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Chromosomal location of esterase, peroxidase and phosphoglucomutase isozyme structural genes in cultivated rye (*Secale cereale* L.)

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Summary. Isoelectric focusing of esterase (EST), peroxidase (PRX), and phosphoglucomutase (PGM) isozymes in 'Chinese Spring' wheat, 'Imperial' rye and several 'Chinese Spring'/'Imperial' and 'Holdfast'/'King II' addition, translocation and substitution lines revealed the chromosomal location of nine *Est* loci previously described and of one *Prx* and *Pgm* locus. Loci *Est1, Est2, Est3, Est5, Est6* and *Est7* were found on chromosome arm 5RL, *Est8* and *Est9* on chromosome 6R in 'Imperial' rye, and the *Est10* locus on chromosome arm 4RL in 'Imperial' rye and 'King II' rye. A discrepancy was found between the chromosomal location of the *Prx* locus in 'Imperial' where chromosome 2R was responsible for the expression of the peroxidase enzyme, and 'King II' with chromosome 1R carrying the *Prx* gene. As a possible explanation, the occurrence of translocation events during the production of wheat/rye aneuploid lines is discussed. The rye *Pgm* locus could be associated with chromosome 4RS in 'Imperial' and 'King II' rye. Except for the location of *Est* loci on chromosome 5RL, the results reported in this paper lend further evidence for the assumed homoeology relationships between the chromosomes of Triticinae and for the conservation of gene synteny groups during the evolution of the Triticeae tribe.

Key words: *Secale cereale* L. – Isozyme loci – Chromosomal location – Homoeology relationships

Introduction

Investigation of strains of hexaploid wheat, *Triticum aestivum,* containing added, translocated or substituted alien chromosome material from Triticeae relatives is an elegant and commonly applied method for easy chromosomal localization of alien genes. A main point of interest of these investigations is the study of homoeology relationships and evolutionary genetics of Triticeae species. Data on the arrangement of alien isozyme loci, namely in *Hordeum, Secale* and *Agropyron* species, that are orthologous to genes with known chromosomal location in *Triticum aestivum,* have contributed a considerable part to our knowledge of the homoeology relationships and the highly conservative synteny of genes within the Triticeae tribe.

Biochemical markers, in addition to cytological evidence, will be needed also in applied genetics and plant breeding for rapidly and reliably controlling the transfer of genetic material from wild Triticeae relatives into the cultivated crops of *Triticum, Hordeum* and *Secale.*

Identification and chromosomal localization of isozyme structural genes by the means of aneuploid strains were most successfully performed in the economically important crops of maize (for a review see Goodman et al. 1982), wheat (Hart 1983), barley (Hart et al. 1980; Islam et al. 1981; Powling et al. 1981) and rye. In rye, all the seven chromosomes have been marked by a total of approximately 25 isozyme loci (Barber et al. 1968; Tang and Hart 1975; Hart 1978; Rao and Rao 1980; Chojecki and Gale 1982; Hsam et al. 1982; Salinas and Benito 1983; Schmidt et al. 1984).

In the present paper some results obtained during the investigation of ten polymorphic enzyme systems in cultivated rye are described. Identification and linkage relationships of ten esterase loci were previously reported (Schmidt-Stohn 1979b; Schmidt-Stohn and Wehling 1983; Wehling and Schmidt-Stohn 1984). The chromosomal location of these esterase (E.C. 3.1.1.1.) loci, and of a peroxidase (E.C. 1.11.1.7.) and phosphoglucomutase (E.C. 2.7.5.1.) locus are presented in this paper.

Materials and methods

The disomic and ditelosomic addition, translocation and substitution lines used in this study are listed in Table 1. The 'Chinese Spring'/'Imperial' (CS/I) lines and lines no. 6, 7, 13, 15–17 and 22 were obtained from F. J. Zeller (München). 'Holdfast'/'King II' (H/K) lines were supplied by G. Röbbelen (Göttingen). Rye chromosomes and telosomes are named according to the nomenclature proposed by Koller and Zeller (1976). Since no 'Holdfast' wheat and 'King II' rye parents were available zymograms of H/K lines are presented only for special purpose.

