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Association of four isozyme loci with a reciprocal translocation between *1R/4R* chromosomes in cultivated rye (*Secale cereale* L.)

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Summary. The progeny of four crosses between a structural heterozygote for a reciprocal translocation and a homozygote for the standard chromosome arrangement were analyzed in rye (*Secale cereale* L. cv "Ailés") for the electrophoretic patterns of eight different leaf and endosperm isozymes and also for the meiotic configuration at metaphase I. The *Pgi-1, 6-Pgd-2* and *Mdh-1* loci are linked to each other and also to the reciprocal translocation. These loci have been located on chromosome *1R*. The *Mdh-1* locus is located in the interstitial segment of chromosome *1R*, between the centromere and the breakpoint. The *Pgm-1* locus has been located on chromosome arm *4RS* and is linked to *Pgi-1, 6-Pgd-2, Mdh-1* and the reciprocal translocation. The estimated distance between the *Pgm-1* locus and the centromere is 14.98 ± 2.27 cM. Therefore, the reciprocal translocation involves the *1R* and *4R* chromosomes. Other linked loci detected have been *Mdh-2b* and *Est-2* (7.40 ± 2.90 cM) and *Got-3* and *Est-2* (5.62 ± 3.07 cM). These three last loci are located on chromosome *3R* and their order most probably is *Mdh-2b – Est-2 – Got-3*.

Key words: Translocation – Isozymes – Rye – Cytogenetic maps

Introduction

Several rye cultivars showing polymorphisms for reciprocal translocations have been observed. In these cultivars, the structural heterozygote frequencies occur between 1.4% and 4.7% (Figueiras et al. 1983). In the case of the cultivar Ailés this frequency is surprisingly high (15%–20%) and shows many different reciprocal translocations (Candela et al. 1979).

Several isozymes markers have been located in rye chromosome arms (Barber et al. 1968; Bergman and Maan 1973; Tang and Hart 1975; Hart 1979; Rao and Rao 1980; Chojecki and Gale 1982; Salinas and Benito 1984a, b; 1985a, b; Lawrence and Appels 1986; Koebner 1987; Chenicek and Hart 1987). However, genetic and cytogenetic maps for isozyme markers and translocations are poorly developed. Linkage maps for isozyme markers have only been reported in rye for esterases (Wehling and Schmidt-Stohn 1984; Wehling et al. 1985), peroxidases (García et al. 1982; Rebordinos and Pérez de la Vega 1987), 6-phosphogluconate dehydrogenase and glucose phosphate isomerase (Lawrence and Appels 1986), malate dehydrogenase and glutamate oxalacetate transaminase (Figueiras et al. 1985), malate dehydrogenase and 6-phosphogluconate dehydrogenase (Figueiras et al. 1985) and endosperm alkaline phosphatases (Figueiras et al. 1987).

Cytogenetic maps with translocations and morphological markers have been reported by De Vries and Sybenga (1984) and with transloctions and isozyme markers by Figueiras et al. (1985).

The genic control, the monomeric or dimeric behaviour and the isozymic patterns of the different isozymes analyzed in this work have already been described by Pérez de la Vega and Allard (1984), Vaquero et al. (1982) and Figueiras et al. (1985).

In the present work, we have tried to relate a particular structural translocation with specific isozymes in order to identify the different chromosomes involved in the reciprocal translocation, to obtain genetic and cytogenetic maps, and to elucidate the possible relevance of such associations in the maintenance of chromosomal polymorphisms for reciprocal translocations in natural plant populations.

Materials and methods

In the *Secale cereale* L. cv Ailés (polymorphic for reciprocal translocations), the offsprings of four different crosses (F-1, F-2,

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F-3 and F-4) between a heterozygous plant for a reciprocal translocation (HT, 1IV ± 5II) and another plant of the same cultivar, homozygous for the standard chromosome arrangement (HM, 7II), were analyzed. Both the chromosome constitution and the isozyme patterns were analyzed in the progeny of these crosses.

