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Genetic control of triosephosphate isomerase isoenzymes in wheat and rye

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Summary. The patterns of chloroplastic and cytosolic isoenzymes of triosephosphate isomerase were analysed by immunoblotting in leaves of rye, wheat, and some species of *Aegilops* or *Agropyrum*. While rye contained solely one chloroplastic and one cytosolic isoenzyme, wheat had a much more complex pattern which can be explained by the presence of three genomes in 6× wheats (*AABBDD*) with distinct triosephosphate isomerase genes that provided different subunit species for the dimeric isoenzyme molecules. The 6× wheats contained five, the 4× wheats three, and the 2× wheats only one chloroplastic isoenzyme band. The isoenzyme patterns were in accordance with a potential origin of one of the three chloroplastic triosephosphate isomerase genes of 6× wheats from an *Aegilops* ancestor. The descent of the other two genes was, however, not in accordance with common contentions on the general evolution of cultural wheats. In the reciprocal intergeneric hybrids *Secalotricum* and *Triticale* both the chloroplastic and the cytosolic isoenzyme patterns of rye and wheat were biparentally inherited, indicating that both isoenzymes were controlled by nuclear genes. When monitored by immunoblotting the chloroplastic triosephosphate isomerase isoenzymes may provide useful genetic markers.

Key words: Evolution (wheat) – Isozymes – *Secale* – Triosephosphate isomerase – *Triticum*

Introduction

Leaves of higher plants (Kurzok and Feierabend 1984a; Pichersky and Gottlieb 1984) and cells of *Euglena* (Mo et al. 1973) are known to contain distinct isoenzymes of triosephosphate isomerase in the chloroplast and in the cytoplasm. Each isoenzyme consists of two subunit polypeptides. Previous studies (Kurzok and Feierabend 1984b; Pichersky and Gottlieb 1984) have shown that the polypeptides of the two compartment-specific isoenzymes differed in their primary structures and were presumably produced by distinct genes. In order to compare the genetic controls of the chloroplastic and the cytosolic isoenzymes we have, in the present investigation, compared their patterns in leaves of rye and wheat. Because the isoenzyme patterns differed quite markedly in the two genera, their mode of inheritance could be analyzed in the reciprocal intergeneric hybrids between rye and wheat, *Secalotricum* and *Triticale,* which are available. It was of particular interest whether the chloroplastic isoenzyme showed an uniparental-maternal inheritance, as expected for an organellar gene, or a biparental inheritance, characteristic for a nuclear gene.

Compared to other plants, the pattern of triosephosphate isomerase isoenzymes observed in hexaploid wheats was rather complex. The origin of the complex isoenzyme pattern of wheat can be understood from its evolution which combined three different genomes in 6× cultural wheats (Riley 1965; Sears 1974; Kasarda et al. 1976). Therefore, we have, for comparison, also analysed triosephosphate isomerase patterns in potential ancestors of present hexaploid wheat.

Isoenzyme patterns obtained by activity staining are often used as genetic markers. Comparisons of activity stains suffer, however, from severe uncertainties, because the isoenzyme proteins are not specifically identified, their ativities do not reliably reflect the amounts of enzyme protein present and different isoenzyme species may be differentially affected by inactivation in vitro. In addition, the analysis of staining patterns is partic-

ularly hampered when groups of compartment-specific isoenzymes overlap, as in wheat. Therefore, we have in the present investigation analysed the isoenzyme patterns after immunoblotting ("Western blot") with antisera raised against individual compartment-specific isoenzymes. By this procedure isoenzyme bands are identified through the antisera by structural homologies and the intensity of the reaction is, at least roughly, related to the amount of isoenzyme protein present.

