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ELECTROPHORETIC ENZYME STUDIES IN THE GENUS *SECALE* L.

This study has been undertaken in a series of our continuing efforts to use electrophoretic isoenzyme patterns to elucidate phylogenetic relationships in the grass tribe *Triticeae* Dum. Our previous isoenzyme studies in the *Triticum-Aegilops* group demonstrated (Jaaska, 1969, 1971) the usefulness of the method in establishing the diploid species which have contributed their genomes and characteristic isoenzymes to the polyploid species.

The genus *Secale* L., together with the genera *Triticum* L., *Aegilops* L., *Haynaldia* Shur., belongs to the subtribe *Triticinae* Benth. of the tribe *Triticeae* Dum. (Цвелев, 1968). However, the karyotype evolution in the genus *Secale* L. has followed a somewhat different route than in the *Triticum-Aegilops* group, entirely avoiding the polyploidy in nature and experimenting mainly at the level of chromosome translocations and gene mutations, as revealed by a series of thorough cytogenetic studies (Khush, 1962, 1963; Khush, Stebbins, 1961; Kostoff, 1937; Kranz, 1963; Nürnberg-Krüger, 1960; Riley, 1955; etc.). The genus has been treated monographically from the morpho-ecological point of view by R. Rozhevich (Рожевич, 1947). Recently, a chemotaxonomic study using thin-layer chromatographic patterns of fluorescent leaf compounds has been published (Dedio et al., 1969). All these studies have significantly contributed to our present comparatively good knowledge of the genus.

In this paper we report upon polyacrylamide gel electrophoretic studies of nine different enzymes, four hydrolases, four dehydrogenases and peroxidase, in the etiolated seedlings of different taxa (species) of the genus *Secale* L. For comparison, three related species of the subtribe, *Haynaldia villosa* (L.) Schur., *Triticum boeoticum* Boiss. and *Triticum aestivum* L., are also involved.

Materials and methods

Plant material. Most of the seed samples were received from the Vavilov All-Union Institute of Plant Industry in Leningrad and were reproductions of the World Collection. These involved the following taxa and accessions: *Secale cereale* L. var. *vulgare* Körn. cv. Vjatka, accession K-9441; *Secale cereale* ssp. *segetale* Zhuk., syn. *Secale segetale* (Zhuk.) Roshev., accessions K-5867, K-7684, K-7745, K-5836, K-7756, K-7984, and K-10036, all derived from the Soviet Caucasian Republics; *Secale cereale* var. *afghanicum* Vav., syn. *Secale afghanicum* (Vav.) Roshev., K-10221 from the Armenian SSR; *Secale cereale* ssp. *dighoricum* Vav., syn. *Secale dighoricum* (Vav.) Roshev., K-9655 from the Ossetian ASSR. *Secale africanum* Stapf., K-10289 from South Africa; *Secale anatolicum* Boiss., strains

K-10086 (Armenian SSR), K-10220 (Nakhitchevan ASSR), K-10237, and K-10325; *Secale montanum* Guss., accessions K-9598 (Stavropol) and K-10269 (Czekhoslovakia); *Secale kuprijanovii* Grossh., accessions K-9585 and K-9586 (both from the Krasnoyarsk region), and K-9670 from the Azerbaijanian SSR; *Triticum boeoticum* Boiss., K-27184.

The seeds of *Secale silvestre* Host. were collected by the author in nature near Yevpatoria in the Crimea, at the Apsheron peninsula in the Azerbaijanian SSR and near Ashkhabad in the Turkmenian SSR. A sample of *Haynaldia villosa* (L.) Schur. of Georgian origin was a gift from Dr. V. Yemelyanova (Baku, Azerbaijanian SSR). *Triticum aestivum* L. cv. Pikker was obtained from the Plant Breeding Station in Jõgeva, Estonian SSR.

Tissue extracts and electrophoresis. Dehusked seeds were sterilized for 10 minutes in 7 per cent hydrogen peroxide and sown in Koch dishes on two sheets of filter paper saturated with 5 ml of 2 mM $Ca(NO_3)_2 \cdot 4H_2O$ and 1 mM EDTA-Na_2Mg. The seeds were germinated in the dark, in a thermostat at 25 °C for 4—5 days.

A 400—500 mg sample of etiolated first internodes was homogenized in a pre-chilled mortar with quartz sand and 2.0 ml of cold buffer mixture at a pH of about 7.8, containing 0.05 M tris-hydroxymethylaminomethane (Tris), 0.04 M ascorbic acid and 1 mM EDTA-Na_2Mg. The resulting homogenate was centrifuged at 18,000 *g* for 30 minutes. Sucrose was added to the supernatants in small vials to a final concentration of about 20 per cent, together with about 20 mg/ml Sephadex G-200 as an inert protein carrier. The protein extracts were stored frozen at —10°.

Electrophoretic separations were performed in a polyacrylamide gel slab (60×45×3 mm) made in a vertical plexiglas cathode chamber by photopolymerizing between two fluorescent lamps of a freshly prepared mixture composed of 10 per cent acrylamide, 0.12 per cent N, N'-methylenebisacrylamide, 0.25 M Tris, 0.075 M HCl, 0.2 per cent triethanolamine and 0.5 mg per cent riboflavine-5-phosphate. The upper cathode buffer contained 0.01 M Tris and 0.08 M glycine, whereas the lower anode buffer was 0.1 M Tris-acetate at a pH of about 8.9. Up to six protein samples were layered in succession between the rubber holders on the top of the small-pore gel, and electrophoresis was carried out in a vertical refrigerated apparatus at about 100—120 mA per cm^2 of gel surface for about 2—3 hours, until the marker dye, bromphenol blue, reached the end of gel.

Enzyme staining. The hydrolases, acid phosphatase, esterase, β-glucosidase and leucine aminopeptidase, were detected histochemically by incubating of the gel slabs in 0,1 M sodium maleate buffer, pH 6, containing 0.5 mg/ml 1-naphthyl phosphate, 1-naphthyl acetate, 2-naphthyl-β-D-glycopyranoside or N-leucyl-2-naphthylamide as substrate, respectively, and 0.02 M freshly diazotized *o*-dianizidine as the dye coupler.

