrylamide gel electrophoretic patterns of seedling esterases for various wheat taxa. Designations of the enzymograms see under Fig. 1.

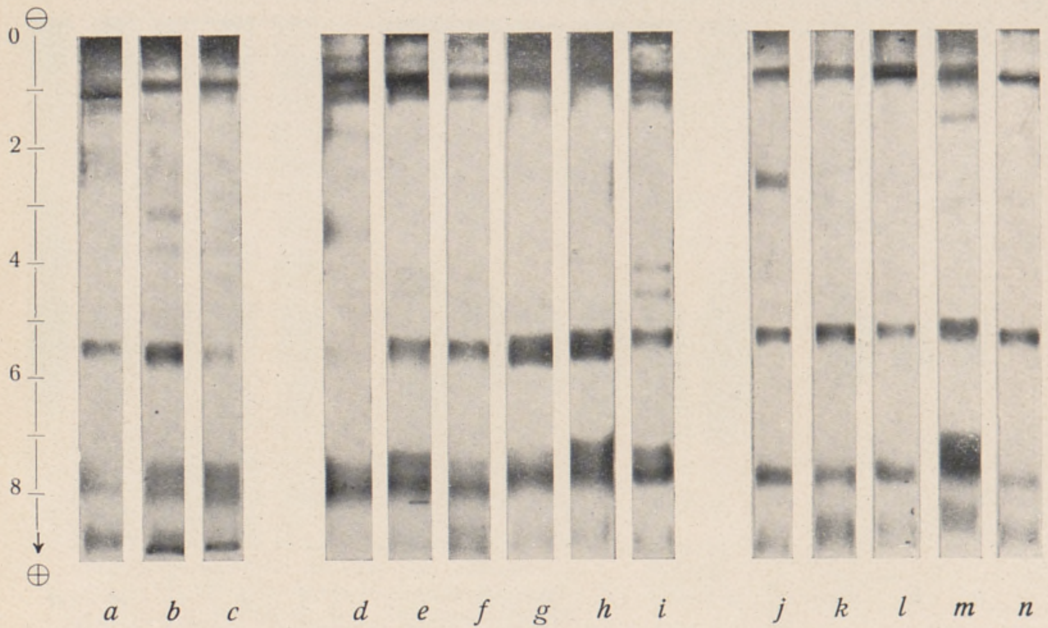

Fig. 3. Polyacrylamide gel electrophoretic patterns of seedling peroxidases for various wheat taxa. Designations of the enzymograms see under Fig. 1.

Fig. 4. The artificial synthesis of acid phosphatase patterns of the tetraploid and hexaploid wheats. Enzymograms: *a* — *T. boeoticum* Boiss., *b* — *T. thaoudar* Reut., *c* — *A. speltoides* Tausch, *d* — *T. boeoticum* + *A. speltoides*, *e* — *T. thaoudar* + *A. speltoides*, *f* — *T. dicoccum* Schrank, *g* — *T. persicum* Vav., *h* — *A. squarrosa* L., *i* — *T. dicoccum* + *A. squarrosa*, *j* — *T. persicum* + *A. squarrosa*, *k* — *T. sphaerococcum* Perc., *l* — *T. aestivum* L.

ethyl alcohol. The sites of peroxidase activity were visualized by adding to the incubation mixture 0.2 ml 1.5 per cent hydrogen peroxide.

Photography. The gels, after staining, were photographed in a transmitting light for a permanent record. The enzymograms were photographically enlarged to a convenient length, using the buffer front as a marker and the enzyme patterns of *Triticum aestivum* L. as reference standards. In several cases, the equivalency of the electrophoretic mobility of enzyme bands was verified by comparing with the pattern obtained from a mixture of samples.

Results

Acid phosphatases. Fig. 1 presents a photograph of enzymograms of anodically migrating acid phosphatases from etiolated seedlings of three diploid, six tetraploid and five hexaploid wheat strains, each of which represents a separate taxon. To facilitate description, the bands have been designated by the distances of migration from the origin to the anode given in arbitrary units.

