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C-Banding Pattern and Meiotic Pairing in Five Rye Chromosomes of Hexaploid Triticale

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Summary. The meiotic behaviour of rye chromosomes 1R, 2R, 3R, 6R and 7R/4R of hexaploid triticale 'Cachirulo' is analyzed using the C-banding technique. These chromosomes show different C-banding patterns and present different pairing levels at metaphase I. A decreasing effect of large telomeric heterochromatin bands on pairing is deduced from the following two main facts: i) The chromosome 7R/4R shows the highest pairing associated with the smallest amount of heterochromatin, ii) pairing levels of 2R short arm and 3R long arm which carry large telomeric bands are less than their respective long and short arms lacking telomeric heterochromatin. Possible desynaptic effects of heterochromatin are discussed although an asynaptic effect cannot be rejected.

Key words: Heterochromatin – Pairing – Rye – Triticale

Introduction

First metaphase is one of the standard stages used to analyze meiotic irregularites in triticale. The origin and causes of univalents observed at metaphase I have long interested triticale workers. Different hypotheses have been suggested to explain univalency in these materials (for reviews see Kaltsikes 1974; Scoles and Kaltsikes 1974).

C-banding staining techniques have revealed differences in localization of heterochromatin between rye and wheat chromosomes. Heterochromatic bands are mainly located at telomeres in rye, in contrast to the more proximal centromeric position in wheat (Naranjo et al. 1979). Thomas and Kaltsikes (1974, 1976), Merker (1976), and Roupakias and Kaltsikes (1977) showed that univalents were mostly rye chromosomes, the pairing failure being associated with the presence of large terminal heterochromatic bands in triticale. Our results (Naranjo and Lacadena 1980) obtained in several wheat-rye derivatives were in agreement with theirs. We observed that the chromosome 1R pairing is reduced in heterozygotes for thick telomeric bands in relation to homozygotes lacking thick bands in this particular chromosome.

If telomeric bands play a role on rye chromosome pairing in the presence of wheat chromosomes, rye homologous pairs with different amounts of heterochromatin will show different pairing levels. In this paper meiotic pairing of five rye homologous pairs of 'Cachirulo' triticale is analyzed using C-banding technique.

Materials and Methods

The material used in this work was hexaploid triticale (AABBRR genome constitution) cv. 'Cachirulo' obtained by Sánchez-Monge (1969). Cytological analysis was carried out on root tips and anthers previously fixed in 1:3 acetic alcohol. Prior to fixation, root tips were immersed in tap water at 0 °C for 24 h in order to shorten the chromosomes. C-banding technique was used in both cases according to Giráldez, Cermeño and Orellana's method (1979).

Results

1 Characterization of Rye Chromosomes

Examination of the heterochromatic banding of rye chromosomes in somatic metaphases indicates that the individual rye chromosomes have characteristic banding patterns and other morphological markers making it possible to distinguish clearly the seven rye chromosomes (Fig. 1, a, b).

The C-banded karyotype and the nomenclature system for rye chromosomes are based on the similarities found between the banding pattern presented in this

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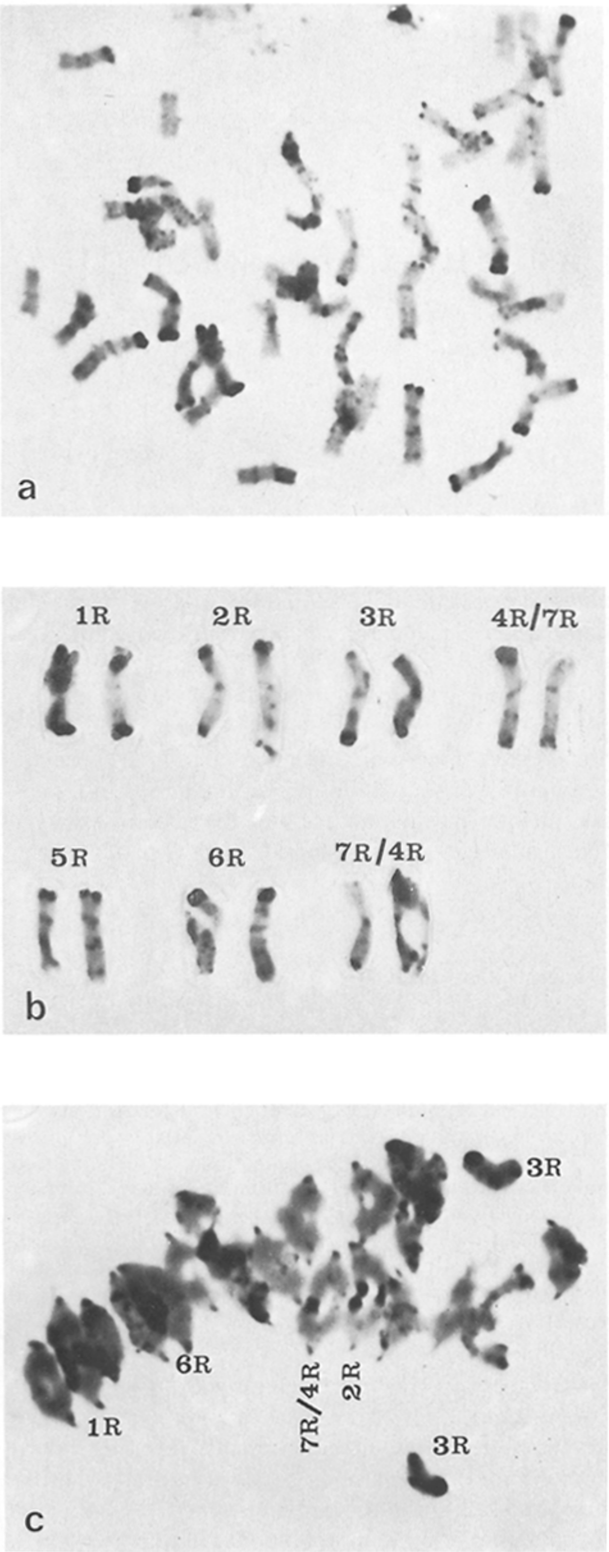

