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Inheritance, linkage relationship and chromosomal localization of the glutamate oxaloacetate transaminase, acid phosphatase and diaphorase isozyme genes in *Secale cereale* L.

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Summary. Genetic analysis of the inheritance of glutamate oxaloacetate transaminase (GOT), acid phosphatase (ACP) and diaphorase (DIA) in leaf tissue of rye revealed the involvement of four *Got*, four *Acp* and three *Dia* loci. Linkage analysis led to the arrangement of these and other previously described isozyme loci into four linkage groups located on chromosomes 3Rq, 4Rq, 6R, and 7Rq, respectively. Implications of these findings for possible translocation differences between chromosomes of *S. cereale* and *S. montanum* are discussed. A χ^2 component analysis which makes use of the entire potential of the information provided by codominantly inherited traits such as isozymes is described.

Key words: *Secale cereale* – Isozymes – χ^2 component analysis – Linkage relationships – Chromosomal location

Introduction

An optimal exploitation of isozyme markers in genetics and breeding requires the genetic analysis of electrophoretic phenotypes. Conclusions based merely on phenotypes, and not genotypes, may be misleading. Furthermore, an efficient usage of marker genes in breeding research is dependent on dense linkage maps for the different chromosomes and has to make use of linkages of marker loci to genes that are of interest for the breeder.

Relative to the number of studies that have been carried out on important cereal crops such as maize, wheat and barley, those providing linkage data in rye are relatively scare (Garcia et al. 1982; Wehling and Schmidt-Stohn 1984; Figueras et al. 1985; Wehling 1985; Lawrence and Appels 1986; Wehling and Uphoff 1990; Vaquero et al. 1990). This may be due to the restricted economical importance of this crop as well as to the fact that *Secale cereale* is an outbreeding species with a very effective self-incompatibility system that may complicate genetic studies. To date more than 120 morphological and biochemical traits in rye have been localized chromosomically (for a review see Schlegel et al. 1986). In most cases, however, a merely phenotypically based localization of traits to chromosomes in aneuploid wheat/rye lines or trisomics has been conducted without any genetic and linkage analysis. An isozyme linkage map of genetically analyzed loci which includes all of the seven rye chromosomes and which enabled the marking and chromosomal assignment of the two self-incompatibility loci (Wricke and Wehling 1985; Gertz and Wricke 1989) has been presented (Wehling 1985; Wehling and Uphoff 1990).

The present paper presents a re-evaluation of the inheritance of GOT enzymes that have been reported to be controlled by three loci (Salinas and Benito 1985; Vaquero et al. 1990) as well as a genetic analysis of acid phosphatase and diaphorase isozymes, the latter of which have not yet been described in rye.

Materials and methods

For segregation and linkage analysis inbred lines ranging from the I_1 to I_5 generation of heterozygous selfed parents were used since data from selfed progeny are equivalent to F_2 data in terms of statistical information for linkage analysis and thus are superior to the data from the backcross type in the case of codominantly inherited traits (Allard 1956). For the production of inbred lines, selfed parents were selected which were heterozygous at a maximum number of isozyme loci, thus allowing the evaluation of multiple-point linkage data against identical genetic backgrounds. The selfing of the rye plants was performed using a self-fertility gene from the cultivar 'Dakold' as well as by

Table 1. χ^2 component analysis in the case of codominance at both loci. **A** Matrix of coefficients, k_{ij_2} for the orthogonal comparison by χ^2 component analysis. **B** Genetic interpretation of χ^2 components

A

	Genotypes, observed frequencies z_i								
	AA BB	*AA Bb*	*AA bb*	*Aa BB*	*Aa Bb*	*Aa bb*	*aa BB*	*aa Bb*	*aa bb*
					i				
	1	2	3	4	5	6	7	8	9
1	1	1	1	−1	−1	−1	1	1	1
2	1	1	1	0	0	0	−1	−1	−1
3	1	−1	1	1	−1	1	1	−1	1
4	1	0	−1	1	0	−1	1	0	−1
j 5	1	−1	1	−1	1	−1	1	−1	1
6	1	0	−1	0	0	0	−1	0	1
7	1	0	−1	−1	0	1	1	0	−1
8	1	−1	1	0	0	0	−1	1	−1
$16 * p_i$	1	2	1	2	4	2	1	2	1

χ^2 components are calculated by:

$$\chi^2 = \frac{(\sum z_i k_{ij})^2}{n \sum p_i k_{ij}^2}$$

where p_i and z_i are the expected and observed frequency of genotypes, respectively; k_{ij} is the coefficient of orthogonality; g is the number of genotypic classes; n is the total number of the individuals