The figure shows that polyacrylamide gel electrophoresis resolved acid phosphatases of the diploid wheats into four or five electrophoretically distinct fractions. The patterns of all the three diploid wheat strains studied were on the whole similar, except a weakly stained band at 4.6 which appeared in the enzymograms of two wild-growing wheats (*T. boeoticum* Boiss. and *T. thaoudar* Reut.), but was absent in the enzymogram of the cultivated strain (*T. monococcum* L.). Specific for the diploid pattern were two closely spaced bands with the migration distances at 5.6 and 5.1, respectively. The other two bands showed low electrophoretic mobilities: one remained near the origin and another showed the mobility at about 1.

In enzymograms from the seedlings of the tetraploid wheats, six or seven phosphatase fractions could be distinguished. Of the six tetraploid wheats studied, five (*T. dicoccum* Schrank, *T. durum* Desf., *T. persicum* Vav., *T. turgidum* L. and *T. polonicum* L.) showed similar phosphatase patterns with four successive bands of intermediate electrophoretic mobility at 6.6, 6.2, 5.6 and 5.1, respectively, as the most characteristic ones for the tetraploid pattern. The fourth band at 6.5—6.8 was much weaker compared with other three fractions and was detected only if the amount of the sample applied and the incubation times in the reaction mixture were sufficient for its appearance. In some enzymograms an additional faint band at about 3.3 could be distinguished.

Phosphatase pattern obtained for a strain of *T. timopheevi* Zhuk. was clearly different from the patterns of the rest five taxa of tetraploid wheats. Although the total number of bands remained the same as in other tetraploids, the mobility of some fractions, their appearance and relative staining intensities were clearly different in the pattern of *T. timopheevi*. Band at 5.1 was absent in *T. timopheevi*, and instead of it a new more diffuse band with a slightly lower mobility appeared.

The acid phosphatase enzymograms for the hexaploid wheats showed the presence of up to eight fractions. Again, the patterns for all the five hexaploids studied (*T. macha* Dek. et Men., *T. spelta* L., *T. vavilovii* Jakubz., *T. sphaerococcum* Perc. and *T. aestivum* L.) were essentially identical, except for slight differences in the appearance of two slow-moving fractions.

When comparing the enzymograms obtained for different ploidy levels, certain similarities as well as differences in the enzyme patterns can be ascertained. Two bands of small electrophoretic mobility were found in

all the wheat taxa studied. These bands, however, showed some variation in the staining intensity and electrophoretic mobility depending on some minor uncontrolled differences in the preparing of tissue extracts or in performing of electrophoresis, and, therefore, have no taxonomic value. The remaining fractions showed no variation and were easily reproducible in different electrophoretic runs of the same homogenate as well as from separate ones.

Two intensely stained bands with the migration distances at 5.1 and 5.6, respectively, were in common to all the taxa studied, except *T. timopheevi* Zhuk., which had the faster band only. Thus, bands at 5.1 and 5.6 can be considered as specific for the genome *A* of the diploid wheats, present also in the tetraploids and hexaploids. The third weaker band (at 4.6) of diploid wheats was seen in some tetraploids only, remaining undetected in hexaploid wheats.

The tetraploid wheats showed the presence of two additional bands, an intensely stained band at 6.2 and a less distinct one at 6.5—6.8, which were absent in enzymograms of diploid wheats, but were present in all the hexaploids. These two bands seem to be introduced in the phosphatase patterns of the tetraploid and hexaploid wheats by the genetic information contained in the genome *B*.

In the hexaploid wheat patterns, another distinct doublet of phosphatase bands not present in the tetraploids and diploids could be found. The most logical interpretation of this phenomenon is that the bands showing the migration distances at about 3.5 and 4.0 were introduced in the hexaploids by the genetic information contributed by the genome *D*.

We also studied phosphatase patterns of five different cultivars of *T. aestivum* L. All the patterns proved to be identical, showing no electrophoretic variation.

Esterases. From Fig. 2 it is seen that polyacrylamide gel electrophoresis distinguished in etiolated wheat seedlings a rather complex system of anodically moving esterases. The exact number of esterase fractions could not be estimated since those of intermediate electrophoretic mobility were diffuse and not sufficiently separated by the electrophoretic procedure adopted to count them. These esterases, as well as the fractions of slight electrophoretic mobility, which remained near the origin and were present in all the samples studied, therefore, had no taxonomic value.

The most distinctive and characteristic patterns were shown by fast-moving series of esterases. The number of fast-moving esterases varied from four to five in the diploid group, and from six to eight in the tetraploid and hexaploid groups.

