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Location of genes coding isozyme markers on *Aegilops umbellulata* chromosomes adds data on homoeology among Triticeae chromosomes

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Summary. Zymogram analysis was used to identify the *Aegilops umbellulata* chromosomes that carry the structural genes for particular isozymes. Wheat, *Aegilops* and wheat-*Aegilops* hybrid derivative lines (which contained identified *Aegilops* chromosomes) were tested by gel electrophoresis for isozymes of particular enzymes. It was found that *Aegilops* chromosome A (nomenclature according to G. Kimber 1967) carries a structural gene for 6-phosphogluconate dehydrogenase, *Aegilops* chromosome B carries structural genes for glucose phosphate isomerase and phosphoglucose mutase, *Aegilops* chromosome D carries genes for leaf peroxidases, *Aegilops* chromosome E carries structural genes for endosperm peroxidases, acid phosphatases and leaf esterases, *Aegilops* chromosome F carries a gene for embryo plus scutellum peroxidases and *Aegilops* chromosome G carries structural genes for endosperm alkaline phosphatases, leaf alkaline phosphatases and leaf esterases. The results obtained indicate that chromosome B is partially homoeologous of the wheat chromosomes of group 1 and 4, and chromosome E is partially homoeologous of wheat chromosomes of groups 7 and 4. Circumstantial evidence is also provided about the possible association between chromosomes C, D and A of *A. umbellulata* respectively with chromosomes 5, 2 and 1 of wheat.

Key words: Chromosomal location – Isozyme markers – *Aegilops umbellulata*

Introduction

A considerable number of wheat lines which contain alien genetic material (added or substituted) have been produced by cytogeneticists taking advantage of the ability of hexaploid wheat to admit aneuploidy. By zymogram analyses of these lines the genes involved in the production of isozymes can be located on specific chromosomes.

The chromosomal location of structural genes for isozymes can provide information for chromosome homology and homoeology as well as genetic relationships among related species (Tang and Hart 1975; Hart and Langston 1977; Hart et al. 1980; Powling et al. 1981; Hart and Tuleen 1983 a).

The genetics of wheat isozymes and characterization of alien genetic material using isozyme markers have been recently reviewed by Hart (1983, 1984) and Hart and Tuleen (1983 b).

The present paper reports on the chromosomal location of various structural genes controlling different isozymes of *Aegilops umbellulata*. Also, biochemical evidence of homoeology among hexaploid wheat, rye, barley and *Aegilops* chromosomes are discussed.

Material and methods

The study was conducted with material produced by Dr. G. Kimber (1967). The materials used were *Triticum aestivum* L. cv. 'Chinese Spring' (CS), *Aegilops umbellulata* L. (AU) (the particular line of *Aegilops umbellulata* which was used in the original cross was not available) and the CS-AU disomic addition lines. The correspondence with the chromosome terminologies used in previous works based on C-banding techniques carried out in Plant Breeding Institute, Cambridge, UK (Teoh and Hutchinson 1983), C-banding and Ag-staining techniques carried out in our laboratory (Cermeño et al. 1984; Lacadena and Cermeño 1985) as well as in homoeology relationships (Martini et al. 1982) are indicated below.

In this paper we have used the chromosome nomenclature described by Gordon Kimber (1967).

The analyses were carried out with parts of individual kernels, specifically embryo plus scutellum (S), and endosperm (E), and also with 12-day-old seedling leaves (L). The zymo-

Chromosome terminology		Homoeologous nomenclature
Kimber	P.B.I., Cambridge	Lacadena and Cermeño (1985)
A	F	Telocentric
B	G	1U
C	D	5U
D	E?	Comparison of C-banding patterns is not conclusive
E	C?	
F	A?	
G	B?	

gram phenotype of the peroxidases (CPXS, CPXE, PERL), acid and alkaline phosphatases (PHE, ALPH, and ACPH), esterases (ESTL), phosphoglucose mutase (PGM), glucose phosphate isomerase (GPI), glutamate oxaloacetate transaminase (GOT) and 6-phosphogluconate dehydrogenase (6-PGD) isozymes were determined for each line examined in this study.