Analyses were carried out with 12–14-day-old seedling leaf blades which were extracted with an aqueous solution of 5% sucrose. Extracts were centrifuged at 30,000×g and −1 °C for 15 min. The supernatant was directly used for isoelectric focusing (IEF). IEF was carried out in 0.5 mm thick polyacrylamide flat gels with a gel width of 120 mm and 100 mm distance between the electrodes. Acrylamide concentration was 6% T/3% C for separation of esterases and 5% T/3% C for peroxidase and phosphoglucomutase. Electrode solutions were 0.5 M H_3PO_4 (anode) and 1 M NaOH (cathode). Ten µl of extract per individual were applicated directly onto the gel surface using a lucite stencil as described by Schmidt-Stohn (1979a). IEF was performed in a pH 3.5–10 gradient (LKB ampholytes).

Esterase activity was visualized as previously described (Schmidt-Stohn and Wehling 1983). Peroxidase staining was performed using the combination o-dianisidine/β-naphtol (Endo 1972) and phosphoglucomutase was detected following the method of Kahl and Stegemann (1973).

Table 1. Disomic and ditelosomic wheat/rye addition and translocation lines used in this study (CS = 'Chinese Spring' wheat, I = 'Imperial' rye, K = 'King' rye)

No. of	Lines
1	*Triticum aestivum* cv. 'Chinese Spring' (CS)
2	*Secale cereale* cv. 'Imperial' (I)
3	CS/I: CS+1R
4	H/K: H+1R
5	CS/I: CS+2R
6	CS/Rosen: CS+2RL
7	Transec: 4A/2R translocation (Driscoll and Jensen 1964)
8	H/K: H+2R
9	CS/I: CS+3R
10	H/K: H+3R
11	CS/I: CS+4R
12	CS/I: CS+4RL
13	T 22: 7BL/4RL translocation (Zeller and Koller 1981)
14	H/K: H+4R
15	Viking 4A/5R translocation
16	Viking 4A/5RL translocation
17	205/70 5A/5RL substitution
18	H/K: H+5R
19	H/K: H+5RS
20	H/K: H+5RL
21	CS/I: CS+6R
22	R 60 6B/6R translocation
23	H/K: H+6R
24	CS/I: CS+7R
25	H/K:H+7R

Alleles of enzyme loci are indicated by the isoelectric point (IEP) of their enzyme product which is given as an index of the locus designation.

Results

Esterases

Figure 1 presents the total esterase pattern of CS wheat, I rye, and – for orientation – four individual plants of the rye material which was used for segregation and linkage analysis of rye esterases (see Schmidt-Stohn and Wehling 1983; Wehling and Schmidt-Stohn 1984).

In Fig. 2 the zymogram of 4A/5R translocation and 4A/5RL substitution lines (see Table 1) is shown. These lines exhibit three rye esterases with alkaline IEP's which are known as alleles of the *Est2, Est6* and *Est7* locus, respectively, and one unknown band above the origin which did not occur in our rye material studied. *Est6* and *Est7* alleles as well as the unknown acidic band do not occur in 'Imperial' rye. Instead of these bands, however, 'Imperial' exhibits an *Est2, Est3* and *Est5* allele. *Est3* and *Est5* are both linked with *Est2, Est6* and *Est7* which are expressed in the CS/I translocation and substitution lines. In H/K 5R addition