The patterns for all the tetraploids and hexaploids showed a high degree of similarity as compared with the minor, but definite differences found in some of the strains. The most characteristic fractions for both tetraploids and hexaploids were seven fractions with the migration distances at about 5.5, 6.3, 6.8, 7.3, 7.7, 8.1 and 8.5. Of these fractions, those at 5.5, 6.3, 6.8 and 7.7 were likewise found to be present in the diploid wheats, and thus they may be contributed to the genome *A*. Band at 8.1 of the tetraploids and hexaploids was never found in the diploids and seems to be specific for the genome *B*. The esterase pattern of *T. timopheevi* Zhuk., in respect to the main bands, was identical with those of other tetraploids, showing, however, some (probably three) additional less intense fractions localized in the region from 4.5 to 5.3.

The feature to be pointed out is that the hexaploid wheats showed no additional band missing in the tetraploids. Apparently, the genome *D*

of hexaploids adds no new genetic information for any esterase different from those controlled by the genomes *A* and *B*.

In addition to the genome-specific features, esterases revealed certain intra-genomal variability in respect to some fractions. Thus, a band at 6.8 was clearly absent in the strain of *T. sphaerococcum* Perc. studied, although present in all the remaining hexaploid and tetraploid strains. A weak band at 7.3 was not found in *T. durum* Desf. and *T. vavilovii* Jakubz. Among the three diploid taxa, variation in esterase bands positioned at about 6.8 and 8.9 could be observed. In contrast to the described variation, the patterns for all the five cultivars of *T. aestivum* L. studied by us were identical, showing no esterase variation.

P e r o x y d a s e s. Polyacrylamide gel electrophoretic patterns of anodically moving peroxidases from the seedlings of different wheat taxa are presented in Fig. 3. As it was in the case of esterases, the exact number of peroxidase fractions could not be estimated, since some bands were excessively diffuse for distinguishing closely spaced fractions, and several weaker bands appeared only after prolonged reaction times, simultaneously with background staining when the main fractions had already over-developed. Furthermore, it was found that the staining intensity of peroxidase fractions depended on the composition of the reaction mixture.

The number of major peroxidase fractions varied from five to eight among the taxa studied. Most of the peroxidase fractions were in common to all the wheat taxa, independent of their ploidy level. These were the bands localized at about 0.1, 1,0, 5.3, 7.5 and 8.5. No band specific for only one particular ploidy level could be established. However, some enzymograms showed the presence of fractions not found in others. Thus, the pattern of *T. macha* var. *coeruleum* (enzymogram *j*) is distinguished by the occurrence of a strong band at about 2.6, that of *T. sphaerococcum* var. *globosum* (enzymogram *m*), by the band at about 1.6.

A certain variation among the patterns could be established for a series of weakly stained fractions of intermediate mobility, localized in the region from 3.0 to 4.5. Thus, the pattern of *T. monococcum* (enzymogram *a*) showed no detectable peroxidase activity in this region, the pattern of *T. boeoticum* (enzymogram *b*) revealed bands at 3.0 and 3.6, while that of *T. thaoudar* (enzymogram *c*), bands at 3.6 and 4.1. The tetraploid strains showed in this region bands at 4.1 and 4.5 which were comparatively intensely stained in enzymogram of *T. timopheevi*, but were absent or appeared faint in enzymograms of the other tetraploids. The hexaploids revealed no detectable band in this region.

Peroxidases located in the region from 7.0 to 7.6 also showed variation among the wheat taxa. Enzymograms for *T. macha*, *T. spelta*, *T. vavilovii* and *T. aestivum* showed the presence of only one fraction in this region, while the rest of the taxa revealed two closely spaced bands. The same situation can be suspected for the fastest-moving zone of peroxidase activity.

T h e a r t i f i c i a l s y n t h e s i s o f p h o s p h a t a s e p a t t e r n s o f t h e t e t r a p l o i d s a n d h e x a p l o i d s. The results of electrophoretic studies of enzyme patterns described above showed that wheats of different ploidy level could be distinguished most clearly by patterns of acid phosphatases. The patterns of esterases and peroxidases revealed most fractions in common to all the three ploidy levels, showing few differences only. Therefore, acid phosphatases could be considered to be most suitable for the establishing of the diploid ancestors from which the polyploid wheats have been evolved. In view of these data, it seemed

desirable to investigate acid phosphatase patterns for *Aegilops speltoides* Tausch and *Aegilops squarrosa* L. which have been considered by various investigators (McFadden, Sears, 1946; Riley et al., 1958; Sarkar, Stebbins, 1956) as the most probable genome donors to the polyploid wheats.

