

# Chromosome factors affecting pairing in progenies of 6x-triticale × *Triticum turgidum* L. ssp. *turgidum* conv. *durum* (Desf.)

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The chromosome constitution and meiotic behaviour of 15 plants of F2 and BC1 progenies from the hybrid of 6x-triticale × *T. turgidum* (L.) ssp. *turgidum* conv. *durum* (Desf.) (F1:  $2n = 5x = 35$ ; AABBRR) were studied using C-banding patterns of both wheat and rye chromosomes as identifying features. Patterns in PMCs at first metaphase permitted the individual identification of the chromosomes of nine of the wheat and all of rye in all plants. The external length and internal heterochromatin of chromosomes were analysed and found to influence the meiotic behaviour of each chromosome pair. The existence of effects of both arm length and amount and distribution of heterochromatin on pairing are assumed. The results seem to demonstrate that of the two mentioned factors the first is the most important.

## INTRODUCTION

It has been reported that constitutive heterochromatin present in wheat chromosomes influences meiotic pairing. Interstitial and subtelomeric heterochromatin seems to facilitate the maintenance of chiasmata. Santos and Giraldez (1978), Loidl (1979), Naranjo and Lacadena (1982), Ferrer *et al.* (1984b), and Dvorak and McGuire (1981) suggested that the differential pairing among genomes of *T. aestivum* could be related to the amount of DNA and heterochromatin present in chromosomes. In general, heterochromatin has been associated with a detrimental effect on chromosome pairing at first metaphase (Yamamoto, 1979; John and Miklos, 1979; Bostock, 1980). It has been speculated that late DNA-replicating heterochromatin interferes in chromosome pairing when rye chromosomes are placed in a wheat genetic background. Modifications in rye chromosomes through the removal of telomeric heterochromatin could improve their level of pairing in triticale (Bennett, 1974, 1980, Darvey and Gustafson, 1975; Gustafson, 1976; Schlegel and Huelgenhof, 1985).

Moreover, pairing and chiasma frequencies have been related to relative arm length in wheat chromosomes. Thus, Sallee and Kimber (1979), concluded that pairing and chiasma formation are

correlated with the relative length, in an analysis of chromosome behaviour at first metaphase of meiosis in telocentrics of common wheat "Chinese Spring".

This paper deals with the analysis of the effect of both external (length) and internal (heterochromatin) on homologous chromosome pairing in plants derived from 6x-triticale × *T. turgidum* L. hybrids where the C-banding technique was used to identify individual chromosomes.

## MATERIALS AND METHODS

F1 hybrids ( $2n = 5x = 35$ ) between 6x-triticale and durum wheats (genomes AABBRR) were self-pollinated and backcrossed to both triticale and *T. turgidum* (L) ssp. *turgidum* conv. *durum* parents to obtain F2 and BC1. From the progenies the following plants were analysed: 11 from the backcross to the durum wheat; 1 from backcross of the hybrid to the 6x-triticale; 3 from the selfed F1. The Spanish 6x-triticale cultivars "Camarma" (A), "S-14" (B) and "Torote" (C) and the selected durum wheats "Arcipreste" (G), "Victor Vz \* Flamingo 'S'" (H), "Alaga \* (Bidi 17 \* Marfed ERT 1) \* (Garza 'S' \* Candeal)" (P) and "Edmore" (J) served as the parents to obtain the F1.

In order to quantify both the amount and the arm lengths in somatic chromosomes, karyological analysis was carried out on the triticale variety "Camarma". This cultivar was used because its utilisation as a parent in the majority of plants analysed. Twenty well spread somatic metaphases in which all chromosomes were identifiable by C-banding were examined and studied. Measurements of the lengths of chromosome arms and of their euchromatin and heterochromatin regions were made from enlarged photographs produced using a BH2 Olympus photomicroscope. To compensate for differences in the degree of condensation of the chromosomes in different cells, the relative lengths with respect to the short arm of chromosome 4B in each cell were used. Rye chromosomes of "Camarma" and segregant plant material were identified according to the internationally agreed karyotype of rye (Sybenga, 1983). The identification of the wheat chromosomes (genomes A and B) was carried out on the