Material and methods

For *Secale cereale* L. the cultivars 'Petkus Kustro' or 'Halo' were used; for 6× *Triticum aestivum* L. the cultivars 'Kolibri', 'Diplomat' and 'Götz' were used. *Secalotricum* Rth 80, *Secalotricum* Rta 52 (summer rye 'Petkus'×'Chinese spring') and 8× *Triticale* cv. 'Meister' (Trh 38) were obtained from the Institut für Pflanzenbau und Pflanzenzüchtung, Göttingen; 8× *Triticale 'Götz' (644×657 (77)) and 6× Triticale* cv. 'Bokolo' were obtained from F. von Lochow-Petkus GmbH, Bergen; *Aegilops tauschii* Coss. (Kuckuck 332/82), 2× *Triticum sinskajae* Filat & Kurk (Kuckuck 334/82), and the hybrid *Triticum dicoccoides×Aegilops squarrosa* (Kuckuck 304/82) had been supplied by Prof. Kuckuck, Hanover. Other seed materials from the collection of the Botanical Garden of the University of Frankfurt were: *Triticum macha* Dekaprel & Menabde, *Triticum spelta* L., *Triticum durum* Desf., *Triticum dicoccon* Schuebl., *Triticum dicoccoides* Koern., *Triticum boeoticum* Boiss., *Triticum monococcum* L., *Agropyrum cristatum* Gaertner ssp. *pectinatum, Aegilops speltoides* Tausch, *Aegilops squarrosa* L., *Aegilops geniculata* Roth (ovata L.), *Aegilops crassa* Boiss. (6×), *Aegilops triuncialis* L. (4×).

Seedlings were grown on Vermiculite or compost either at 22 °C in continuous white light, or in a greenhouse. Cell-free extracts were prepared by grinding the leaves with mortar and pestle at 4 °C with 25 mM K-phosphate buffer, pH 8.6, containing 20 mM 2-mercaptoethanol and 0.5 mM phenylmethylsulfonyl fluoride and 15 min centrifugation at 48 000×g and 4 °C. Before electrophoresis 0.33 vol. 250 mM Tris-HCl (pH 6.8)/40% (w/v) glycerol/0.1% (w/v) bromphenol blue were added. The final extracts contained 1.5 ml medium per g fresh weight. Chloroplasts were isolated from leaf homogenates by differential centrifugation (Kurzok and Feierabend 1984a).

Preparation and partial purification of rabbit antisera has been described (Kurzok and Feierabend 1984b). Polyacrylamide gel electrophoresis under non-denaturing conditions was performed on 12.5% homogeneous polyacrylamide slab gels for 20 h at 10 mA as previously described (Kurzok and Feierabend 1984a), except that the proportion of bisacrylamide was increased (acrylamide-bisacrylamide 30 : 1.25). For immunoblotting the gels were, after termination of non-denaturing electrophoresis, presoaked for 20 min at 50 °C in 25 mM Tris, 192 mM glycine, 1% (w/v) sodium dodecyl sulfate. Immunoblotting was then performed as previously described for gels electrophoresed in the presence of sodium dodecyl sulfate (Kurzok and Feierabend 1984b). After transfer to the nitrocellulose paper, the triosephosphate isomerase polypeptides reacted not only with their corresponding antiserum but also with the antiserum against the other isoenzyme (see Kurzok and Feierabend 1984b). Antisera exhibiting minimal cross-reaction were selected, so that the cytosolic isoenzyme was undetectable after immunoblotting with the anti-chloroplast isoenzyme-serum. However, after immunoblotting with antiserum against the cytosolic isoenzyme cross-reactions with the chloroplast isoenzyme bands were slightly visible.

The activity stain for triosephosphate isomerase was described previously (Kurzok and Feierabend 1984a).

Results and discussion

Rye leaves contain one cytosolic and one chloroplast isoenzyme of triosephosphate isomerase (Kurzok and Feierabend 1984a). However, wheat leaves have a much more complex pattern of seven bands resolved by stain for enzyme activity (Fig. 1b). The five forms of highest mobility (bands 3–7) were found in isolated wheat chloroplasts and visualized after immunoblotting with an antiserum against the rye chloroplast isoenzyme (Fig. 1a). They can, therefore, be regarded as chloroplastic forms and the remaining two bands were presumably cytosolic. Immunoblotting with an antiserum against the cytosolic isoenzyme from rye suggested that wheat leaves contained altogether three cytosolic triosephosphate isomerase isoenzymes (Fig. 2b, lane 3, bands 3′–5′). However, the cytosolic form of highest mobility appeared to overlap with the chloroplast form of lowest mobility and was, therefore, not to be distinguished in the stain pattern (Fig. 1b). Immunoblotting with the antiserum against the cytosolic isoenzyme was not as specific as immunoblotting with the anti-chloroplast isoenzyme-serum because, due to some cross-reaction, chloroplast forms were also always slightly visible (Fig. 2b). It is to be emphasized that only immunoblotting enabled an unequivocal resolution of the chloroplastic isoenzymes in total leaf extracts. The pattern of five bands of chloroplastic triosephosphate isomerase forms was without any variation observed in several different 6× wheat species (*Triticum aestivum, spelta, macha*) and in different cultivars of *Triticum aestivum* ('Diplomat', 'Götz', 'Kolibri').