B

	χ^2_j component	Test for null hypothesis (H_0) versus alternative hypothesis (H_a)
Locus *A* components	χ^2_1	H_0: frequency of $AA+aa=Aa$ H_a: genotype selection at locus *A*
	χ^2_2	H_0: frequency of $AA=aa$ H_a: gamete selection at locus *A*
Locus *B* components	χ^2_3	H_0: frequency of $BB+bb=Bb$ H_a: genotype selection at locus *B*
	χ^2_4	H_0: frequency of $BB=bb$ H_a: gamete selection at locus *B*
$A \times B$ components	χ^2_5	H_0: independent segregation and lack of genotype selection H_a: linkage or/and genotype selection
	χ^2_6	H_0: independent segregation H_a: linkage
	χ^2_7	H_0: no epistasis H_a: genotype selection at locus *A* dependent on locus *B* or/and gamete selection at locus *B* dependent on locus *A*
	χ^2_8	H_0: no epistasis H_a: genotype selection at locus *B* dependent on locus *A* or/and gamete selection at locus *A* dependent on locus *B*

means of spontaneous mutations to self-fertility in German rye cultivars. In addition, genetically self-incompatible plants from different cultivars were selfed in the growth chamber by exploiting the ocurrence of pseudo-compatibility under elevated temperature (Wricke 1978). Chromosomal localization was performed using 'Chinese Spring'/'Imperial' and 'Holdfast'/'King II' wheat/rye addition, substitution and translocation lines kindly provided by T.E. Miller, Cambridge and F.J. Zeller, Munich, and by detection of linkage to previously located loci (Wehling and Schmidt-Stohn 1984; Wehling 1985).

Tests for linkage were performed by χ^2 component analysis for two-locus segregations. In the case of codominant segregation at both loci the total χ^2 value was divided into eight orthogonal components that allow for the testing of specific genetic situations (Table 1, Wehling 1986). A specific test component for the detection of linkage was derived (χ^2_6 in Table 1) that is devoid of other interactive factors. In a similar way, five χ^2 components were derived in the case that one of the two loci segregated with a null allelle in a 3:1 ratio (not shown). Recombination values were calculated using the formulas and tables provided by Allard (1956).

The electrophoretic separation and staining of isozymes were performed as previously described (Wehling 1985).

Results

GOT

Three main zones of GOT activity controlled by four loci could be distinguished after PAGE (see Fig. 3). The activ-

Table 2. Pooled single-locus segregation data in selfed progeny for *Got*, *Acp* and *Dia* loci

Parental genotype Allele 1+2	Segregation in selfed progeny			Number of plants	Number of lines	χ^2 observed versus expected[a]	χ^2 het (*df*)
	11	12	22				
$Got1_{S+F}$	162	263	155	580	6	5.20	3.80 (10)
$Got2_{S+F}$	75	143	59	277	4	2.14	2.23 (6)
$Got2_{F+n}$	218		76	294	3	0.11	1.46 (2)
$Got4_{S+F}$	243	505	278	1026	14	2.64	24.50 (26)
$Acp1_{S+F}$	117	291	146	554	6	4.45	7.31 (10)
$Acp2_{B+n}$	667		233	900	8	0.38	3.25 (7)
$Acp2_{B+n}$	61	115	60	236	3	0.16	3.99 (4)
$Acp3_{S+F}$	29	76	40	145	3	2.01	2.59 (4)
$Dia1_{4.8+4.9}$	195	315	159	705	10	3.69	9.31 (18)
$Dia1_{4.8+5.2}$	29	78	42	149	2	2.60	0.04 (1)
$Dia2_{5.0+n}$	355		115	470	6	0.07	4.23 (5)
$Dia3_{4.2+4.3}$	83	169	98	350	5	1.70	6.71 (8)

[a] Expected ratio was either 1:2:1 or 3:1

ity zone with the slowest electrophoretic mobility is controlled by a single locus, *Got4*. This zone has been described previously as GOT3. Two active alleles, $Got4_S$ (slow) and $Got4_F$ (fast), could be identified (Table 2). Heterozygous plants displayed an intermediate hybrid band of doubled intensity, which indicates that the GOT4 enzymes have a dimeric nature.

A second locus, *Got3*, can be postulated for the expression of the intensely stained activity zone with intermediate mobility. With the exception of a rare "slow" allele in Californian and Spanish rye material (Vaquero et al. 1990), this locus displays no polymorphism in the rye material studied.

The fast-mobility zone, which consists of a complex banding pattern in a number of inbred lines, is governed by two loci, *Got1* and *Got2* (Figs. 1 and 2). At the *Got1* locus two alleles, $Got1_S$ and $Got1_F$, could be identified. The *Got2* locus is polymorphic with three alleles, $Got2_S$, $Got2_F$, and a null allele, $Got2_n$. In the case of the $Got2_n$ allele a 3:1 ratio was observed in segregating progeny, whereas a 1:2:1 ratio was obtained for active alleles (Table 2). Both loci encode enzymes having a dimeric behavior that form intragenic as well as intergenic hybrid enzymes. Thus, $Got1_{SF}/Got2_{SF}$ heterozygotes display a complex banding pattern composed of the homodimeric parental bands $\alpha_s\alpha_S$ and $\alpha_f\alpha_f$ encoded by *Got1*, $\beta_s\beta_s$ and $\beta_f\beta_f$ encoded by *Got2*, intragenic heterodimers of $\alpha_s\alpha_f$ and $\beta_s\beta_f$ and intergenic heterodimers of the constitution $\alpha_s\beta_s$, $\alpha_s\beta_f$, $\alpha_f\beta_s$ and $\alpha_f\beta_f$ (Fig. 1). Lines segregating at both loci display a total number of nine banding phenotypes in the GOT1/GOT2 zone (Fig. 1). Nine out of the total of ten dimeric forms expected could be separated as distinct bands in different genotypes. In the double heterozygote, however, where all ten dimeric forms are present, only five bands are visualized due to partial overlapping of different dimers (bracketed in Fig. 1). Figure 2 shows two different inbred lines with a single-locus segregation at *Got1* and *Got2*, respectively. In both cases, the three different genotypes of a monohybrid segregation are observed with intergenic GOT1/GOT2 hybrid enzymes in the homozygotes and both intra- and intergenic heterodimers in the heterozygotes. Heterodimers formed between GOT1 and GOT2 subunits are always less intensely stained than the homo- and heterodimers within loci.