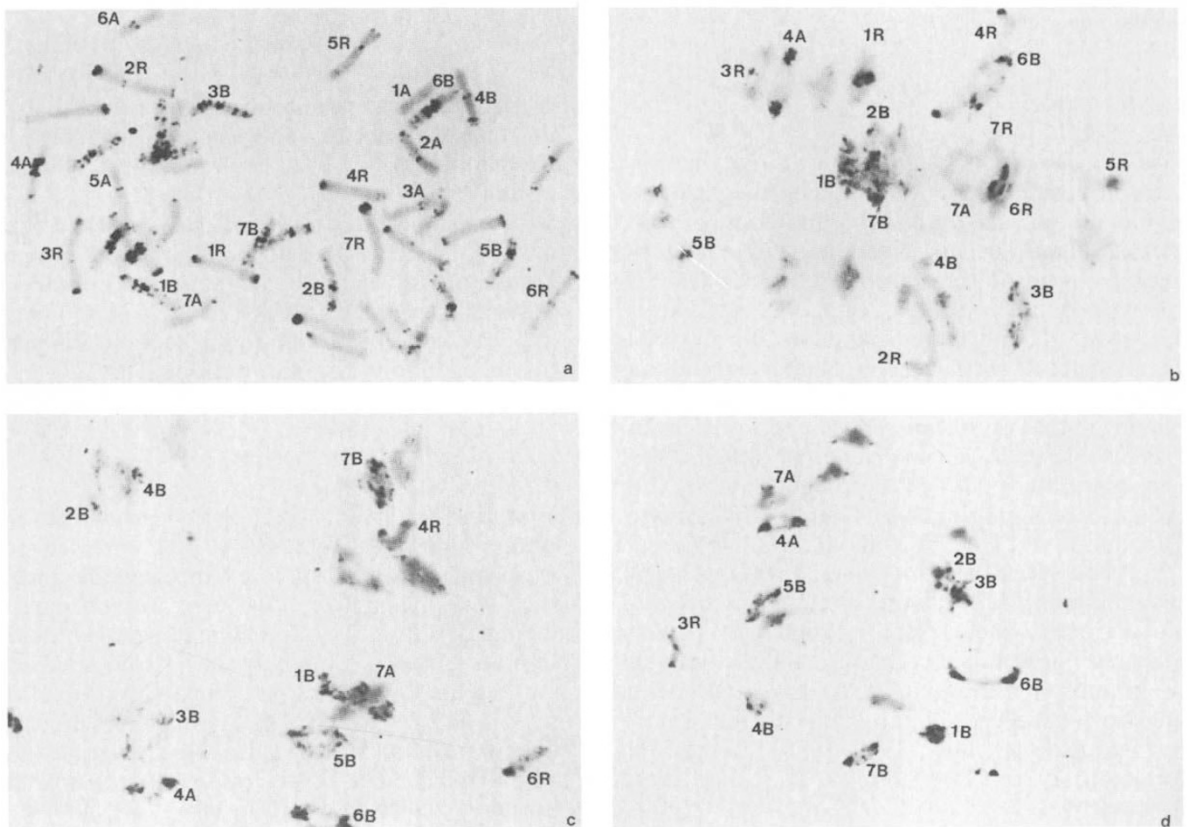
basis of their individual pattern of bands previously reported for hexaploid wheat *T. aestivum* L. (Gill and Kimber, 1974*a, b*; Ferrer *et al.*, 1984*a*).

The chromosome number and constitution, and the meiotic pairing of each plant were examined in PMCs at first metaphase using the C-banding technique described previously (Jouve *et al.*, 1980). The anthers were fixed in Carnoy's solution and preparations were made following excision of pollen mother cell sacks from the anthers.