The dehydrogenases, malate dehydrogenase (MDH), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GDH) and glucose 6-phosphate dehydrogenase (G6PDH), were located histochemically by incubating of the gel slabs in the dark at 30—35 °C in the reaction mixture containing 30 ml of 0.1 M Tris-KH_2PO_4 buffer, pH about 8.6, with 0.5 mM $MgSO_4 \cdot 7H_2O$, 2 ml of a neutralized 0.1 M substrate solution, 2 ml 0.5 per cent NAD (or NADP, for G6PDH), 2 ml nitroblue tetrazolium (100 mg dissolved in 2 ml dimethylformamide and diluted to 50 ml with distilled water), and 2 ml phenazine methosulfate (0.25 mg/ml).

Peroxidase isoenzymes were localized by incubating the gels for about 20 min in a reaction mixture made of 36 ml 0.2 M sodium acetate buffer, pH 3.6, 4 ml 0.01 M *o*-dianizidine dihydrochloride water solution and 0.2 ml 0.2 M catechol in 0.05 M oxalic acid. The sites of peroxidase activity were visualized by adding to the mixture of 0.2 ml 1.5 per cent hydrogen peroxide. Polyphenol oxidase (*o*-diphenol oxidase) activity, if present, was detectable in the same reaction mixture before the addition of hydrogen peroxide.

The gels were photographed in a diffuse transmitting light for a permanent record. The enzymograms were photographically enlarged to a convenient length, using the buffer front or selected isoenzyme bands as markers.

Results

Acid phosphatases. Fig. 1 presents photographs of enzymograms of anodically moving acid phosphatases from etiolated seedlings of nine different rye taxa. Enzymograms for *Haynaldia villosa* and *Triticum boeoticum* are also represented for comparison. To facilitate description, the bands will be designated by the distances of migration from the origin to the anode expressed in arbitrary units on the scale in the figure.

All the enzymograms in the figure show staining area near the origin at the site of the sample application. This activity is presumably due to structurally bound enzyme aggregates which fail to migrate significantly in the gel matrix. This staining is characteristic of many enzymes and of most tissue extracts, and we shall leave it out of consideration as a nonspecific one.

The next staining area due to phosphatase activity appears at the migration distance of about 1.3—1.8. This activity zone which is actually comprised of several closely spaced bands showed considerable variability in the staining intensity among the taxa studied and was totally absent in many enzymograms (*5*, *7*, *8*, and *11* in Fig. 1*A*). It was present in all the three strains of *S. kuprijanovii* which we studied, being especially dominant in the enzymograms for the strain K-9586.

Isoenzymes with the migration distances from 2 to 4 revealed the most striking variation among the rye taxa which fell in three groups according to the arrangement of their phosphatase isoenzymes. Four taxa, *S. anatolicum*, *S. montanum*, *S. africanum* and *S. kuprijanovii*, have either one or two major bands with the migration distances at 2.1 and 2.5 (enzymograms *3—6* in Fig. 1*A*). All these taxa have been assigned (Рожевич, 1947) in the same section (range) *Kuprijanovia* Roshev. on the morpho-ecological grounds. The next four rye taxa, *S. dighoricum*, *S. afghanicum*, *S. segetale* and *S. cereale*, show the presence of up to four successive phosphatase isoenzymes at 2.5, 2.8, 3.3 and 3.7, as seen in enzymograms *7—10* in Fig. 1*A*. These taxa have been included (Рожевич, 1947) in a separate section (range) *Cerealia* Roshev. or have been suggested (Khush, 1963; Nürnberg-Krüger, 1960) to be considered as intraspecific taxa of a polymorphous species, *S. cereale* L. The comparison of respective enzymograms in Fig. 1*A* clearly demonstrates differences between the two groups by their phosphatase isoenzyme electrophoretic patterns. The isoenzymes at 2.8 and 3.7 which are characteristic of the section *Cerealia* are clearly absent in the enzymograms of the taxa belonging to the section *Kuprijanovia*, while the band at 3.3 is scarcely distinguishable. The isoenzyme at 2.1, in its turn, was absent in the taxa of the section *Cerealia*. Fig. 1*B* demonstrates that the acid phosphatase electrophoretic pattern is subjected to characteristic variation between different accessions of *S. segetale* in the staining intensity of individual isoenzyme bands. However, the general features of the pattern remained unchanged.

It should be mentioned that our electrophoretic enzyme patterns characterize the whole populations of the accessions, since the homogenates were made of about 10—20 seedlings. Our preliminary studies of individual seedlings revealed phosphatase isoenzyme variation characteristic of genetic polymorphism. The isoenzymes at 2.5 and 2.8 are apparently two genetic variants at one phosphatase locus, and the isoenzymes at 3.3 and 3.7 represent genetic variants at the second locus. The variation between different accessions of *S. segetale* in the staining intensities of individual isoenzyme bands, as demonstrated in Fig. 1*B*, is presumably due to differences in the frequencies of homozygous and heterozygous phenotypes in

the seedling samples analyzed. The individual variation in phosphatase isoenzymes in different populations of ryes is under more detailed study at present. Preliminary data show that some annual and perennial rye individuals possess indistinguishable phosphatase patterns.

The acid phosphatase pattern of *S. silvestre*, the only species assigned (Рожевич, 1947) in a separate section *Silvestria* Roshev., consisted of two major bands at about 2.9 and 3.2. It can be seen in Fig. 1*C* that these two phosphatase isoenzymes were present in all five collections of *S. silvestre* from the Crimea, Apsheron and Turkmenia, showing no intraspecific variation. The isoenzyme bands of closely similar electrophoretic mobility are also present in the enzymograms characteristic of the section *Cerealia*, but it seems to be an accidental similarity of the electrophoretic mobilities. Similarly, *Triticum boeoticum* Boiss. (enzymogram *11* in Fig. 1*A*) possesses a minor isoenzyme, electrophoretically identical to the fastest isoenzyme of *S. cereale*. However, the two major phosphatases of *T. boeoticum* are clearly absent in all species of rye.