GOT banding patterns obtained with wheat/rye addition lines are shown in Fig. 3. The more intensive activity staining of the slowest GOT4 band in the CS/I 3R addition than in CS wheat and remaining addition lines indicates the involvement of an 'Imperial' *Got* gene and thereby confirms the chromosomal location of *Got4* on chromosome 3R as reported by Tang and Hart (1975). In the GOT3 zone, the 6R addition line displays a pattern of five closely spaced bands, the slowest of which displays the same mobility as the 'Imperial' band, whereas the remaining bands are formed by heterodimers between the three wheat *Got* loci and the rye *Got3* locus (Tang and Hart 1975). *Got3* can thus be localized on chromosome 6R.

In 4R and 7R additions the fast-moving GOT1/GOT2 zone is stained more intensely and appears to be broader than in wheat and remaining addition lines. The broadened zone in 4Rq and 7R lines cannot be resolved properly into distinct bands. Thus, the correct appointment of loci *Got1* and *Got2* to chromosomes 4Rq and 7R is not possible by means of addition lines. However, the chromosomal location of both loci could be determined by linkage analysis (see below).

Acid phosphatase, ACP

ACP isozymes of rye leaf can be separated into four distinct zones, ACP1, 2, 3, and 4 (Fig. 4A). ACP1 is

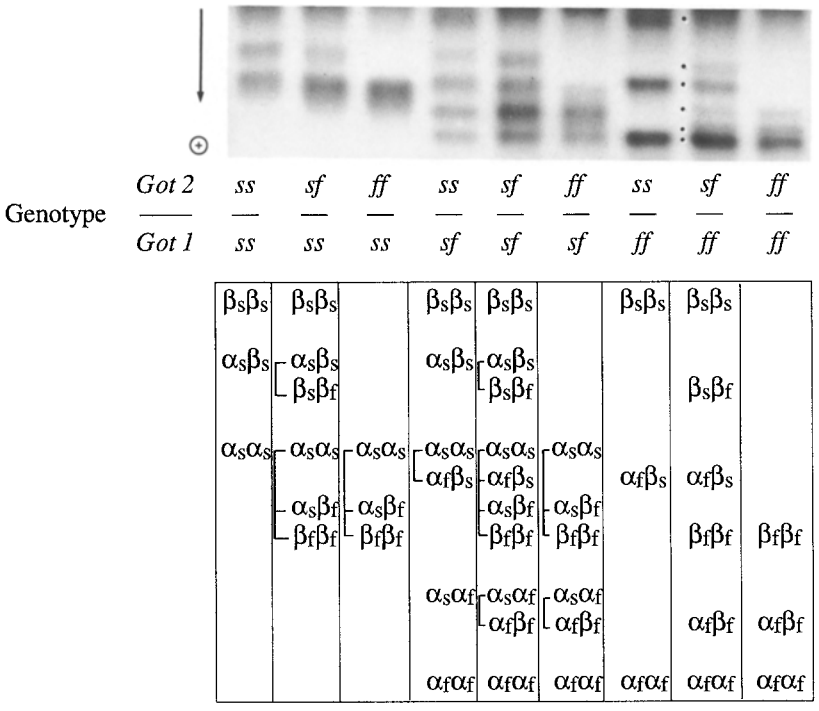

Fig. 1. Dihybrid segregation at loci *Got1* and *Got2* in an inbred line displaying the nine different banding phenotypes. *Upper part*, close-up of the GOT1/GOT2 activity zone. Some homogenic bands (*lane 8*) are *dotted* for better identification. *Lower part*, schematic representation of putative subunit composition of dimeric forms in the nine genotypes. Dimers overlapping in the zymogram above are indicated by a *bracket*. α GOT1 subunits, β GOT2 subunits