## RESULTS AND DISCUSSION

### *C-banding of somatic chromosomes in 6x-Triticale*

The C-banding technique was applied to analyse the amount and distribution of heterochromatin in somatic chromosomes of the 6x-triticale variety "Camarma" (A). This is shown in fig. 1. An



**Figure 1** C-banding microphotographs of metaphase plates. (a) somatic metaphase in triticale variety "Camarma". (b) F2 plant: cell showing 14 wheat bivalents+7 rye bivalents. (c) BC1 plant: cell showing 14 wheat bivalents+4R and 6R univalents. (d) BC1 plant: cell showing 14 wheat bivalents+3R univalent.

idiogram for each somatic chromosome is presented in fig. 2, together with the "cyclograms" representing the results of meiotic pairing. Identification of chromosomes is based on the standard karyotypes of wheat and rye chromosomes previously reported (Gill and Kimber, 1974a, b). The triticale line "Camarma" contained all seven pairs of rye chromosomes. The results on relative chromosome lengths and heterochromatin amounts are given in table 1.

The amount of heterochromatin present per chromosome varied both within and between genomes. Thus, the mean amount of heterochromatin for each genome was of: A = 14.3 per cent (range 0-36.0), B = 32.6 per cent (range 16.2-44.0), and R = 14.1 per cent (range 9.5-18.7).

The distribution of heterochromatin was quite different for the wheat and rye chromosomes. Some wheat chromosomes were characterised by the presence of heavy proximal (7 of the B genome and 4A) or interstitial (2A, 3A, 5A and 6A) bands. Chromosome 1A appeared to lack heterochromatic regions and 7A presented small and terminal bands. Rye chromosomes showed mainly telomeric heterochromatin with the exception of the 5RL and 6RL arms.

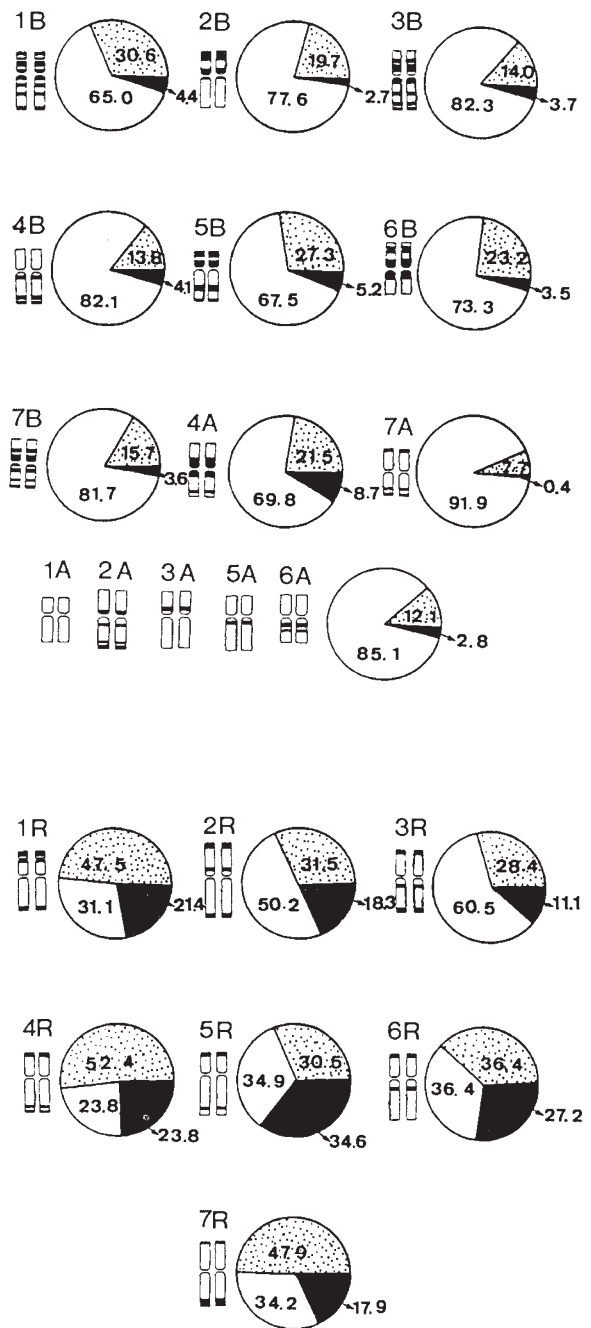
*Chromosome number and constitution in the F2 and BC1 progenies*

All plants analysed possessed 14 wheat chromosomes (7"AA+7"BB). Two plants had only this chromosome constitution. The chromosome composition of the remaining plants was very variable and there were different monosomic and polysomic additions of rye chromosomes (29 to 40: 7"AA+7"BB+1-12R/RR). Three plants showed a triticale chromosome composition (2n = 42; 7"AA+7"BB+7"RR). These results are shown in table 2.

