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The genetic control of triosephosphate isomerase of hexaploid wheat and other Triticeae species

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SUMMARY

The zymogram phenotypes of triosephosphate isomerase (TPI) were determined for a large number of aneuploid derivatives of *Triticum aestivum* cv. 'Chinese Spring' and for six wheat-alien species chromosome addition series. Examination of the available compensating nullisomic-tetrasomic and homoeologous groups 3 and 5 ditelosomic lines of Chinese Spring disclosed that *T. aestivum* possesses two systems of dimeric TPI isozymes, designated TPI-1 and TPI-2. The genes *Tpi-A1*, *Tpi-B1* and *Tpi-D1* were located in Chinese Spring chromosome arms 3Ap, 3Bp and 3Dp, respectively, and the genes *Tpi-A2*, *Tpi-B2* and *Tpi-D2* in chromosome arms 5Aq, 5Bq and 5Dq, respectively. *Tpi-1* genes were also located in *Hordeum vulgare* cv. Betzes chromosome 3H, *T. longissimum* chromosome G, *Elytrigia elongata* chromosome 3E, and *Secale cereale* cvs. Imperial and Dakold chromosome 3R. *Tpi-2* genes were found in Betzes chromosome 5H, *T. umbellulatum* chromosome 5U, *T. longissimum* chromosome F, and Imperial and Dakold chromosome 5R. These gene locations provide evidence of homoeology between the alien chromosomes in which the genes are located and the chromosomes of homoeologous groups 3 and 5 of Chinese Spring, respectively. Evidence was obtained for the presence of a *Tpi-R2* gene in each of the *T. aestivum* cv. Kharkov-*S. cereale* cv. Dakold chromosome addition lines studied suggesting that this gene is present in the wheat genome in each member of this addition series.

INTRODUCTION

As genetic knowledge of the cultivated wheats and their relatives in the Gramineae tribe Triticeae increases, the ability to efficiently manipulate the genetic material present in these species for purposes of crop improvement also increases. The chromosomal locations of a large number of enzyme structural genes have been determined in *Triticum aestivum* ($2n = 6x = 42$) cv. 'Chinese Spring' by zymogram studies of aneuploid lines (Hart, 1979, 1984). By study of wheat-alien chromosome addition lines, the locations of homologous genes have been determined in other Triticeae species (Hart & Tuleen, 1983*a*). These structural genes can serve as excellent markers for chromosomes and chromosome segments during genetic manipulations of the cultivated wheats and their relatives.

Triosephosphate isomerase (TPI; E.C. 5.3.1.1) catalyses the equilibrium

reaction between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This paper reports the results of studies designed to determine the manner of genetic control and the subunit composition of the multiple forms of TPI in the tribe Triticeae. The chromosomal arm locations of the genes which encode TPI in *T. aestivum* cv. Chinese Spring and the chromosomal locations of homologous TPI genes in several wheat relatives were determined. The results obtained indicate that the TPI enzymes of the tribe Triticeae exist functionally as dimers.

2. MATERIALS AND METHODS

Chinese Spring aneuploids examined included all possible compensating nullisomic-tetrasomic lines, except nulli 2A-tetra 2B, 2A–2D, 4A–4B, and 4A–4D, all possible homoeologous group 3 ditelosomic lines and the three homoeologous group 5 *q* arm (= long arm) ditelosomic lines.

Table 1. *Lines examined along with recipient varieties, donors, and sources*

Recipient	Donor	Lines examined*	Original source
Chinese Spring	*Hordeum vulgare* cv. Betzes	Addns. 2H–7H	Islam, Shepherd & Sparrow (1981)
Chinese Spring	*T. longissimum*	Addns. A–G	Feldman (1979*a*, *b*) Hart & Tuleen (1983*c*)
Chinese Spring	*T. umbellulatum*	Addns. 1U, 5U, 7U, A, D, F, CSU-31	Kimber (1967)
Chinese Spring	*Elytrigia elongata*	Addns. 1E–7E	Dvorak & Knott (1974) Dvorak (1980) Hart & Tuleen (1983*b*)
Chinese Spring	*Secale cereale* cv. Imperial	Addns. 1R, 2R, 3R, 4/7R, 5R, 6/7R, 7/4/6R	Driscoll & Sears (1971)
Kharkov	*S. cereale* cv. Dakold	Addns. 1R, 3R, 4/7R, 5R, 6/7R	Evans & Jenkins (1960)

* Only one added alien chromosome is present in line F of the *T. umbellulatum* series. Lines B & E of the *T. longissimum* series each contain two added pairs of alien chromosomes and 20 pairs of wheat chromosomes (Hart & Tuleen, 1984). All of the other lines examined are disomic addition lines.

Six chromosome addition series maintained at Texas A & M University were analysed. The addition lines studied and the recipient varieties, alien species, and original sources are given in Table 1. In addition to the chromosome addition lines, cvs. Chinese Spring, Kharkov, Betzes, Imperial and Dakold, several accessions each of *T. longissimum* and *T. umbellulatum*, and the Chinese Spring-*T. longissimum*, Chinese Spring-*E. elongata*, and Chinese Spring-*S. cereale* cv. Imperial amphiploids were studied.

Tissue extracts containing TPI were prepared for electrophoresis from shoots of 8 to 14-day-old green seedlings grown in petri dishes on moist filter paper. Each shoot was macerated in a mortar containing a small amount of sea sand and 50 μl of a pH 7·5 buffer containing 0·1 M Tris, 0·1 M-KCl, 0·005 M-EDTA, 0·04 M-β-mercaptoethanol, and 0·1 M sucrose. The slurry obtained was centrifuged at

10000 rev/min for 15 min in 1 ml tubes in an SS-34 rotor in a Sorvall RC2-B centrifuge and the resulting supernatant used directly for electrophoresis. Electrophoresis was performed in 12% horizontal Electrostarch gels using a pH 8·1, 0·028 M lithium hydroxide-0·188 M boric acid electrode buffer and a gel buffer composed of one part electrode buffer and nine parts pH 8·3, 0·05 M Tris-0·007 M citric acid buffer (Gottlieb, 1981). A constant current of 45 mA was maintained during electrophoresis until the voltage rose to 200 after which this voltage was maintained until the borate front had migrated 10 cm from the origin at which time electrophoresis was stopped.