Fig. 1a–c. C-banding pattern of 'Cachirulo' triticale. **a** Somatic metaphase with $2n = 6x = 42$ chromosomes. **b** Karyotype of the rye chromosomes derived from the cell showed in (a). **c** Metaphase I showing homologous pairs 1R, 2R, 6R, and 7R/4R as ring bivalents and 3R as a pair of univalents

work and that observed by Darvey and Gustafson (1975) and by Giráldez et al. (1979). The characteristics of each chromosome can be described as follows:

Chromosome 1R is a nucleolar organizer chromosome. It is submetacentric and has a prominent band on both telomeres. The short arm carries a second prominent band just adjacent to the satellite.

Chromosome 2R is nearly metacentric. It is characterized by a terminal band on each telomere, the most prominent of the two bands being on the short arm.

Chromosome 3R is nearly metacentric. It carries a prominent band at the end of the long arm and a thin band at the end of the short arm.

Chromosome 4R/7R is submetacentric with a thin terminal band on the short arm. The long arm has three weak bands; an interstitial band located in the middle of the arm and two almost terminal bands.

Chromosome 5R is submetacentric with a terminal band on the short arm and two interstitial bands on the long arm.

Chromosome 6R is submetacentric. It carries a prominent terminal band on the short arm and several weak bands on the long arm.

Chromosome 7R/4R is metacentric carrying terminal bands of different intensity at the two ends, the thinnest corresponding to the telomere of the short arm.

From the seven rye chromosomes above described, it has only been possible to identify the homologous pairs 1R, 2R, 3R, 6R, and 7R/4R in 152 PMCs observed at metaphase I of meiosis. Identification of these bivalents was based on both the C-banding pattern and centromere position (Fig. 1c)

2 *Metaphase I Pairing*

The chromosomes 1R, 2R, 3R, 6R, and 7R/4R generate more univalent pairs (5.33%) than the remainder do (0.86%), including all the wheat chromosomes and the rye chromosomes 5R and 4R/7R. Frequencies of bivalents and univalent pairs are given in Table 1. Comparison made by a contingency chi-square test indicates that

Table 1. Frequencies of bivalents and univalent pairs produced by the rye chromosomes identified at metaphase I (1R, 2R, 3R, 6R, and 7R/4R) and the unidentified chromosomes (A and B wheat genomes and rye chromosomes 4R/7R and 5R) in 'Cachirulo' triticale

Chromosomes	Total bivalents	Total univalent pairs
1R, 2R, 3R, 6R, 7R/4R	718	42
Wheat genomes A and B, 4R/7R, 5R	2 411	21

there are significant differences ($X^2 = 65.07$; d.f. = 1; $p < 0.001$) with respect to the number of univalents between both groups of chromosomes.