It is seen in Fig. 4 that acid phosphatase pattern of *A. speltoides* (enzymogram *c*) clearly differed from those of the diploid wheats (enzymogram *a* and *b*) in the electrophoretic mobility of the fractions. Two bands at 5.6 and 5.1, characteristic for the einkorn wheats, were absent in the *A. speltoides* pattern, and, instead of these, another doublet of faster-moving closely-spaced bands localized at about 6.6 and 6.2 was observed. Electrophoresis of mixtures of seedling extracts from a diploid wheat (*T. boeoticum*, *T. thaoudar*) with that from *A. speltoides* resulted in obtaining phosphatase patterns (enzymograms *d* and *e*) which contained all the bands of both the diploid components and proved to be identical with the patterns of natural tetraploid wheats (see enzymograms *f* and *g*, for *T. dicoccum* and *T. persicum,* respectively). In other words, phosphatase patterns of natural tetraploid wheats have been reproduced artificially by electrophoresis of tissue extract mixtures of their progenitor diploid species

Phosphatase pattern of *A. squarrosa*, presented in enzymogram *h* in Fig. 4, in addition to three bands common to all wheat taxa, revealed two succesive bands with migration rates at about 3.5 and 4.0 which were characteristic for the hexaploid pattern, but were absent in enzymograms of the diploid and tetraploid wheats. One band of the einkorn wheat and two bands specific for *A. speltoides* were clearly absent in the pattern of *A. squarrosa*. Electrophoresis of mixtures of seedling tissue extracts from a tetraploid wheat (*T. dicoccum*, *T. persicum*) with that of *A. squarrosa* yielded phosphatase patterns (enzymograms *i* and *j* in Fig. 4) which appeared identical with the patterns of natural hexaploid wheats (enzymograms *k* and *l*, for *T. sphaerococcum* and *T. aestivum*, respectively). Accordingly, the biochemical evidence obtained supports the view that *A. squarrosa* might be the diploid contributor of the genome *D* to the hexaploid wheat.

Discussion

The results of the present study demonstrate that among the wheat taxa three major groups could be distinguished from the electrophoretic patterns of seedling acid phosphatases. The same three groups in the genus *Triticum* L. have been distinguished by earlier investigators (see Percival, 1921, for numerous references) on the basis of morphological characters, geographical distribution, hybridization relationships and karyological data. These include the diploid "einkorn" wheats, the tetraploid "emmer" wheats and the hexaploid "dinkel" or "aestivum" wheats, with 14, 28 and 42 somatic chromosomes constituting the genomes *AA*, *AABB* and *AABBDD*, respectively.

The origin of polyploid wheats has long been a matter of considerable speculation and extensive investigation (see a review by Sears, 1959). According to the modern concept, the tetraploid emmer wheats are considered to have emerged by spontaneous diploidization in the nature of a hybrid between a wild diploid wheat having genome *A* and a diploid species of *Aegilops* contributing genome *B*. Among earlier investigators, considerable disagreement had existed regarding the possible donors of the *B* genome in the tetraploid wheats. Recent comparative morphological (Sarkar, Stebbins, 1956) and cytogenetical (Riley et al., 1958) studies of

related diploid species of *Aegilops* and *Agropyron* have, however, reached the conclusion that *A. speltoides* Tausch. should be considered as the most probable donor of the *B* genome in polyploid wheats.

Our present investigation provides further evidence in support of this suggestion, showing that the genome of *A. speltoides* contains genetical information controlling the synthesis of acid phosphatases which were identical in electrophoretic properties with the fractions found in the polyploid wheats, but absent in the diploid wheats. Electrophoresis of tissue extract mixtures from wild diploid wheats and *A. speltoides* resulted in full artificial reproduction of acid phosphatase patterns found for the natural tetraploid wheats. Of course, the evidence obtained here does not yet exclude the possibility that some other diploid species of *Aegilops* could serve as the genome donor in the polyploid wheat. To establish this, further studies of acid phosphatase patterns of all diploid species of *Aegilops* will be needed. This work is in progress at present, and in the course of preliminary communication it can be announced that acid phosphatase patterns for *A. mutica* Boiss., *A. sharonensis* Eig, and *A. longissima* (Schw. et Muschl.) Eig proved to be distinct from that of *A. speltoides* Tausch, showing that the former three species can be ruled out as possible *B* genome donors.