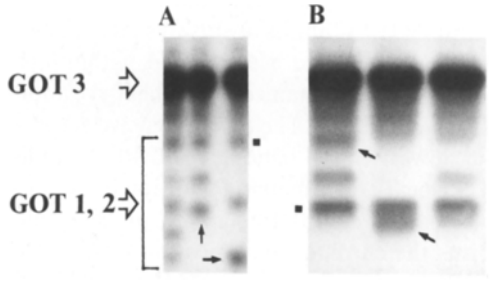

Fig. 2 A, B. Monohybrid segregation at *Got1* and *Got2* locus, respectively. **A** *Got1* segregating for the *s* and *f* allele. Homodimeric GOT1 bands are indicated by an *arrow*; homodimeric band of the non-segregating *Got2* locus is indicated by a *dark square* (■). **B** *Got2* segregation for the *s* and *f* allele. Homodimeric GOT2 bands are indicated by an *arrow;* the homodimeric product of the non-segregating *Got1* locus is indicated by a *dark square* (■)

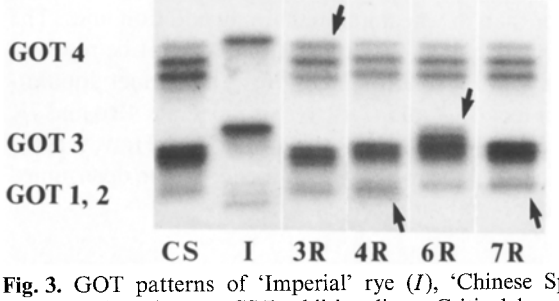

Fig. 3. GOT patterns of 'Imperial' rye (*I*), 'Chinese Spring' wheat (*CS*) and some CS/I addition lines. Critical bands are indicated by *arrows*. 1R, 2R and 5R additions (not shown) are similar to CS wheat

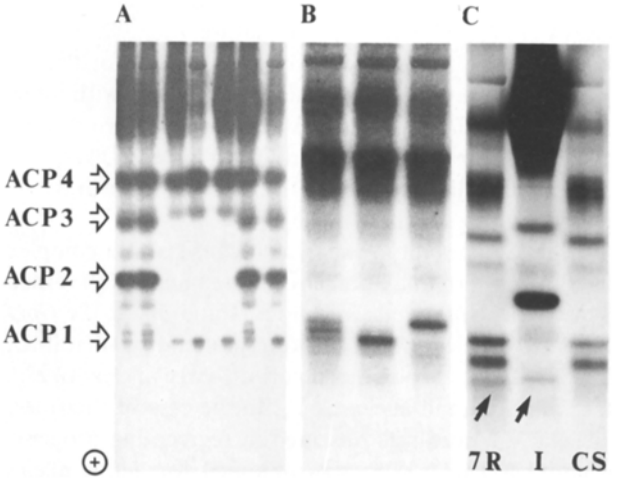

Fig. 4. **A** ACP isozyme pattern of rye leaf tissue displaying the four activity zones. **B** Segregation at the *Acp1* locus in an inbred line. **C** Chromosomal localization of *Acp1* on chromosome 7R. *I* 'Imperial' rye, *CS* 'Chinese Spring' wheat and CS/I additions other than the 7R line

controlled by a single locus with two alleles, $Acp1_S$ and $Acp1_F$. Heterozygotes display the triple-banded pattern expected of dimeric enzymes (Fig. 4B). Two other loci, *Acp2* and *Acp3*, govern the expression of the successive activity zones. Segregations occur in the expected 3:1 or 1:2:1 ratio (Table 2) with an active and null allele at *Acp2* and two active alleles at the *Acp3* locus (Fig. 4A). In some cases, segregants at the *Acp2* locus could be separated into all three genotypic classes on the basis of

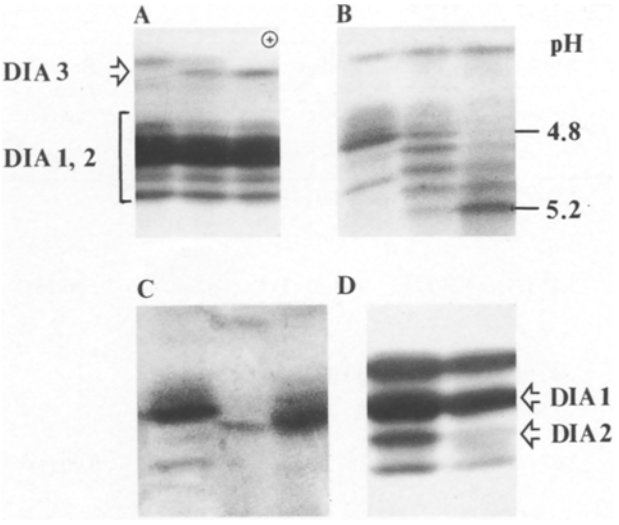

Fig. 5. **A** Diaphorase zymogram after IEF. **B** Segregation with alleles $Dia1_{4.8}$ and $Dia1_{5.2}$. *Lane 1* $Dial_{4.8+4.8}$, *lane 2* $Dia1_{4.8+5.2}$, *lane 3* $Dia_{5.2+5.2}$. **C** Segregation with alleles $Dia1_{4.8}$ and $Dia1_{4.9}$. *Lane 1* $Dia1_{4.8+4.8}$, *lane 2* $Dia1_{4.9+4.9}$, *lane 3* $Dia1_{4.8+4.9}$. **D** Segregation at the *Dia2* locus with alleles $Dia2_{5.0}$ and $Dia2_n$. *Lane 2* $Dia2_{n+n}$ homozygote; *Dia1* is not segregating

band intensity (Table 2). ACP2 and ACP3 enzymes behave as monomers. An additional locus, *Acp4*, is postulated for the slow activity zone. Segregation data for this zone is not yet available.