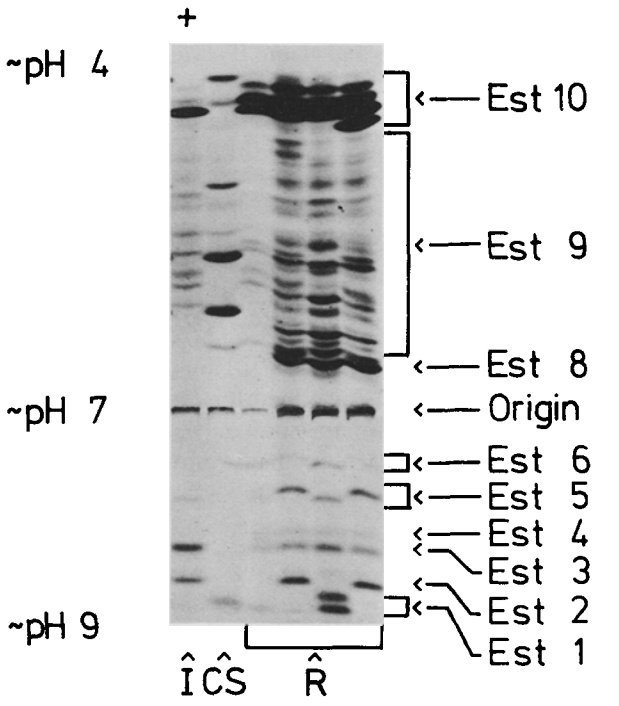

Fig. 1. Total IEF esterase pattern of 'Chinese Spring' wheat and 'Imperial' rye. For orientation, four individual plants of a rye line are also presented at the right side of the gel. CS = 'Chinese Spring' wheat, I = 'Imperial' rye, R Individual plants of a rye inbred line

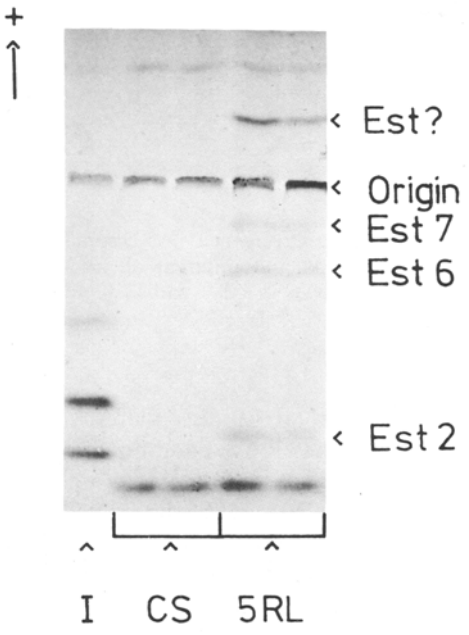

Fig. 2. Enlargement of the alkaline esterase zymogram region of CS wheat (CS), I rye (I) and 4A/5R translocation and 4A/5RL substitution lines (5RL). Note the four additional bands in the 5RL lines. Est? indicates the unknown esterase band above the origin

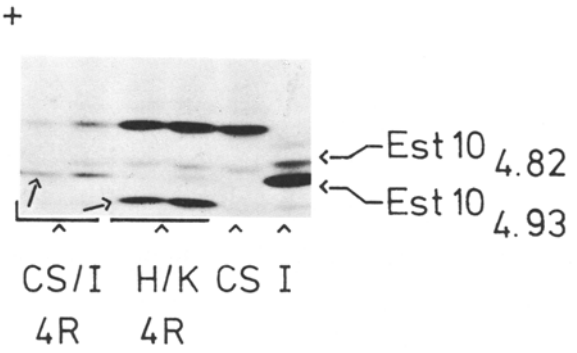

Fig. 4. *Est10* alleles expressed in 4R CS/I and H/K addition and translocation lines. CS/I-4R = lines 11–13; H/K-4R = line 14