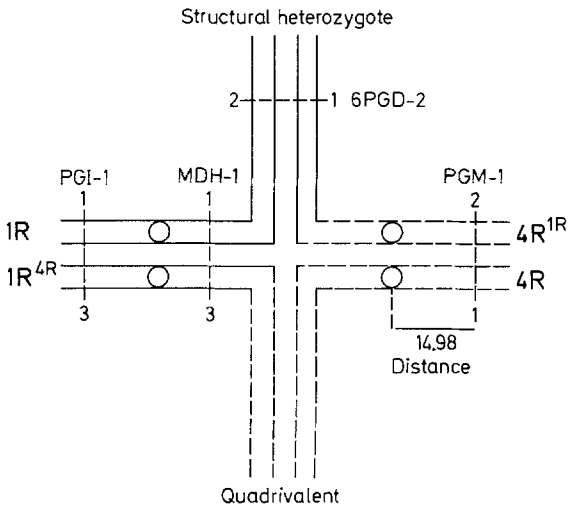
The cytological study was made on the pollen mothers cells (PMCs) at metaphase I (MI) following the aceto-orcein stain procedure. Those plants showing 1IV + 5II were classified as structural heterozygotes for the reciprocal translocation and the plants showing 7II were classified as structural homozygotes. To analyze whether the nucleolar organizer chromosome (*1R*) was involved in the interchange of the structural heterozygote, PMCs at diplotene-diakinesis were stained with ammonium-ferric-sulfate and hematoxylin, in order to make the nucleolus visible and ascertain whether or not it was associated with the quadrivalent.

The seed set from the four different progenies were allowed to germinate. The biochemical analyses were carried out with the 12-day-old leaves and with a part of the dry endosperms. The isozymic systems were studied following the protocols described in Figueiras et al. (1985) and were: glutamate oxalacetate transaminase (GOT), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), esterase (EST), malate dehydrogenase (MDH), 6-phosphogluconate dehygrogenase (6-PGD), endosperm alkaline phosphatases (ALK), and endosperm cathodal peroxidases (PER).

Results

The hematoxylin analysis of PMCs at the diplotene-diakinesis stage reveals that the nucleolus is associated with the quadrivalent observed in the structural heterozygotes (HT) obtained in the four crosses HT × HM (Fig. 1). Therefore, the nucleolar organizer chromosome (*1R*) would be implicated in the translocation. In the four crosses studied, the segregation observed for the quadrivalent at MI was the expected 1:1 (1IV + 5II:7II).

The segregation for the individual isozymic loci was as expected in the four crosses analyzed. In Table 1, two-point linkage tests for the isozymic loci in the HT × HM crosses are summarized; only those loci showing linkage relationships are present. In Table 2, the loci having linkage with reciprocal translocation are shown. It can be seen that *Pgm-1* and *Pgi-1*, *Pgm-1* and *6-Pgd-2*, *Pgm-1* and *Mdh-1* and *6-Pgd-2* and *Mdh-1* loci were linked. The *Got-3* and *Est-2* and *Mdh-2b* and *Est-2* loci were also linked. The *Mdh-1* locus was located on chromosome *1R*, the *Pgi-1* and *6-Pgd-2* loci were located on chromosome arms *1RS* and *1RL*, respectively, the *Mdh-2b*, *Got-3* and *Est-2* loci were located on chromosome *3R*, and the *Pgm-1* locus was located on chromosome arm *4RS* (see introduction for the references). On the other hand, the loci *Pgi-1*, *Mdh-1*, *6-Pgd-2* and *Pgm-1* were associated with the translocation (Table 2).



