Theor Appl Genet (1984) 67:457–461

Chiasma distribution in rye chromosomes of diploid rye and in wheat/rye addition lines in relation to C-heterochromatin *

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Received July 22, 1983
Communicated by R. Riley

Summary. In five genetically different inbred lines of rye and in the seven 'Chinese Spring'/'Imperial' wheat-rye addition lines, chiasma distribution in rye chromosomes was studied with respect to the amount and position of constitutive heterochromatin (Giemsa C-bands). In all inbred lines, rye chromosomes with one primary terminal band were more frequently found as univalents than those with primary bands on both telomeres. These chromosomes were most probably 5R and/or 6R. In the addition lines a highly significant reduction in the number of arms bound by chiasmata was found for rye chromosomes 5R and 6R. Because of the similar chiasma distribution in the inbred lines and in the rye chromosomes of the addition lines, no effect of the wheat genome on the number of chiasmata in the rye chromosomes can be ascertained. However, a relationship between chiasma frequency and chromosome arm length seems to exist, since under reduced chiasma conditions the two shortest arms of the rye complement, those of chromosomes 5R and 6R, frequently fail to form a chiasma. No effect of the large blocks of constitutive heterochromatin in the telomeres of the rye chromosomes on the position of chiasmata within a bivalent could be established.

Key words: Chiasmata – C-heterochromatin – Rye – Wheat-rye addition lines

Introduction

In triticale, univalents often occur at first meiotic metaphase. It has been long suspected that chromosomes which fail to pair usually originate from the rye genome (Müntzing 1979).

Unequivocal evidence for this assumption was provided by the use of the C-banding technique (Thomas and Kaltsikes 1974, 1976; Lelley 1975, 1981). This enabled rye chromosomes having large blocks of terminally located constitutive heterochromatin to be easily distinguished from wheat chromosomes in which there is much less C-heterochromatin, situated mostly in intercalary position.

DNA replication in rye telomeres is started and completed later than in the euchromatic parts of the chromosomes (Lima-de-Faria and Jaworska 1972). Since duration of meiosis was found to last much longer in diploid rye (51 h) than in hexaploid triticale (34 to 37 h) it has been suggested that meiotic disturbances of rye chromosomes in triticale are caused by the overlapping of DNA replication in the C-heterochromatic regions with the beginning of meiosis, i.e., the synapsis of homologous chromosomes (Bennett 1973; Bennett and Kaltsikes 1973; Thomas and Kaltsikes 1974). This is important in view of the proposal that selection for a reduced amount of heterochromatin will improve nuclear stability in early endosperm development and so give rise to increased kernel weight and yield in triticale (Bennett 1977; Bennett and Gustafson 1982).

The constitutive heterochromatin in rye chromosomes is not evenly distributed. Therefore, a study of the meiotic behaviour of individual rye chromosomes differing in heterochromatin content and distribution could provide useful additional information on the influence of C-heterochromatin on the meiotic behaviour of these chromosomes. Unfortunately, identification of individual rye chromosomes by C-bands is difficult in meiosis since secondary and tertiary bands which are crucial for somatic differentiation are seldom visible in meiotic chromosomes. One exception is chromosome 1R, the meiotic identification of which is possible by the regular occurrence of a second primary band on the nucleolus organizing arm.

Unambiguous identification of single rye chromosomes by the Giemsa technique is possible, however, in wheat-rye addition lines. Several such series using different wheat and rye varieties have been developed

* This study was financially supported by the Deutsche Forschungsgemeinschaft

in the last 40 years. The purpose of this study was to analyse the pairing behaviour of diploid rye and the single pairs of rye chromosomes in a series of wheat-rye addition lines in relation to the amount and position of constitutive heterochromatin.

Materials and methods

Five highly inbred lines of rye with varying average chiasma frequencies were used to study the number of univalents with different C-banding patterns. These lines have been investigated thoroughly in previous cytological and karyomorphological studies by Lelley (1978) and Lelley et al. (1978).

In order to analyse the individual rye chromosomes in a hexaploid wheat background, the seven 'Chinese Spring'/'Imperial' wheat-rye addition lines were used. This series has been adopted by an international workshop on rye chromosomes held in March, 1982, in Wageningen, the Netherlands, as a standard for the rye chromosome set (Sybenga 1983).

Plants were grown in the greenhouse. For the determination of chromosome numbers in root tip mitosis, the conventional Feulgen-staining technique was applied. For differential staining of chromosomes in mitosis as well as in meiosis, the Giemsa-staining procedure (C-banding) was used as described by Gustafson et al. (1976). Giemsa bands, referred to as primary or major, are invariably terminal ones, except in the case of chromosome 1R as described earlier. Bands called secondary and tertiary are small in comparison to primary bands and usually occur in intercalary positions with the exception of the telomeres of chromosomes 4R and 6R. For analysing meiosis, only pollen mother cells (PMC's) were selected in which the pairing configuration for all chromosomes was clearly discernible.