From enzymogram *1* in Fig. 1*A* it is seen that *Haynaldia villosa*, in addition to the staining near the origin and at about 1.5, has one distinct phosphatase isoenzyme at about 3.5.

Esterases, as it is seen in Fig. 2*A*, are represented in the rye seedlings by a rather complex series of fractions, the exact number of which is impossible to determine in the enzymograms. Esterases of intermediate electrophoretic mobilities with the migration distances from 2.5 to 4 show high enzymatic activities, as evidenced by their intense staining. They are represented by a number of closely spaced bands which tend to fuse when overstained in order to reveal weaker bands. The taxa belonging to the section *Kuprijanovia* (enzymograms *3—6* in Fig. 2*A*) show generally similar pattern of this group of esterases, but *H. villosa* (enzymogram *1*) and *S. silvestre* (enzymogram *2*) are distinct.

The most distinctive banding patterns are shown by fast-moving esterases, ranging from the mobilities at 5 to 7. All the rye taxa studied revealed one or two intensely stained bands in the region between 6 and 7, except for two samples of *S. silvestre* collected in Turkmenia (enzymogram *2*). It can be seen in Fig. 2*B* that the samples of *S. silvestre* collected in the Apsheron peninsula and in the Crimea possess an intense esterase band at about 6.3, characteristic of all rye species. However, this band is totally lacking in enzymograms of *S. silvestre* from Turkmenia (enzymogram *2* in Fig. 2*A* and enzymogram *4* in Fig. 2*B*), and instead of this isoenzyme a new less intense band at about 5.4 is evident. One of the samples collected in the Crimea possessed both bands (enzymogram *3* in Fig. 2*B*).

The four taxa comprising *S. cereale* L. s. lat. (or the section *Cerealia*) showed essentially similar esterase enzymograms. Enzymogram in Fig. 2*C* demonstrates general intraspecific constancy of the esterase patterns in the geographically different populations of *S. segetale*. The patterns for the taxa of the *Kuprijanovia* and *Cerealia* sections were also highly similar with major fractions in common, so that the two sections could not be distinguished by esterase enzymograms. *S. dighoricum* K-9655 of the section *Cerealia* (enzymogram not presented) and *S. anatolicum* K-10237 (enzymogram *3* in Fig. 2*A*) of the section *Kuprijanovia* were similar in having three electrophoretically similar fast-moving isoenzyme bands. The three accessions of *S. kuprijanovii* revealed, in their esterase enzymograms, two fast-moving isoenzymes (enzymogram *6* in Fig. 2*A*), while the remaining taxa showed the presence of only one of them. A preliminary study of enzymograms from single seedlings of *S. kuprijanovii* revealed that the population was polymorphous with respect

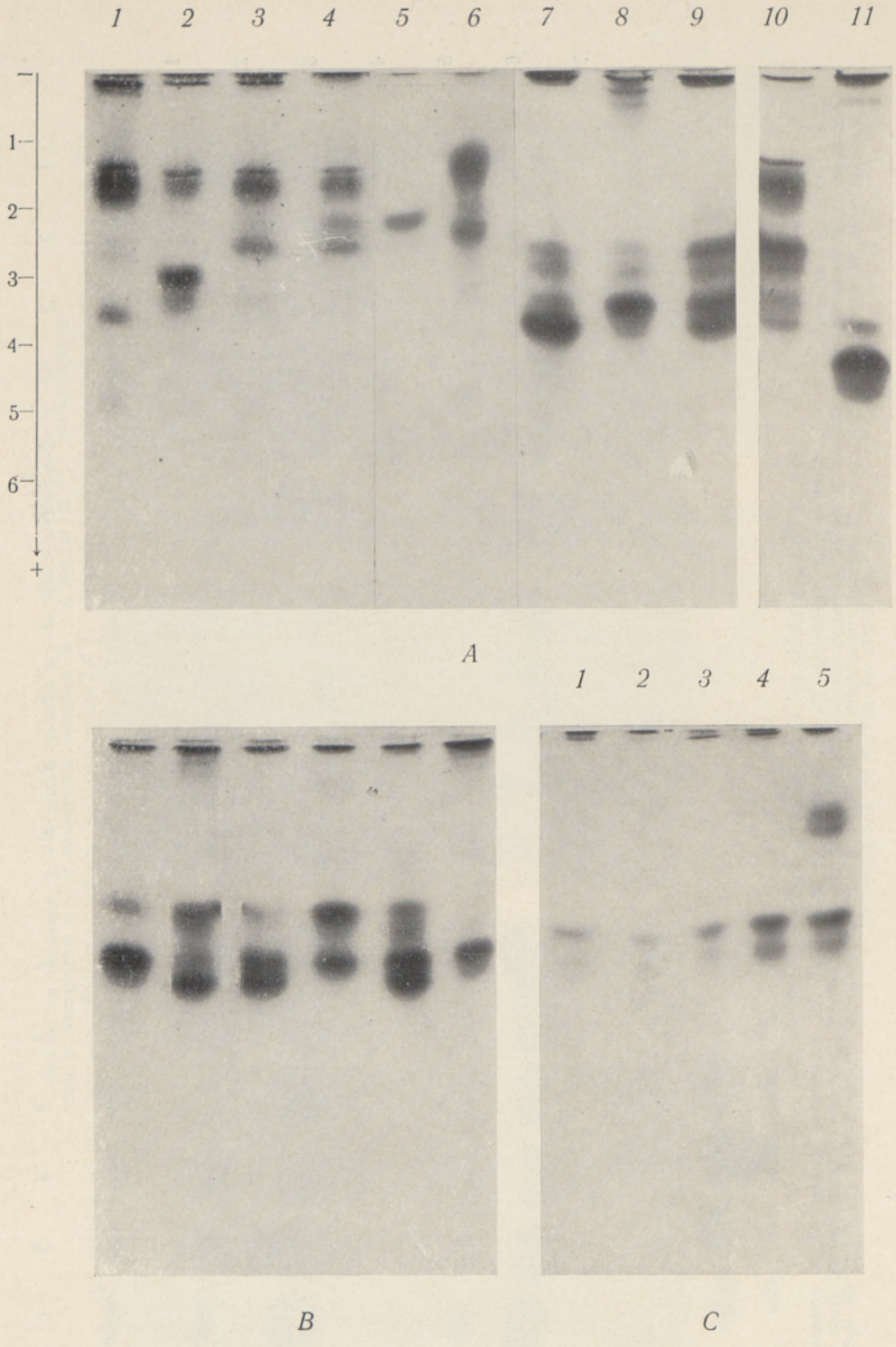

Fig. 1. Polyacrylamide gel electrophoretic patterns of acid phosphatase.