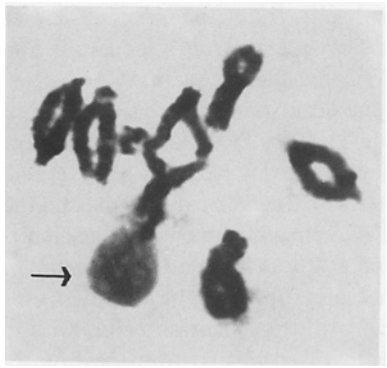


Fig. 1

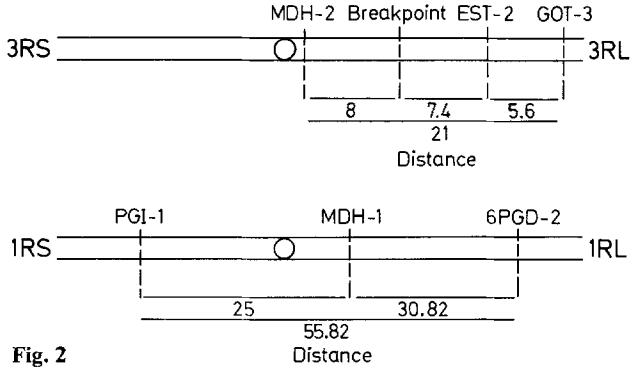


Fig. 2

Fig. 1. PMC at diplotene-diakinesis with hematoxylin of the structural heterozygote (HT) used in the cross F-1. *Arrow* indicates the nucleolus associated with the quadrivalent

Fig. 2. Diagrammatic representation of the quadrivalent observed in the structural heterozygotes used in the crosses F-1, F-2, F-3 and F-4. The *1R* and *4R* chromosomes are involved in the translocation. The isozymic alleles of *PGM-1*, *PGI-1*, *MDH-1* and *6-PGD-2* have been named 1, 2 or 3. Also the genetic distances calculated between *Pgm-1* and the centromere on the *4R* chromosome, among *Pgi-1*, *Mdh-1*, *6-Pgd-2* on the *1R* chromosome and among *Mdh-2b*, breakpoint, *Est-2* and *Got-3* on the *3RL* chromosome are indicated

Table 1. Linkage analyses between two isozymic loci in the crosses F-1, F-2, F-3 and F-4

Loci	Cross	Parental genotypes	Distribution of progeny (phenotype)					1:1:1:1	χ^2 3:1:3:1	1:2:1 1:2:1	χ^2 linkage	Distance (cM)
			11 +	11 −	12 +	12 −						
Got-3, Est-2	F-3	(11×12), (±×±)	14	15	22	1			17.64[b]		16.03[b]	5.62±3.07
Mdh-2b, Est-2	F-3	(12×11), (±×±)	14	16	22	0			21.74[b]		19.52[b]	0.00±0.00
			11 11	11 12	12 11	12 12						
Pgm-1, 6-Pgd-2	F-3	(12×11), (12×11)	6	13	25	8		16.76[b]			11.07[b]	26.92±6.15
			11 11	11 13	12 11	12 13						
Pgm-1, Mdh-1	F-3	(12×11), (13×11)	0	19	29	4		41.69[b]			37.23[b]	7.60±3.69
6-Pgd-2, Mdh-1	F-3	(12×11), (13×11)	24	7	5	16		16.46[b]			13.85[b]	23.07±5.84
Pgm-1, Pgi-1	F-2	(12×11), (13×11)	54	24	28	50		17.74[b]			17.33[b]	33.33±3.77
			1111	1112	1211	1212	1222					
Pgm-1, Pgi-1	F-3	(12×11), (12×12)	1	8	8	20	5			12.92[a]	7.46	

[a] $p<0.05$
[b] $p<0.001$

Discussion

Our results are in agreement with the previous data about chromosome localization of the isozymes analyzed in the present work. The distances between the isozymic loci were estimated by means of the maximum likelihood method.

Our data reveal that the *Got-3* and *Est-2* loci are linked (5.62±3.07 cM), as well as the *Mdh-2b* and *Est-2* loci (0.00 cM) in the cross F-3 (Table 1). These results fit very well with the chromosomal location data indicated by Figueiras et al. 1985. The distance observed between *Got-3* and *Mdh-2b* loci in rye is 21.00±2.00 cM (Figueiras et al. 1985). In addition, other data in the Ailés cultivar indicated that the estimated distance between *Mdh-2b* and *Est-2* loci is at least 7.40±2.90 cM (unpublished). Therefore, the most probable order for these loci is *Mdh-2b – Est-2 – Got-3*. These three loci are located on *3R* chromosome and, in hexaploid wheat, are located on the long arm of homoeologous group 3; thus, the most probable location in rye for these three loci is chromosome arm *3RL*.

Previous data (Figueiras et al. 1985) indicated that the *Mdh-2b* locus is totally linked to a translocation between *1R* and *3R* chromosomes, and is probably located in the interstitial segment between the centromere and the breakpoint. The *Got-3* locus is also partially linked to this translocation (Figueiras et al. 1985).