Since it is often uncertain whether one or two chiasmata are present in a paired chromosome arm, bound chromosome arms were counted rather than chiasmata. Accordingly, a rod bivalent has one bound arm, a ring bivalent two.

The number of PMC's in which a clear determination of number and position of chiasmata is possible is rather low in Giemsa stained preparations in comparison to conventionally stained PMC's. Therefore, numbers of PMC's analysed per plant and per anther are different. But the number of plants was always higher than three except for line 5R in which case only two plants were studied.

Results

Pairing pattern of homologous chromosomes in diploid rye

Since individual rye chromosomes can be identified at meiosis only in rare cases, observation was concentrated only on univalents with detectable Giemsa bands at one or both of the telomeres.

Previous karyotyping of the material (Lelley et al. 1978) showed that in all lines except for 3c, chromosomes 5R and 6R possessed only one primary Giemsa band, located always at the end of the short arm. Secondary and tertiary bands recorded in the long arm of these two chromosomes are rarely visible in meiotic configurations. In all other chromosomes, telomeric heterochromatin exists, although in different quantities, but clearly visible at both ends of the meiotic chromosomes. In line 3c, chromosome 4R, as well as chromosomes 5R and 6R, showed one primary terminal band on the short arm telomere. In the standardized karyotype of *Secale cereale*, adopted by the international workshop on rye chromosomes (Sybenga 1983), these three chromosomes have major terminal Giemsa bands only at the short arm telomeres.

Table 1. Per cent of unpaired chromosomes with terminal constitutive heterochromatic blocks at one and at both of their telomeres in five genetically different inbred lines of rye

Lines	Average chiasma frequency	No. of PMC with univalents	Unpaired chromosomes with heterochromatin at	
			one end	both ends
2a	10.9	34	7.1	8.8
1a	10.4	35	6.1	10.6
5e	9.7	131	5.5	12.0
3c	9.5	55	5.2	13.5
4b	9.0	140	7.7	11.0

As one would expect, a clear increase in pairs of univalents was found as average chiasma frequency decreased in the different inbred lines (Table 1). However, the number of pairs of univalents with only one terminal Giemsa band exceeded those with Giemsa bands at both telomeres at all levels of chiasma frequency, indicating that one of the chromosomes 4R, 5R or 6R, or probably all three of them, were represented disproportionately often in the class of univalents.

Pairing pattern of the single pair of homologous rye chromosones in the seven wheat-rye addition lines

Overall, the frequency with which rye chromosomes occurred as univalents was very low. While the average chiasma frequency in chromosomes 1R, 2R, 3R and 7R did not differ significantly, a sharp drop occurred for chromosomes 5R and 6R (Tables 2 and 3), the difference between 4R and 7R being also significant.

As already mentioned, chromosomes 4R, 5R and 6R differ from the rest of the complement by possessing only one primary Giemsa band at the short arm telomere. Chromosomes 5R and 6R also exhibit the largest arm length difference to give a ratio of approximately 1 : 2. Chromosome 4R occupies, in this respect, an intermediate position between 5R, 6R and those

Table 2. Average number of bound arms of rye chromosomes and percentage of rye univalents in the seven wheat-rye addition lines

Addition lines	PMC's	Bound arms of rye bivalents	Rye univalents %
1	348	1.72	2.8
2	358	1.66	0.8
3	236	1.77	2.9
4	215	1.63	1.8
5	115	1.35	0.8
6	254	1.16	1.1
7	116	1.84	1.7

Table 3. Difference in average frequency of bound arms of rye chromosomes in the seven addition lines. Statistical significance ($P = 1\%$) revealed by a Scheffé-test is underlined

	2	3	4	5	6	7
1	0.06	0.05	0.09	0.37	0.56	0.12
2		0.11	0.03	0.31	0.50	0.18
3			0.14	0.42	0.61	0.07
4				0.28	0.47	0.21
5					0.19	0.49
6						0.68

chromosomes of the rye genome which have close to median centromere positions, i.e. 2R and 3R.

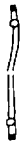
Based on the number and position of chiasmata in relation to telomeric Giemsa bands, rye bivalents were classified into eight categories (Table 4). Such differentiation was possible since in all chromosomes, primary bands, when present at both telomeres, were different in size. In the diagrams of Table 4 only the larger telomeric bands are indicated. Because a clear distinction between terminal and subterminal chiasmata is rarely possible, both were termed distal.