Increased numbers of isoenzyme forms are frequently connected with polyploidy (Gottlieb 1982). The multiplicity of chloroplast triosephosphate isomerases in wheat leaves can also be explained, as for other isoenzymes (e.g. Hart 1970; Tang and Hart 1975), by the contribution of three distinct genes to the subunit composition of the dimeric enzyme molecule. Hexaploid wheats contain three different genomes, designated as *A*, *B* and *D* (Riley 1965; Sears 1974; Kasarda et al. 1976), and the isoenzyme patterns suggest that they possess different genes for chloroplastic triosephosphate isomerase. A model explaining the origin of the five dimeric wheat chloroplast triosephosphate isomerase forms from three different subunit polypeptide species (designated as α, β, δ), and their quantitative

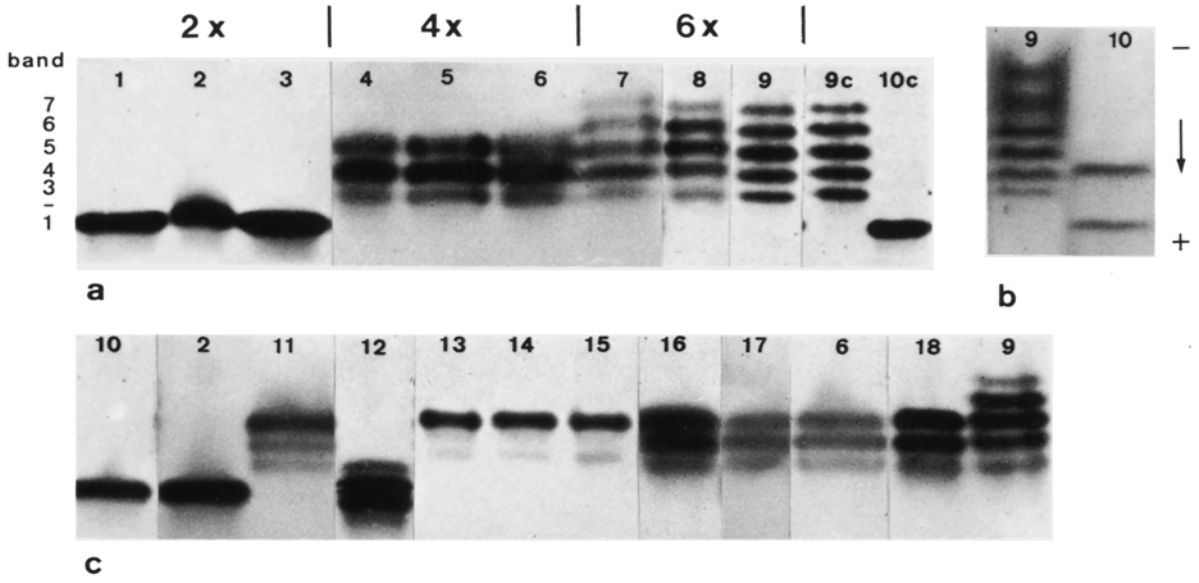

Fig. 1a–c. Non-denaturing polyacrylamide gel electrophoresis of leaf extracts (1–18) or soluble supernatant fractions of isolated chloroplasts (9c, 10c). **a** and **c** Immunoblotting with an antiserum against the chloroplast triosephosphate isomerase; **b** stain for triosephosphate isomerase activity. 1: *Triticum sinskajae;* 2: *Tr. boeoticum;* 3: *Tr. monococcum;* 4: *Tr. durum;* 5: *Tr. dicoccon;* 6: *Tr. dicoccoides;* 7: *Tr. spelta;* 8: *Tr. macha;* 9: *Tr. aestivum* 'Kolibri'; 10: *Secale cereale* 'Halo'; 11: *Agropyrum cristatum;* 12: *Aegilops geniculata;* 13: *Ae. tauschii;* 14: *Ae. speltoides;* 15: *Ae. squarrosa;* 16: *Ae. crassa;* 17: *Ae. triuncialis;* 18: *Tr. dicoccoides* × *Ae. squarrosa*

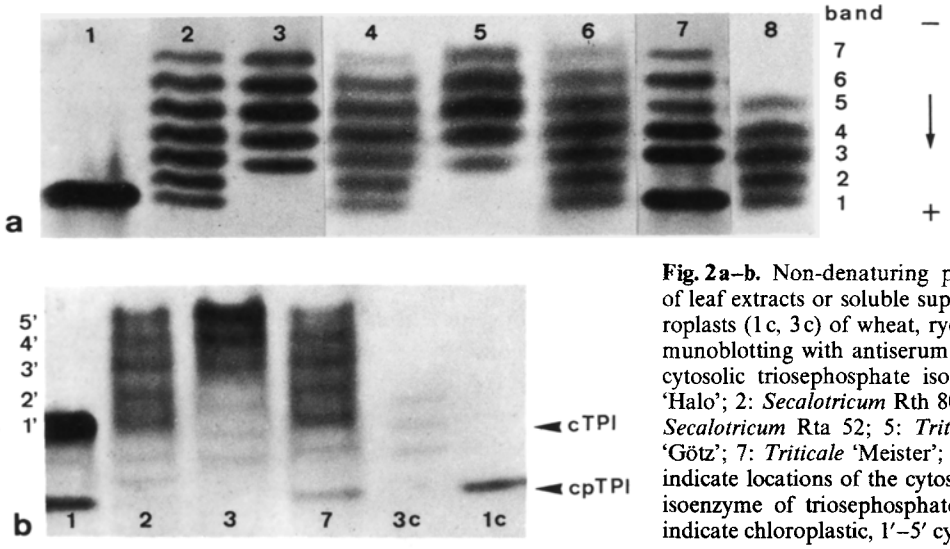