The information on both the cathodal peroxidase CPXS and CPXE of embryo plus scutellum (S) and endosperm (E), respectively, and the alkaline phosphatases of the endosperm (PHE) were obtained using polyacrylamide gel slabs (10%). The protocols described by Benito and Pérez de la Vega (1979) and Salinas and Benito (1984a), respectively, were followed. The nomenclature used for these isozymes was described by Salinas and Benito (1984a, b).

The remaining isozymes analyzed (GOT, PGM, GPI, ESTL, 6-PGD, ALPH and ACPH) were electrophoresed following the protocols described by Benito et al. (1985), Salinas et al. (1985), Figueiras et al. (1985), Salinas and Benito (1984a) and the staining methods described by Brewer and Singh (1970). The nomenclature used for these isozymes is the same described in the preceding works.

In addition lines, all the enzyme systems studied showed the wheat isozymes and, sometimes, also the specific *Aegilops* isozymes.

Results

1 Endosperm alkaline phosphatases (PHE) (Figs. 1 and 2)

The CS-wheat showed nine PHE bands designated from PHE-1 to PHE-9. The *Aegilops* showed four bands named from PHE-1 to PHE-4. The *Aegilops* band PHE-2 and PHE-5 wheat had the same migration rate. The *Aegilops* PHE-1 migrated faster than the PHE-5 wheat band; PHE-3 and PHE-4 bands migrated slower than wheat PHE-9. The CS-AU addition line with the G chromosome showed all the CS-wheat bands and, in addition, the PHE-1 *Aegilops* band. The CS-AU addition line with the E chromosome presented all the bands of CS-wheat and also the PHE-3 and PHE-4 bands of *Aegilops*. In the CS-AU addition line with the D chromosome all the bands of CS were observed, but they were very faint. The remaining addition lines showed the same pattern as CS-wheat.

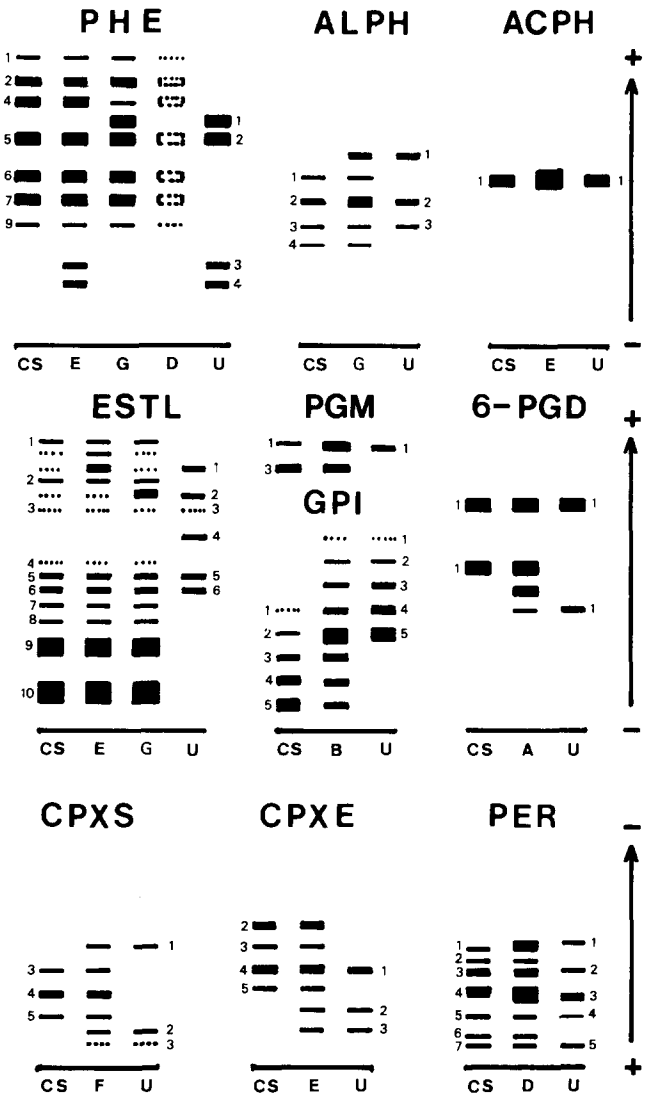

Fig. 1. Diagramatic representation of the zymograms of the 'Chinese Spring' (CS), *Aegilops umbellulata* (U) and the wheat-*Aegilops umbellulata* addition lines with chromosomes A, B, C, D, E, F and G for the isozymes: alkaline phosphatase of the endosperm (PHE), leaf alkaline phosphatases (ALPH), leaf acid phosphatases (ACPH), leaf esterases (ESTL), phosphoglucose mutase (PGM), glucose phosphate isomerase (GPI), 6-phosphogluconate dehydrogenase (6-PGD), embryo plus scutellum peroxidases (CPXS), endosperm peroxidase (CPXE) and leaf peroxidases (PERL). All these isozymes migrated from the cathode to the anode except for CPXS, CPXE and PERL which migrated from the anode to the cathode

