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Linkage relationships of esterase loci in rye (*Secale cereale* L.)

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Summary. Genetic analysis of esterase polymorphism in rye inbred lines with isoelectric focusing in polyacrylamide flat gels yielded evidence for the existence of at least ten esterase loci, *Est 1–Est 10.* The loci can be attributed to four different linkage groups (*Est 1/Est 2/Est 3/Est 5/Est 6/Est 7*), (*Est 4*), (*Est 8/Est 9*), and (*Est 10*). Loci *Est 5/Est 6/Est 7* and *Est 8/Est 9,* respectively, are tightly linked with a maximum recombination frequency of 0.2% and can therefore be regarded as compound loci which possibly originated in tandem duplications.

Key words: *Secale cereale* L. – Esterase polymorphism – Linkage relationships – Compound loci

Introduction

While the genetics of the cereals maize, wheat and barley have been intensively explored, the genome of rye has been only poorly mapped by morphological and biochemical markers. This neglect can be explained to a great extent by the mating system, including an effective gametophytic self-incompatibility mechanism (Wricke 1969), which complicates genetic and breeding research within this species.

On the other hand, *Triticale* research has made the techniques of addition and translocation of chromosomal material useful tools in studying the chromosomal location of isoenzymic markers in rye.

Once the rye chromosomes have been marked, a detailed mapping of the rye genome is easily done by analyzing the linkage relationships of the marker genes with loci detected by the considerable enzyme polymorphism in rye.

The cumulative facilitation of chromosome mapping by a growing number of marker loci with known linkage relationships, and the applications of isoenzymic markers have been broadly discussed by Tanksley and Rick (1980b). In tomato, where nine of the 12 chromosomes have been marked by 22 loci (Tanksley and Rick 1980b), new biochemical marker loci can easily be mapped by performing 20–30 point linkage tests instead of the usual 2 or 3 point tests (Tanksley and Rick 1980a).

Garcia et al. (1982) accomplished the first step in setting up a linkage map of isoenzymic marker loci in *Secale cereale.* They found a total of 13 loci controlling the peroxidase variation in the embryo and endosperm, comprising linkage between all five endosperm loci.

As a second isoenzyme system, rye esterases have recently been genetically analyzed, yielding a total of at least 10 loci which control the esterase pattern in the leaf blades (Schmidt-Stohn and Wehling 1983). The linkage relationships among these loci will be shown in this paper.

Materials and methods

Plant material and electrophoretic methods used for genetic and linkage analysis have already been described (Schmidt-Stohn 1979a, b; Schmidt-Stohn and Wehling 1983). Tissue extracts were taken from the leaf blades of 10 to 14 day old seedlings because this method allows individual plants to be propagated to the next generation.

Linkage was detected by chi-square analysis of two-locus segregations for the different pairs of the 10 esterase loci.

When active alleles with codominant expression are present at both loci of a gene pair, a total of 9 phenotypic classes can be distinguished in the selfed progeny of a heterozygous individual. The total chi-square value can thus be

Table 1. Two-locus segregations between different pairs of the 10 esterase loci *Est 1 –Est 10* and chi-square analysis for the detection of linkage