*Pairing at Metaphase I*

The wheat and rye chromosomes were easily distinguished at meiosis. Nine wheat chromosomes (4A, 7A and the seven of the B genome) and the all rye chromosomes were recognized at first metaphase of meiosis, and the meiotic behaviour was investigated in a sample of PMCs from each F2 and BC1 plant (fig. 1b, c and d).

A configuration of 14 bivalents (7"AA+7"BB) was the most frequent meiotic association observed amongst the wheat chromosomes. The meiotic behaviour of each pair whether univalent, rod bivalent or closed bivalents, differed significantly within the same plant ( $\chi^2 = 16.48$  to 58.91; df = 8;



**Figure 2** Meiotic behaviour of each chromosome pair. Idiogram for somatic chromosomes near to each cyclogram corresponds to Giemsa C-banding studies for the chromosomes of triticale variety "Camarma". Cyclograms represent the meiotic behaviour showed by the nine identified chromosomes of wheat (4A, 7A and the seven of the B genomes), the seven rye chromosomes and overall of the remaining of the A genome. Black sectors = univalents; Dotted sectors = open bivalents; White sectors = ring bivalents.

**Table 1** Arm length (relative to 4BS = 1), amount of heterochromatin (% respect to chromosomal length) of the triticale variety "Camarma" and the mean number of chiasmata in F2 and BC1 plants analysed

	Mean length (relative to 4BS)	Arm ratio (L/S)	Amount of heterochromatin %		Mean number of Xata
			Arms	Total	
<b>Wheat*</b>					
1BS	0.92		18.30		0.68
1BL	1.81	1.98	22.70	41.00	1.09
2BS	1.19		18.47		0.99
2BL	1.57	1.32	2.89	21.36	0.91
3BS	1.24		16.49		0.87
3BL	1.67	1.35	23.71	40.20	1.07
4BS	1.00		0.00		0.90
4BL	1.59	1.59	16.21	16.21	1.03
5BS	0.77		19.74		0.70
5BL	1.56	2.01	13.30	33.04	1.27
6BS	1.14		25.86		0.77
6BL	1.18	1.03	18.10	43.96	1.03
7BS	1.10		15.58		0.85
7BL	1.21	1.09	16.88	32.46	1.06
4AS	1.23		19.37		0.75
4AL	1.35	1.10	16.66	36.03	0.96
7AS	1.11		6.11		1.03
7AL	1.18	1.07	7.86	13.97	0.99
<b>Rye†</b>					
1RS	1.14		11.61		0.31
1RL	1.70	1.49	7.04	18.65	0.89
2RS	1.71		10.72		0.51
2RL	2.02	1.19	5.09	15.81	0.87
3RS	1.33		6.73		0.83
3RL	1.79	1.34	11.21	17.94	0.74
4RS	1.19		6.87		0.26
4RL	1.72	1.44	5.49	12.36	0.79
5RS	1.05		5.88		0.34
5RL	2.01	1.91	3.59	9.47	0.72
6RS	1.16		5.74		0.37
6RL	2.05	1.77	4.53	10.27	0.75
7RS	1.45		2.50		0.61
7RL	1.75	1.21	11.87	14.37	0.59

\* For chiasmata, data from 15 plants: 1069 PMCs.

† For chiasmata, data from 4 plants with the exception of chromosome 2R (3 plants): 215 PMCs.