2 Leaf alkaline phosphatases (ALPH) (Fig. 1)

The CS-wheat showed four ALPH bands named from ALPH-1 to ALPH-4. The *Aegilops* presented three ALPH bands: ALPH-1, ALPH-2 and ALPH-3. The CS-AU addition line with G chromosome showed all the bands of CS-wheat, but the ALPH-1 and ALPH-3 bands always presented a greater staining intensity than those of CS-wheat. The CS-AU addition line with

D chromosome showed the same bands as CS-wheat, but these bands were very faint. The remaining addition lines presented identical patterns to that of CS-wheat.

3 Leaf acid phosphatases (ACPH) (Figs. 1 and 2)

The CS-wheat showed one ACPH-1 band and *Aegilops* also had one ACPH band with the same migration as the ACPH-1 wheat band. In the CS-AU addition line with E chromosome the ACPH-1 band always showed a greater staining intensity than that observed in CS-wheat. The remaining addition lines showed the same pattern than CS-wheat.

4 Leaf esterases (ESTL) (Figs. 1 and 2)

In the leaf of CS-wheat ten ESTL bands, from ESTL-1 to ESTL-10, were observed. *Aegilops* showed six ESTL bands designated from ESTL-1 to ESTL-6. The ESTL-1 and ESTL-2 *Aegilops* bands were located in the CS-AU addition lines with E and G chromosomes respectively. The remaining addition lines showed the same pattern as CS-wheat.

5 Leaf phosphoglucose mutase (PGM) (Figs. 1 and 2)

Two major bands designated PGM-1 and PGM-3 with relative staining intensities of 1:2, respectively, were observed in CS-wheat. *Aegilops* showed only one PGM band with a migration very similar to that of the PGM-1 wheat band. The CS-AU addition line with B chromosome showed all the CS-wheat bands, but the PGM-1 band always presented a greater staining intensity than PGM-1 wheat band. The remaining addition lines showed the same pattern as CS-wheat.

6 Leaf glucose phosphate isomerase (GPI) (Figs. 1 and 2)

The CS-wheat had five bands, from GPI-1 tp GPI-5 (in order of decreasing anodic migration), and *Aegilops* also showed five GPI bands, GPI-1 to GPI-5. The CS-AU addition line with B chromosome presented all the bands of CS-wheat and also the GPI-1, GPI-2 and GPI-3 *Aegilops* bands. Moreover, in this addition line the GPI-1, GPI-2 and GPI-3 wheat bands showed different relative staining intensities. The remaining addition lines presented the same pattern as CS-wheat.

7 Leaf 6-phosphogluconate dehydrogenase (6-PGD) (Figs. 1 and 2)

The 6-PGD zymogram phenotype produced by CS-wheat showed two activity zones. Zone I (the fastest migrating zone) had one band, and zone II contained another band. Two activity zones were also observed in *Aegilops:* zone I having one band with the same migration as wheat band, and zone II having only one band (with a slower migration than the wheat zone II band). All the bands of CS-wheat were observed in the CS-AU addition line with A chromosome, but the zone II showed three bands with relative staining intensities of 9:6:1, respectively (in order of decreasing electrophoretic mobility). The remaining addition lines examined showed the same pattern as CS-wheat.

8 Embryo plus scutellum peroxidases (CPXS) (Figs. 1 and 2)

CS-wheat showed three CPXS bands designated CPXS-3, CPXS-4 and CPXS-5. *Aegilops* also showed three bands in the S of the dry kernel designated CPXS-1, CPXS-2 and CPXS-3. The CPXS-1 band migrated faster thand the CPXS-3 wheat band; CPXS-2 and CPXS-3 *Aegilops* bands migrated slower than the CPXS-5 wheat band. The CS-AU addition line with F chromosome presented all the bands of CS-wheat and also the CPXS-1, CPXS-2 and CPXS-3 *Aegilops* bands. The remaining addition lines examined showed the same pattern as CS-wheat.