Locus combination	No. of lines	Total no. of plants	Segregation locus A × locus B					Single locus segregation (found vs expected)		Joint segregation	Recombination[a] fraction *P*
			Genotype locus A	Genotype locus B							
				BB	B.	Bb	bb bb	Locus A χ^2	Locus B χ^2	χ^2	
Est 1 – Est 2	4	521	A.		268		119	0.05 (1)[b]	0.02 (1)	41.85 (1)*	0.18±0.042 R[c]
			aa		130		4				
Est 1 – Est 3	4	455	A.		247		100	0.39 (1)	0.53 (1)	20.96 (1)*	0.27±0.043 R
			aa		101		7				
Est 1 – Est 4	1	103	A.		51		21	1.43 (1)	0.55 (1)	0.09 (1)	–
			aa		23		8				
	1	99	A.	18		31	20	1.48 (1)	4.94 (2)	5.04 (2)	–
			aa	6		10	14				
Est 1 – Est 5	4	487	A.		312		33	4.49 (1)*	0.01 (1)	162.14 (1)*	0.19±0.020 A
			aa		54		88				
	2	182	A.		93		52	2.12 (1)	1.65 (1)	16.02 (1)*	0.15±0.072 R
			aa		36		1				0.19±0.019
Est 1 – Est 8	2	364	A.		208		67	0.06 (1)	0.53 (1)	3.05 (1)	–
			aa		59		30				
Est 1 – Est 10	2	395	A.	73	152		67	0.24 (1)	4.36 (2)	4.08 (2)	–
			aa	21	65		17				
Est 2 – Est 3	3	355	A.		243		29	0.50 (1)	5.28 (1)*	49.83 (1)*	0.24±0.027 A
			aa		42		41				
Est 2 – Est 5	2	291	A.		152		69	0.14 (1)	0.14 (1)	24.44 (1)*	0.12±0.058 R
			aa		69		1				
Est 2 – Est 8	2	232	A.		126		48	0.00 (1)	0.21 (1)	0.62 (1)	–
			aa		45		13				
Est 2 – Est 10	2	399	A.	72		166	63	0.04 (1)	4.82 (2)	0.02 (2)	–
			aa	23		54	21				
Est 3 – Est 5	4	639	A.		364		147	8.41 (1)*	1.36 (1)	36.95 (1)*	0.00±0.040 R
			aa		128		0				
Est 3 – Est 8	1	105	A.		62		23	1.98 (1)	0.71 (1)	0.31 (1)	–
			aa		13		7				
Est 3 – Est 10	1	232	A.	44		101	40	2.78 (1)	2.31 (2)	0.01 (2)	–
			aa	11		26	10				
Est 4 – Est 5	2	317	A.		180		61	0.18 (1)	0.05 (1)	0.03 (1)	–
			aa		56		20				
Est 4 – Est 8	2	317	A.		173		68	0.18 (1)	3.18 (1)	0.59 (1)	–
					51		25				
Est 4 – Est 10	2	310	A.	51		112	70	0.004 (1)	2.21 (2)	1.39 (2)	–
			aa	19		40	18				
Est 5 – Est 6	7	459	AA	110		0	0	0.44 (2)	0.33 (2)	913.10 (4)*	0.002±0.0015
			Aa	2		227	0				
			aa	0		0	120				
	12	617	A.	0		305	147	1.00 (1)	1.13 (2)	453.04 (2)*	0.00±0.00
			aa	165		0	0				
	6	687	A.		525		0	0.74 (1)	0.74 (1)	635.98 (1)*	0.00±0.00 A
			aa		0		162				0.0009±0.00064
Est 5 – Est 7	10	583	A.		286		140	1.16 (1)	0.30 (1)	69.76 (1)*	0.00±0.041 R
			aa		157		0				

Table 1 (continued)

Locus combination	No. of lines	Total no. of plants	Segregation locus A × locus B					Single locus segregation (found vs expected)		Joint segregation	Recombination[a] fraction *P*
			Genotype locus A	Genotype locus B							
				BB	B.	Bb	bb bb	Locus A χ^2	Locus B χ^2	χ^2	
Est 5 – Est 8	1	99	AA	7		9	5	2.27 (2)	0.51 (2)	1.08 (4)	–
			Aa	15		26	16				
			aa	4		11	6				
	1	243	A.		147		44	1.68 (1)	1.00 (1)	0.20 (1)	–
			aa		42		10				
Est 5 – Est 10	1	232	A.	45		95	36	0.09 (1)	2.31 (2)	1.41 (2)	–
			aa	10		32	14				
Est 6 – Est 7	13	672	A.	189		319	1	0.20 (1)	3.58 (2)	644.14 (2)*	0.00 + 0.0017
			aa	0		0	163				
Est 8 – Est 10	2	334	AA	24		50	14	1.21 (2)	2.21 (2)	7.08 (4)	–
			Aa	36		77	44				
			aa	25		50	14				

[a] Recombination values were calculated only when linkage was confirmed by chi-square analysis. They are given together with their standard deviations. The recombination values were determined using the formulas and tables provided by Allard (1956)
[b] Degrees of freedom
[c] A: Linkage in coupling phase
R: Linkage in repulsion phase
* Significant at the 5% level

divided into 8 components with one degree of freedom, respectively, each representing an orthogonal comparison of phenotypic frequencies. Therefore, progeny showing significant deviations from the expected two-locus segregation pattern but with no disturbances in the single-locus segregations can be investigated. Tests were run to determine whether the deviating two-locus segregations were due either to linkage of the two loci or to selection of gametes or zygotes. Since no evidence was found for selection processes in inbred lines with undisturbed single-locus segregations, the single chi-square components were combined in Table 1. This resulted in two chi-square values for the test of undisturbed single-locus segregation (χ^2-locus A and χ^2-locus B, respectively) and the additive remaining component for the test of linkage (χ^2-joint segregation). When segregation with a null allele occurred at one or both loci, the chi-square analysis was performed using the model of dominance with 6 or 4 phenotypic classes, respectively.