$P < 0.05$  to  $P < 0.001$ ). However, there were no significant differences between the meiotic configurations scored for the same chromosomes in different plants. Correlation coefficients comparing chiasma frequencies amongst all plants were estimated. The  $r$  values of the mean frequencies of metaphase association per chromosome arm were always near 0.9 and in all cases the correlations were significant and positive. Consequently, although differences at the level of pairing amongst plants were found, the variations in the mean number of chiasmata were regularly distributed amongst chromosomes. The variance ratios were not significantly different at the 5 per cent level. This result confirmed the similar tendencies of each

wheat chromosome arm to pair independently from both the rye chromosome additions and genetic background. It can be assumed that, although plants differed among themselves in rye chromosome constitution, pairing intensities of wheat arms were uniformly affected under the effect of different rye additions. Evidence in favour of the effect of rye heterochromatin on wheat chromosome pairing are presented in a separate paper (Bernardo *et al.*, 1987). Chromosome pairing in wheat must therefore be controlled by a balance between several factors. Thus, Miller *et al.* (1983) demonstrated that chromosome pairing is influenced by extra dosage of group 3 chromosomes. The effect of increased dosage of other wheat



chromosomes on chromosome pairing of individual wheat bivalents was also observed (Miller and Reader, 1985).

As a consequence of similar meiotic behaviour of each chromosome pair in different plants, the individual results can be considered as a whole. Fig. 2 illustrates the frequencies of meiotic configurations for the identified pairs of wheat and rye chromosomes, and summarises data for the remaining chromosomes (1A, 2A, 3A, 5A and 6A). The "cyclograms" were obtained by taking data from all the plants analysed, and fig. 3 shows the mean number of chiasmata for the wheat and rye chromosomes. From these results it can be deduced: (a) chromosome 4A and the rye chromosomes carry the largest amount of heterochromatin either around the centromere (4A) or in the telomeres and showed the highest frequency of univalents. (b) wheat chromosomes having the highest arm ratio (1BL/1BS = 1.96; 5BL/5BS = 2.02) also exhibited a higher frequency of open bivalents (chiasmata in the long arm) than the others having similar lengths in both arms. (c) Contrarily, wheat chromosomes having the lowest arm ratio (7AL/7AS = 1.06; 7BL/7BS = 1.1; 3BL/3BS = 1.34) showed the highest frequencies of ring bivalents. (d) chromosome arms 1BL, 3BL, 4BL, 2BS and 7AL, which exhibited subtelomeric or telomeric bands also showed a high level of pairing.

The meiotic behaviour of each chromosome pair in the set of plants analysed may indicate that each chromosome arm seems to pair with a different frequency. Factors upon which pairing intensity seem to depend are heterochromatin and arm size.

Fig. 4 shows the regression lines and correlation coefficients from a comparison of the mean number of chiasmata per chromosome arm with relative amounts of heterochromatin and length. The main observation that can be made is the positive and significant slope for differences in arm length for both wheat and rye chromosomes. The mean number of chiasmata are significantly higher in longer chromosome arms than in short ones. However, there was not a significant correlation between total amount of heterochromatin and mean number of chiasmata either for the wheat or the rye chromosome arms.

These statistical tests confirm previous results on the influence of chromosome size on pairing intensity. For example, Sallee and Kimber (1979) assumed that the different pairing intensity showed by chromosomes of different genomes could be related with their differences in length. Dvorak and