In Fig. 4C the ACP phenotypes of CS wheat, 'Imperial' rye and addition lines are presented. 7R additions show an $ACP1_F$ band that is not observed in CS wheat and remaining addition lines. *Acp1* can thus be assigned to chromosome 7R in 'Imperial' rye.

Diaphorase, DIA

Diaphorase zymograms comprise a complex banding pattern in the acidic region of the isoelectric focusing (IEF) gel. Genetic variation was observed within two separate activity zones. The first zone includes intensely stained bands between approximiate pH values of 4.8 and 5.3. The second more weakly stained zone is focused near the anode at pH 4.2–4.4 (Fig. 5A). In different inbred lines, different patterns of variation can be observed within the first zone. Figure 5B presents one of the segregation patterns that includes three different phenotypes. Two phenotypes display a single intensely stained band at pH 4.8 and 5.2, respectively, which is succeeded by a series of secondary bands of decreasing intensity in the anodal direction. The third phenotype is characterized by a pattern of five bands, the outer two of which are identical to the parental pH 4.8 and 5.2 bands. Selfing of single-banded phenotypes yields uniform single-banded progeny, whereas five-banded phenotypes give rise to segregating selfed progeny as shown in Fig. 5B with 25%

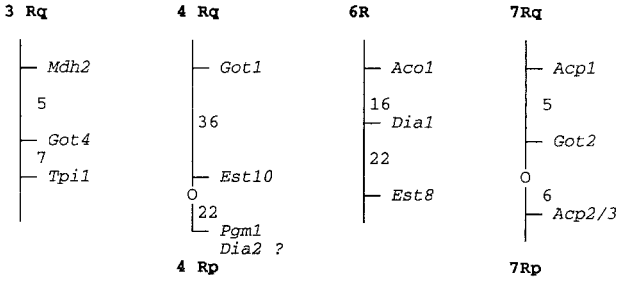

Fig. 6. Partial isozyme linkage map for rye chromosomes 3R, 4R, 6R, and 7R with percentages of recombination

single-banded and 50% five-banded individuals (Table 2). Thus, a *Dia1* locus with the alleles $Dia1_{4.8}$ and $Dia1_{5.2}$ can be postulated for the control of the observed segregation pattern. With respect to the five-banded heterozygotes a tetrameric structure of the DIA1 enzymes can be assumed. A third allele of the *Dia1* locus is observed in some inbred lines that encodes a band at pH 4.9. Fig. 5C presents a segregation pattern that includes the $DIA1_{4.8}$ and $DIA1_{4.9}$ band, the latter displaying a strongly decreased intensity as compared to the former. Homozygous plants are characterized by a sharply focused band at pH 4.8 and 4.9, respectively. In heterozygotes a broadened zone is observed because single homo- and heterotetrameric bands cannot be resolved within the narrow pH 4.8–4.9 interval. As for the $Dia1_{4.8/5.2}$ segregations a 1:2:1 ratio is obtained for the combination of $Dia1_{4.8}$ and $Dia1_{4.9}$ (Table 2).

In some inbred lines additional bands are observed that are distinguishable from the DIA1 products by their isoelectric points of approximately pH 5.0 and 5.2, respectively (Fig. 5D). In some cases, selfed progeny which are homozygous at the *Dia1* locus show a 3:1 segregation with respect to presence versus absence of these bands (Table 2). Since this variation is independent of the *Dia1* locus, a second locus with alleles $Dia2_{5.0}$ and $Dia2_n$ can be postulated. A third locus, *Dia3*, governs the expression of two bands with a monomeric behavior within the anodal activity zone (Fig. 5A). Two alleles are observed, $Dia3_{4.2}$ and $Dia3_{4.3}$, which are segregating in the expected ratio (Table 2).

Linkage relationships

In Table 3 the pooled two-locus segregation data together with recombination estimates are given. Linkage data including markers other than the *Got, Acp* and *Dia* loci have been presented previously (Wehling 1985; Wehling and Uphoff 1990). In Fig. 6 the putative arrangement of loci within linkage groups is given. Four linkage groups were obtained and subsequently located on chromosomes 3Rq, 4Rq, 6R, and 7R, respectively.