9 Endosperm peroxidases (CPXE) (Figs. 1 and 2)

In the E of CS-wheat four CPXE bands from CPXE-2 to CPXE-5 were observed; in *Aegilops,* three CPXE bands (CPXE-1, CPXE-2 and CPXE-3) were observed. The CPXE-2 and CPXE-3 *Aegilops* bands showed a slower cathodic migration than all the wheat CPXE. CPXE-1 *Aegilops* band presented the same migration as the CPXE-4 wheat band. In the CS-AU addition line with E chromosome all that CS wheat bands were observed as well as the CPXE-2 and CPXE-3 *Aeqilops* bands. The remaining addition lines studied presented the same pattern as CS-wheat.

10 Leaf peroxidases (PERL) (Figs. 1 and 2)

The CS-wheat leaves presented seven PERL bands, PERL-1 to PERL-7. In *Aegilops* leaves five PERL bands, designated PERL-1 to PERL-5, were observed. The CS-AU addition line with D chromosome had all the bands of CS-wheat but the PERL-1 and PERL-4 always showed a greater intensity than those bands of CS-wheat. The PERL-1 and PERL-3 *Aegilops* bands showed the same migration as PERL-1 and PERL-4 CS-wheat bands, respectively. The remaining addition lines showed the same pattern as CS-wheat.

11 Leaf glutamate oxaloacetate transaminase (GOT) (Fig. 2)

The CS-wheat showed three activity zones (I, II and III, in order of decreasing electrophoretic mobility). Zone I had one band, zone II had one band, and zone III had