A modification of an electrophoretic procedure described by Rick, Fobes & Holle (1977) produces equivalent resolution and greater staining intensity of TPI-1 isozymes than the procedure described above. However, this method does not resolve all of the TPI-2 isozymes and is therefore unsatisfactory for simultaneous study of both of the TPI isozyme systems (Pietro, 1984).

The TPI isozymes were stained using a modification of a technique developed by Shaw and Prasad (1970). The substrate, dihydroxyacetone-phosphate (DHAP), is prepared by combining 5 ml of pH 8·0, 0·2 M Tris-HCl buffer, 5 ml of 2·0 M-α,β-glycerophosphate (sodium salt), 5 ml of 1·0 M pyruvate (sodium salt), 15 mg NAD^+, 70 units of lactate dehydrogenase and 14 units of α-glycerophosphate dehydrogenase in 35 ml of distilled water. After thorough mixing, the substrate solution is covered and placed in an incubator at 37 °C for 2 h after which the pH of the solution is adjusted to 2·0 with 1 N-HCl and then a few minutes later readjusted to 7·0 with 1·0 M Tris. The staining solution is then prepared by mixing 50 ml of the substrate solution with 20 mg NAD^+, 10 mg (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), 175 mg sodium arsenate, 6 mg phenazine methosulphate (PMS), and 400 units of phosphoglyceraldehyde dehydrogenase. Staining was carried out at 37 °C in the dark.

3. RESULTS

(i) *Aneuploid genetic analyses of hexaploid wheat triosephosphate isomerase*

The triosephosphate isomerase zymogram phenotype of *Triticum aestivum* cv. Chinese Spring consists of eight bands. Aneuploid analyses provided results which indicate that these bands are the products of two genetically independent groups of TPI isozymes (designated TPI-1 and TPI-2) and that the TPI isozymes are dimers.

(*a*) *Genetics of TPI-1*

The three electrophoretically cathodal TPI isozymes compose the TPI-1 system. Studies of the compensating nullisomic-tetrasomic derivatives of Chinese Spring disclosed that the TPI-1 zymogram phenotype varies with the dosage of the homoeologous group 3 chromosomes but not with variation in the dosage of any other chromosome (Fig. 1).

Strains nullisomic for chromosome 3D express only the most cathodal TPI-1 band (II, Fig. 1). The phenotype exhibited by the nulli-3B tetra-3A and nulli-3A tetra-3B strains is indistinguishable from the phenotype of Chinese Spring

(I, Fig. 1). The other group 3 nulli-tetra types, nulli-3A tetra-3D and nulli-3B tetra-3D, exhibit a phenotype in which TPI-1 bands 1 and 2 have greater staining intensity than band 3 (III, Fig. 1). The observed variation in the TPI-1 bands provides evidence that these bands are the sites of a group of enzymes which are encoded by genes located in the homoeologous group 3 chromosomes. The results are consistent with the nullisomic and tetrasomic conditions for each group 3

Fig. 1. Diagrams of TPI-1 zymogram phenotypes produced by the compensating nullisomic-tetrasomic and ditelosomic derivatives of Chinese Spring and by Chinese Spring. (I) Chinese Spring, nulli-3A tetra-3B, nulli-3B tetra-3A, each of the nulli-tetra combinations of homoeologous groups 1, 2, 4, 5, 6, and 7, and ditelo-3Ap, 3Bp, -3Dp. (II) Nulli-3D tetra-3A, nulli-3D tetra-3B, and ditelo-3Dq. (III) Nulli-3A tetra-3D and nulli-3B tetra-3D. (IV) Ditelo-3Aq and -3Bq.

Table 2. *Schematic models for the subunit composition of the TPI-1 isozymes produced by* T. aestivum *cv. Chinese Spring and by each of the homoeologous group 3 compensating nulli-tetra and ditelosomic strains**

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Isozymes	Chinese Spring Ditelo-3Ap, -3Bp and -3Dp	Nulli-3D Tetra-3A or -3B and Ditelo-3Dq	Nulli-3B Tetra-3A	Nulli-3B Tetra-3D	Ditelo-3Bq
TPI-1a	$1/9\,\delta_1\delta_1$		$1/9\,\delta_1\delta_1$	$4/9\,\delta_1\delta_1$	$1/4\,\delta_1\delta_1$
TPI-1b	$4/9\,\alpha_1\delta_1, \beta_1\delta_1$		$4/9\,\alpha_1\delta_1$	$4/9\,\alpha_1\delta_1$	$2/4\,\alpha_1\delta_1$
TPI-1c	$4/9\,\alpha_1\alpha_1, \beta_1\beta_1, \alpha_1\beta_1$	$\alpha_1\alpha_1, \beta_1\beta_1, \alpha_1\beta_1$	$4/9\,\alpha_1\alpha_1$	$1/9\,\alpha_1\alpha_1$	$1/4\,\alpha_1\alpha_1$

* The subunit compositions and predicted quantitative distributions of the nulli-3A tetra-3B, nulli-3A tetra-3D and ditelo-3Aq isozymes are the same as those of the nulli-3B tetra-3A, nulli-3B tetra-3D and ditelo-3Bq isozymes, respectively, except for the presence of β_1, rather than α_1, subunits.

chromosome resulting respectively in the absence and in the doubling in quantity of a TPI-1 gene and of the subunit that the gene produces. A schematic model for the subunit composition of the TPI-1 isozymes of Chinese Spring and the group 3 nulli-tetra strains is shown in Table 2. The three TPI-1 genes located in chromosomes 3A, 3B and 3D are designated *Tpi-A1*, *Tpi-B1* and *Tpi-D1*, respectively, and the subunits they encode as α_1, β_1 and δ_1, respectively. The model assumes that the three types of subunits are produced in equal quantities and that they associate randomly in all possible combinations.

The model for the TPI-1 isozymes predicts that in the absence of chromosome 3D, *Tpi-D1* and consequently the δ_1 subunit is not present and therefore that the one remaining isozyme is composed entirely of α_1 and β_1 subunits, namely, $\alpha_1\alpha_1$

and $\beta_1\beta_1$ homodimers and $\alpha_1\beta_1$ heterodimers. The model predicts, consistent with the results, that when chromosome 3D (and therefore *Tpi-D1*) is present in four doses (and either 3A or 3B is absent) the relative intensity of TPI-1a, produced by $\delta_1\delta_1$ dimers, will increase. An intermediate mobility is expected for $\alpha_1\delta_1$ and $\beta_1\delta_1$ heterodimers since they consist of one electrophoretically fast subunit (δ_1) and one slow subunit (α_1 or β_1). Given the evidence that TPI-1a is composed of $\delta_1\delta_1$ subunits, the absence of TPI-1b when 3D is absent provides direct evidence that TPI-1b is composed of $\alpha_1\delta_1$ and $\beta_1\delta_1$ subunits, since these dimers are expected to be absent when δ_1 is not present.