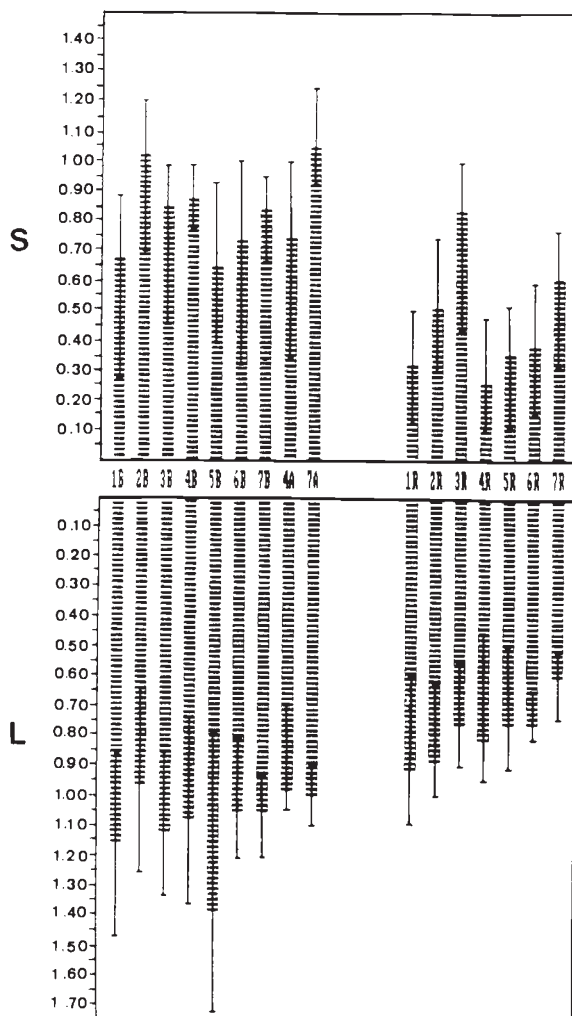
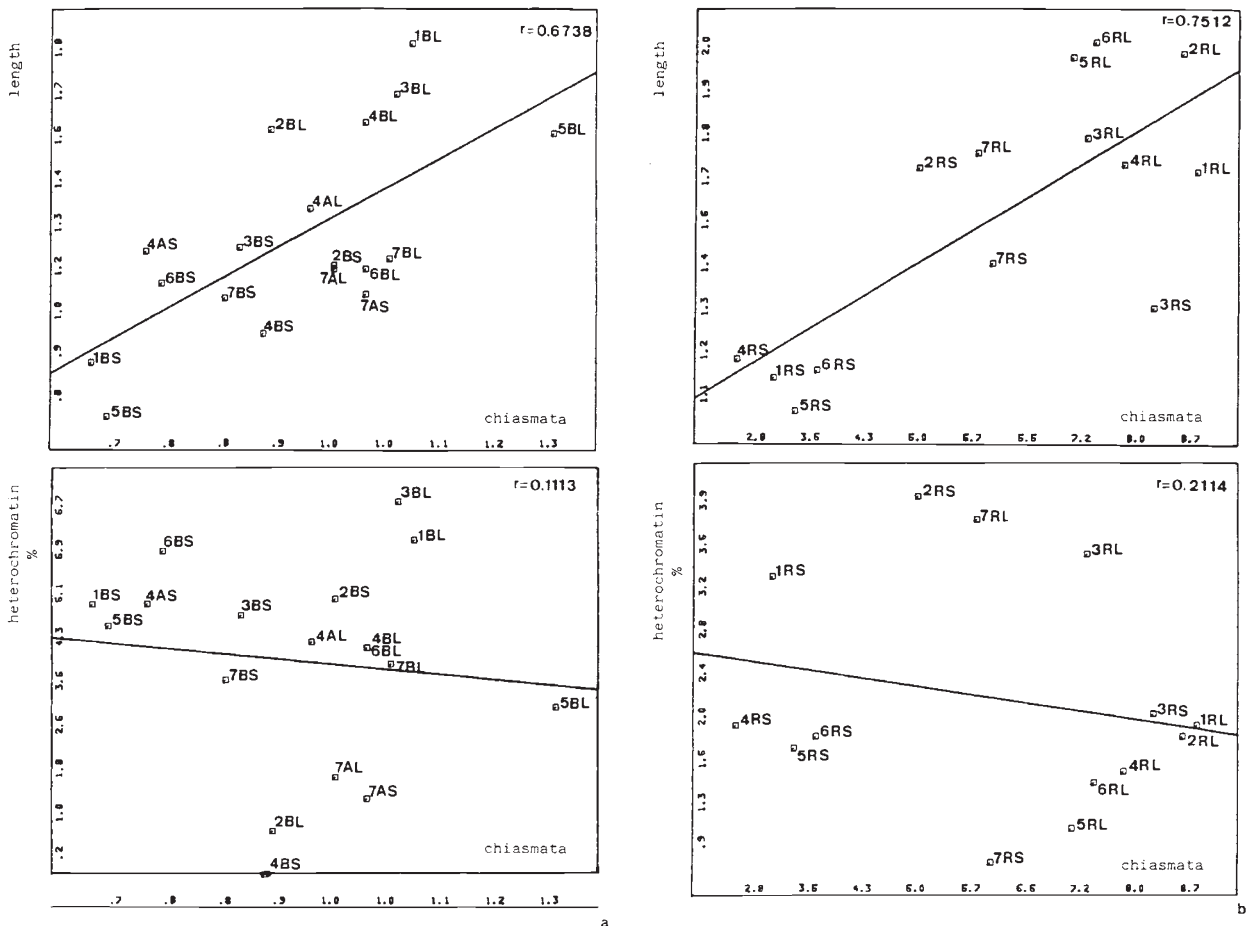