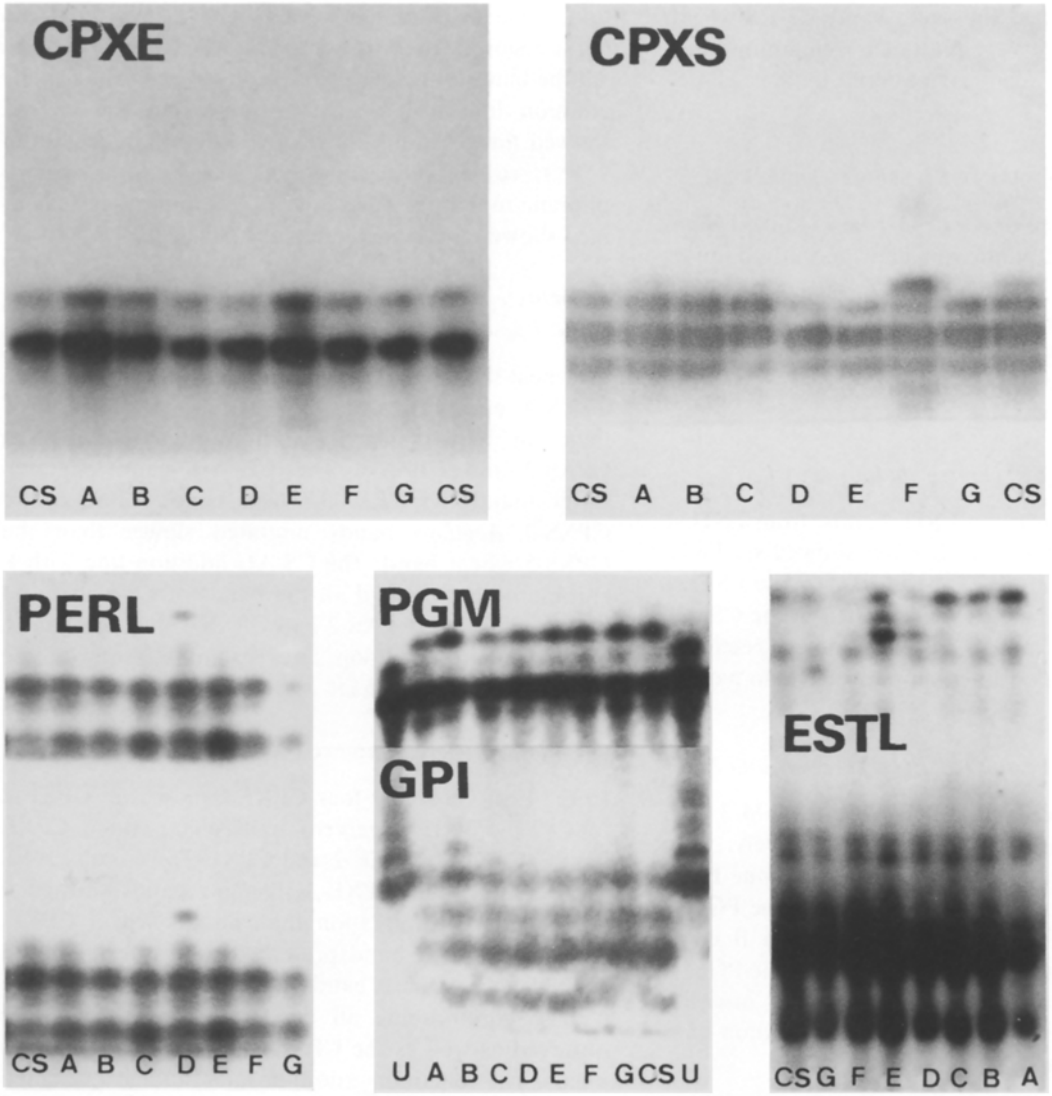

Fig. 2. Zymograms of the 'Chinese Spring' (CS), *Aegilops umbellulata* (U) and the wheat-*Aegilops umbellulata* addition lines with the chromosomes A, B, C, D, E, F and G for the isozymes: endosperm peroxidases (CPXE), embryo plus scutellum peroxidases (CPXS), leaf peroxidases (PERL), phosphoglucose mutase (PGM), glucose phosphate isomerase (GPI), leaf esterases (ESTL), glutamic oxaloacetate transaminase (GOT), leaf acid phosphatases (ACPH), alkaline phosphatases of the endosperm (PHE) and 6-phosphogluconate dehydrogenase (6-PGD). All these these isozymes migrated from the cathode to the anode except for CPXE, CPXS and PERL

three bands named GOT-1, GOT-2 and GOT-3, with relative staining intensities of 4 : 4 : 1, respectively. *Aegilops* also presented three activity zones: zone I with one band, zone II containing another band, and zone III having one band with the same migration as GOT-2 wheat band. The *Aegilops* bands of zones I and II showed the same migration as the wheat bands. All the addition lines showed the same pattern than that found in CS-wheat. Therefore, the *Aegilops* GOT isozymes have not been related to any particular chromosome.

Discussion

The isozyme structural genes that were located on *Aegilops* chromosomes are shown in Table 1. The seven CS-AU addition lines examined in this study can be identified by different isozyme markers. Only the CS-AU addition line with C chromosome does not present any isozyme marker and, therefore, can be identified by elimination. Using C-banding and Ag-staining techniques it has been proposed that chromosome C of