The hexaploid wheats are also considered to be of allopolyploid origin arisen by diploidization of a hybrid between a tetraploid wheat and a diploid species of *Aegilops* possessing the genome *D*. On the basis of similarities in chromosome morphology, Pathak (1940) suggested that *A. squarrosa* L. may be considered as a possible *D*-genome donor. This hypothesis found soon experimental confirmation by McFadden and Sears (1944, 1946), Kihara (1944) and by Kihara and Lilienfeld (1949) who succeeded in the artificial synthesis of an amphidiploid between *T. dicoccoides* and *A. squarrosa* which closely resembled *T. spelta* morphologically and produced with hexaploid wheats fertile hybrids showing a good meiotic chromosome pairing.

Enzymatical investigations reported in the present work showed that the genome of *A. squarrosa* carries genetical information for the synthesis of two acid phosphatases which electrophoretically were identical with two analogous fractions found in the hexaploid wheats. Acid phosphatase patterns obtained by electrophoresis of tissue extract mixtures from the tetraploid wheats and *A. squarrosa* proved to be identical with those of the natural hexaploids. These data provide further evidence in support of the view that *A. squarrosa* could be the diploid ancestor of the hexaploid wheats.

From our data it follows that separate enzymes show different degrees of phylogenetic divergence and, therefore, have different value in establishing taxonomic and phylogenetic relationships. The patterns of peroxidases showed main bands in common for all the three ploidy levels. Therefore, the ploidy level of a wheat taxon could not be established by its peroxidase electrophoretic pattern. Peroxidase enzymograms for *T. monococcum*, *A. speltoides* and *A. squarrosa* (not presented in this report) likewise revealed most bands in common, showing the presence of no genome-specific fractions. Esterase enzymograms for the tetraploid and hexaploid wheats were also indistinguishable in the electrophoretic behaviour of fast-moving fractions. In contrast, enzymograms of acid phosphatases revealed the presence of genome-specific fractions being clearly distinct for each ploidy level and for each of the three diploid parental species.

The above data can be interpreted as indicating the independent diver-

gence of separate enzymes during the phylogenetic differentiation of *Aegilops* and *Triticum* species. Thus, the loci determining the structure of genome-specific acid phosphatases have undergone definite mutational changes which resulted in the appearance of phosphatases of distinct electrophoretic mobility. Conversely, the loci for the enzymes found to be of common occurrence in all the diploid and polyploid taxa have probably maintained their original structure.

The occurrence of enzymes common to all the three diploid ancestors of wheats and to the wheats of different ploidy level suggests that the genomes *A*, *B* and *D* carry identical structural genes determining these enzymes. This is in good line with cytogenetical studies of Sears (1941) and Riley et al. (1958) who observed that the F_1 hybrids of a diploid wheat with *A. speltoides* and *A. squarrosa* show considerable meiotic pairing resulting in the formation of up to seven bivalents in some cells, although the average pairing was less. From these data and from the analysis of inheritance in the nullisomics of the hexaploid wheat, it has been concluded that considerable similarity exists between the chromosomes of the three diploids and that each chromosome in a diploid genome has a genetically corresponding, but only partially homologous, i. e., homoeologous chromosome in each of the other two genomes (Sears, 1952, 1959).

All the above evidence supports the view about the monophyletic origin of the three diploids from a common ancestor through the mutational changes of the initial genome resulting in genetical isolation and structural differentiation of several diploid genomes.

Another noteworthy feature to be considered here is the additiveness of parental enzymes in the patterns of the amphiploids. Acid phosphatase patterns of the polyploid wheats consisted of fractions found in the contemporary diploid species from which the polyploids had arisen. This evidence indicates that the loci determining the genome-specific acid phosphatases have maintained their original structure since their incorporation in the genome of amphiploids. The same data also suggest that the differentiation of the loci for the genome-specific acid phosphatases had occurred in the past at the diploid level, before the appearance of the initial amphiploids. Further evolutionary differentiation of polyploid wheats has occurred due to a divergence of certain other loci, while those determining acid phosphatases showed no further divergence. This is evidenced by the general similarity of acid phosphatase enzymograms for all wheat taxa at the same ploidy level, *T. timopheevi* Zhuk. being the only exception.