The model is also supported by the observed relative staining intensities of the bands which compose the Chinese Spring and nulli-tetra phenotypes. Expansion of the trinomial $(p+q+r)^2$, in which p, q and r are the predicted frequencies of α_1, β_1, and δ_1 respectively, for Chinese Spring and the various nulli-tetra strains generates the theoretical frequencies presented in Table 2. The relative staining intensities of the bands produced by each of these strains are in good agreement with the predicted frequencies of the isozymes.

To determine the chromosomal arm locations of the *Tpi-1* genes, the group 3 ditelosomic strains were analyzed. The absence of any one of the q arms of chromosomes 3A, 3B or 3D has no distinguishable effect on the Chinese Spring zymogram phenotype (I, Fig. 1). However, the absence of any one of the p arms of the homoeologous group 3 chromosomes causes the production of a phenotype which differs from that of Chinese Spring. The phenotype of the ditelo-3Dq strain is indistinguishable from the phenotype of the nulli-tetra strains which lack chromosome 3D (II, Fig. 1). This confirms that 3D contains one or more TPI-1 structural genes and locates the gene or genes in the p arm of the chromosome. The absence of the short arm of either 3A or 3B causes the production of a phenotype in which the intermediate band stains with greater intensity than the approximately equally intense anodal and cathodal bands (IV, Fig. 1). This result is predicted by the model when either *Tpi-A1* or *Tpi-B1* is absent, namely, a 1:2:1 distribution among the TPI-1a, -1b and -1c isozymes. These results thus confirm the location of *Tpi-A1* and *Tpi-B1* in 3A and 3B, respectively, and indicate that the genes are located in the p arms of the chromosomes.

(*b*) *Genetics of TPI-2*

The TPI-2 system is composed of the five electrophoretically anodal TPI isozymes. The TPI-2 zymogram phenotype varies with variation in the dosage of the homoeologous group 5 chromosomes but not with the dosage of any other chromosome (Fig. 2). Nullisomy for each homoeologous group 5 chromosome causes the absence of a pair of TPI-2 bands. Specifically, strains nullisomic for chromosome 5A do not express bands 1 and 2 (II and III, Fig. 2), strains lacking 5D do not express bands 4 and 5 (VI and VII, Fig. 2), and strains nullisomic for 5B do not express bands 2 and 4 (IV and V, Fig. 2). Also, tetrasomy for each group 5 chromosome causes an increase in the relative staining intensity of two bands, at least one of which is absent when the same chromosome is absent. Since plants nullisomic for 5A, 5B and 5D lack bands 1 and 2, 2 and 4, and 4 and 5, respectively, it is apparent that a gene (or genes) located in each group 5 chromosome encodes

a product(s) located at the sites of these respective bands. It is also apparent that chromosomes 5A and 5B jointly produce TPI-2b, that 5B and 5D jointly produce TPI-2d, that 5A, 5B and 5D jointly produce TPI-2c, and that TPI-2a is produced by 5A alone and TPI-2e by 5D alone. On the assumption that the TPI-2 isozymes are dimers, these findings indicate that TPI-2a is composed of homodimers produced by a 5A gene(s), that TPI-2e is composed of homodimers produced by a 5D gene(s), that Tpi-2b is composed of heterodimers produced by 5A and 5B genes, that TPI-2d is composed of heterodimers produced by 5B and 5D genes, and that TPI-2c is composed of homodimers produced by a 5B gene(s) and of heterodimers produced by 5A and 5D genes.

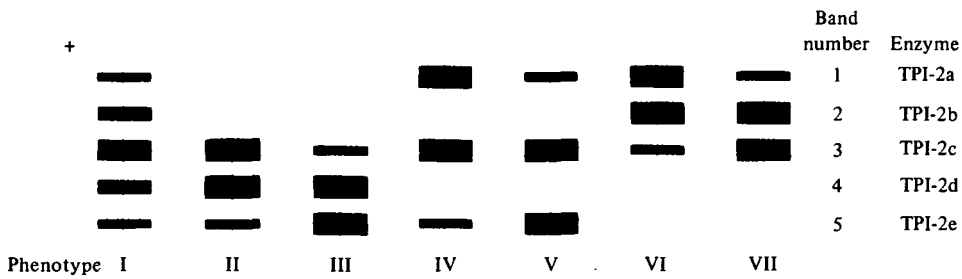

Fig. 2. Diagrams of TPI-2 zymogram phenotypes produced by the compensating nullisomic-tetrasomic derivatives of Chinese Spring and by Chinese Spring. (I) Chinese Spring, each of the nulli-tetra combinations of homoeologous groups 1, 2, 3, 4, 6 and 7, and ditelo-5Aq, -5Bq, -5Dq. (II) Nulli-5A tetra-5B. (III) Nulli-5A tetra-5D. (IV) Nulli-5B tetra-5A. (V) Nulli-5B tetra-5D. (VI) Nulli-5D tetra-5A. (VII) Nulli-5D tetra-5B.

The simplest hypothesis consistent with the results obtained is that chromosomes 5A, 5B and 5D each contain one TPI-2 structural gene. These genes are designated *Tpi-A2*, *Tpi-B2* and *Tpi-D2*, respectively, and the products they encode as α_2, β_2, and δ_2, respectively. A schematic model for the subunit composition of the TPI-2 isozymes of Chinese Spring and of each of the homoeologous group 5 nulli-tetra lines is shown in Table 3. The model makes the same assumptions as described earlier for the TPI-1 model.

The expected quantitative distributions of the TPI-2 isozymes of Chinese Spring and of each of the group 5 compensating nulli-tetra lines, based on the model just described, are shown in Table 3. These distributions are in good agreement with the observed relative staining intensities of the TPI-2 bands of the Chinese Spring and nulli-tetra zymogram phenotypes and therefore provide additional support for the model of genetic control and subunit composition of TPI-2 that is proposed here.