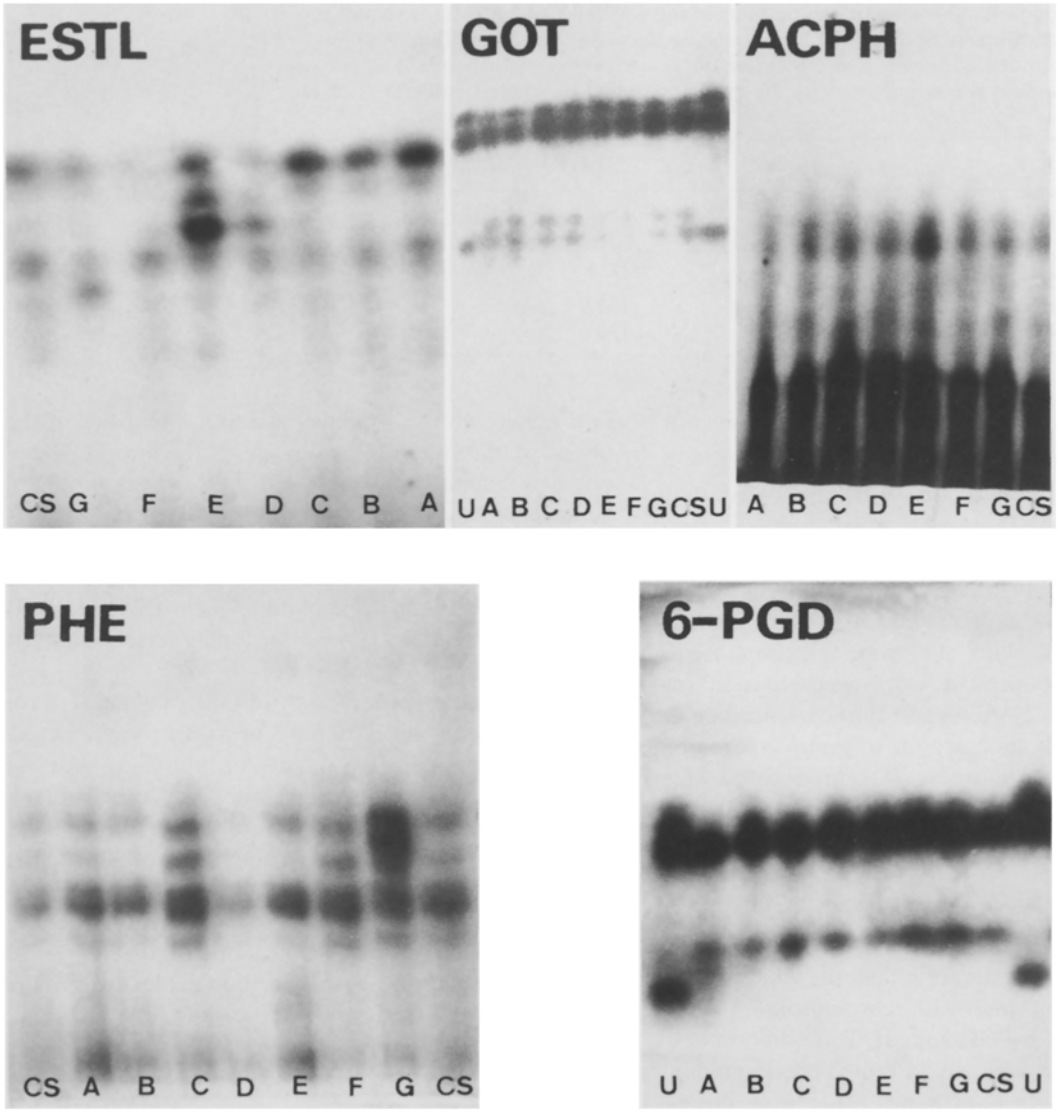

Fig. 2 (continued)

Table 1. Chromosomal locations of isozyme structural genes in *Aegilops umbellulata*

Aegilops umbellulata chromosome nomenclature of G. Kimber	Structural genes
A	*6-Pgd-2*
B	*Pgm-1, Gpi-1*
D	*Per-1,3*
E	*Cpxe-2,3; Acph*(L); *Phe-3,4; Estl* (L)-1
F	*Cpxs-1,2,3*
G	*Alph*(L); *Phe*(E)-1; *Estl*(L)-2
D	Decreasing intensity of wheat PHE

Ae. umbellulata is homoeologous to group 5 chromosomes of the CS wheat (Lacadena and Cermeño 1985). None of the isozyme structural genes studied in our work were located on *Aegilops* chromosome C. Moreover, none of these isozyme structural genes are located on homoeologous group 5 in the Triticinae. Therefore, our results are not in disagreement with the hypothesis that *Aegilops* chromosome C would be homoeologous to the group 5 chromosomes. On the basis of chromosomal location of structural genes for shikimate dehydrogenase and triosephosphate isomerase-2 in chromosome 5 (Koebner and Shepherd 1982; Pietro and Hart 1985, respectively), the homoeology of group 5 chromosomes in some Triticeae species, including *Ae.*

Table 2. Schematic models for the subunit composition of the 6-PGD-2 and PGM-1 isozymes produced by *T. aestivum* cv. 'Chinese Spring' (CS), *Aegilops umbellulata* (AU) and the 'Chinese Spring' – *Aegilops umbellulata* addition lines with A and B chromosomes, respectively. The expected quantitative distribution of the isozymes is indicated by the ratios preceding the dimers (6-PGD-2) or the monomers (PGM-1)