Besides the genome-specific enzymes and the enzymes common to all the three genomes of the hexaploid wheat, several enzyme fractions showed clear intra-genomal variation among the wheat taxa of the same ploidy level. Three different types of intra-genomal enzyme variation could be distinguished. The first type of variation showed the absence of a separate enzyme band in the enzymogram. This kind of variation was found for some esterase (Fig. 2) and peroxidase (Fig. 3) fractions, and probably for a faint phosphatase fraction (Fig. 1) (enzymogram *a*). The second variation pattern involved the alteration of the electrophoretic mobility of an enzyme fraction. This kind of variation was found for a pair of weakly stained peroxidases (Fig. 3, enzymograms *b* and *c*). The third type lay in the appearance of an additional band which was electrophoretically distinct from the others, as it was found for some peroxidase fractions (Fig. 3, enzymograms *j* and *m*).

The first two types of enzyme variation can be explained by the action

of distinct alleles of mutant origin resulting in the synthesis of an inactive enzyme analogue, or of an active enzyme of altered electrophoretic mobility (Shaw, 1965). The appearance of an additional enzyme fraction in a wheat strain, however, cannot be explained so easily. One might assume the presence of an additional structural gene resulting in the synthesis of a new enzyme fraction. In this case, however, it is difficult to explain the origin of the additional structural gene in the genome composition. Therefore, other possible explanations must be searched for. Our present data provide no experimental evidence for the genetic causes of the intra-genomal enzyme variation.

The results of enzyme pattern studies in *T. timopheevi* Zhuk. need separate discussion. It has been found that *T. timopheevi* shared a unique position among the tetraploid wheats in respect of its acid phosphatase pattern which proved to be clearly distinct from the patterns of other tetraploids of the Emmer group.

T. timopheevi Zhuk. is a tetraploid wheat species endemic to a narrow territory in Western Georgia (Dekaprelevich, Menabde, 1932; Zhukovsky, 1928). Hybridization studies carried out by many investigators (Dekaprelevich, Menabde, 1932; Lilienfeld, Kihara, 1934; Sachs, 1953, etc.) showed that *T. timopheevi* is genetically isolated from the tetraploids of the Emmer group. Its F_1 hybrids with the tetraploid Emmer wheats showed a decreased and irregular chromosome pairing in meiotic metaphase and were almost completely sterile, or of low fertility.

Cytological studies of Kihara and Lilienfeld (1934) on meiotic chromosome pairing in the hybrids *T. aegilopoides*×*T. timopheevi*, *T. timopheevi* ×*T. dicoccum*, and *T. timopheevi*×*T. persicum* showed that the genome *A* of the diploid wheat paired with one genome of *T. timopheevi*, while the another genome of *T. timopheevi* was not fully homologous with the genome *B* of the Emmer group, resulting in reduced and irregular chromosome pairing. On these grounds the authors proposed to designate the second genome of *T. timopheevi* as the genome *G* so as to distinguish it from the genome *B*.

In contrast to the above cytogenetical data, our enzymological studies revealed a difference between *T. timopheevi* and other tetraploid wheats attributable to the genome *A*. An acid phosphatase fraction specific to the genome *A* was found to be absent in the pattern of *T. timopheevi*. However, it is possible that some other differences in enzyme patterns can be attributed to the genome *B*. Esterase and peroxidase enzymograms for *T. timopheevi* and other tetraploids revealed most bands in common, although they showed the presence of some additional esterase fractions. This suggests that the genome of *T. timopheevi* exhibits a high degree of similarity with the *AB* genome, showing differences in the structure of separate genes. The degree of the biochemical divergence of *T. timopheevi* from the other tetraploid wheats, however, remains to be studied.

The present study convincingly demonstrated the existence of a close correlation between enzymological data and the results of other approaches in wheat systematics. This suggests that electrophoretic studies of enzymes can be used to estimate the degree of genome homology and to elucidate the phylogenetic relationships existing among the species of *Aegilops* and *Triticum*.

Summary

Polyacrylamide gel electrophoretic patterns of acid phosphatases, esterases and peroxidases from etiolated seedlings of three diploid, six tetraploid and five hexaploid wheat taxa have been investigated. In addition, acid phosphatase and peroxidase patterns for *Aegilops speltoides* Tausch and *A. squarrosa* L. were obtained and compared with the patterns of wheat taxa.