Fig. 2a–b. Non-denaturing polyacrylamide gel electrophoresis of leaf extracts or soluble supernatant fractions of isolated chloroplasts (1c, 3c) of wheat, rye, or intergeneric hybrids, and immunoblotting with antiserum against **a** the chloroplast, or **b** the cytosolic triosephosphate isomerases of rye. 1: *Secale cereale* 'Halo'; 2: *Secalotricum* Rth 80; 3: *Triticum aestivum* 'Kolibri'; 4: *Secalotricum* Rta 52; 5: *Triticum aestivum* 'Götz'; 6: *Triticale* 'Götz'; 7: *Triticale* 'Meister'; 8: *Triticale* 'Bokolo' (6×). *Arrows* indicate locations of the cytosolic (cTPI) or chloroplast (cp TpI) isoenzyme of triosephosphate isomerase from rye leaves. 1–7 indicate chloroplastic, 1′–5′ cytosolic isoenzyme bands

proportions is presented in Table 1. The model of Table 1 implicates that three homodimers and three heterodimers are produced and that the heterodimers are expected in the middle between the bands of related homodimers in twofold concentrations when all three subunit species are equally expressed. The validity of the model and particularly its assumption that each of the three subunit species has to be assigned to one of the three genomes is greatly supported by the observation that all 4x wheat species (*Tr. dicoccoides, dicoccon,* and *durum*) containing the genomes *A* and *B*, possessed only three chloroplast isoenzyme bands (Fig. 1a), while the 2× wheats (*Tr. boeoticum, monococcum, sinskajae*) containing solely the *A* genome possessed only one chloroplast triosephosphate isomerase form (Fig. 1a). From the comparison of 4× and 6× wheats chloroplast triosephosphate isomerase bands 6 and 7 should be those containing the δ polypeptide contributed by the *D* genome. Though *Aegilops squarrosa* is regarded as the potential donor of the *D* genome (Riley 1965; Sears 1974; Kasarda et al. 1976), a chloroplast isoenzyme form corresponding to band 7