The TPI-2 zymogram phenotypes of the homoeologous group 5 *q* arm ditelosomic lines are identical to the phenotype of Chinese Spring, indicating that each line contains each of the TPI genes. It is therefore concluded that the *Tpi-2* genes are located in the *q* arms of the group 5 chromosomes of Chinese Spring.

Additional evidence that the TPI-1 and TPI-2 isozymes are encoded by different sets of paralogous genes consists of the finding that the TPI-1 isozymes of Chinese Spring are located in the cytosol and the TPI-2 isozymes in the chloroplasts (Pietro, 1984).

Table 3. *Schematic models for the subunit composition of the TPI-2 isozymes produced by* T. aestivum *cv. Chinese Spring and by each of the homoeologous group 5 compensating nulli-tetra strains*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Isozymes	Chinese Spring	Nulli-5A Tetra-5B	Nulli-5A Tetra-5D	Nulli-5B Tetra-5A	Nulli-5B Tetra-5D	Nulli-5D Tetra-5A	Nulli-5D Tetra-5B
TPI-2a	$1/9\ \alpha_2\alpha_2$	—	—	$4/9\ \alpha_2\alpha_2$	$1/9\ \alpha_2\alpha_2$	$4/9\ \alpha_2\alpha_2$	$1/9\ \alpha_2\alpha_2$
TPI-2b	$2/9\ \alpha_2\beta_2$	—	—	—	—	$4/9\ \alpha_2\beta_2$	$4/9\ \alpha_2\beta_2$
TPI-2c	$3/9\ \beta_2\beta_2$, $\alpha_2\delta_2$	$4/9\ \beta_2\beta_2$	$1/9\ \beta_2\beta_2$	$4/9\alpha_2\delta_2$	$4/9\ \alpha_2\delta_2$	$1/9\ \beta_2\beta_2$	$4/9\ \beta_2\beta_2$
TPI-2d	$2/9\ \beta_2\delta_2$	$4/9\ \beta_2\delta_2$	$4/9\ \beta_2\delta_2$	—	—	—	—
TPI-2e	$1/9\ \delta_2\delta_2$	$1/9\ \delta_2\delta_2$	$4/9\ \delta_2\delta_2$	$1/9\ \delta_2\delta_2$	$4/9\ \delta_2\delta_2$	—	—

(ii) *Aneuploid genetic analyses of triosephosphate isomerase of relatives of hexaploid wheat*

For a dimeric enzyme, one or the other of two types of variant zymogram phenotypes is commonly produced by one member of a wheat-alien chromosome addition line series (Hart & Tuleen, 1983*a*). In one type, two or more new bands that do not overlap the existing zymogram bands of the recipient wheat strain are formed due to the production of alien homodimers and of one or more novel heterodimers (formed by dimerization of alien subunits with wheat subunits) which differ in mobility from the wheat isozymes. The second common form of variant phenotype occurs due to the coincident electrophoretic mobilities of the newly formed homo- and heterodimers with wheat isozymes. In this case, the zymogram phenotype produced differs from that of the recipient wheat strain only in the relative staining intensities of the bands which compose it.

The latter of the two aforementioned types of variant phenotypes was observed in the study of the *T. aestivum* cv. Chinese Spring–*H. vulgare* cv. Betzes addition line series. *H. vulgare* cv. Betzes exhibits a TPI zymogram phenotype which consists of one band equal in mobility to the most anodal band of the TPI-1 system of Chinese Spring and one band with the same mobility as the intermediate band of the TPI-2 isozyme system.

Electrophoresis of tissue extracts from each of the six available Chinese Spring–Betzes disomic chromosome addition lines (a disomic Betzes chromosome 1H addition line is not available) disclosed that only the chromosome 3H addition line expresses a TPI-1 zymogram phenotype (II, Fig. 3) that differs from the phenotype of Chinese Spring. This suggests that Betzes chromosome 3H contains a TPI-1 structural gene(s). A schematic model for the subunit composition of TPI-1 of the chromosome 3H addition line is shown in Table 4. This model assumes that chromosome 3H carries a TPI-1 gene, designated *Tpi-H1*, which encodes a subunit, designated θ_1, and that the four types of TPI-1 subunits of addition line 3 are produced in equal quantities and associate randomly in all possible combinations. The expected distribution of the isozymes shown in Table 4 is obtained by expanding the tetranomial $(p+q+r+s)^2$ with $p = q = r = s = 1/4$ where p, q, r and s represent the predicted frequencies of the four subunits present. Good evidence

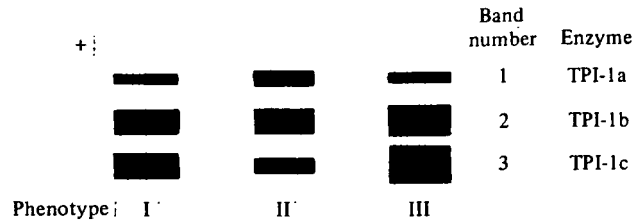

Fig. 3. Diagrams of wheat-alien chromosome addition line TPI-1 zymogram phenotypes. (I) Chinese Spring, addition lines containing Betzes chromosomes 2H, 4H, 5H, 6H and 7H, *T. longissimum* chromosome addition lines A, B, C, D, E and F, *T. umbellulatum* addition lines 1U, 5U, 7U, A, D, F and CSU-31 and *E. elongata* chromosome addition lines 1E, 2E, 4E, 5E, 6E and 7E. (II) Betzes chromosome 3H addition line. (III) *T. longissimum* chromosome addition line G, Chinese Spring–*T. longissimum* amphiploid, *E. elongata* chromosome addition line 3E, and Chinese Spring–*E. elongata* amphiploid.

