Isozymes in *Aegilops kotschyi* and *Ae. biuncialis* × *Secale cereale* hybrids and *Ae. kotschyi* × *S. cereale* amphiploids in relation to their parents

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**Abstract.** Seven enzymatic systems in F₁ *Aegilops kotschyi* and *Ae. biuncialis* × *Secale cereale* hybrids, *Aegilops kotschyi* × *S. cereale* amphiploids and their parental species (*Ae. kotschyi*, *Ae. biuncialis* and *S. cereale*) were analysed by starch and polyacrylamide gel electrophoresis. Five of them (phosphoglucose isomerase, glutamic oxalacetic transaminase, esterase, acid phosphatase, and diaphorase) were polymorphic and two (malic dehydrogenase and superoxide dismutase) were monomorphic. Several isophorms of phosphoglucose isomerase, esterase, acid phosphatase, and diaphorase were detected in some hybrids and amphiploids, but absent in the parents. The role of regulators, translocations and recombination is discussed in relation to the origin of these new isophorms. Some parental isozymes were absent both in hybrids and amphiploids, probably as a result of the suppression of structural genes in new combinations of the three genomes.

**Key words:** *Aegilops biuncialis*, *Aegilops kotschyi*, amphiploids, electrophoresis, intergeneric hybrids, isozymes, *Secale cereale*.

**Introduction**

Intergeneric hybrids are interesting in the theoretical aspect in evolutionary, taxonomic and cytological studies. However, the introduction of new genes into the cultivated taxa and their monitoring has been frequently used as tools in practical plant breeding. Electrophoretical analyses of isozymes are the most popular methods for the detection of gene products. Many papers presented the enzymatic analysis of different wheat and rye species, including the localisation of genes on chromosomes (i.e. CUBADDA et al. 1975, JAASKA 1976, 1980, 1983, CHOJECKI,
Recently Aegilops–Secale amphiploids have been characterised by pollen morphology and spectra of mature pollen proteins after two-dimensional gel electrophoresis (KALINOWSKI et al. 2001).

Cytological analysis proved that the U genome of Aegilops sp. polyploids is very closely related to the U diploid genome of Ae. umbellulata and it has not been substantially modified during their evolution, whereas the S genome of Ae. kotschyi (UUSS) and the M genome of Ae. biuncialis (UUMM) have undergone substantial changes (KIMBER et al. 1988, KIMBER, YEN 1989, YEN, KIMBER 1990). For these reasons combinations of two different genomes of Aegilops sp. with the Secale cereale genome (RR) seemed to be interesting material for molecular investigation.

The aim of the present report was the electrophoretical analysis of malate dehydrogenase, superoxide dismutase, phosphoglucose isomerase, glutamic oxalacetic transaminase, esterase, acid phosphatase, and diaphorase of Ae. kotschyi and Ae. biuncialis × S. cereale F1 hybrids and Ae. kotschyi × S. cereale amphiploids in relation to their parents: Ae. kotschyi, Ae. biuncialis and S. cereale.

Material and methods

Plant material

The plant material consisted of: (1) the parental species – Aegilops kotschyi (accessions: TKKO1, 14805, 14808, in this paper referred to as AK-0, AK-2, AK-3, respectively), Ae. biuncialis (accessions: 14712, 14714, 14716, 14718), S. cereale inbred line form (in the paper referred to as IL) and three self-compatible lines (in the paper referred to as Sc); (2) 9 F1 hybrids Ae. kotschyi × S. cereale (Sc); (3) 46 F1 hybrids Ae. biuncialis × S. cereale – 23 plants with inbred rye (IL) and 23 plants with self-compatible rye (Sc); (4) 7 Ae. kotschyi × S. cereale (Sc) amphiploids.

The F1 hybrids were produced via embryo culture, while the amphiploids were obtained through colchicine treatment and in vitro propagation (WOJCIECHOWSKA, PUDELSKA 2002). Malate dehydrogenase, superoxide dismutase, phosphoglucose isomerase, glutamic oxalacetic transaminase, esterase, acid phosphatase and diaphorase were analysed in the hybrids, amphiploids and their parental species using gel electrophoresis.