Table 1. Schematic model of the subunit polypeptide compositions and quantitative proportions of the chloroplast triosephosphate isomerase isoenzyme bands of rye, wheat, and their hybrids *Secalotricum* and *Triticale*. Band numbers refer to Figs. 1a and 2b. Underlining indicates overexpression of isoenzyme forms containing the rye subunit polypeptide ϱ

Band no.	*Secale* (2×)	*Triticum* (4×)	*Triticum* (6×)	*Secalotricum*, *Triticale* (8×)	*Triticale* 'Meister' (8×)	*Triticale* 'Bokolo' (6×)
7	–	–	$\delta\delta$	$\delta\delta$	$\delta\delta$	–
6	–	–	$2\beta\delta$	$2\beta\delta$	$2\beta\delta$	–
5	–	$\beta\beta$	$\beta\beta$, $2\alpha'\delta$	$\beta\beta$, $2\alpha'\delta$	$\beta\beta$	$\beta\beta$
4	–	$2\alpha'\beta$	$2\alpha'\beta$	$2\alpha'\beta$, $2\delta\varrho$	$\underline{2\delta\varrho}$	$2\alpha'\beta$
3	–	$\alpha'\alpha'$	$\alpha'\alpha'$	$\alpha'\alpha'$, $2\beta\varrho$	$\underline{2\beta\varrho}$	$\alpha'\alpha'$, $2\beta\varrho$
2	–	–	–	$2\alpha'\varrho$	–	$2\alpha'\varrho$
1	$\varrho\varrho$	–	–	$\varrho\varrho$	$\underline{\varrho\varrho}$	$\varrho\varrho$

($\delta\delta$) of 6× wheats was not found in the contemporary accession of this species. *Aegilops squarrosa* contained a chloroplast isoenzyme of the same mobility as that found in *Aegilops speltoides*, a hypothetical donor of the *B* genome. This isoenzyme form found in both *Aegilops* species corresponded to band 5 of 6× wheat and may represent a potential source of the β polypeptide. Consequently, in a hybrid between *Triticum dicoccoides* and *Aegilops squarrosa* no δ-containing isoenzyme form appeared but the $\beta\beta$ and $\alpha'\beta$ bands were intensified, thus confirming a potential descent of the subunit designated β from an *Aegilops* species. The cytosolic forms of triosephosphate isomerase appeared, however, to differ in *Aegilops squarrosa* and *speltoides* (not shown) and the pattern of 6× wheats conceivably contained the forms of both species. The chloroplast triosephosphate isomerase form found in contemporary 2× wheats, the presumptive donors of the *A* genome, was different from band 3, the expected homodimer contributed by the *A* genome in 4× or 6× wheats, but had the same mobility as the chloroplast isoenzyme from rye (Fig. 1). Because the polypeptide expected to be derived from the *A* genome was not identical with the enzyme form of contemporary 2× wheat species regarded as ancestors of the *A* genome, it was designated as α' (Table 1).

While the chloroplast isoenzyme patterns were quite constant in different species of *Triticum* and changed only with the ploidy level, greater variations were observed among different species of *Aegilops* or of *Agropyrum* which has also been discussed as a potential ancestor in the evolution of wheat (Riley 1965; Kasarda et al. 1976). Some of them, e.g. *Aegilops crassa, triuncialis* or *geniculata,* and *Agropyrum cristatum,* contained similar chloroplast triosephosphate isomerase patterns or forms as the 4× wheats and might represent potential sources for the α' gene (Fig. 1c). The pattern of 6× *Aegilops crassa* closely resembled that of the hybrid *Triticum dicoccoides* × *Aegilops squarrosa.*