Table 4. *Schematic models for the subunit composition of the TPI-1 and TPI-2 isozymes of* T. aestivum *cv. Chinese Spring and of certain Chinese Spring*–Hordeum vulgare *cv. Betzes*,–T. longissimum,–T. umbellulatum *and*–E. elytrigia *addition lines*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Isozymes	Chinese Spring	Betzes 3H	*T. longissimum G**
TPI-1a	$1/9\ \delta_1\delta_1$	$1/4\ \theta_1\theta_1, \delta_1\delta_1, \delta_1\theta_1$	$1/16\ \delta_1\delta_1$
TPI-1b	$4/9\ \alpha_1\delta_1, \beta_1\delta_1$	$2/4\ \alpha_1\theta_1, \alpha_1\delta_1, \beta_1\theta_1, \beta_1\delta_1$	$6/16\ \alpha_1\delta_1, \beta_1\delta_1, \delta_1\lambda_1$
TPI-1c	$4/9\ \alpha_1\alpha_1, \beta_1\beta_1, \alpha_1\beta_1$	$1/4\ \alpha_1\alpha_1, \beta_1\beta_1, \alpha_1\beta_1$	$9/16\ \alpha_1\alpha_1, \beta_1\beta_1, \lambda_1\lambda_1, \alpha_1\beta_1, \alpha_1\lambda_1, \beta_1\lambda_1$
	Chinese Spring	Betzes 5H†	*T. umbellulatum 5U*
TPI-2a	$1/9\ \alpha_2\alpha_2$	$1/16\ \alpha_2\alpha_2$	$1/16\ \alpha_2\alpha_2$
TPI-2b	$2/9\ \alpha_2\beta_2$	$4/16\ \alpha_2\beta_2, \alpha_2\theta_2$	$2/16\ \alpha_2\beta_2$
TPI-2c	$3/9\ \beta_2\beta_2, \alpha_2\delta_2$	$6/16\ \beta_2\beta_2, \alpha_2\delta_2, \beta_2\theta_2, \theta_2\theta_2$	$5/16\ \beta_2\beta_2, \alpha_2\delta_2, \alpha_2\mu_2$
TPI-2d	$2/9\ \beta_2\delta_2$	$4/16\ \beta_2\delta_2, \delta_2\theta_2$	$4/16\ \beta_2\delta_2, \beta_2\mu_2$
TPI-2e	$1/9\ \delta_2\delta_2$	$1/16\ \delta_2\delta_2$	$4/16\ \delta_2\delta_2, \delta_2\mu_2, \mu_2\mu_2$

* The subunit composition and predicted quantitative distribution of the TPI-1 isozymes of the *E. elongata* chromosome 3E addition line is the same as that of the *T. longissimum* chromosome G addition line except for the presence of ϵ_1 rather than λ_1 subunits.

† The subunit composition and predicted quantitative distribution of the TPI-2 isozymes of the *T. longissimum* chromosome F addition line is the same as that of the Betzes chromosome 5H addition line except for the presence of λ_2 rather than θ_2 subunits.

in support of the proposed model was obtained, since the observed relative staining intensities of the bands of the Betzes chromosome 3H addition line zymogram phenotype are in agreement with the 1:2:1 distribution of isozymes predicted by the model.

The TPI-2 zymogram phenotypes observed for the Chinese Spring–Betzes chromosome addition series are shown in Figure 4. Among the six chromosome addition lines, only the chromosome 5H addition line produced a phenotype which differs from that of Chinese Spring (II, Fig. 4). This suggest that a TPI-2 structural gene or genes is located in Betzes chromosome 5H. A schematic model for the

subunit composition of the TPI-2 isozymes of the chromosome 5H addition line is shown in Table 4. This model assumes that Betzes chromosome 5H contains a gene, designated *Tpi-H2*, which encodes a subunit, designated θ_2, and that the four subunits present in addition line 5H are produced in equal quantities and associate randomly in all possible combinations. The model predicts a difference in the

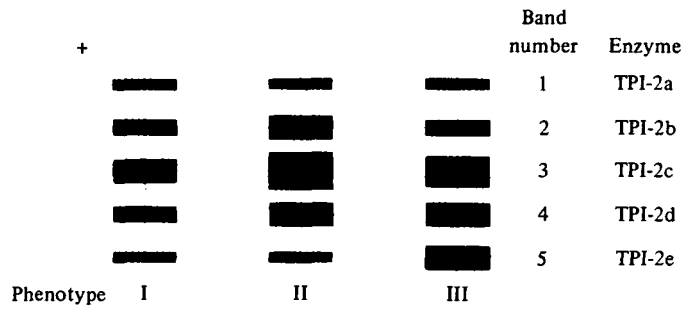

Fig. 4. Diagrams of wheat-alien chromosome addition line TPI-2 zymogram phenotypes. (I) Chinese Spring, Betzes chromosome addition lines 2H, 3H, 4H, 6H and 7H, *T. longissimum* chromosome addition lines A, B, C, D, E and G, *T. umbellulatum* addition lines 1U, 7U, A, D, F, and CSU-31, *E. elongata* chromosome addition lines 1E–7E and the Chinese Spring–*E. elongata* amphiploid. (II) Betzes chromosome addition line 5H and *T. longissimum* addition line F. (III) *T. umbellulatum* addition line 5U.

relative quantities of the five isozymes produced by Chinese Spring and the chromosome 5H addition line that is of lesser magnitude than the difference in quantity predicted between the TPI-1 isozymes of Chinese Spring and the chromosome 3H addition line (see above). Nevertheless, the good agreement observed between the relative staining intensities of the chromosome 5H addition line zymogram phenotype and that predicted for the line provides good support for the model.

Other addition line series studied in which evidence was obtained for expression of novel isozymes coincident in electrophoretic mobility to the Chinese Spring isozymes include the *T. aestivum* cv. Chinese Spring–*T. longissimum*, –*E. elongata* and –*T. umbellulatum* addition series. Only the chromosome G addition line among the seven Chinese Spring–*T. longissimum* lines studied exhibited a different TPI-1 zymogram phenotype than Chinese Spring (III, Fig. 3). The TPI-1 and TPI-2 genes of *T. longissimum* are designated *Tpi-S^{l}1* and *Tpi-S^{l}2*, respectively, and the protomers they encode as μ_1 and μ_2, respectively. The expected relative frequencies of TPI-1a, -1b, and -1c of chromosome addition line G, assuming equal production and random association of protomers and that the alien homodimer has an electrophoretic mobility coincident to the wheat TPI-1c isozyme, is 1:6:9 (III, Fig. 3). The finding that the ratio corresponds closely to the relative intensities of the line G zymogram bands provides evidence that the TPI-1 gene of *T. longissimum* is located in the *T. longissimum* chromosome present in line G. Chromosome addition line F of this series produces a TPI-2 phenotype identical to that of the *T. aestivum* cv. Chinese Spring–*H. vulgare* cv. Betzes disomic chromosome 5H addition line (II, Fig. 4). Agreement between the corresponding

model and the observed TPI-2 zymogram phenotype of the chromosome F addition line provides evidence that *Tpi-S^{l}2* is located in the *T. longissimum* chromosome contained in line F.