Different recombination values were obtained for the locus pair *Got4* – *Mdh2*, namely, P=0.05 and 0.16, re-

Table 3. Linkage relationships of *Got*, *Acp* and *Dia* loci in rye

Pair of loci *A* – *B*	Two-locus segregation					Number of plants	Number of lines	Single loci		Linkage Loci *A* × *B* χ^2 (*df*)	Recombination $p \pm s$	Heterogeneity χ^2 (*df*)
	Genotype, locus *A*	Genotype, locus *B*						Locus *A* χ^2 (*df*)	Locus *B* χ^2 (*df*)			
		BB	*B*	*Bb*	*bb*							
Got4 – Tpi1	*AA*	0		5	16	90	1	1.20 (2)	3.60 (2)	42.71 (1)*	0.07 ±0.020	–
	Aa	2		47	1							
	aa	16		2	1							
Got4 – Mdh2	*AA*	56		5	1	268	4	3.55 (2)	4.30 (2)	164.55 (1)*	$p_1 = 0.05$ ±0.010	5.61 (3)
	Aa	7		138	4							
	aa	0		7	50							
Got4 – Mdh2	*AA*	15		4	3	90	1	1.47 (2)	2.62 (2)	30.04 (1)*	$p_2 = 0.16$ ±0.030	–
	Aa	2		38	10							
	aa	2		0	16							
Tpi1 – Mdh2	*AA*	0		7	32	175	2	3.67 (2)	4.39 (2)	87.86 (1)*	0.09 ±0.016	0.00 (1)
	Aa	0		84	16							
	aa	32		2	2							
Got1 – Est10	*AA*	1		15	14	139	1	0.07 (2)	5.78 (2)	15.22 (1)*	0.36 ±0.038	–
	Aa	19		29	14							
	aa	16		25	6							
Dia2 – Got1	*A–*	24		115	73	285	3	0.06 (1)	4.38 (2)	125.90 (1)*	0.13 ±0.021	0.62 (2)
	aa	61		12	0							
Est10 – Pgm1	*AA*	13		8	0	69	1	3.26 (2)	0.71 (2)	36.23 (1)*	0.22 ±0.041	–
	Aa	5		16	6							
	aa	1		7	13							
Dia1 – Aco1	*AA*	2		0	11	57	1	0.20 (2)	2.12 (2)	22.74 (1)*	0.16 ±0.038	–
	Aa	4		22	4							
	aa	11		1	2							
Est8 – Dia1	*AA*	58		40	3	477	5	4.40 (2)	2.10 (2)	128.94 (1)*	$p_1 = 0.22$ ±0.016	8.11 (4)
	Aa	42		167	49							
	aa	6		37	75							
Est8 – Dia1	*A–*	26		49	4	108	2	0.20 (1)	0.07 (2)	54.54 (1)	$p_2 = 0.08$ ±0.027	0.01 (1)
	aa	0		5	24							
Est8 – Aco1	*AA*	15		7	6	143	2	3.15 (2)	2.03 (2)	17.48 (1)*	0.33 ±0.036	0.46 (1)
	Aa	15		38	19							
	aa	2		23	18							
Got2 – Acp1	*AA*	0		0	11	67	1	2.79 (2)	2.79 (2)	57.37 (1)*	$p_1 = 0.00$ ±0.000	–
	Aa	0		36	0							
	aa	20		0	0							
Got2 – Acp1	*A–*	18		44	3	84	1	0.25 (1)	0.64 (2)	37.79 (17)*	$p_2 = 0.05$ ±0.024	–
	aa	0		1	18							
Got2 – Acp2	*A–*	19		32	2	79	1	2.64 (1)	1.94 (2)	62.41 (1)*	0.06 ±0.026	0.15 (2)
	aa	0		3	23							
Got2 – Acp2	*A*		94		52	191	2	0.21 (1)	0.50 (1)	22.58 (1)*		
	aa		45		0							
Acp1 – Acp2	*A*	1		39	21	84	1	0.25 (1)	0.64 (2)	40.01 (1)*	0.08 ±0.031	–
	aa	17		6	0							
Acp2 – Acp3	*A–*	13		28	0	55	1	0.01 (1)	0.06 (2)	36.67 (1)*	0.00 ±0.000	
	aa	0		0	14							

χ^2 components given in this table do not sum up to the total χ^2 value. Components other than those for single-locus segregations and for linkage are omitted because they were all non-significant

spectively (Table 3). Four of the five inbred lines, however, were consistent for the lower estimate. We thus give the most probable order of loci as being *Mdh2 – Got4 – Tpi1*.

The recombination value of $P = 0.09 \pm 0.02$ that was directly measured between *Mdh2* and *Tpi1* is close to the expected $P = 0.11$ when interference is absent (Trow 1913). Since *Mdh2* and *Got4* (which is named *Got3* by other authors) have been reported to be located on the long arm of chromosome 3R (Schlegel et al. 1986), *Tpi1* is probably also located on 3Rq because it is closely linked to *Got4*.

On chromosome 4R the *Est10* locus is located on the long arm, 4Rq, whereas *Pgm1* can be assigned to 4Rp (Figueras et al. 1985; Wehling et al. 1985). Linkage analysis reveals that *Got1* is linked to *Est10* and is thus situated on chromosome 4R. Since 4Rq addition lines as well as 7R additions display an altered banding pattern in the GOT1/GOT2 zone (Fig. 3), it is concluded that *Got1* is located on the long arm of 4R. The *Dia2* locus is linked to *Got1*. However, no three-point linkage data is available for *Dia2* and *Pgm1* to date. Thus, it is not possible to deduce the arrangement of these loci within linkage groups nor can the arm location for *Dia2* be determined.