A. H. villosa (enzymogram *1*), *S. silvestre*, Turkmenia (*2*), *S. anatolicum* K-10086 (*3*), *S. montanum* K-9598 (*4*), *S. africanum* K-10221 (*5*), *S. kuprijanovi* K-9670 (*6*), *S. dighoricum* K-9655 (*7*), *S. afghanicum* K-10221 (*8*), *S. segetale* K-7684 (*9*), *S. cereale* K-9441 (*10*), *T. boeoticum* K-27184 (*11*). *B. Secale segetale*, strains K-10036 (*1*), K-7984 (*2*), K-7745 (*3*), K-7756 (*4*), K-7784 (*5*), and K-5836 (*6*). *C. Secale silvestre*, strains originating from the peninsula Apsheron (enzymogram *1*), from the Crimea, near Yevpatoria (enzymograms *2* and *3*), from Turkmenia, near Ashkhabad (enzymograms *4* and *5*).

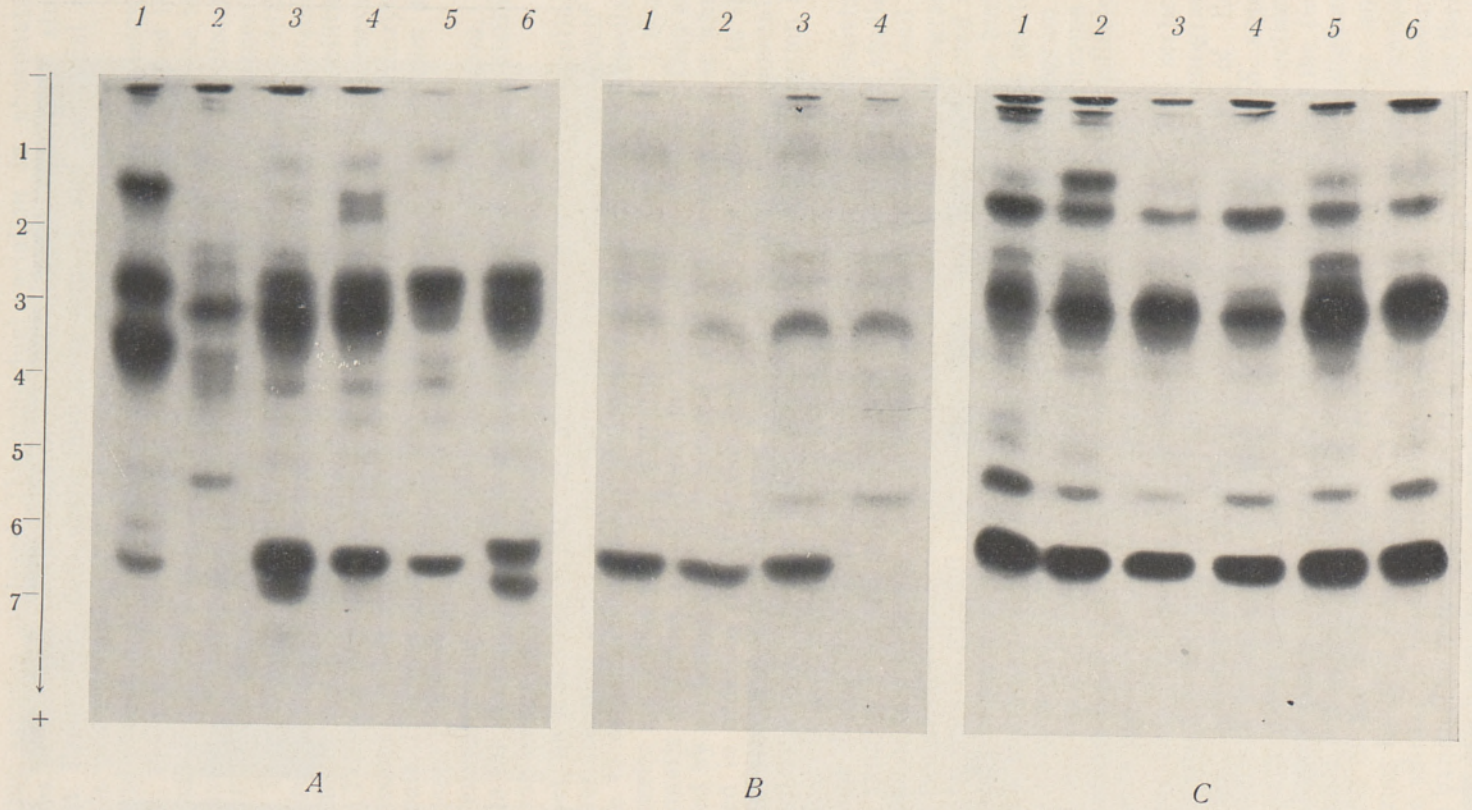

Fig. 2. Polyacrylamide gel electrophoretic patterns of esterase.

A. H. villcsa (enzymogram *1*), *S. silvestre* from Turkmenia (*2*), *S. anatolicum* K-10237 (*3*), *S. montanum* K-9598 (*4*), *S. africanum* K-10239 (*5*), *S. kuprijanovii* K-9670 (*6*). *B. Secale silvestre*, strains originating from the peninsula Apsheron (enzymogram *1*), from the Crimea, near Yevpatoria (*2* and *3*), from Turkmenia, near Ashkhabad (*4* and *5*). C. *Secale segetale*, strains K-10036 (*1*), K-7984 (*2*), K-7745 (*3*), K-7756 (*4*), K-7784 (*5*), and K-5836 (*6*).