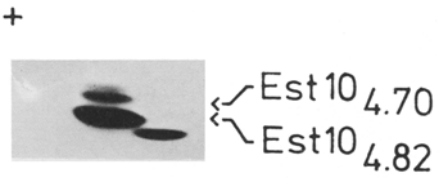

Fig. 5. Different *Est10* alleles among 'Imperial' rye individuals

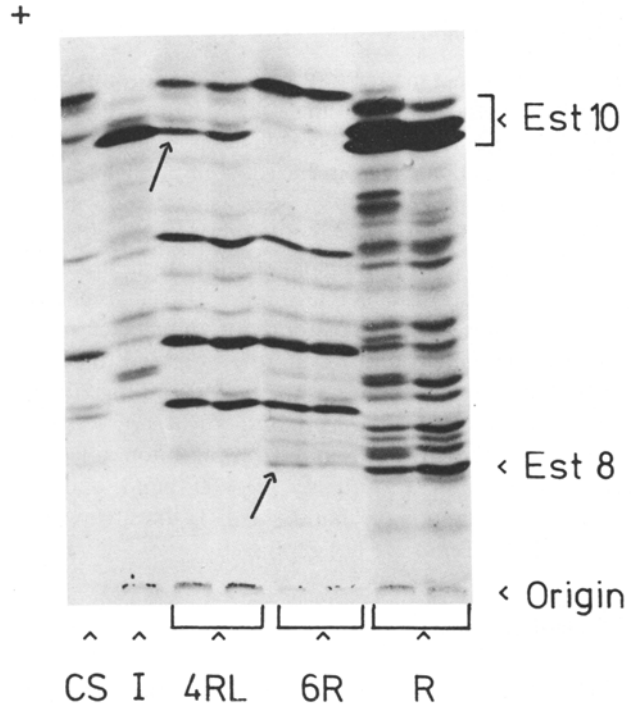

Fig. 3. Acidic esterase zymogram region of wheat, I rye and 4R (lines 11–13 in Table 1) and 6R (lines 21, 23) CS/I lines. For orientation, two individual plants of a rye inbred line are also presented (R). Critical bands are indicated by *arrow*

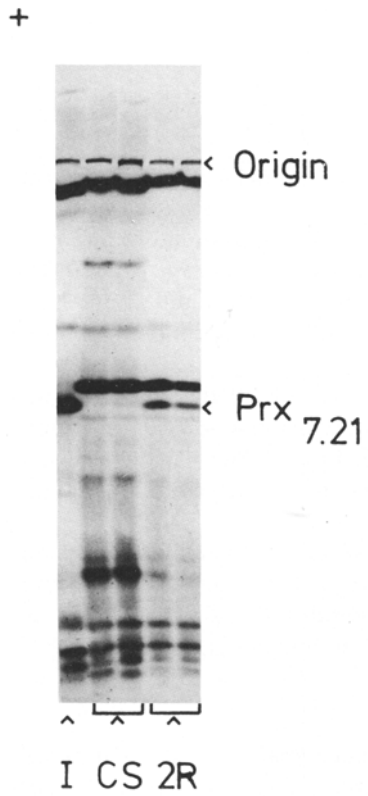

Fig. 6. Total IEF peroxidase pattern of CS wheat, I rye and the CS/I 2R line (line 5 in Table 1)

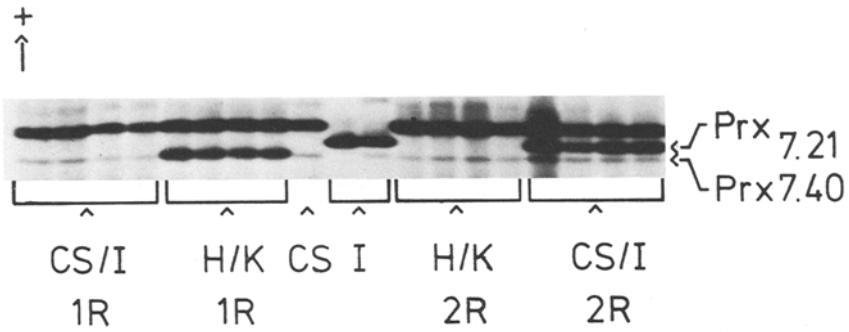

Fig. 7. Divergence in chromosomal location and different alleles of the *Prx* locus in CS/I and H/K addition lines

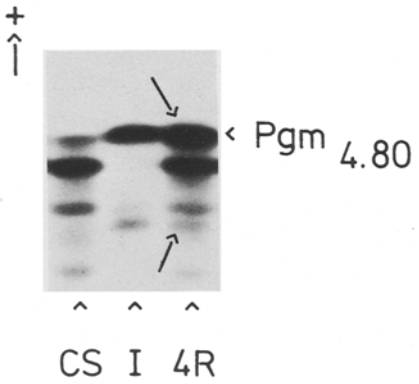

Fig. 8. IEF zymogram of PGM isozymes in CS wheat, I rye and wheat/rye addition, translocation and substitution lines. CS = CS wheat and all lines other than lines 11 and 14; I = 'Imperial' rye; 4R = lines 11 and 14

lines only the acidic band at pH 6.55 is present (not shown).

The 6R CS/I addition line has an additional band which is encoded by the *Est8* locus in rye (Fig. 3). This band is also present in the 6R H/K addition line but is absent in the R60 6B/6R translocation line (not shown).