Recombination data obtained for the *Dia1* locus suggest an arrangement between the *Est8* and *Aco1* loci.

For gene pairs *Est8 – Dia1* and *Got2 – Acp1* non-homogenous groups of recombination estimates were found (0.22/0.08 and 0.00/0.05, respectively).

The *Acp1* locus, which encodes dimeric enzymes, could be localized on chromosome 7R. Two other *Acp* loci, *Acp2* and *Acp3*, are linked to *Acp1*. Hart (1978) localized an *Acp* locus on the short arm of 7R. Salinas and Benito (1984) also found three "monomeric" *Acp* loci on 7Rp, whereas the "dimeric" locus, which is named *Acp1* in the present paper, was localized on 7Rq. Schmidt et al. (1984) reported an *Acp* locus encoding a fast isozyme which is probably identical to our ACP1 product on 7Rq. Thus, the *Acp1* locus appears to be situated on the long arm of chromosome 7R, whereas *Acp2* and *Acp3* are located its short arm. The *Got2* locus can be assigned to 7R because it is linked to *Acp* loci (Table 3). Furthermore, 7R addition lines show an altered banding phenotype in the GOT1/GOT2 zone (Fig. 3). Tang and Hart (1975) found that the long arm of chromosome 7R is involved in the inheritance of "GOT zone 1". Thus, we may conclude that *Got2* is situated on 7Rq and that it can be arranged between *Acp2*/*Acp3* and *Acp1*.

Discussion

GOT in rye has been reported to be controlled by three loci, *Got1, Got2* and *Got3* (Salinas and Benito 1985; Vaquero et al. 1990). Genetic analysis of the GOT system by use of inbred lines reveals, however, that variation of the GOT pattern in rye leaf tissue is governed by four unlinked loci that all encode dimeric enzymes. These loci may be designated in the order of the decreasing electrophoretic mobility of their products as *Got1, Got2, Got3* and *Got4*. Given this designation, *Got1* and *Got2* would be synonymous to the "GOT zone 1" described by Tang and Hart (1975) and "*Got-1*" by Vaquero et al. (1990), *Got3* would be identical to "*Got-R2*" and "*Got-2*", and *Got4* synonymous to "*Got-R3*" and "*Got-3*" described by the same authors. Two of the four loci, *Got3* and *Got4*, could be located by Tang and Hart (1975) on chromosomes 3R and 6R, respectively. Unequivocal chromosomal assignment of "GOT zone 1" genes could not be performed by these and other authors (Schmidt et al. 1984; Salinas and Benito 1985). The latter authors postulated one locus for the genetic control of this zone, which they located on chromosome arm 7Rq. Tang and Hart (1975), however, observed various bands in this activity zone, the genetic basis of which could not be exactly determined. Their suggestion was that chromosomes 4R and 7R are involved in the expression of these GOT bands. Our genetic data confirms the supposition of Tang and Hart and clarifies the genetic control of fast-moving "GOT zone 1" isozymes. Segregation analysis reveals the involvement of two unlinked loci, *Got1* and *Got2*, which are situated on chromosome 4Rq and 7Rq, respectively.

The formation of enzymatically active hybrid dimers between GOT1 and GOT2 subunits hints at a close genetic relationship between *Got1* and *Got2* loci, which is to be expected if these loci have evolved from a recent duplication event. Besides tandem duplication, duplicate loci can be generated by reciprocal translocation by which the duplicate units are distributed to different chromosomes (see, e.g., Weeden 1983). The reduced intensity of intergenic GOT1/GOT2 hybrid bands relative to the intragenic homo- and heterodimers (Fig. 2) may be due to a reduced specific enzyme activity of intergenic hybrid dimers of to a non-random association among GOT1 and GOT2 subunits and thus may hint at a progressive genetic diversification between *Got1* and *Got2* alleles.

In addition to the chromosomal location of two phosphogluconate dehydrogenase loci on chromosome arms 4Rq and 6Rq (Rao and Rao 1980; Hsam et al. 1982), the location of *Got1* and *Got2* on chromosomes 4Rq and 7Rq provides further biochemical evidence for the translocation events in *Secale cereale* between chromosomes 4R, 6R and 7R as was inferred cytologically by Koller and Zeller (1976). According to these authors chromosome 4R of *S. cereale* 'Imperial' is a translocation product from chromosomal segments 4Rq, 4Rq, and 7Rp of *S. montanum*, chromosome $6R^{cer}$ is a translocation product from $7Rq^{mon}/6Rq^{mon}/6Rp^{mon}$ and chromo-

some $7R^{cer}$ contains 4Rq/7Rp//7Rq/6Rq *montanum* segments. Thus, both chromosomes 4R and 7R in *S. cereale* contain parts of the original *montanum* 7Rp chromosome arm. This view is confirmed by the fact that both chromosomes in *S. cereale* share genes for GOT enzymes that are still similar enough to interact at the subunit level by formation of active hybrid dimers.