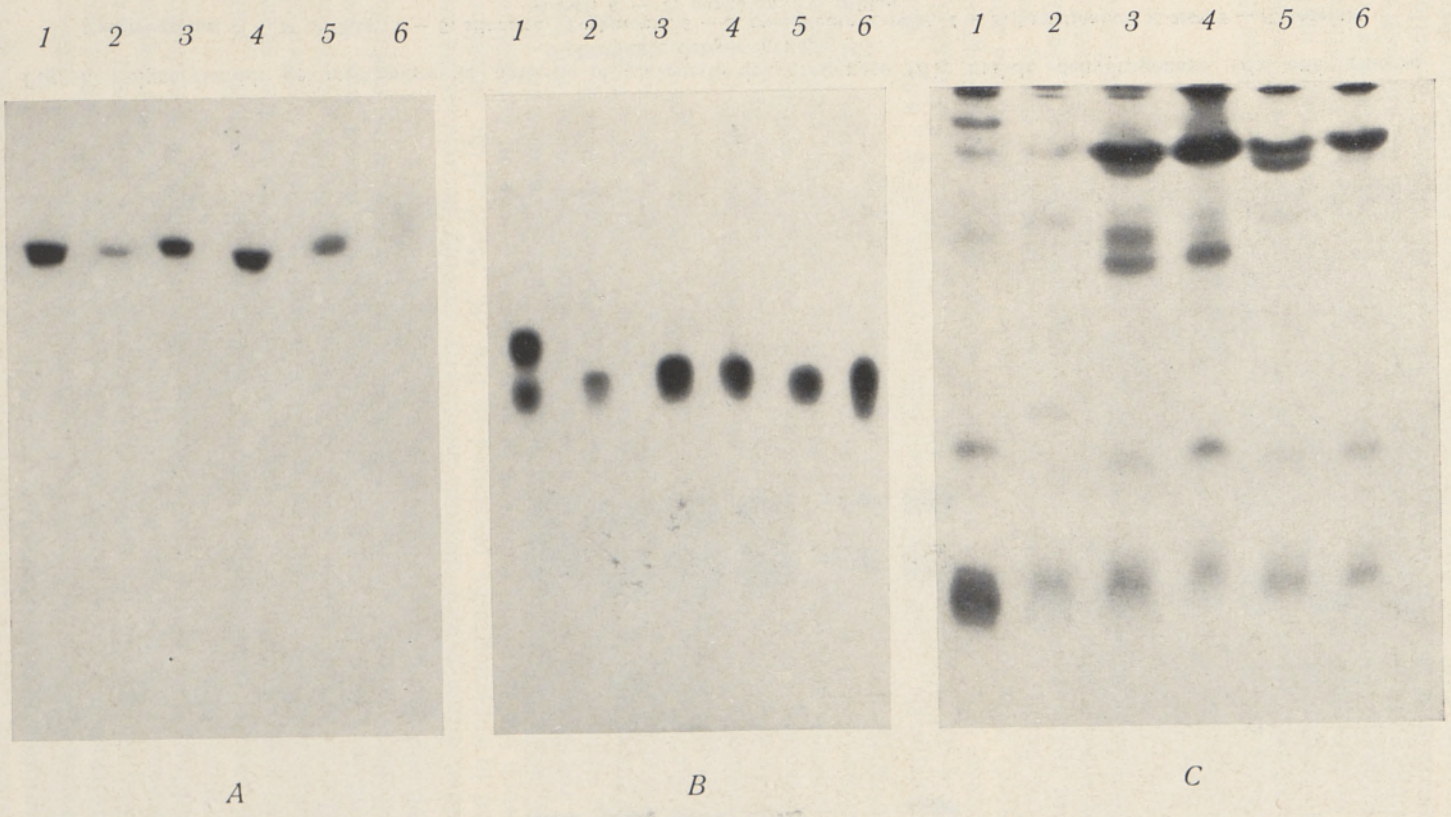

Fig. 3. Polyacrylamide gel electrophoretic patterns of β-glucosidase (*A*), leucine aminopeptidase (*B*), and peroxidase (*C*).
Enzymograms: *1* — *H. villosa*, *2* — *S. silvestre*, Turkmenia; *3* — *S. anatolicum* K-10237, *4* — *S. kuprijanovii* K-9586, *5* — *S. segetale* K-5867, *6* — *T. aestivum*, cv. Pikker.