The most frequent type of chiasma distribution on chromosome 1R with three primary C-bands was one distal chiasma on the short arm and one intercalary chiasma on the long arm (Category 2 in Table 4).

In the case of chromosomes 2R and 3R the most common configuration was a ring bivalent with a distal chiasma on the arm with less heterochromatin and with an intercalary chiasma on the arm with the large terminal heterochromatic segment. These two chromosomes have very similar arm ratios and they are comparable with respect to the sizes of their two terminal C-bands.

Chromosomes 4R, 5R and 6R appeared most frequently in category 5, i.e. with one chiasma located mostly on the long arm in intercalary position.

Table. 4. Number and distribution of chiasmata within a bivalent of the seven rye chromosomes of the 'Chinese Spring'/'Imperial' addition series

Addition line	PMC's	Ring bivalents (%)	Category[a] 1	2	3	4	Rod bivalents (%)	Category 5	6	7	8
											
1[b]	348	75.0	4.2	92.3	0.7	2.6	22.1	7.7	35.1	57.1	–
2	358	66.2	10.5	78.4	8.0	3.0	32.9	13.5	82.2	4.2	–
3	236	80.0	2.1	84.1	13.7	–	16.9	17.5	35.0	47.5	–
4	215	64.6	2.8	1.4	31.6	64.0	33.4	63.8	18.4	9.7	6.9
5	115	35.6	21.9	2.4	34.1	41.4	63.4	82.1	16.4	–	1.3
6	254	16.9	4.6	11.6	53.4	30.2	81.8	83.6	16.3	–	–
7	116	85.3	28.2	61.6	10.1	–	12.9	26.6	40.0	20.0	13.3

[a] Category 1: Two chiasmata which are intercalary on both arms (ring bivalent)
2: Chiasma is intercalary on the arm with the large band and distal on the arm with the smaller band
3: Chiasmata are distal on both arms
4: Chiasma is intercalary on the arms with the smaller band and distal on the arm with the larger band
5: Only one chiasma which is intercalary on the arm with the smaller band (rod bivalent)
6: Chiasma is distal on the arm with the smaller band
7: Chiasma is intercalary on the arm with the larger band
8: Chiasma is distal on the arm with the larger band

[b] The banded telomere represents the long arm of this chromosome

The distribution of chiasma on chromosome 7R was similar to that of chromosomes 2R and 3R where a distal chiasma in the arm with less heterochromatin regularly occurred while in the arm with the primary Giemsa band the chiasma was mostly intercalary.

Discussion

It has been previously suggested that in diploid rye all seven pairs of homologous chromosomes have similar chiasma frequencies (Schlegel and Friedrich 1975; Giraldez and Lacadena 1978).

Our study of five genetically diverse rye inbred lines by means of Giemsa staining, however, revealed that under conditions of reduced chiasma frequency, chromosomes with only one terminal Giemsa band occur more frequently as univalents than chromosomes with Giemsa bands at both telomeres (Table 1). Since in four of the lines only two chromosomes, 5R and 6R, exist with one major terminal Giemsa band, it appears that in this material a differential behaviour between individual chromosomes of the rye complement is present with regard to chiasma formation.

The fact that chiasmata were more often absent in chromosome arms without large blocks of heterochromatin than with heterochromatin suggests that, in diploid rye, in spite of a remarkable amount of C-heterochromatin at most of the telomeres, no significant connection between telomeric C-heterochromatin and chiasma formation exists.

A similar conclusion was reached by Naranjo and Lacadena (1980) after studying the meiotic behaviour of chromosome 1R in diploid rye. This assumption is also supported by the observation that F_1-hybrids between inbred lines invariably display a highly significant increase in chiasma frequency. In the F_2-generations, a clear segregation for this character can be observed (Rees 1955).

When, however, single chromosomes of rye are added to wheat, interactions may occur between the wheat genotype and the larger telomeric heterochromatic blocks of the rye chromosomes (Merker 1967; Bennett 1977). In derivatives of a second backcross generation between a hexaploid triticale and a cultivated variety of diploid rye, Naranjo and Lacadena (1980) observed a significant decrease of homologous pairing for chromosome 1R. The authors suggest that this was due to the presence of the wheat chromosomes in which case "the telomeric heterochromatin of rye plays an important role" in the pairing reduction at least in 1R. But this is clearly not the case for the wheat-rye addition lines studied here.

The average chiasma number in chromsome 1R with three primary Giemsa bands does not differ significantly from that in chromosome 7R with only one major Giemsa band which has the highest average chiasma frequency (Tables 2 and 3).

A relationship can, however, be observed between arm length and chiasma frequency, since the two shortest chromosome arms of 5R and of 6R showed the greatest reduction in chiasma frequency. In this respect the behaviour of chromosomes in the addition lines and in the inbred lines appears to be similar.