The four possible configurations, namely, ring bivalent (R), open bivalent in which the long arm was bound (OS), open bivalent in which the short arm was bound (OL), and pair of univalents (U) could be identified in the five rye homologous pairs 1R, 2R, 3R, 6R, and 7R/4R at metaphse I. Table 2 shows the frequencies of these configurations for the five homologous couples. These results are graphically summarized in Fig. 2. From the analysis of this figure one can deduce that: (i) The chromosome 7R/4R carries the smallest amount of heterochromatin (as was found by visual inspection in a number of somatic metaphase plates and in the 152 PMCs analyzed) and it shows the highest frequency of ring bivalents; (ii) the chromosomes 1R and 6R are submetacentric and both have large telomeric bands in the short arm, while long arm telomeres are differently banded. However, configuration frequencies are similar in both chromosomes. When these frequencies were compared by means of a contingency chi-square test no significant differences were found, although chromosome 1R generates more univalent pairs than 6R does ($X^2 = 5.21$; d.f. = 3; $0.10 < p < 0.20$); (iii) the almost metacentric chromosomes 2R and 3R, carrying the large telomeric heterochromatic bands in different positions (2Rs and 3R1), have in addition a different behaviour in relation to meiotic pairing. While the number of ring bivalents and univalent pairs is the same, the higher frequency of bound arms in the open bivalents of both chromosomes is shown by the arms with smaller telomeric bands. Comparison made by a contingency chi-square test indicates that chromosomes 2R and 3R are significantly different in meiotic pairing ($X^2 = 32.22$; d.f. = 3; $p < 0.001$). On the other hand, the meiotic pairing of chromosome 2R is similar to those of chromosomes 1R and 6R ($X^2 = 11.70$; d.f. = 6; $0.05 < p < 0.10$) which are submetacentric and have prominent telomeric bands on their short arms.

Table 2. Frequencies of the four configurations observed in 152 PMCs at metaphase I for the chromosomes 1R, 2R, 3R, 6R, and 7R/4R of 'Cachirulo' triticale

Configurations^b	Chromosome				
	1R	2R	3R	6R	7R/4R
R	87	102	104	82	121
OS	47	35	8	51	11
OL	4	8	33	11	14
U	14	7	7	8	6

R = Ring bivalent, OS = Rod bivalent open by its short arm, OL = Rod bivalent open by its long arm, U = Pair of univalents

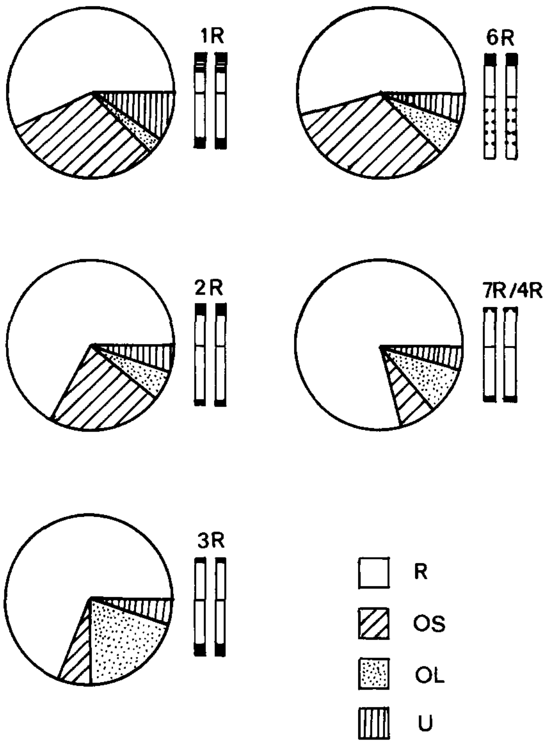

Fig. 2. C-banding pattern of the five rye chromosomes analyzed, and relative frequencies (represented as circular segments) of the four possible metaphase I configurations. R = Ring bivalent; OS = Rod bivalent open by its short arm; OL = Rod bivalent open by its long arm; U = Pair of univalents

Discussion

From the meiotic pairing observed in 'Cachirulo' triticale it can be deduced that there is a higher contribution of rye than wheat chromosomes to generate univalents at metaphase I (Table 1). This agrees with the previous observations of many authors (Müntzing 1957; Sánchez-Monge 1958; Larter et al. 1968; Pieritz 1970; Thomas and Kaltsikes 1974, 1976; Merker 1976). In order to ascertain the cause of univalency Müntzing (1939), based on the fact that rye inbred lines showed a reduction in mean number of chiasmata, suggested that the rye genome in triticale was being inbred, as triticale is predominantly self pollinating, and this led to homozygosis of deleterious genes in the rye genome, resulting in univalents at metaphase I. Since the five chromosomes 1R, 2R, 3R, 6R and 7R/4R of 'Cachirulo' triticale show univalent frequencies similar to those obtained for the seven rye chromosomes in 'Cachirulo' triticalex *Secale cereale* hybrids where inbreeding has been broken (6.5%, Naranjo et al. 1979), irregularities observed at meiosis cannot be attributed to homozygosis in the rye genome. Many other reports show opposite results to the Müntzing's hypothesis (see Scoles and Kaltsikes 1974). Other suggestions have been made to explain the meiotic instability of triticale and they were classified by Scoles and Kaltsikes (1974) as: a) interaction of genes of the parental species that affect meiotic pairing, b) cytoplasmic factors, and c) genomic allocycly.