The Chinese Spring–*E. elongata* amphiploid and the *T. aestivum* cv. Chinese Spring–*E. elongata* chromosome 3E addition line produce the same TPI-1 zymogram phenotype, a phenotype different from that of Chinese Spring and the other addition lines in this series. This variant phenotype is identical to that of addition line G of the Chinese Spring–*T. longissimum* addition series (III, Fig. 3). Agreement between the observed TPI-1 zymogram phenotype of the 3E line and its respective model provides evidence that the *E. elongata* gene, *Tpi-E1*, is located in chromosome 3E (Table 4). All of the addition lines in this series and the Chinese Spring–*E. elongata* amphiploid produced a TPI-2 phenotype indistinguishable from the Chinese Spring TPI-2 phenotype, thus the chromosomal location of an *E. elongata Tpi-2* gene could not be determined.

None of the Chinese Spring–*T. umbellulatum* addition lines exhibited a TPI-1 zymogram phenotype different from that of Chinese Spring. However, the Chinese Spring–*T. umbellulatum* disomic chromosome 5U addition line TPI-2 phenotype differs from the phenotype of Chinese Spring (III, Fig. 4) while all of the other addition lines expressed the same TPI-2 phenotype as Chinese Spring. The agreement between the observed staining intensities of the chromosome 5U addition line zymogram phenotype and the corresponding model (Table 4), which is based on the same assumptions as were stated earlier, indicates the *T. umbellulatum* TPI-2 structural gene, designated *Tpi-U2*, is located in chromosome 5U.

In contrast to the findings described thus far, certain of the *T. aestivum* cv. Chinese Spring–*S. cereale* cv. Imperial and *T. aestivum* cv. Kharkov–*S. cereale* cv. Dakold addition lines expressed novel TPI isozymes whose electrophoretic mobilities differ from the homologous isozymes of the recipient strains.

The TPI zymogram phenotype of the Chinese Spring–Imperial disomic chromosome 3R addition line differs from that of Chinese Spring, most noticeably by a major increase in the relative staining intensity of bands 7 and 8 (II, Fig. 5). This is consistent with the production of two TPI-1 isozymes by this line that are not produced by Chinese Spring, namely, TPI-1d and TPI-1e, which coincide in electrophoretic mobility with TPI-2d and -2e, respectively. A schematic model for the TPI-1 isozymes of the chromosome 3R addition line is shown in Table 5. The product of the rye gene is designated ρ_1 and the gene is designated *Tpi-R1*. The model is based on the assumptions of equal production and random association of protomers described above. The model predicts a major increase in the relative staining intensity of TPI band 7 (due to the presence of $\rho_1\rho_1$ homodimers) and especially of band 8 (due to the production of $\alpha_1\rho_1$ and $\beta_1\rho_1$ heterodimers) of the chromosome 3R addition line relative to that of Chinese Spring. The expected distribution of the five TPI-1 isozymes which are located at the sites of bands 6 through 10 is 1:2:5:4:4, respectively (Table 5). However, TPI-2d and TPI-2e are also located at the sites of bands 6 and 7, respectively, and have a predicted distribution of 2:1, respectively. Assuming that TPI-1 and TPI-2 subunits are produced in equal quantities, combining the predicted distributions of the TPI-1

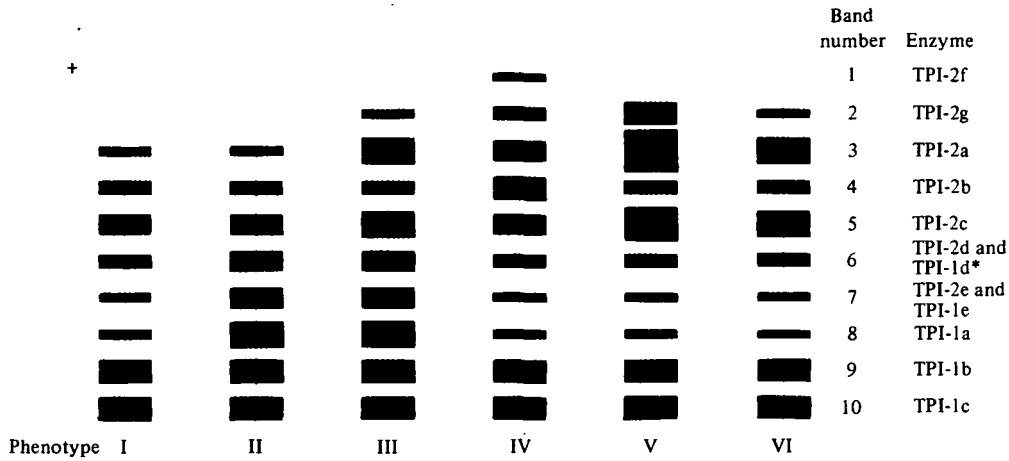

Fig. 5. Diagrams of the TPI-1 and TPI-2 zymogram phenotypes observed in the study of the *T. aestivum* cv. Chinese Spring–*S. cereale* cv. Imperial and *T. aestivum* cv. Kharkov–*S. cereale* cv. Dakold chromosome addition series. (I) Chinese Spring, Kharkov, and Imperial chromosome addition lines 1R, 2R, 4/7R, 6/7R, and 7/4/6R. (II) Imperial chromosome addition line 3R. (III) Dakold chromosome addition line 3R. (IV) Imperial chromosome addition line 5R. (V) Dakold chromosome addition line 5R. (VI) Dakold chromosome addition lines 1R, 4/7R, and 6/7R. * TPI-1d and TPI-1e do not exist in phenotypes I, IV, V, and VI.