All these results indicated that the *Mdh-2b* locus is near the centromere and that the order in the *3RL* chromosome arm is: centromere – *Mdh-2b – Est-2 – Got-3*.

From the cross F-2, the obtained distance between *Pgm-1* and *Pgi-1* was 33.33±3.77 cM (Table 1). In the cross F-3, the χ^2 linkage for these loci is not significant (the segregation of the *Pgm-1* locus is on the limit, $\chi^2=3.77$) but when the distance is calculated, the value obtained is 25.00±4.24 cM. The obtained distance between *Pgm-1* and *6-Pgd-2* from the cross F-3 was 26.92±6.15 cM. The *Pgm-1* and *Mdh-1* loci were also linked (7.69±3.69 cM) and this is again the case for *6-Pgd-2* and *Mdh-1*, linked at an estimated distance of 23.07±5.84 cM (Table 1). The *Pgi-1* and *6-Pgd-2* loci were located on chromosome arms *1RS* and *1RL*, respectively. The *Mdh-1* locus is on the *1R* chromosome and the *Pgm-1* locus is on the *4RS* chromosome arm. The results obtained here indicated that loci known to be located in different chromosomes are shown to be linked in our material due to a reciprocal translocation being *1R* and *4R*, the chromosomes involved in that translocation.

In the crosses F-1, F-2, F-3 and F-4, the distances obtained between the *Pgm-1* locus and the reciprocal translocation (breakpoint) were 17.28±4.20 cM, 18.18 ±0.17 cM, 7.69±3.69 cM and 11.53±6.26 cM, respectively (Table 2). The data from these progenies can be pooled, since the heterogeneity test was not significant at

Table 2. Linkage analyses between two isozymic loci with the reciprocal translocation (7 II or 1 IV + 5 II) in four different crosses (F-1, F-2, F-3 and F-4)

Loci	Cross	Parental genotypes	Meiotic configuration (phenotype)				χ^2 1:1:1:1	χ^2 linkage	Distance (cM)
			7 II		1 IV + 5 II				
			11	12	11	12			
Pgm-1	F-1	(12 × 11)	30	7	7	37	38.25[b]	37.34[b]	17.28 ± 4.20
Pgm-1	F-2	(12 × 11)	35	10	6	37	36.09[b]	36.05[b]	18.18 ± 0.17
Pgm-1	F-3	(12 × 11)	19	4	0	29	41.69[b]	37.23[b]	7.69 ± 3.69
Pgm-1	F-4	(12 × 11)	11	1	2	12	15.53[a]	15.00[b]	11.53 ± 6.26
6-Pgd-2	F-3	(12 × 11)	7	16	24	5	17.69[b]	15.08[b]	23.07 ± 5.84
			12	22	12	22			
6-Pgd-2	F-1	(12 × 22)	14	23	29	15	7.44		
			11	13	11	13			
Pgi-1	F-2	(13 × 11)	35	10	12	31	22.45[b]	22.00[b]	25.00 ± 4.61
Mdh-1	F-3	(13 × 11)	0	23	29	0	53.38[b]	52.00[b]	0.00 ± 0.00

[a] $p < 0.01$
[b] $p < 0.001$

the 5% level. With the pooled data, the estimated distance was 14.98 ± 2.27 cM. The estimated distance between the *Pgi-1* locus and the reciprocal translocation was 25.00 ± 4.61 cM in F-2 (Table 2). In the cross F-1, although the χ^2 for independence between the *6-Pgd-2* locus and the translocation is not significant (7.44), if the χ^2 linkage is calculated with the same data the resulting value is significant (6.53), that being the estimated distance 35.80 ± 2.83 cM. This distance was 23.07 ± 5.84 cM in F-3 (Table 2). The data from these progenies (F-1 and F-3) can be pooled, since the heterogeneity test was not significant at the 5% level and the estimated distance (with the pooled data) between the *6-Pgd-2* locus and the breakpoint was 30.82 ± 4.00 cM. The *Mdh-1* locus appears to be totally linked to the translocation, as can be inferred by the absence of recombinant plants (F-3) (Table 2). The distances between *6-Pdg-2* and *Mdh-1*, *Pgm-1* and *Mdh-1* were 23.07 ± 5.84 and 7.69 ± 3.69 cM, respectively (F-3) (Table 1). Exactly the same values were observed when the linkage relationships between *6-Pgd-2* and the reciprocal translocation, and *Pgm-1* and the reciprocal translocation were analyzed. Therefore, the results obtained indicated that the *Pgi-1*, *6-Pgd-2*, *Mdh-1* (located in chromosome *1R*) and *Pgm-1* (located in chromosome *4R*) loci are linked to the translocation. These linkage relationships between isozymic loci conform very well with the cytogenetic data on the reciprocal translocation, indicating that the chromosomes involved in this translocation are *1R* and *4R*.