In several grasshopper genera, as well as in *Drosophila*, it has been found that the appearance of a large telomeric heterochromatic segment leads to a marked internal redistribution of chiasmata within the bivalents. Whether in heterozygous or in homozygous condition, the chiasmata invariably move away from the heterochromatic segment (for lit. review see John and Miklos 1979).

No such effect of the terminal heterochromatin blocks in the rye chromosomes on the positional distribution of chiasmata could be observed in the seven addition lines. The general tendency was that in long chromosome arms intercalary chiasmata were most frequent irrespective of the presence or absence of a C-band at the end of these chromosome arms, whereas in the short arm, i.e. 1R, 4R, 5R and 6R, chiasmata occurred mostly in terminal or subterminal positions.

In conclusion, the results of this study strongly support the assumption that in cultivated rye the genetic mechanisms regulating the number and position of chiasmata act independently from the large blocks of constitutive heterochromatin in the telomeres both in diploid rye and in wheat-rye addition lines.

Acknowledgements. We wish to thank Mrs. Nina Hoffmann for her excellent technical assistance and Prof. Dr. Dr. h.c. G. Röbbelen for critically reading the manuscript.

References

Bennett MD (1973) Meiotic, gametophytic and early endosperm development in Triticale. In: MayIntyre R, Campbell M (eds) Triticale. IDRC, Ottawa, pp 137–148

Bennett MD (1977) Heterochromatin, aberrant endosperm nuclei and grain shrivelling in wheat-rye genotypes. Heredity 39:411–419

Bennett MD, Kaltsikes PJ (1973) The duration of meiosis in a diploid rye, a tetraploid wheat and the hexaploid triticale derived from them. Can J Genet Cytol 15:671–694

Bennett MD, Gustafson JP (1982) The effect of telomeric heterochromatin from *Secale cereale* on triticale (×Triticosecale). 2. The presence or absence of blocks of heterochromatin in isogenic backgrounds. Can J Genet Cytol 24:93–100

Giraldez R, Lacadena JR (1978) Relationships between frequency, localization and errors in chiasma formation in desynaptic rye. Chromosoma 66:193–204

Gustafson JP, Evans LE, Josifek K (1976) Identification of chromosomes in *Secale montanum* and individual *S. montanum* additions to 'Kharkov' wheat by heterochromatin bands and chromosome morphology. Can J Genet Cytol 18:339–343

John B, Miklos GLG (1979) Functional aspects of satellite DNA and heterochromatin. Int Rev Cytol 58:1–114

Lelley T (1975) Identification of univalents and rod bivalents in triticale with Giemsa. Z Pflanzenzücht 75:252–256

Lelley T (1978) Genetic control of chiasma frequency and distribution in rye, *Secale cereale*. Can J Genet Cytol 20: 471–474

Lelley T (1981) Meiotic behaviour of the rye genome in triticale. In: Induced variability in plant breeding. Int Symp Sec Mutat Polyploidy EUCARPIA. PUDOC, Wageningen, pp 101–105

Lelley T, Josifek K, Kaltsikes PJ (1978) Polymorphism in the Giemsa C-banding pattern of rye chromosomes. Can J Genet Cytol 20:307–312

Lima-de-Faria A, Javorska H (1972) The relationship between chromosome size gradient and the sequence of DNA replication in rye. Hereditas 70:39–58

Merker A (1976) The cytogenetic effect of heterochromatin in hexaploid triticale. Hereditas 83:215–222

Müntzing A (1979) Triticale, results and problems. Fortschritte der Pflanzenzüchtung. Z Pflanzenzücht (Suppl) 10:1–103

Naranjo T, Lacadena JR (1980) Interaction between wheat chromosomes and rye telomeric heterochromatin on meiotic pairing of chromosome pair 1R of rye in wheat-rye derivatives. Chromosoma 81:249–261

Rees H (1955) Heterosis in chromosome behaviour. Proc R Soc London, Ser B 144:140–159

Schlegel J, Friedrich I (1975) Erste Untersuchungen zum meiotischen Paarungsverhalten Giemsa-markierter Chromosomen des diploiden Roggens (*Secale cereale* L.). Biol Rundsch 13:178–179

Sybenga J (1983) Rye chromosome nomenclature and homoeology relationships. Workshop Rep, Z Pflanzenzücht (in press)

Thomas J, Kaltsikes PJ (1974) A possible effect of heterochromatin on chromosome pairing. Proc Natl Acad Sci USA 71:2787–2790

Thomas J, Kaltsikes PJ (1976) The genomic origin of unpaired chromosomes in triticale. Can J Genet Cytol 18:687–700