Protein extraction

Two young leaves were collected from each plant and kept at −70°C. The proteins were extracted from leaves homogenised for 30 min at 4°C with 0.1 M TRIS-HCl
buffer of pH 7.2 containing 1% dithiothreitol and 0.1% Triton X-100. After centrifugation, crude extracts were absorbed onto Whatman 3 MM (4 × 12 mm, 9 µg protein) and applied onto starch gels. The same crude extracts as for starch gel electrophoresis were used for polyacrylamide gel electrophoresis (25 µl extract, containing ~17 µg of proteins was applied into the pocket of stacking gel).

Electrophoresis

Starch gel electrophoresis was conducted in 12.5% gel (Conaught) with 0.2 M lithium-borate with pH of 8.3 as electrode buffer. The gel buffer was composed of nine parts of 0.05 M TRIS-citric acid with pH of 8.3 and one part of the electrode buffer (GRENECHE et al. 1991). Slices of the gel were incubated in the stain solution for the activity of phosphate glucose isomerase – PGI and glutamate oxaloacetate transaminase (GOT).

Polyacrylamide gel electrophoresis (PAGE) in 10% polyacrylamide slabs (1 mm in thickness, 8-cm path of separation) was run under the constant power of 10 mA/cm². The proteins in gels were specifically stained for esterase (EST), acid phosphatase (ACPH) and diaphorase (DIA).

Results

Among the analysed enzymatic systems, malate dehydrogenase and superoxide dismutase were monomorphic and they are not described in this paper.

Phosphoglucone isomerase (PGI)

Phosphoglucone isomerase, separated in starch gel, showed three (ab) and five band (abc) phenotype patterns (Figure 1). Five bands were detected only in the hybrids *Ae. kotschyi* AK-3 × *S. cereale* and their amphiploids. However, in the remaining hybrids and amphiploids, as well as the parental plants, three-band phenotypes “ab” were observed.

Glutamic oxaloacetic transaminase (GOT)

Glutamic oxaloacetic transaminase was separated in the starch gel. One and three isozyme patterns were detected corresponding to “a”, “b” and “ab” phenotypes (Figure 2). Three accessions of the *Ae. kotschyi* showed “ab” phenotypes, whereas *Ae. biuncialis* accessions had all three phenotypes (“a”, “b” and “ab”). Two phenotypes, “a” and “ab”, were found in self-compatible rye, whereas the inbred rye (IL) had the “ab” phenotype. A total of 70% of *Ae. biuncialis* × *S. cereale* hybrids showed three band patterns (“ab”), while the remaining hybrids had the “b” phenotype. The *Ae. kotschyi* (“ab” phenotype) × *S. cereale* (“a” and “ab” phenotypes) hybrids and their amphiploids showed a one band pattern (phenotype “b”), except for three *Ae. kotschyi* AK-3 × *S. cereale* hybrids, which had the “ab” phenotype.
Esterase (EST)

Three fastest moving isoesterases (EST) 1, 2 and 3 were detected with high frequency in all the analysed samples, as opposed to the remaining seven bands observed with lower frequencies. Band 8 (present in the J phenotype) was detected only in one *Ae. kotschyi* AK-2 × *S. cereale* hybrid. The ten band patterns of isoesterases formed thirteen phenotypes (Figure 3). In the leaves of *Ae. kotschyi* AK-0 seven active isoesterases showed phenotype A, whereas in *Ae. kotschyi* both

\[
\begin{array}{cccccccccc}
A & B & C & D & E & F & G & H & I & J \\
\text{-----} & \text{-----} & \text{-----} & \text{-----} & \text{-----} & \text{-----} & \text{-----} & \text{-----} & \text{-----} & \text{-----} \\
\end{array}
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Figure 3. Phenotypes of esterases (EST) found in *Ae. kotschyi*, *Ae. biuncialis*, *S. cereale*, *F*₁ hybrids and amphiploids
AK-2 and AK-3 accessions phenotype B was found. Either three band patterns (2 × phenotype C) or a five band pattern (1 × phenotype D) were present in the leaves of all samples of *Ae. biuncialis*. In self-compatible rye two isoesterase bands were detected, which formed phenotypes E and F, whereas the inbred rye had five isoesterases: phenotype G. The *Ae. kotschyi × S. cereale* hybrids showed five band patterns which formed five phenotypes (C, H, I, J and K) independently of the phenotypes of the maternal accessions. The *Ae. kotschyi* AK-2 × *S. cereale* amphiploids, similarly to four *Ae. biuncialis* accessions, had three isoesterases 1, 2, 3 (phenotype C). Isoesterases 4 and 5 were present only in *Ae. kotschyi* AK-0 (phenotype A) and in the *Ae. kotschyi* AK-3 × *S. cereale* amphiploids (phenotype M). In seven *Ae. kotschyi × S. cereale* hybrids isoesterases 6 and 7 were detected and an additional band 8 (one plant, see phenotype K), absent in the parents.