The CS/I 4RL addition and all the other 4R addition and translocation lines express an acid rye esterase which has been identified as the $Est10_{4.82}$ allele (Fig. 3). The H/K addition line is characterized by another allele, $Est10_{4.93}$ (Fig. 4).

The comparison of several individuals of 'Imperial' rye reveals that the latter is heterogenous in respect to the *Est10* genotype. Besides the $Est10_{4.82}$ allele the $Est10_{4.70}$ was also found in 'Imperial' (Fig. 5).

Peroxidases

As our results from genetic analysis of rye leaf peroxidases have not been published yet the critical *Prx* locus mentioned below is characterized merely by the IEP of its alleles but not by a serial number.

Figure 6 presents the total banding pattern of CS wheat, 'Imperial' rye and the 2R CS/I addition line.

Whereas a chromosomal localization of the peroxidases in the alkaline (cathodal) and acidic (anodal) gel region was not possible by the means of aneuploid lines, the $PRX_{7.21}$ band is clearly expressed in the CS/I 2R addition line, but not in the 2RL addition (line 6, Table 1). This band represents one of at least four different alleles of a peroxidase locus which was genetically analyzed in rye inbred lines (not published). The 'Holdfast'/'King II' 2R addition line does not exhibit any rye peroxidase. However, in the H/K 1R addition a band electrophoretically identical with another known allele of the above mentioned *Prx* locus, $Prx_{7.40}$, is expressed (Fig. 7).

Phosphoglucomutase

Rye exhibits two main PGM bands the more intensive of which cofocuses with the wheat band at pH 4.80 (Fig. 8) and belongs to a locus that is polymorphic with at least two active alleles in rye (data unpublished). The second band focusing at a less acidic pH value is followed by a faint anodal band and does not show any variation in rye. Preliminary studies using the two-dimensional titration gel technique (Rosengren et al. 1977) indicate that the possibility of an artefact nature of these two latter bands cannot be excluded (data unpublished). Figure 8 presents the three banding patterns found among CS wheat, I rye and wheat/rye 4R addition lines. The 4R CS/I and H/K addition lines are characterized by an intense pH 4.80 band and the additional cathodal rye band. In 4RL lines, however, the rye PGM bands are not expressed.

Discussion

IEF zymograms reveal that the rye esterases, especially those encoded by the *Est10* locus, and the peroxidases around pH 7 are suited very well for chromosomal markers. The *Pgm* band is more difficult to detect due to overlapping with the wheat band.

Analysis of addition, translocation and substitution lines proves that nine out of ten genetically investigated rye esterase loci can be attributed to three rye chromosomes. As presented above, localization of loci *Est2, Est6, Est7* (chromosome 5RL), *Est8* (chromosome 6R) and *Est10* (chromosome 4RL) could be performed directly by the use of aneuploid lines. Thus, linkage groups *Est1/Est2/Est3/Est5/Est6/Est7, Est8/Est9* and the unlinked *Est10* locus are situated on chromosome arm 5RL, 6R and 4RL, respectively. Locus *Est4* remains unlocalized. The unknown esterase band focusing anodally from the origin at pH 6.55 is present in all 5R lines. However, the same band was found in a H/K 5RS as well as in a H/K 5RL line. This could hint at duplicate loci located on both arms of chromosome 5R and encoding for esterase with the same electrophoretic mobility. This interpretation, however, remains speculative although Rao and Rao (1980) found duplicate loci for 6-phosphogluconate dehydrogenase on two different chromosomes in rye.

Barber et al. (1968) reported the location of a rye esterase locus on chromosome G (=3R according to Koller and Zeller 1976) in 'Imperial' rye and suggested a dimeric nature of the esterase enzyme concerned. From our results obtained by isoelectric focusing we could not gain evidence for an involvement of chromosome 3R in esterase production or for a dimeric nature of rye esterases in the leaf blade.

The *Prx* locus encoding for the $Prx_{7.21}$ band can unequivocally be located in chromosome BR in 'Imperial' rye which is homologous to chromosome III in 'King II' and is designated 2R by Koller and Zeller (1976). Since of the 2RL addition line no allele of this *Prx* locus is expressed the latter may be situated on the short arm of the 2R chromosome in the CS/I 2R line. In 'King II', however, this locus is found on chromosome 1R. Schmidt et al. (1984) attributed a peroxidase band Per-13 to chromosome 1R in 'King II' by using the IEF technique. Although the isoelectric point of this band was not given we assume that Per-13 is identical to our $Prx_{7.40}$ band in 'King II'.