Isozymes	CS	AU	CS-AU-A
6-PGD-2a	$\alpha\alpha$ $\alpha\beta$ $\beta\beta$ $\alpha\delta$ $\beta\delta$ $\delta\delta$		9/16 $\alpha\alpha$ $\alpha\beta$ $\beta\beta$ $\beta\delta$ $\alpha\delta$ $\delta\delta$
6-PGD-2b			6/16 $\gamma\alpha$ $\gamma\delta$ $\gamma\beta$
6-PGD-2c		$\gamma\gamma$	1/16 $\gamma\gamma$
PGM-1	1/3 β	γ	1/2 β γ
PGM-3	2/3 α δ		1/2 α δ

umbellulata has been suggested (these authors do not name explicitly the chromosome C of *Aegilops*).

The results obtained in this work indicated that *Aegilops* chromosome D carries genes for leaf peroxidases. Leaf peroxidase structural genes have been related with homoeologous group 1 and 2 in wheat (Ainsworth et al. 1984; Bosch et al. 1985). Since the leaf peroxidases revealed with the same electrophoretic method used in the present work were located on homoeologous group 2, we suggest that chromosome D would probably be homoeologous to group 2 chromosomes. Also, this chromosome (D) produces a decreasing intensity of wheat PHE, and a similar behaviour has been found for 2R chromosome (Salinas and Benito 1984a).

The wheat zone II of 6-PGD showed one band and 6-PGD zone II of *Ae. umbellulata* also showed one band, but with a slower migration than the wheat band. The CS-AU addition line with chromosome A presented three bands in 6-PGD zone II. This addition line showed a new band with an intermediate migration between wheat and *Aegilops* bands. Therefore, the 6-PGD isozymes of wheat and *Aegilops* are probably dimers. The relative staining intensities 9:6:1, observed in the CS-AU addition line with chromosome A could be explained if the 6-Pgd *Aegilops* structural gene codes a subunit designated γ^2 that could form dimers with the α^2, β^2 and δ^2 subunits coded by CS wheat. A schematic model for the subunit composition of 6-PGD zone of CS, Aegilops and the addition line with the chromosome A is shown in Table 2. The expected distribution of the possible dimeric molecules of the addition line A would be based on $(p+q+r+s)^2$, where p, q, r and s represent the frequencies of α^2, β^2, δ^2 and γ^2 subunits, respectively. When the tetranomial is expanded and the proportions for those dimers (which are of coincident electrophoretic mobility) are combined, the expected distribution of the isozymes assumed to be responsible for the production of the three bands of 6-PGD (zone II) of the addition line with chromosome A, is 9:6:1. This theoretical proportion agrees with the relative staining intensities of the observed three bands in our case. The proposed hypothesis assumes that there are three wheat structural genes encoding three subunits, designated α, β and δ, respectively. The random association of these subunits results in the production of six types of dimers, which are expressed in only one band of the 6-PGD (zone II) (the six dimers would be the same electrophoretic mobility).

The structural genes for 6-PGD of zone II have been located on chromosome 5 of barley (Nielsen et al. 1982; Benito et al. 1985) and on the long arm of chromosome 1R in rye (Lawrence and Appels 1986). The chromosome 5 of barley and 1R of rye are homoeologous to the group 1 chromosomes of wheat. The results obtained in this work indicate that *Ae. umbellulata* chromosome A (telocentric) carries structural genes for 6-PGD isozymes of zone II. These results suggest that chromosome A is homoeologous to the barley chromosome 5, rye chromosome arm 1RL and, probably, to long arms of the group 1 chromosomes of wheat.

A previous report, placed the chromosomal location of a structural gene for 6-PGD isozymes of rye, named *6-Pdg-2* (Salinas and Benito 1983), on chromosome 2R. This structural gene would be different from that described by Lawrence and Appels (1986), and would control the isozymes in the faster-moving zone (zone I). On the other hand, the *Mdh-1* locus (corresponding to the faster-moving zone) had been located on chromosome 5 of barley (Powling et al. 1981). In our case, the results of MDH isozyme pattern analyses were not taken in account because of the poor resolution obtained. Therefore, the loci encoding 6-PGD isozymes of the slower-moving zone (zone II) and the MDH (Figueiras et al. 1985) would be on chromosome 1R, instead of 2R as had been proposed.