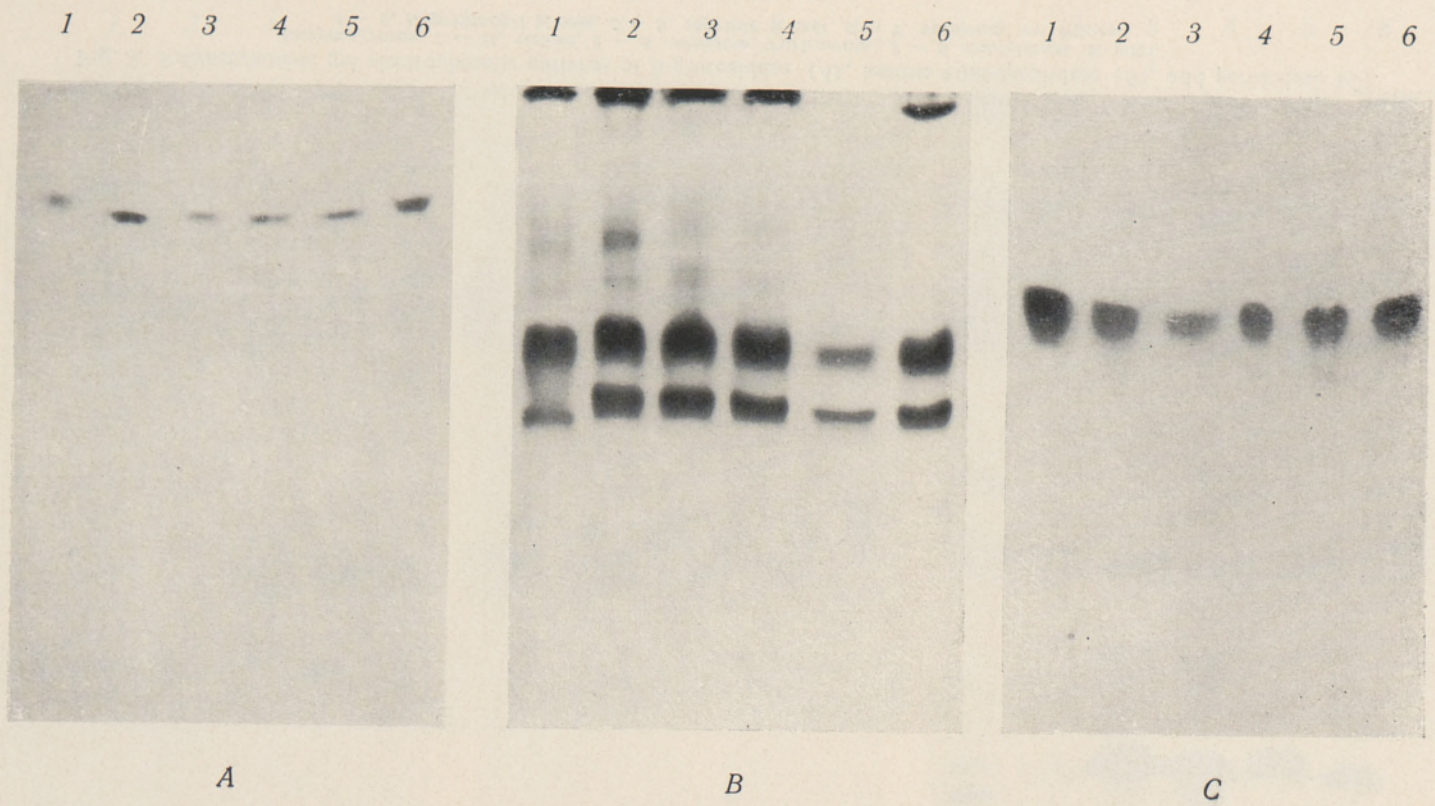

Fig. 4. Polyacrylamide gel electrophoretic patterns of glutamate dehydrogenase (*A*), malate dehydrogenase (*B*) and glucose 6-phosphate dehydrogenase (*C*).

Enzymograms: *1* — *H. villosa*; *2* — *S. silvestre*, Turkmenia, *3* — *S. anatolicum* K-10237, *4* — *S. kuprijanovii* K-9586, *5* — *S. segetale* K-5867, *6* — *T. aestivum*, cv. Pikker.

to the fast-moving esterase and consisted of homozygous and heterozygous individuals with one or two bands, respectively.

β-Glucosidase. Only one band of β-glucosidase activity of similar electrophoretic mobility was found in all rye taxa and in *H. villosa*, as demonstrated in Fig. 3*A*. *T. aestivum* (enzymogram *6*) possessed two closely spaced scarcely distinguishable bands of much lesser activity than in the rye seedlings. One of the bands has the same electrophoretic mobility as the rye glucosidase.

Leucine aminopeptidase. One zone of amidohydrolase activity was found in all ryes as well as in *T. aestivum*, and it has about the same electrophoretic mobility as seen in Fig. 3*B*. This zone actually consisted of two very closely spaced bands which soon fused together in the course of staining and look like one band in the photograph. *H. villosa* has two distinct activity zones, one of which coincides in its electrophoretic mobility with the rye and wheat amidohydrolase.

Peroxidase. From 5 to 7 distinct anodically moving peroxidase bands can be seen in enzymograms presented in Fig. 3*C*. Most of the variation found between the taxa was inherent to slow-moving series of peroxidase isoenzymes, while the two major fast-moving peroxidase bands were common to all rye taxa, *H. villosa* and *T. aestivum*, except for one of the two peroxidases which had changed (slower) electrophoretic mobility in *S. silvestre* (enzymogram *2*). The three accessions of *S. silvestre* (from the Crimea, Apsheron and Turkmenia) possessed similar peroxidase patterns, so that the change in the electrophoretic mobility of one major peroxidase seems to be characteristic of this rye species.

The fastest-moving broad peroxidase band actually consisted of three to four closely spaced isoenzymes which fused upon prolonged incubation aimed at detecting less active isoenzymes, and could not be distinguished in the photograph presented in Fig. 3*C*. A slight shift in the mobility of this peroxidase series in *H. villosa* (enzymogram *1*) with respect to the corresponding rye peroxidases can also be ascertained Fig. 3*C*.

Dehydrogenases. One glutamate dehydrogenase (Fig. 4*A*), two major malate dehydrogenases (Fig. 4*B*), one major glucose 6-phosphate dehydrogenase (Fig. 4*C*), and one sorbitol dehydrogenase (enzymogram not presented) were found to be in common in all rye taxa, *H. villosa* and *T. aestivum*, being electrophoretically essentially invariable among the species and taxa studied. The four dehydrogenases, however, clearly differed between themselves in the electrophoretic mobility of the bands, showing that the enzyme activities were due to structurally different proteins.

Discussion

The results of the present study clearly demonstrate that separate enzymes and even separate isoenzymes of the same enzyme show different degrees of evolutionary variation within the limits of a taxonomic unit. This may be considered as a phenomenon of heterobatmy (mosaic evolution) at the macromolecular level which is indicative of different stability of individual loci controlling the structure of separate enzymes. An alternative explanation is that these differences may be due to different selection pressure on individual loci, since the proteins or the enzymes coded by them are of different significance in the functioning of the organism. This explanation, however, is unsatisfactory for explaining the total absence of variation in some enzymes. Even if electrophoretic protein or enzyme

variants are encountered in the species populations, their number and kind is strikingly limited and characteristic of the species or species groups.