More recently, results obtained by Thomas and Kaltsikes (1974, 1976), Merker (1976), and Roupakias and Kaltsikes (1977) suggested a role of rye telomeric heterochromatin in the pairing failure. However, the heterochromatin effect on rye meiotic pairing has been found only when wheat chromosomes are also present (Naranjo and Lacadena 1980).

The pairing levels found for the chromosomes 1R, 2R, 3R, 6R, and 7R/4R of 'Cachirulo' triticale clearly suggest an influence of heterochromatin on meiotic pairing for two main reasons, namely: a) the chromosome 7R/4R shows the highest level of pairing associated with the smallest heterochromatin amount, b) the levels of pairing of the short arm 2R and the long arm 3R carrying prominent telomeric bands are lower than those of their respective 2R1 and 3Rs arms, the decrease of pairing being similar in both cases. Assuming that chromosomes 2R and 3R are nearly metacentric and have similar relative lengths (Giráldez et al. 1979), one can expect the same number of chiasmata in the four arms of these chromosomes. As it is not the case, the pairing failure observed can be attributed to the presence of telomeric heterochromatin.

The fact that heterochromatin effect on chromosome 2R and 3R pairing is similar does not mean that all chromosomes (chromosome arms) carrying telomeric bands have to behave in the same way. Thus, in comparing the pairing failure in plants heterozygotes for C-bands at the short arm 1R telomere and plants heterozygotes for C-bands at the long arm 1R telomere, Naranjo and Lacadena (1980) found that influence of telomeric heterochromatin on pairing failure is higher in the short arm 1R than in the long arm 1R. Only a small effect of heterochromatin located on the long arm 1R is also found in 'Cachirulo' triticale. This special behaviour of the long arm of 1R could explain the similarity observed between the chromosome 6R (which lacks telomeric band on its long arm) and even the chromosome 2R (which has a rather thin telomeric band on its long arm) and the chromosome 1R carrying bands at the two telomeres.

Thomas and Kaltsikes (1974) suggested that the effect of heterochromatin could arise from an overlap between the processes of DNA replication and chromosome pairing, since terminal bands of rye chromosomes are late replicating. From Merker's (1976) standpoint this hypothesis seems very appealing, but it is inconsistent with the cytological observations that show that the pairing failure of triticale is of a desynaptic rather an asynaptic type. He proposed that desynapsis could proceed from an interference between heterochromatin and chiasma terminalisation. However, he concluded that heterochromatin may have both desynaptic and asynaptic effects and, therefore, the hypothesis of interference with prophase pairing and with terminalisation of chiasmata has some validity.

However, in contrast with Merker's suggestion, no interference of heterochromatin with chiasma terminalisation was detected in the metaphase I analysis of telomeric heterochromatin heterozygotes for chromosome 1R (Naranjo and Lacadena 1980), since a recombinant arm (which should show each chromatid with a different telomeric constitution) did not appear between the unpaired arms of open bivalents and/or univalent pairs. In addition, it has been reported in *Chorthippus biguttulus* (Santos and Giráldez 1978) and in *Allium flavum* (Loidl 1979) that interstitial heterochromatin can act as a barrier preventing chiasma terminalisation.

Desynapsis can also arise from an inhibition of chiasma formation. In this case, chromosomes will show a quite regular behaviour at diakinesis when other mechanisms in addition to crossing-over maintain adhered homologous chromosome arms during this stage. If these mechanisms cease to act at metaphase I homologous couples will form open bivalents and univalents pairs besides ring bivalents. A mechanism of this type has been observed in the achiasmatic meiosis of *Bithynia* by Debus (1978). He demonstrated the formation of synaptonemal complexes with nodules in spermatocytes of achiasmatic meiosis of *B. leachi* and *B. tentaculata.* Although no crossing-over took place, the separating bivalents adhered terminally in late meiotic prophase, so that the chromosomes appeared ring-like during diakinesis. Since these connections are not attributable to real terminalized chiasmata, Debus suggested that the nodules could be responsible for the chiasmalike adhesion.

If an adhesion of this type takes place during late prophase in triticales, interference of heterochromatin with chiasma formation would produce formation of univalents at metaphase I. However, with the data available by the present, an asynaptic effect of heterochromatin cannot be rejected.

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