The results obtained suggest that the most probable order for the loci located on chromosome *1R* is *Pgi-1* – centromere – *Mdh-1* – *6-Pdg-2*. This fits very well with previous data about linkage relationships between the loci located on chromosome *1R*. The estimated distance between the *Mdh-1* and *6-Pdg-2* loci in cultivar Ailés without translocation was 16.26 ± 3.32 cM (Figueiras et al. 1985), and the distance obtained by Lawrence and Appels (1986) between the *Pgi-1* and *6-Pgd-2* loci was 22.30 cM. The *Pgi-1* and *6-Pgd-2* loci show independent behaviour, since the distance between the *Pgi-1* and *Mdh-1* loci and between the *6-Pdg-2* and *Mdh-1* loci were 25.00 cM and 30.82 cM, respectively. Our estimated distance results are greater than others previously described. The reason for this might be a translocation involving chromosome *1R*. When the distance between the *6-Pgd-2* and *Pgm-1* loci is calculated, the obtained value is 45.11 ± 4.31 cM. The obtained partial distances *Pgm-1* – breakpoint and the breakpoint – *6-Pgd-2* are 13.53 cM and 30.82 cM, respectively, in the crosses F-1 and F-3 (Table 2). Therefore, there is a strong positive interference, calculated as 1-c, c being the coincident coefficient (0.09 in our case).

The rye cultivar Ailés is highly polymorphic for reciprocal translocations. Several different translocations have been observed in this cultivar (Candela et al. 1979); in two of them, involving chromosomes *4RL/5RL* and *4RL/5RS*, it has been possible to identify the chromosome arm translocated (*4RL*). The estimated distances between the *Pgm-1* locus and the translocation were 13.63 cM and 14.63 cM, respectively (unpublished data). These distances are similar and agree with the distance obtained in this work (14.57 cM). The *Pgm-1* locus is located on the *4RS* chromosome arm and probably the *4RL* chromosome arm is translocated in our case. There-

fore, the estimated distance between the *Pgm-1* locus and the translocation is in fact the distance between the *Pgm-1* locus and the centromere.

The positive interference between the *Pgm-1* locus (*4RS* chromosome arm, non-translocated arm) and the *6-Pgd-2* locus (*1RL* chromosome arm) suggests that the *1RL* chromosome arm could be the translocated arm, because there is a negative interference between opposite arms and a positive one between adjacent arms (Sybenga and Mastenbroek 1980). Therefore, the chromosome arms *1RL* and *4RL* could probably be those involved in the reciprocal translocation (Fig. 2).

It is very well known that reciprocal translocations have been relevant for the evolution of genus *Secale*, *4RL* and *7RS* being the chromosome arms very often involved. The fact that in our case, the *4RL* chromosome arm is again involved in at least three different translocations indicates that this chromosome arm is very unstable in structural rearrangements. On the other hand, the chromosome *1R* is present with a normal frequency in the arrangements of the Ailés cultivar.

The alleles located in the translocated chromosomes are 2 for *Pgm-1* locus, 2 or possibly 3 for *Pgi-1* locus, 3 for *Mdh-1* locus and 1 for *6-Pgd-2* locus. The most frequent allele in the Ailés cultivar is always allele 1 in all the loci. The Ailés cultivar showed greater genic heterozygosity than other cultivars without interchanges (Figueiras et al. 1988). If the genic heterozygosity for certain loci had a selective advantage, the structural heterozygetes would be favoured by natural selection. If the alleles with a low frequency are located (totally or partially linked) on the translocated chromosomes, this could explain the correlation between the structural and genic heterozygosities found, and also the maintenance of the chromosomal polymorphism for reciprocal translocation.

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