Genetic analysis of acid phosphatases in rye leaf led to the identification of three polymorphic loci, *Acp1, 2*, and *3*, and a monomorphic locus, *Acp4*. Due to close linkage in repulsion of *Acp2* and *Acp3* and to limited number of individuals the recombination estimate for these loci is 0.00. Nevertheless, *Acp2* and *Acp3* appear to constitute different genetic units because in some inbred lines there was coincidence of segregation at one of the two loci and homozygosity for an active or null allele at the other locus. Thus, recombination must have occurred within the rye populations the selfed parents were derived from.

Among the diaphorase, loci, *Dia1* is the first marker locus in rye reported to code for tetrameric products. Observations (not shown) on inbred lines that segregate at both *Dia1* and *Dia2* suggest that the subunits encoded by the active $Dia2_{5.0}$ allele also associate to form tetramers with the DIA1 subunits, thereby creating a very complex banding pattern that is difficult to evaluate. In soybean, *Glycine soja*, and *G. max*. there are also monomeric and tetrameric diaphorase isozymes. Also, two loci interact to form intergenic heterotetramers (Gorman et al. 1982a, b, 1983).

References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Figueras AM, González-Jaén MT, Salinas J, Benito C (1985) Association of isozymes with a reciprocal translocation in cultivated rye (*Secale cereale*). Genetics 109:177–193

García P, Pérez de la Vega M, Benito C (1982) The inheritance of rye seed peroxidases. Theor Appl Genet 61:341–351

Gertz A, Wricke G (1989) Linkage between the incompatibility locus Z and a β-glucosidase locus in rye. Plant Breed 102:255–259

Gorman MB, Kiang KT, Chiang YC, Palmer RG (1982a) Preliminary electrophoretic observations from several soybean enzymes. Soybean Genet Newsl 9:140–143

Gorman MB, Kiang YT, Chiang YC, Palmer RG (1982b) Electrophoretic classification of the early maturity groups of named soybean cultivars. Soybean Genet Newsl 9:143–156

Gorman MB, Kiang YT, Palmer RG, Chiang YC (1983) Inheritance of soybean electrophoretic variants. Soybean Genet Newsl 10:67–84

Hart GE (1978) Chromosomal arm locations of *Adh-R1* and an acid phosphatase structural gene in Imperial rye. Cereal Res Commun 6:123–133

Koller OL, Zeller FJ (1976) The homoeologous relationships of rye chromosomes 4R and 7R with wheat chromosomes. Genet Res 28:177–188

Lawrence GJ, Appels R (1986) Mapping of the nucleolus organizer region, seed protein loci and isozyme loci on chromosome 1R in rye. Theor Appl Genet 71:742–749

Salinas J, Benito C (1984) Phosphatase isozymes in rye. Characterization, genetic control and chromosomal location. Z Pflanzenzücht 93:115–136

Salinas J, Benito C (1985) Chromosomal locations of phosphoglucomutase, phosphoglucose isomerase, and glutamate oxaloacetate transaminase structural genes in different rye cultivars. Can J Genet Cytol 27:105–113

Schlegel R, Melz G, Mettin D (1986) Rye cytology, cytogenetics and genetics – Current status. Theor Appl Genet 72:721–734

Schmidt JC, Seliger P, Schlegel R (1984) Isoenzyme als biochemische Markerfaktoren für Roggenchromosomen. Biochem Physiol Pflanz 179:197–210

Tang KS, Hart GE (1975) Use of isoenzymes as chromosome markers in wheat-rye addition lines and in triticales. Genet Res 26:187–201

Trow AH (1913) Forms of reduplication: primary and secondary. J Genet 2:313–324

Vaquero F, Rebordinos L, Vences FJ, Pérez de la Vega M (1990) Genetic mapping of isozyme loci in *Secale cereale* L. Theor Appl Genet 80:88–94

Weeden NF (1983) Evolution of plant isozymes. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, part A. Elsevier Sci Publ, Amsterdam, Oxford, New York, pp 175–205

Wehling P (1985) Electrophoretic analysis of 10 enzyme systems in rye: linkage relationships and chromosomal location of isozyme loci. In: Proc EUCARPIA Meeting of the cereal section on rye. Svalöf, Sweden, pp 101–124

Wehling P (1986) Genetische Analyse und chromosomale Lokalisation von Isoenzymloci beim Roggen. PhD thesis, University of Hannover

Wehling P, Schmidt-Stohn G (1984) Linkage relationships of esterase loci in rye (*Secale cereale* L.). Theor Appl Genet 67:149–153

Wehling P, Uphoff H (1990) Untersuchungen zur Markierung von Identifizierung der Inkompatibilitätsloci beim Roggen. Vortr Pflanzenzücht 18:45–69

Wehling P, Schmidt-Stohn G, Wricke G (1985) Chromosomal location of esterase, peroxidase and phosphoglucomutase isozyme structural genes in cultivated rye (*Secale cereale* L.). Theor Appl Genet 70:377–382

Wricke G (1978) Pseudo-Selbstkompatibilität beim Roggen und ihre Ausnutzung in der Züchtung. Z Pflanzenzücht 81:140–148

Wricke G, Wehling P (1985) Linkage between an incompatibility locus and a peroxidase isozyme locus (*Prx7*) in rye. Theor Appl Genet 71:289–291