For determination of recombination values and pooling of different data sets (inbred lines) the formulas and tables provided by Allard (1956) were used.

Tests for heterogeneity showed that the inbred lines scored for each locus pair were homogeneous. Thus, only the combined data together with a common recombination value are given in Table 1.

Lines showing slightly disturbed single-locus segregation were considered for linkage analysis only when there was no doubt about linkage between the concerned loci, and when the maximum likelihood estimate of the recombination fraction was not remarkably affected by the disturbance.

Results

The two-locus segregations between pairs of 10 esterase loci *Est 1 – Est 10* and the chi-square analysis for the detection of linkage are listed in Table 1.

In lines segregating for the group of the three loci *Est 5, Est 6,* and *Est 7* and other loci, no recombination was detected between these three loci. Therefore, the recombination frequencies between loci *Est 6* and *Est 7* and all other segregating loci were the same as those found for recombination between *Est 5* and the other loci and thus are not listed separately.

Linkage data for all analyzed pairs of loci support the hypothesis that the ten esterase loci belong to four different linkage groups: 1. *Est 1/Est 2/Est 3/Est 5/Est 6/Est 7*; 2. *Est 4*; 3. *Est 8/Est 9*; 4. *Est 10.*

A remarkable phenomenon is the very close linkage between loci *Est 5, Est 6, Est 7* (Table 1) and between *Est 8* and *Est 9* (0 recombinants in 1,030 individuals). However, there is evidence for two separate loci, *Est 8* and *Est 9*, since in some cases segregation at one of both loci was observed while the other locus was non-segregating, respectively.

Loci *Est 1, Est 2, Est 3, Est 5, Est 6,* and *Est 7* are arranged along the chromosome as shown in Fig. 1.

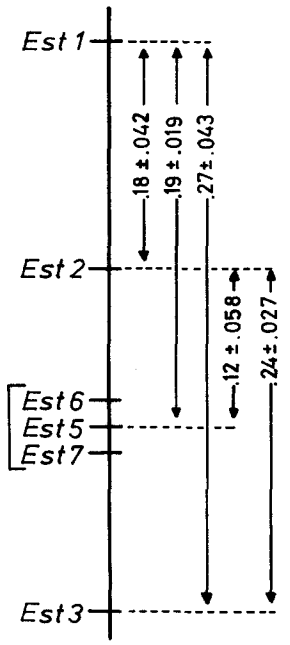

Fig. 1. Possible arrangement of the alkaline esterase loci *Est 1, Est 2, Est 3, Est 5, Est 6,* and *Est 7* along the chromosome. Map distances are directly evaluated as recombination fraction together with standard deviation. Loci *Est 5, Est 6* and *Est 7* are very tightly linked and are therefore marked as a block. Note that the arrangement *Est 6–Est 7–Est 5* is also likely (see text)

However, the low recombination frequency between loci *Est 5, Est 6,* and *Est 7* does not allow a precise arrangement of these three loci. They are thus presented as a block in Fig. 1. Since in the only recombinant individual found between *Est 6* and *Est 7* recombination also occurred between *Est 5* and *Est 6,* arrangements in the order *Est 6–5–7* or *Est 6–7–5* are probable. The order *Est 5–6–7* is unlikely because recombination between both *Est 5–Est 6* and *Est 6–Est 7* would only be possible if double-crossing-over had occurred between *Est 5* and *Est 6* and *Est 6* and *Est 7* simultaneously. This is unlikely to happen because of the close vicinity of the three loci.

Loci *Est 3* and *Est 5* obviously are closely linked with each other (Table 1). However, since recombination data for this gene pair are only based on linkage in the repulsion phase, the true recombination fraction is probably higher than the value obtained on our studies.

Between loci *Est 8* and *Est 9* no confirmed recombination was observed among 1,030 individuals of different inbred lines. The data are not listed in Table 1. Within the complex banding pattern of zone 9 described previously (Schmidt-Stohn and Wehling 1983), only one recombinant between the bands 9/10 and the complex of bands 3, 4, 5 occurred in 1,030 progeny, representing a recombination frequency of 0.08%.

Discussion

The remarkable series of tightly linked loci *Est 3/Est 5/Est 6/Est 7* and *Est 8/Est 9*, respectively, with a maximum recombination rate of 0.2% is of great interest. The only recombination observed within the *Est 9* region hints at the existence of at least two individual genes at the *Est 9* locus. However, the data available are not yet sufficient for further mapping of the *Est 9* region. It seems, however, appropriate to regard the esterases of *Est 8* and *Est 9* as encoded by a compound locus.