Table 5. *Schematic models for the subunit composition of the TPI-1 and TPI-2 isozymes produced by* T. aestivum *cvs. Chinese Spring and Kharkov and by the Chinese Spring–*S. cereale *cv. Imperial and the Kharkov–*S. cereale *cv. Dakold chromosome 3R addition lines.*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Isozymes*	Chinese Spring and Kharkov	Imperial 3R TPI-2	Imperial 3R & Dakold 3R TPI-1	Dakold 3R TPI-2
TPI-2g .	—	—	—	$1/16\,\rho_2\rho_2$
TPI-2a	$1/9\,\alpha_2\alpha_2$	$1/9\,\alpha_2\alpha_2$	—	$5/16\,\alpha_2\alpha_2, \beta_2\rho_2, \alpha_2\rho_2$
TPI-2b	$2/9\,\alpha_2\beta_2$	$2/9\,\alpha_2\beta_2$	—	$2/16\,\alpha_2\beta_2$
TPI-2c	$3/9\,\beta_2\beta_2, \alpha_2\delta_2$	$3/9\,\beta_2\beta_2, \alpha_2\delta_2$	—	$5/16\,\beta_2\beta_2, \alpha_2\delta_2, \delta_2\rho_2$
TPI-1d & TPI-2d	$2/9\,\beta_2\delta_2$	$2/9\,\beta_2\delta_2$	$1/16\,\rho_1\rho_1$	$2/16\,\beta_2\delta_2$
TpI-1e & TPI-2e	$1/9\,\delta_2\delta_2$	$1/9\,\delta_2\delta_2$	$2/16\,\delta_1\rho_1$	$1/16\,\delta_2\delta_2$
TPI-1a	$1/9\,\delta_1\delta_1$	—	$5/16\,\delta_1\delta_1, \beta_1\rho_1, \alpha_1\rho_1$	—
TPI-1b	$4/9\,\alpha_1\delta_1, \beta_1\delta_1$	—	$4/16\,\alpha_1\delta_1, \beta_1\delta_1$	—
TPI-1c	$4/9\,\alpha_1\alpha_1, \beta_1\beta_1, \alpha_1\beta_1$	—	$4/16\,\alpha_1\alpha_1, \beta_1\beta_1, \alpha_1\beta_1$	—

* TPI-1d and TPI-1e are not present in Chinese Spring and Kharkov.

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and TPI-2 isozymes which are located at the sites of bands 6–10, the theoretical distribution of the isozymes becomes 3:3:5:4:4, respectively. This corresponds closely to the relative staining intensities of bands 6 through 10 of the chromosome 3R addition line zymogram phenotype and the Chinese Spring–Imperial amphiploid. This agreement between model and observations provides good evidence that *Tpi-R1* is located in Imperial chromosome 3R.

The TPI zymogram phenotype of the Imperial chromosome 5R alien addition line consists of 10 bands, including the three cathodal TPI-1 bands and seven anodal TPI-2 bands (IV, Fig. 5). The designation ρ_2 is assigned to the *S. cereale* TPI-2 subunit and the gene which encodes the subunit is designated *Tpi-R2*. A schematic model for the subunit composition of the TPI-2 isozymes of the 5R addition line is shown in Table 6. It assumes that TPI-2f is composed of homodimeric ρ_2 subunits and TPI-2g of $\alpha_2\rho_2$ heterodimers. The expected distribution of the TPI-2 isozymes of the 5R addition line is 1:2:3:4:3:2:1; this distribution is derived by expanding the equation $(p+q+r+s)^2$ where, as for the other addition line isozymes described above, $p = q = r = s = \frac{1}{4}$ and p, q, r and s represent the frequencies of α_2, β_2, δ_2, and ρ_2 subunits. The agreement between the relative staining intensities of the chromosome 5R addition line zymogram phenotype and this model provide good support for the hypothesis that *Tpi-R2* is located in Imperial chromosome 5R.

The TPI-1 and TPI-2 zymogram phenotypes of Kharkov are identical to those of Chinese Spring. However, the Dakold TPI-2 isozyme has lesser electrophoretic mobility than the Imperial TPI-2 isozyme, being coincident in mobility to the wheat TPI-2g isozyme. Surprisingly, *each* of the Kharkov–Dakold chromosome addition lines exhibits a zymogram phenotype which differs from that of Kharkov. The phenotype consists of eight TPI bands that are electrophoretically homologous with the eight bands of the Kharkov phenotype plus one additional band coincident in mobility with the Dakold TPI-2 band (Fig. 5, III, V and VI).

Since the TPI-1 zymogram phenotype of the Kharkov–Dakold chromosome 3R addition line is the same as that for the Chinese Spring–Imperial 3R addition line, they are subject to the same isozyme subunit (Table 4) and genetic interpretations. The findings obtained therefore indicate that *Tpi-R1* of Dakold is located, as is the *Tpi-R1* gene of Imperial, in chromosome 3R.

Interpretation of the genetics and the subunit structure of the TPI-2 isozymes of the Kharkov–Dakold series is complicated by two factors. Firstly, Dakold TPI-2 migrates to a position less anodal and therefore closer to TPI-2a of wheat than does Imperial TPI-2. Consequently none of the heterodimers formed by association of ρ_2 with wheat subunits is expected to be precisely coincident in electrophoretic mobility with any wheat isozyme (see below). However, no discrete bands were observed that might be the sites of these heterodimers. Secondly, TPI-2g, presumably composed of $\rho_2\rho_2$ homodimers, was present on the zymogram produced by *each* chromosome addition line (see III, V and VI, Fig. 5).

The relative staining intensities of the bands which compose the 5R addition line zymogram phenotype differ from those of the other addition lines in a manner consistent with the presence of an increased quantity of ρ_2 subunits. Consequently, it appears likely that a *Tpi-R2* locus is present in the Kharkov genome in each

of the addition lines and that the chromosome 5R addition line carries four *Tpi-R2* genes, as opposed to two in each of the other addition lines. An alternative possibility is that a Kharkov *Tpi-2* locus is duplicated, but the presence of the same allele at the added locus as is present in Dakold makes the presence of a *Tpi-R2* locus in the Kharkov genome more likely. Since findings similar to these have been

Table 6. *Schematic models for the subunit composition of the TPI-2 isozymes produced by* T. aestivum *cvs. Chinese Spring and Kharkov, by the Chinese Spring–*S. cereale *cv. Imperial chromosome 5R addition line and by the Kharkov–*S. cereale *cv. Dakold chromosomes 1R, 3R, 4/7R, 5R, and 6/7R addition lines.*

(The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers.)