Figure 3 Histograms showing the mean number of chiasmata for each identified wheat and rye chromosome arms (L = long; S = short).

McGuire (1981) suggested that the variation in pairing frequencies in wheat genomes could be related to the amount of DNA and heterochromatin present in chromosomes. There is a large amount of evidence on the influence of the telomeric heterochromatin of rye chromosomes on meiotic pairing in triticale. Thus, Thomas and Kaltsikes (1974) suggested that the heterochromatin present in the telomeres can affect the processes of DNA replication and prophase pairing observed in the rye chromosomes. This effect becomes critical for rye chromosomes both in triticale and plants with mono- and polysomic additions where a shorter meiotic cycle is forced upon the rye chromosomes (Bennett and Kaltsikes, 1973).

**Table 2** Chromosome constitution of the plants analysed. Plant numbers 1 to 16 are derived from the cross (Triticale \* *T. turgidum*) \* *T. turgidum*; plant number 17, from (Triticale \* *T. turgidum*) \* Triticale, and plant numbers 18 to 20 from selfpollinated (Triticale \* *T. turgidum*)

Materials	2n	Maximum bivalents	Chromosome composition
1 (A * G) * G	29	14"	7"AA+7"BB+6R'
3 (A * G) * S	31	14"	7"AA+7"BB+2R'+6R'+7R mtl-L
4 (A * G) * S	29	14"	7"AA+7"BB+6R'
7 (A * H) * H	29	14"	7"AA+7"BB+1R'
8 (A * H) * H	30	14"	7"AA+7"BB+4R'+7R'
9 (A * H) * H	28	14"	7"AA+7"BB
11 (A * H) * H	31	14"	7"AA+7"BB+1R'+6R'+7R'
12 (A * H) * H	28	14"	7"AA+7"BB
13 (A * H) * H	29	14"	7"AA+7"BB+1R'
15 (A * P) * U	31	14"	7"AA+7"BB+1R mtl-S+2R'+7R'
16 (A * P) * U	31	14"	7"AA+7"BB+1R'+5R mtl-S+7R iso-L
17 (A * G) * E	42	21"	7"AA+7"BB+7"RR
18 (B * G)	40	20"	7"AA+7"BB+6"RR
19 (B * G)	42	21"	7"AA+7"BB+7"RR
20 (B * G)	42	21"	7"AA+7"BB+7"RR



**Figure 4** Regression lines comparing the mean number of chiasmata and both relative amount of heterochromatin and length in wheat (A) and rye (B) chromosome arms. The pairing frequencies were also checked against data on chromosome length of Sallee and Kimber (1979) with similar statistical result.

Interstitial and subtelomeric C-bands of heterochromatin can facilitate the maintenance of chiasmata (Santos and Giraldez, 1978; Loidl, 1979; Naranjo and Lacadena, 1982; Ferrer *et al.*, 1984). This effect could explain the increased pairing observed in the wheat chromosome arms above mentioned. In this case, the increased level of pairing could be explained in terms of heterochromatin interfering with the terminalisation of chiasmata. In any case, meiotic pairing in wheat chromosomes may be influenced by an overlapping effect of both arm length and amount and distribution of heterochromatin. Our results seem to demonstrate that of the two factors the chromosome length is the most important.

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