It is well established that only a limited number of amino acids are involved in the "active centre" of the protein molecule controlling its functional activity. Therefore, many electrophoretically detectable changes in the amino acid composition of a protein outside its active centre could occur without any or substantial change in its functional activity and in the viability or selectional fitness of the organism as a whole. In other words, a significant amount of point mutations produced by nucleotide replacement and causing changes in the amino acid sequences of the proteins would theoretically be totally or almost neutral in natural selection. Although some proteins presumably have more rigid structural requirements for functioning normally than others, tolerating structural changes in a lesser degree, our present knowledge suggests that the degree of permissible amino acid changes still remains significant in all proteins, constituting the basis for further evolution. The older views about the harmfulness of the overwhelming majority of gene mutations need to be revised in the light of the biochemical evidence about the structural basis of the functional activity of proteins. Even in those cases when differential selection pressure on individual loci is presumable or when separate enzyme variants have a slightly different selection value, additional intrinsic control over the rate and kind of mutations in a given locus seems to be operating. The nature of this internal variability control is almost unknown at present, but certain structural and conformational peculiarities of the genome DNA might well appear to be the main factors.

The four dehydrogenases studied here were the most conservative of the enzymes considered, showing no significant variation between the species of the whole subtribe *Triticinae* Benth. They had, therefore, no taxonomic value in establishing phylogenetic relationships within the subtribe. Dehydrogenases, however, may have useful application in the phylogenetic studies at higher taxonomic rank. It seems probable that main evolutionary changes in the loci controlling dehydrogenases had occurred in more remote times and the loci have become stabilized, at least in the group of cereals. Our preliminary data (in preparation) suggest that some dehydrogenases, glutamate dehydrogenase and sorbitol dehydrogenase, have remained, as a rule, invariant within the limits of the whole tribe *Triticeae* Dum. The constancy of the dehydrogenases in the polyploid species is especially impressive, since the presence of the same locus in multiplicity in a polyploid would release some of the homologous loci out of the control by natural selection. The absence of increased variation of certain dehydrogenases in the polyploid species of the tribe is an additional indication of internal control of genetic stability.

Leucine aminopeptidase (LAP) and β-glucosidase were also quite conservative enzymes, showing, however, electrophoretically distinguishable divergence in the subtribe. LAP showed one major activity zone of common electrophoretic mobility to all rye species. *H. villosa*, however, had an additional band of LAP activity of distinct electrophoretic mobility which was not encountered in other species. Since only one accession of *H. villosa* was under study, it would be premature at present to consider the two-banded LAP pattern as characteristic of this species, and this point needs further clarification. Similarly, β-glucosidase showed a single fraction of coinciding electrophoretic mobility in *H. villosa*, rye species and in *T. aestivum*. However, the hexaploid wheat had an additional closely-spaced β-glucosidase band, and both bands were of much lesser intensity

than in the rye species, witnessing of smaller soluble glucosidase activity in wheat.

Peroxidase, esterase and phosphatase electrophoretic patterns were more variable, showing clear variation with respect to some isoenzyme fractions between different rye taxa, intra-specific differences and even intra-populational individual genetic polymorphism.

The only enzyme among those studied by us which had certain taxonomic value in establishing phylogenetic relationships between the taxa of the genus *Secale* was acid phosphatase. The examination of acid phosphatase enzymograms permits to arrange the rye taxa in three groups which exactly coincide with the three sections established on the basis of morpho-physiological characteristics by R. Rozhevich (Рожевич, 1947) and later revised on the basis of cytogenetical evidence by U. Nürnberg-Krüger (1960). Our "micro-morphological" data concerning acid phosphatase isoenzymes and classical "macromorphological" data, thus, enable to reach the same basic conclusion with respect to sub-grouping of the genus *Secale* L.

Our isoenzyme data confirm the high degree of genetic closeness of the taxa belonging to the same section. This is in good line with the available cytogenetic evidence. Thus, all the taxa belonging to the section *Cerealia — cereale, segetale, afghanicum, dighoricum*, and *ancestrale*, were found (Khush, 1963; Nürnberg-Krüger, 1960) to be interfertile, producing vigorous F_1 hybrids with normal meiosis and high pollen fertility. Similarly, the taxa of the section *Kuprijanovia — montanum, africanum, kuprijanovii*, and *anatolicum*, have the same chromosome arrangement and can be readily crossed, yielding hybrids of good fertility (Khush, 1962). The hybridization studies give no reason for recognizing these taxa as independent biological species as proposed by R. Rozhevich (Рожевич, 1947), but rather suggest to consider them as subspecies or varieties of the two species, *S. cereale* L. s. l. and *S. montanum* Guss. s. l., as treated earlier by N. Vavilov (1917, 1926) and P. Zhukovsky (Жуковский, 1928). The isoenzyme data yielded no additional valuable information about further intraspecific differentiation within the two species, and the intra-populational genetic polymorphism of phosphatase isoenzymes which is under more detailed study at present is the most interesting feature discovered.

S. montanum and *S. cereale* were shown (Khush, Stebbins, 1961; Nürnberg-Krüger, 1960; Riley, 1955) to differ from each other by two large translocations involving three pairs of chromosomes. As a result, the F_1 hybrids between various forms of the two species reveal meiotic disturbances and reduced fertility. The perennial rye, *S. montanum*, is considered to possess the most primitive characters, and the annuals, *S. silvestre*, *S. cereale* and *S. vavilovii*, are thought to have originated from different ancestral forms (or populations) of this species through a fixation of certain chromosome rearrangements involving reciprocal translocations and genic mutations (Khush, 1962; Nürnberg-Krüger, 1960). Our data do not yield any conclusive evidence for considering which species is the most primitive one and ancestral to others. However, evolutionary divergence in the genus *Secale* L. has involved changes in the structure of genes controlling electrophoretic properties of the phosphatase isoenzymes, leaving the genes for many other enzymes (e. g., dehydrogenases, leucine aminopeptidase, β-glucosidase) unchanged. When assuming that *S. montanum* is the ancestral species, then the emergence of *S. cereale* has been accompanied by an appearance of new phosphatase electrophoretic variants that have become fixed and maintained at high frequencies in the species populations. Similarly, major phosphatase isoenzymes characteristic of

various taxa of *S. montanum* were absent from *S. silvestre*, and at least one new, electrophoretically distinct major phosphatase isoenzyme, not found in *S. montanum*, appeared in *S. silvestre*. However, our present isoenzyme data alone, without additional amino acid sequence or "fingerprint" data about phosphatase isoenzymes, are insufficient for solving the problem of the ancestor in the rye genus.