A compound locus, probably formed by several tightly linked loci (0 recombinants among 579 testcross progeny), was found in *Stephanomeria exigua* at the Adh_1 locus (Roose and Gottlieb 1980), the composition of which presumably comprises between one to three single genes in different individuals. Two other compound loci, Adh_1 (Schwartz and Endo 1966) and E_5 (Macdonald and Brewbaker 1974) were found in maize. Close linkage was also observed in *Hordeum* for the esterase loci *EA, EB,* and *EC* with 0.23% recombination between *EA* and *EB* and 0.48% recombination between *EA* and *EC* (Kahler and Allard 1970). Similar to the situation in barley and rye, a series of closely linked esterase loci (*Est 1, Est 5, Est 6, Est 7*) within a chromosomal segment of 2 cM was observed in tomato by Tanksley and Rick (1980a).

Rye esterases controlled by loci of the same linkage group, especially those of the *Est 8/Est 9* group, exhibit very similar electrophoretic behaviour, expressed by their only slightly differing isoelectric points. This, and the fact that eight loci out of a total of ten esterase genes in rye can be appointed to two linkage groups with close linkage between the single loci, provides evidence for the duplicate nature of the series of linked esterase loci (Tanksley and Rick 1980a).

Tanksley and Rick suggest a phylogenetic connection on the grounds of similar linkage relationships and electrophoretic mobilities between the esterases in tomato (cited above) and barley (Kahler and Allard 1970). The authors point out that in both species a total of seven esterase loci were observed, four in tomato and three in barley, which encoded for the enzymes with the least electrophoretic mobility and were tightly linked with each other.

According to our studies, which revealed linkage between the six alkaline esterase loci *Est 1, Est 2, Est 3, Est 5, Est 6,* and *Est 7*, with very tight linkage between the latter four loci, the situation in rye resembles that reported for barley and tomato and thus does not

contradict the phylogenetical speculations of Tanksley and Rick.

Linkage analysis of rye esterase loci reveals that at most four of the seven rye chromosomes can be marked by esterase loci. As will be shown in another publication, studies with wheat/rye addition lines provide evidence that at least two linkage groups (*Est 1/Est 2/Est 3/Est 5/Est 6/Est 7*) and (*Est 10*) are located on different chromosomes. Further mapping studies are in progress using various isoenzyme systems.

Enzymic markers in rye could facilitate the identification of single rye chromosomes for the production of certain wheat/rye addition and translocation lines and for taxonomical research.

Cornish et al. (1980) detected linkage between the *Pgi2*-locus and the *S* locus in *Lolium perenne*. If linkage could also be detected between isoenzymic marker loci and those genes controlling self-incompatibility in rye the handling of self-incompatibility in rye breeding could be improved.

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References

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

Cornish MA, Hayward MD, Lawrence MJ (1980) Self-incompatibility in ryegrass III. The joint segregation of *S* and *Pgi-2* in *Lolium perenne* L. Heredity 44:55–62

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

Kahler AL, Allard RW (1970) Genetics of isoenzyme variants in barley I. Esterases. Crop Sci 10:444–448

Macdonald T, Brewbaker JL (1974) Isoenzyme polymorphism in flowering plants IX. The E_5-E_{10} esterase loci of maize. J Hered 65:37–42

Roose ML, Gottlieb LD (1980) Alcohol dehydrogenase in the diploid plant *Stephanomeria exigua* (Compositae): gene duplication, mode of inheritance and linkage. Genetics 95:171–186

Schmidt-Stohn G (1979a) Eine Methode zur isoelektrischen Fokussierung von Proteinen in Flachgelen für Serienuntersuchungen in der Genetik und Züchtungsforschung. Z Pflanzenzücht 83:81–90

Schmidt-Stohn G (1979b) Genetische Analysen von Esterase-Loci beim Roggen (*Secale cereale* L.) mit Hilfe der isoelektrischen Fokussierung in Flachgelen. Z Pflanzenzücht 83: 155–162

Schmidt-Stohn G, Wehling P (1983) Genetic control of esterase isoenzymes in rye (*Secale cereale* L.). Theor Appl Genet 64:109–115

Schwartz D, Endo T (1966) Alcohol dehydrogenase polymorphism in maize, simple and compound loci. Genetics 53:709–715

Tanksley SD, Rick CM (1980a) Genetics of esterases in species of *Lycopersicon*. Theor Appl Genet 56:209–219

Tanksley SD, Rick CM (1980b) Isozymic gene linkage map of the tomato: applications in genetics and breeding. Theor Appl Genet 57:161–170

Wricke G (1969) Untersuchungen zur Vererbung der Selbstfertilität beim Roggen (*Secale cereale*). Theor Appl Genet 39:371–378