Sears (1973) found that during meiosis, a simultaneous misdivision of two univalent chromosomes in monosomic-6B/monosomic-5R wheat/rye lines always led to a fusion of two telocentrics when resulting in two telocentric chromosomes in the same meiocyte. Thus a translocated chromosome having one arm from each of the parental univalents was formed. Using isozyme markers, Hart and Tuleen (1982) demonstrated that chromosomes of *Elytrigia elongata* translocation products consisted of two different *Elytrigia* chromosomes in three of the seven 'Chinese Spring'/*E. elongata* addition lines. They concluded that translocation between alien chromosomes as a result of simultaneous misdividing and subsequent fusion of nonhomologous telocentric chromosomes may often happen during the development of wheat/alien chromosome addition lines (Hart and Tuleen 1983). The discrepancy between the chromosomal location of the *Prx* locus in CS/I and H/K addition lines may thus have its origin in such a translocation. Cytological evidence for translocation involving chromosomes 3R and 7R in a 'Holdfast'/'King II' addition line was obtained by Koller and Zeller (1976).

Linkage data (unpublished) clearly indicate joint segregation of the above mentioned *Prx* locus and the phosphoglucoisomerase (*Pgi*) locus in rye. Thus, in 'Imperial' additions to wheat, the *Pgi* locus may also be located on chromosome 2R whereas in 'King II' additions to CS' wheat this locus was found on chromosome 1R (Chojecki and Gale 1982). Overlapping of 'Chinese Spring' wheat and 'Imperial' rye PGI bands embarrassed us to unequivocally locate the *Pgi* locus via CS/I aneuploid lines. Addition lines with a null-*Pgi*-D1 Chinese Spring (called CS') wheat recipient as were used by Chojecki and Gale were not available.

The *Pgm* locus can be appointed to rye chromosome 4R in 'Imperial' and 'King II'. Since the 4RL addition and translocation lines did not exhibit rye PGM bands the *Pgm* locus may be situated on the short arm of 4R.

A gene coding for phosphoglucomutase was detected on chromosome 4H in barley (Brown and Munday 1982) and on the short arms of homologous group 4 in 'Chinese Spring' wheat (Benito 1982).

In wheat cv. 'Chinese Spring' an esterase gene is located on the group 6 and 7 chromosomes, respectively (May et al. 1973; Jaaska 1980). In rye, loci *Est8* and *Est9* are situated on chromosome 6R and *Est10* on chromosome 4RL. On the basis of cytological and biochemical evidence the 4RL chromosome arm has been demonstrated to be homoeologous to group 7 chromosomes in wheat due to a translocation between chromosome 4R and 7R in *Secale cereale* (Koller and Zeller 1976; Hsam et al. 1982). In addition to the present list of orthologous genes within the Triticeae tribe (for a review see Hart and Tuleen 1983) the chromosomal location of *Pgm, Est8/Est9* and *Est10* loci in rye provide further biochemical evidence for the conservation of the synteny of genes within the Triticeae, as assumed by Hart et al. (1980).

The location of the group of 'alkaline' esterase loci on chromosome 5RL, however, is outstanding in that there is no analogy in wheat as far as we know. In addition to group 6 and 7 wheat chromosomes only group 3 chromosomes have also been reported to carry two esterase loci in immature grains, roots, leaves and coleoptiles (Barber et al. 1968; Jaaska 1980) and a compound locus in the grain (Ainsworth et al. 1984). In *Hordeum,* five out of ten esterase loci are situated on chromosome 3H (Hvid and Nielson 1977; Brown and Munday 1982). Thus, it would not be surprising if in rye a further translocational interchange involving chromosomes 3R and 5R had taken place. However, data on the chromosomal location of loci encoding unspecific enzymes such as esterases must be judged with great care with respect to the identification of homoeology relationships between different genomes.

Cytological work and a further mapping of specific rye chromosome arms by linkage analysis of isozyme

genes has to be conducted to decide whether there are more chromosomal rearrangements in the rye genome than reported to date.

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