While our comparisons have provided convincing evidence that the pattern of chloroplast triosephosphate isomerase forms is related to the evolution of 6× wheat and generated by the presence of three distinct genes, only the possible descent of the β polypeptide is in accord with present contentions about the evolution of cultural wheats. The question of the phylogenetic origins of the α' and δ polypeptides has to remain unsettled and would require much more refined methods for a detailed comparison of gene structures. Our results do not question existing general conclusions about the origins of the *A* and *D* genomes. However, when the latter are correct, either the phylogenetic origins of the α' and δ genes must differ from the general path of the evolution of wheat or they must have diverged from their ancestral forms in the contemporary accessions of *Aegilops squarrosa* and in 2× and 6× *Triticum.*

The constancy and clear difference of the chloroplast triosephosphate isomerase patterns of 6× or 4× wheat and 2× rye enabled an analysis of their inheritance in the reciprocal hybrids *Triticale* (*Triticum* ♀ × *Secale* ♂) and *Secalotricum* (*Secale* ♀ × *Triticum* ♂; Fig. 2a). Four different lines of 8× *Triticale* or *Secalotricum* contained both the five isoenzyme bands of 6× *Triticum* and the one isoenzyme band of rye. The 6× *Triticale* 'Bokolo' contained the three isoenzyme bands of 4× *Triticum* as well as that of *Secale.* In all intergeneric hybrids, except for *Triticale* 'Meister', an extra isoenzyme band (band 2, Fig. 2a) appeared, in addition to those present in the parents, which has to be regarded as a heterodimer of wheat and rye polypeptides ($\alpha'\varrho$, Table 1). The quantitative distribution of the chloroplast isoenzyme forms indicates that, except for *Triticale* 'Meister', the four distinct subunit species of the 8× hybrids, or the three subunit species of the 6× hybrid were equally expressed. This has also been observed for other isoenzymes in hybrids or addition

lines (Tang and Hart 1975). In the 8× *Triticale* 'Meister', band 2 ($\alpha' \varrho$) was virtually absent and the distribution of the intensities of the chloroplast isoenzymes differed greatly from that of *Triticale* 'Götz' or *Secalotricum* (Fig. 2a). The pattern of *Triticale* 'Meister' suggests that in this cultivar the α' gene is either missing or greatly suppressed while the gene from *Secale* seems to be greatly overexpressed, as compared to the other subunit genes (Table 1). Irrespective of such peculiarities, the results obtained with all hybrids (Fig. 2a) show, however, that the chloroplast triosephosphate isomerases were clearly biparentally inherited. Inasmuch as only *Triticum* has a strictly maternal, Secale, however, a biparental plastid transmission (Tilney-Basset 1978), the main convincing evidence which indicates a nuclear control of the chloroplast triosephosphate isomerase comes from the fact that it was also transmitted from a male wheat parent to *Secalotricum*. This is in accord with observations about the inheritance of different chloroplast triosephosphate isomerase forms assayed by activity stain in investigations on gene duplications in *Stephanomeria* and *Clarkia* which also indicated that the chloroplast forms were controlled by nuclear genes (Gallez and Gottlieb 1982; Pichersky and Gottlieb 1983). Therefore, the chloroplastic triosephosphate isomerases would be expected to be synthesized on cytoplasmic 80S ribosomes as already reported in preliminary form for rye (Feierabend 1979), whereas Mo et al. (1973) had concluded from inhibitor experiments that the chloroplastic triosephosphate isomerase was in *Euglena* produced on 70S ribosomes.

Cytosolic triosephosphate isomerase was also biparentally inherited. The isoenzyme patterns of *Secalotricum* and 8× *Triticale* were identical (Fig. 2b). Besides the three isoenzyme forms from wheat and one from rye, they contained an additional intermediate isoenzyme band, presumably composed of both a wheat and a rye subunit polypeptide. However, the immunological cross-reaction with the chloroplast isoenzyme and the lower number of separable isoenzyme bands made immunoblotting analysis of the cytosolic triosephosphate isomerase less unequivocal and less indicative for the genotypic composition of the plant than immunoblotting of the chloroplast isoenzymes. Analysis of the chloroplast triosephosphate isomerase pattern by immunoblotting might, however, serve as convenient aid for monitoring genetic constitutions of plants in wheat breeding, inasmuch as only a small amount of vegetative leaf material is needed.

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