**Acid phosphatase (ACPH)**

Nine isozymes of ACPH were detected after separation in polyacrylamide gels. Isozyme 1 was present in all accessions of *Ae. kotschyi* and *S. cereale* IL, in two forms of *S. cereale* Sc, and in the three *Ae. biuncialis × S. cereale* hybrids. Band 2 was present in all the analysed samples, except for the *Ae. kotschyi* AK-2 × *S. cereale* hybrids. Band 3 was present in *Ae. kotschyi* AK-0 × *S. cereale* and some of *Ae. biuncialis × S. cereale* (Sc, IL) hybrids. Isoform 4 was detected in *Ae. kotschyi* AK-2 × *S. cereale* and in *Ae. biuncialis × S. cereale* (Sc) hybrids. These bands were absent in the phenotypes of the parents. The nine isozymes of ACPH formed seventeen phenotypes (Figure 4). Three accessions of *Ae. kotschyi* showed three different phenotypes (A, B, C), in which three fast migrating isozymes (1, 2, 3) were present (Figure 4), and they differed in the presence or absence of slowly migrating isozymes 6-9. Three phenotypes A, D and E

|   | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
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Figure 4. Phenotypes of acid phosphatases (ACPH) found in *Ae. kotschyi*, *Ae. biuncialis*, *S. cereale*, their F1 hybrids and amphiploids

in *Ae. kotschyi* AK-2 × *S. cereale* and 5 in *Ae. biuncialis × S. cereale* (Sc) hybrids. These bands were absent in the phenotypes of the parents. The nine isozymes of ACPH formed seventeen phenotypes (Figure 4). Three accessions of *Ae. kotschyi* showed three different phenotypes (A, B, C), in which three fast migrating isozymes (1, 2, 3) were present (Figure 4), and they differed in the presence or absence of slowly migrating isozymes 6-9. Three phenotypes A, D and E
were found in \textit{S. cereale} (SC). The \textit{Ae. kotschyi} AK-0 \(\times\) \textit{S. cereale} showed the same phenotypes as the parental forms (phenotype A) and \textit{Ae. kotschyi} AK-3 \(\times\) \textit{S. cereale} hybrids had a one band pattern (phenotype D), identical to the paternal form. The \textit{Ae. kotschyi} AK-3 \(\times\) \textit{S. cereale} hybrids were represented by one band phenotype (F), while their amphiploids had three isophorms (phenotype E). Out of the twenty three \textit{Ae. biuncialis} \(\times\) \textit{S. cereale} (Sc) hybrids, three had the paternal phenotype (E), whereas the remaining showed ten different phenotypes (G, H, I, J, K, L, M, N, O and P). The hybrids of \textit{Ae. biuncialis} with the inbred rye showed eight phenotypes: E, I, J, K, O, Q, R and S, absent in the parents.

\textbf{Diaphorase (DIA)}

Slow and fast migrating zones, each consisting of four DIA isozyme bands, were visualised on the gels in the analysed samples (Figure 5). Isophorm 4 (absent in the parents) was detected in five \textit{Ae. biuncialis} \(\times\) \textit{S. cereale} (Sc) hybrids. Some paternal isophorms of diaphorase were absent in \textit{Ae. kotschyi} \(\times\) \textit{S. cereale} hybrids and their amphiploids. Three phenotypes (A, B, C) were found in \textit{Ae. kotschyi} accessions, whereas in \textit{S. cereale} (Sc) only two (F and G) were found. The \textit{Ae. kotschyi} \(\times\) \textit{S. cereale} hybrids showed E, D, I and K phenotypes, absent in the parental species. The \textit{Ae. kotschyi} AK-2 \(\times\) \textit{S. cereale} amphiploids showed three fast moving (1, 2, 3) and two slow moving (6, 7) bands, creating in all the cases phenotype D (absent in both parents). The \textit{Ae. kotschyi} AK-3 \(\times\) \textit{S. cereale} amphiploids had phenotype T not observed in the parents. The \textit{Ae. biuncialis} (phenotypes A, D) \(\times\) \textit{S. cereale} (Sc, phenotypes F, G) hybrids showed five phenotypes: K, L, M, O and T, except for one plant with phenotype A. In \textit{Ae. biuncialis} \(\times\) \textit{S. cereale} (IL) hybrids the maternal phenotype D was found in four plants, while in the remaining phenotypes K, L, M, N, O, P, Q, R, and S were observed, which were absent in the parents.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure5.png}
\caption{Phenotypes of diaphorase (DIA) found in \textit{Ae. kotschyi}, \textit{Ae. biuncialis}, \textit{S. cereale}, \textit{F1} hybrids and amphiploids}
\end{figure}
Discussion