Separate enzymes were shown to exhibit different degrees of variation and evolutionary divergence, and, therefore, they are of different value for establishing taxonomic and phylogenetic relations. Three different types of enzymes were distinguished, depending on the degree of divergence: 1) the enzymes of common occurrence in all the wheat taxa and in their diploid ancestor species, 2) the genome-specific enzymes, 3) the enzymes showing intra-genomal variation. The intra-genomal enzyme variation was of three different kinds: 1) the absence of an enzyme band in enzymogram, 2) the alteration of the electrophoretic mobility of a fraction, 3) the appearance of an additional, electrophoretically distinct enzyme.

Phosphatase enzymograms showed the presence of genome-specific fractions which were characteristic for the separate ploidy levels, in addition to fractions common to all wheat taxa. Esterase patterns of the tetraploid and hexaploid wheats were indistinguishable, showing only minor intra-genomal variation. In the diploid group, the number of distinguishable esterase fractions was lower than in the tetraploid group, and some of them showed intra-genomal variation. Peroxidase enzymograms revealed major bands being in common to all the wheat taxa, independent of their ploidy level. No genome-specific peroxidase fraction could be found, but some fractions showed intra-genomal variation.

Phosphatase pattern obtained for a strain of *T. timopheevi* Zhuk. was distinctly different from the patterns of the rest of the five tetraploid wheats. Esterase and peroxidase patterns of *T. timopheevi* Zhuk., however, were similar to those of the other tetraploids, except higher activity of two peroxidases and some additional esterase fractions present in *T. timopheevi*.

Phosphatase patterns of the polyploid wheats consisted of fractions found in the contemporary diploid species from which the polyploids have presumably been arisen. Electrophoresis of tissue extract mixtures from a wild diploid wheat and *A. speltoides* Tausch, and from a tetraploid wheat and *A. squarrosa* L. resulted in full artificial reproduction of acid phosphatase patterns found for the natural tetraploid and hexaploid wheats, respectively. Accordingly, the enzymological evidence presented supports the view that *A. speltoides* Tausch and *A. squarrosa* L. might be the diploid contributors of genomes *B* and *D*, respectively, to the polyploid wheat.

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VELLO JAASKA

IDANDITE FOSFATAASIDE, ESTERAASIDE JA PEROKSÜDAASIDE ELEKTROFOREETILINE UURIMINE PEREKONNAS *TRITICUM* L.

Resümee

Polüakrüülamiidgeelelektroforeetiliselt uuriti kolme diploidse, kuue tetraploidse ja viie heksaploidse nisu idandite happelisi fosfataase, esteraase ja peroksüdaase, samuti *Aegilops speltoides* Tausch ja *A. squarrosa* L. fosfataase ja peroksüdaase.

Leiti, et eri fermendid erinevad oma varieeruvuse ja fülogeneetilise divergentsi astme poolest, mille tõttu neil on erinev tähtsus taksonoomiliste ja fülogeneetiliste seoste selgitamisel. Fülogeneetilise divergentsi astme alusel eristati kolme tüüpi fermente: 1) kõigile nisutaksoonidele ja nende diploidsetele eellastele ühised fermendid, 2) genoomi-spetsiifilised fermendid ja 3) genoomisisese varieeruvusega fermendid. Fermentide genoomisisesest varieeruvusest täheldati 1) fraktsiooni puudumist, 2) fraktsiooni elektroforeetilise liikuvuse muutumist ja 3) täiendava fraktsiooni ilmnemist ensümogrammil.

Happelistel fosfataasidel leiti nii kõigile nisutaksoonidele ühiseid kui ka genoomispetsiifilisi fraktsioone. Tetraploidsete ja heksaploidsete nisude esteraaside ensümogrammid olid ühesugused, kuid mõningatel fraktsioonidel täheldati genoomisisest varieeruvust. Diploidsete nisude esteraaside arv oli väiksem kui tetraploididel ja mõnedel fraktsioonidel esines genoomisisene varieeruvus. Põhilised peroksüdaasi fraktsioonid olid kõigile nisudele ühised, olenemata taksooni ploidsuse astmest. Ühtegi genoomi-spetsiifilist peroksüdaasi ei leitud, kuid üksikutel fraktsioonidel esines genoomisisest varieeruvust.