Isozymes	Chinese Spring and Kharkov	Imperial 5R	Dakold 1R, 3R, 4/7R, 6/7R	Dakold 5R
TPI-2f	—	$1/16\ \rho_2\rho_2$	—	—
TPI-2g	—	$2/16\ \alpha_2\rho_2$	$1/16\ \rho_2\rho_2$	$4/25\ \rho_2\rho_2$
TPI-2a	$1/9\ \alpha_2\alpha_2$	$3/16\ \alpha_2\alpha_2, \beta_2\rho_2$	$5/16\ \alpha_2\alpha_2, \beta_2\rho_2, \alpha_2\rho_2$	$9/25\ \alpha_2\alpha_2, \beta_2\rho_2, \alpha_2\rho_2$
TPI-2b	$2/9\ \alpha_2\beta_2$	$4/16\ \alpha_2\beta_2, \delta_2\rho_2$	$2/16\ \alpha_2\beta_2$	$2/25\ \alpha_2\beta_2$
TPI-2c	$3/9\ \beta_2\beta_2, \alpha_2\delta_2$	$3/16\ \beta_2\beta_2, \alpha_2\delta_2$	$5/16\ \beta_2\beta_2, \alpha_2\delta_2, \delta_2\rho_2$	$7/25\ \beta_2\beta_2, \alpha_2\delta_2, \delta_2\rho_2$
TPI-2d	$2/9\ \beta_2\delta_2$	$2/16\ \beta_2\delta_2$	$2/16\ \beta_2\delta_2$	$2/25\ \beta_2\delta_2$
TPI-2e	$1/9\ \delta_2\delta_2$	$1/16\ \delta_2\delta_2$	$1/16\ \delta_2\delta_2$	$1/25\ \delta_2\delta_2$

obtained in analysis of the *T. aestivum* cv. Kharkov–*S. montanum* addition series (unpublished results), it is likely that the *Tpi-R2* locus was incorporated into the Kharkov accession used in the development of these two chromosome addition series prior to the initiation of their development. There is no evidence that the Kharkov accession used in this study contains a *Tpi-R2* gene. As noted above, the Kharkov plants studied express a TPI zymogram phenotype identical to that of Chinese Spring.

Schematic models for the subunit composition of the TPI-2 isozymes of the Kharkov–Dakold chromosome addition lines are shown in Table 6. These are based on the same assumptions as those of other subunit models described above, except in this case it is assumed that the electrophoretic mobilities of the three heterodimers which include ρ_2 subunits are sufficiently similar to those of wheat isozymes to be indistinguishable from them on zymograms. The agreement between models and observations provides evidence that Dakold chromosome 5R contains a *Tpi-R2* gene and, as stated above, that a second *Tpi-R2* locus is present in the Kharkov genome of each addition line in this series, presumably incorporated by a translocation prior to development of this addition series.

DISCUSSION

The chromosomal locations of homologous genes in different Triticeae genomes provide evidence for homoeologies among specific Triticeae chromosomes. The chromosomal location of a TPI-1 structural gene in Triticeae genomes A, B, D, E, H, R, and S^l and of a TPI-2 structural gene in genomes A, B, D, H, R, S^l, and

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U is reported in this paper. The results obtained indicate that the TPI-1 protomers produced by Chinese Spring genomes A, B, and D associate approximately randomly in all possible combinations to form approximately equally active dimeric molecules and also that the genome E, H, R, and S^l TPI-1 protomers, when present in a wheat-alien-chromosome addition line or a wheat-alien-species amphiploid, associate with the three wheat protomers in a similar manner. Consequently, the evidence for homology of the seven identified genes which encode TPI-1 protomers is strong. Similar evidence was obtained for homology of the seven identified TPI-2 genes.

Chinese Spring homoeologous group 3 enzyme structural gene sets that are orthologous to genes whose chromosomal locations have been determined in one or more alien species include, in addition to a *Tpi-1* set, an *Est-1*, a *Got-3*, and a *Pde*-1 gene set (Hart, 1984, contains a compilation of the chromosomal locations of Chinese Spring enzyme and protein structural genes). The *Got-3* genes are located in the *q* arms of the Chinese Spring group 3 chromosomes; the other genes are located in the *p* arms. A *Tpi-1* gene has been located in chromosomes 3E, 3H, 3R, and in *T. longissimum* chromosome G, a chromosome tentatively designated $3S^l$ by Hart & Tuleen (1983*c*). A *Got-3* gene has also been located in each of these chromosomes (Brown & Munday, 1982; Tang & Hart, 1975; Hart & Tuleen, 1983*b*, *c*) and an *Est-1* gene in each of these chromosomes except 3H (Barber *et al.* 1968, 1969; Hart & Tuleen, 1983*b*, *c*). Also, Ceoloni & Galili (1982) have located a *Pde-1* gene in *T. longissimum* chromosome G and Ainsworth (1983) has located a hexokinase gene designated *Hk-E1* in 3E which may be orthologous to a 3B gene. Chromosomal arm locations have been determined for four of these genes, namely, *Est-E1* and *Hk-E1* in 3ES, *Got-E3* in 3EL, and *Pde-*S^l*1* in the short arm of *T. longissimum* chromosome G. These findings provide good evidence for homoeology of *E. elongata* chromosome 3H, *H. vulgare* chromosome 3H, *S. cereale* chromosome 3R, and *T. longissimum* chromosome G with each other and with the homoeologous group 3 chromosomes of Chinese Spring.

Chinese Spring homoeologous group 5 enzyme structural gene sets that are orthologous to genes whose chromosomal locations have been determined in one or more alien species include, in addition to a *Tpi-1* set in the *q* arms, a *Skdh-1* gene set in the *p* arms and *Aco-2*, *Adh-2* and *Lpx-2* gene sets in the *q* arms (Hart, 1984).

As reported in this paper, a *Tpi-2* gene is present in chromosomes 5H, 5R, 5U and in chromosome F of *T. longissimum*. Also present in the latter chromsome, which has been tentatively designated $5S^l$ (Hart & Tuleen, 1983*c*) is a gene orthologous to each of the other aforementioned homoeologous group 5 genes (Hart & Tuleen, 1983*c*; Chernicek, 1984) while among these genes only an *Lpx-2* gene has not been located in chromosome 5R (Koebner & Shepherd, 1983; Chenicek, 1984; Hart & Tuleen, unpublished). In addition to a *Tpi-2* gene, a *Skdh-1* gene has been located in chromosomes 5U (Koebner & Shepherd, 1983) and 5H (Hart & Tuleen, unpublished). Consequently, the enzyme structural gene locations reported in *H. vulgare* chromosome 5H, *S. cereale* chromosome 5R, *T. umbellulatum* chromosome 5U and *T. longissimum* chromosome F support the designation of these chromosomes as being homoeologous with each other and with the homoeologous group 5 chromosomes of Chinese Spring.

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