The PGM has been described as a monomer in both *Triticum aestivum* (Benito et al. 1984) and other related species. The CS cultivar showed two bands with a relative staining intensities of 1:2. The CS-AU addition line with B chromosome showed the same two bands, but the relative staining intensities observed were 1:1. If the PGM isozymes of CS and AU are monomers and if the *Pgm-1 Aegilops* structural gene codes for a subunit γ with the same migration does the β subunit of CS (Table 2), the addition line with the chromosome that carries the *Pgm-1* structural gene would show the relative staining intensities 1:1.

The GPI structural genes have been located on the short arms of the homoeologous group 1 chromosomes in CS wheat and, also, in the short arm of 1R chromosome of rye. Moreover, the nucleolar organizer regions have been located in the short arm of the 1B wheat chromosome (6B, 5D and 1A are other nucleolar organizer chromosomes) and in the 1R rye chromosome. In our work, the GPI structural genes have been related to the *Aegilops* chromosome B; the use of C-banding and Ag-staining techniques suggests that this chromosome is a nucleolar organizer chromosome (Lacadena and Cermeño 1985).

The ESTL isozymes have been related to chromosomes E and G of *Ae. umbellulata.* The loci that control esterases have been located on the homoeologous group 3 and 6 in wheat, rye and barley (Bergman 1972; Bergman and Maan 1973; Hart et al. 1980; Salinas and Benito 1985). Also, it was demonstrated that there is information for esterases in rye chromosomes 4R and 5R (Schmidt-Stone and Wehling 1985) and in barley chromosome 1 (Hart et al. 1980). Therefore, it is difficult to establish homoeology relationships with these biochemical markers.

The chromosomal locations of the isozyme structural genes studied in this paper have been carried out in hexaploid wheat cultivar 'Chinese Spring', in rye cultivars 'Imperial', 'Dakold' and 'King II', and in barley cultivar 'Betzes'. The results obtained in these works indicate that the wheat chromosomes of groups 1, 2, 3, 4, 5, 6 and 7 are homoeologous to rye chromosomes 1R, 2R, 3R, 4R, 5R, 6R and 7R (translocation 7R/4R), respectively, and also are homoeologous of barley chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H, respectively (Hart 1983; Hart and Tuleen 1983a; Benito et al. 1984, 1985; Bosch et al. 1986).

The structural genes for PGM and GPI are, respectively, located on the short arms of homoeologous group 4 (Benito et al. 1984) and the short arms of homoeologous group 1 (Chojecki and Gale 1982) in hexaploid wheat. The results obtained in this work indicate that *Ae. umbellulata* chromosome B carries structural genes for PGM and GPI. These results suggest that chromosome B is partially homoeologous to the 4 and 1 wheat chromosome groups. Netzle and Zeller (1984) have found cytogenetic evidence that *Ae. longissima* chromosome C is partially homoeologous to the wheat chromosomes of groups 1 and 4.

The structural genes for CPXE, ACPH and PHE are respectively located on the 7AS, 4BL and 7DS wheat chromosome arms (Kobrehel and Feillet 1975; Benito and Pérez de la Vega 1979), the 4AL, 4BS and 4DL chromosome arms (Hart and Langston 1977) and the 4AL, 4BS and 4DL chromosome arms (Salinas et al. 1984) in wheat. The *Ae. umbellulata* chromosome E carries structural genes for CPXE, PHE and ACPH. These results suggest that chromosome E is partially homoeologous to the wheat chromosomes of groups 4 and 7. In *Ae. longissima,* cytogenetic evidence that chromosome D is partially homoeologous to the wheat chromosomes of groups 4 and 7 has been found by Netzle and Zeller (1984).

The translocation 4/7 has been found in CS wheat (4BL/7BS) using endosperm peroxidases (Kobrehel and Feillet 1975; Benito and Pérez de la Vega 1979), in rye (4RL/7RS) by means of endosperm peroxidases and phosphatases (Salinas et al. 1984a, b) and, also, in barley (4H/7H) with leaf acid phosphatase and endosperm phosphatase isozymes (Powling et al. 1981; Salinas et al. 1985). Therefore, this interchange probably occurred a long time ago, at least before the evolutive divergence of these species.

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