Our previous pollen protein analysis of *Aegilops* sp. × *Secale cereale* by two-dimensional gel electrophoresis showed that some parental peptides were absent in the amphiploids of *Ae. variabilis* and *Ae. kotschyi* with *S. cereale*. However, some of the pollen proteins were characteristic only for the amphiploids. Differences in the structure of exine and the shape of pollen grains between the parent and amphiploid pollen were also observed (KALINOWSKI et al. 2001).

In this report some isozymes active only in the leaves of hybrids and amphiploids were detected as the two additional PGI bands forming the “abc” phenotype. This phenotype was found in the *Ae. kotschyi* AK-O and AK-3 × *S. cereale* hybrids and the *Ae. kotschyi* AK-3 with *S. cereale* amphiploids. Similarly, ACPH bands 6 and 7 were present in the *Ae. kotschyi* × *S. cereale* hybrids only. The appearance of ACPH isophorm 4 in 30% of *Ae. kotschyi* AK-2 × *S. cereale* and 61% of *Ae. biuncialis* × *S. cereale* (Sc) and isophorm 5 in 35% *Ae. biuncialis* × *S. cereale* (IL) hybrids may be also connected to the expression of new genes. Isophorm 4 of DIA, absent in the parental plants, was detected in *Ae. biuncialis* × *S. cereale* hybrids only. The detection of new isozymes in both the F1 hybrids and the amphiploids, not expressed in the parents, may be a result of the recombination between different alleles. In the hybrid population the intragenic recombination significantly increases the variation and number of alleles including unique alleles. It has been shown that unique alleles could be formed also by intragenic recombination (STROBECK, MORGAN 1978, MORGAN, STROBECK 1979, GOLDING, STROBECK 1983). These all observations probably may also be a result of regulators controlling the expression of structural genes (COLAS DES FRANCES, THIELLEMENT 1985, ZIVY et al. 1992). It is possible that parental species possess enzymatic silent genes activated in the new combinations of different genomes USR, UMR and UUSSRR as a result of the neutralisation of suppression. New isozymes patterns may also result from chromosome translocations, which have been observed to occur with high frequency in intergeneric hybrids between wheat and rye (LUKASZEWSKI et al. 1984, MARAIS, MARAIS 1994, APOLINARSKA 1996). The creation of intergeneric hybrids not only allowed for the introduction of new beneficial genes into cultivated taxa, but also activated silent structural genes. Parental PGI (ab) were expressed in F1 hybrids, whereas paternal GOT (a) phenotype was absent in hybrids and amphiploids, which had “ab” and “b” phenotypes only. The F1 hybrids were characterised with a low frequency of ACPH isophorms 1 and 3. Diaphorase 6 was absent in F1, whereas 2 and 3 were detected in low frequencies. Probably it may be a result of gene suppression (GALILI, FELDMAN 1984). Sometimes, for example in the case of diaphorase, more isohorms were detected in the hybrids than in the amphiploids, and this may be a result of gene dosage (ARAGONCILLO et al. 1978).
Conclusions

Intergeneric *Ae. kotschyi* and *Ae. biuncialis × S. cereale* F$_1$ hybrids and *Ae. kotschyi × S. cereale* amphiploids show new isozymes. These new isozymes occur to be active as a result of the recombination of alleles from different genomes. It may also be a result of acting regulators activating silent genes and translations. The intergeneric hybridisation between wild wheats and rye facilitate the introduction of new beneficial genes into cultivated taxa.

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