Teistest tetraploidsetest nisudest erines *T. timopheevi* Zhuk. märgatavalt happelise fosfataasi ensümogrammi poolest. Esteraaside ja peroksüdaasi ensümogrammid olid kõigil tetraploididel sarnased, välja arvatud mõnede täiendavate esteraaside ja kahe aktiivsema peroksüdaasi esinemine *T. timopheevi* Zhuk. idandites.

Polüploidsete nisude fosfataaside ensümogrammid koosnesid tänapäeval esinevate diploidsete liikide — polüploidsete nisude eellaste fosfataaside fraktsioonide summast. Diploidse nisu ja *A. speltoides* Tausch koeekstraktide segu elektroforeesil saadi tetraploidsele nisule iseloomulik fosfataasi ensümogramm, kuna tetraploidse nisu ja *A. squarrosa* L. ekstraktide segu elektrofores andis heksaploidsele nisule iseloomuliku ensümogrammi. Seega ühtuvad ensümoloogilised andmed seisukohaga, mille kohaselt polüploidsed nisud tekkisid diploididest ning genoomide *B* ja *D* doonoriteks olid vastavalt *A. speltoides* Tausch ja *A. squarrosa* L.

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ВЕЛЛО ЯАСКА

ЭЛЕКТРОФОРЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ ФОСФАТАЗ, ЭСТЕРАЗ И ПЕРОКСИДАЗ ПРОРОСТКОВ В РОДЕ *TRITICUM* L.

Резюме

Методом электрофореза в полиакриламидном геле изучались кислые фосфатазы, эстеразы и пероксидазы этиолированных проростков трех диплоидных, шести тетраплоидных и пяти гексаплоидных таксонов пшеницы. Кроме того, получены энзимограммы кислых фосфатаз и пероксидаз для *Aegilops speltoides* Tausch и *A. squarrosa* L.

Показано, что отдельные ферменты проявляют различную степень варьирования и филогенетической дивергенции, в результате чего они имеют разную ценность при выявлении таксономических и филогенетических взаимоотношений. По степени филогенетической дивергенции выделено три типа ферментов: 1) ферменты, общие для всех таксонов пшениц и их диплоидных предшественников, 2) геном-специфические ферменты, 3) ферменты, проявляющие внутригеномную изменчивость. При этом установлено три вида внутригеномной изменчивости ферментов: 1) отсутствие фракции фермента, 2) изменение ее электрофоретической подвижности, 3) появление дополнительной фракции на энзимограмме.

Наряду с общими для всех таксонов пшениц фракциями выявлены геном-специфические кислые фосфатазы, характерные для отдельных уровней плоидности. Энзимограммы эстераз тетраплоидных и гексаплоидных пшениц оказались одинаковыми, обнаружив только некоторую внутригеномную изменчивость. Число различаемых фракций эстераз у диплоидных пшениц было ниже, чем у тетраплоидов, и некоторые из них проявляли внутригеномную изменчивость. Основные фракции пероксидаз оказались общими для всех таксонов пшениц вне зависимости от плоидности. Не обнаружено ни одной геном-специфической пероксидазы, однако некоторые фракции показали внутригеномную изменчивость.

T. timopheevi Zhuk. четко отличалась от остальных пяти тетраплоидных пшениц по энзимограмме кислых фосфатаз, но энзимограммы эстераз и пероксидаз были в основном сходными у всех тетраплоидов, за исключением наличия нескольких дополнительных эстераз и двух более активных пероксидаз у *T. timopheevi* Zhuk.

Энзимограммы фосфатаз полиплоидных пшениц состояли из суммы фракций, найденных у современных диплоидных видов — предшественников полиплоидных пшениц. Энзимограммы фосфатаз, полученные при электрофорезе смеси тканевых экстрактов диплоидной пшеницы и *A. speltoides* Tausch, полностью соответствовали энзимограммам фосфатаз тетраплоидных пшениц, а электрофорез смеси экстрактов тетраплоидной пшеницы и *A. squarrosa* L. дал энзимограммы, характерные для гексаплоидных пшениц. Таким образом, энзимологические данные согласуются с представлениями о происхождении полиплоидных пшениц из диплоидов с участием *A. speltoides* Tausch и *A. squarrosa* L. в качестве доноров геномов *B* и *D* соответственно.

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