Summary

Polyacrylamide gel electrophoresis was applied to study the isoenzyme composition of nine different enzymes in the etiolated seedlings of various taxa of the genus *Secale* L. Three related species belonging to the same subtribe, *Haynaldia villosa* (L.) Schur., *Triticum boeoticum* Boiss., and *T. aestivum* L., were also compared enzymologically.

One glutamate dehydrogenase, two major malate dehydrogenases, one sorbitol dehydrogenase, and one major glucose 6-phosphate dehydrogenase were found to be electrophoretically essentially invariable and common to all rye taxa, *H. villosa* and *T. aestivum*. Leucine aminopeptidase and β-glucosidase both revealed a single activity zone of coinciding electrophoretic activity in the taxa of the subtribe *Triticinae* Benth. An additional leucine aminopeptidase band not encountered in other species was, however, present in *H. villosa*, while *T. aestivum* revealed two closely spaced β-glucosidase bands of much lesser intensity than in the rye seedlings.

Peroxidase, esterase and phosphatase electrophoretic enzymograms exhibited distinct variation between the rye taxa in some isoenzymes, while the major acid phosphatase isoenzymes showed intra-populational individual genetic polymorphism. On the basis of acid phosphatase patterns, the rye taxa studied fall in the same three sections or polymorphous biological species as previously established on the basis of morpho-ecological characteristics and cytogenetic studies.

The enzymes differed considerably in the degree of phylogenetic variation within the limits of the subtribe *Triticinae* Benth., presumably reflecting different stability of individual loci to mutational changes.

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PEREKOND *SECALE* L. ENSÜÜMIDE ELEKTROFOREETILINE UURIMINE

Resümee

Perekond *Secale* L. erinevate taksoonide ja samasse alamtriibusesse *Triticinae* Benth. kuuluvate liikide *Haynaldia villosa* (L.) Schur., *Triticum boeoticum* Boiss. ja *T. aestivum* L. etioleeritud tõusmete üheksa fermendi fraktsioonilist (isoensüümset) koostist uuriti polüakrüülamiidgeelelektroforeesimeetodi abil.

Glutamaadi, sorbitooli ja glükooso-6-fosfaadi dehüdrogenaasid, samuti leutsiini aminopeptidaas ja β-glükosidaas andsid elektroforeesil tavaliselt igaüks ühe põhifraktsiooni, mis omavahel erinesid elektroforeetilise liikuvuse poolest, olid aga muutumatud ja ühised kõigi rukkitaksoonide, *H. villosa* ja *T. aestivum*'i tõusmetel, nagu ka kaks malaadi dehüdrogenaasi põhifraktsiooni. Erandina sisaldasid *H. villosa* tõusmed veel ühe leutsiini aminopeptidaasi fraktsiooni, mis puudus ülejäänud liikidel, *T. aestivum*'i tõusmete mõlemad β-glükosidaasi fraktsioonid aga olid märgatavalt nõrgema intensiivsusega kui rukkil.

Peroksüdaas, esteraas ja fosfataas jaotusid elektroforeesil paljudeks isoensüümideks, millest mitmed varieerusid erinevail rukkitaksoonidel, fosfataasi põhiliste isofermentide osas aga täheldati *S. cereale* L. taksoonidel populatsioonisisest individuaalset geneetilist polümorfismi. Uuritud rukkitaksoonid jaotusid happelise fosfataasi isofermentse koostise alusel samasse kolme sektsiooni või polümorfsesse bioloogilisse liiki, mis on fikseeritud morfo-ökoloogilistest ja tsütoloogilistest uurimistest lähtudes.

Alamtriibuse *Triticinae* Benth. piires erinesid üksikud ensüümid tunduvalt fülogeneetilise varieeruvuse poolest. Arvatavasti tuleneb see vastavate lookuste erinevast stabiilsusest mutatsiooniliste muutuste suhtes.

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ВЕЛЛО ЯАСКА

ЭЛЕКТРОФОРЕТИЧЕСКОЕ ИЗУЧЕНИЕ ФЕРМЕНТОВ В РОДЕ *SECALE* L.

Резюме

Методом электрофореза в полиакриламидном геле изучался фракционный (изоферментный) состав девяти ферментов в этиолированных проростках различных таксонов рода *Secale* L., а также трех родственных видов — *Haynaldia villosa* (L.) Schur., *Triticum boeoticum* Boiss. и *T. aestivum* L., принадлежащих к подтрибе *Triticinae* Benth.

Дегидрогеназы глутаминовой кислоты, сорбитола и глюкозо-6-фосфата, а также лейцинаминопептидаза и β-глюкозидаза встречались в виде одной фракции с различной и характерной для каждого фермента электрофоретической подвижностью, которая, как правило, оказывалась постоянной в пределах всей субтрибы, как и две основные фракции малат-дегидрогеназы. Как исключение в проростках *H. villosa* встречалась

дополнительная фракция лейцинаминопептидазы, отсутствующая у всех остальных видов. Обе фракции β-глюкозидазы у *T. aestivum* оказались намного менее активными, чем в проростках ржи.

Пероксидаза, эстераза и фосфатаза разделились при электрофорезе на множество изоферментов, многие из которых выявили характерное варьирование среди таксонов ржи. Таксоны *S. cereale* обнаружили внутрипопуляционный индивидуальный генетический полиморфизм основных изоферментов кислой фосфатазы. По изоферментному составу кислой фосфатазы таксоны ржи подразделились в те же три секции или полиморфных биологических вида, которые ранее были выявлены на основании морфофизиологических и цитогенетических исследований.

Отдельные ферменты существенно различались по степени филогенетической изменчивости в пределах субтрибы, что, возможно, обусловлено различной стабильностью отдельных локусов к мутационным изменениям.

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