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Meiotic pairing of the amphiploid *Hordeum chilense* × *Triticum turgidum* conv. *durum* studied by means of Giemsa C-banding technique

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Summary. The meiotic behaviour of the amphiploid *Hordeum chilense* × *Triticum turgidum* conv. *durum* using a C-banding staining method is studied. Nine pairs of chromosomes at metaphase-1 (4A, 7A and the seven of the B genome) were identified and the remaining wheat chromosomes (1A, 2A, 3A, 5A and 6A) and seven of the *chilense* (1 to 7 H^{ch} chromosomes) were assigned to its particular genome. A similar mean number of univalents from parental genomes (wheat and wild barley) were found. No meiotic pairing between *chilense* and *turgidum* chromosomes was detected. Differences in the meiotic behaviour per chromosome and amongst genomes are explained on the basis of cytomorphological and heterochromatin characteristics.

Key words: Heterochromatin – *Hordeum chilense* – Tritordeum – C-banding – Meiosis – *Triticum* – Amphiploid

Introduction

Interspecific hybrids between *Hordeum chilense* (2n = 2x = 14, and genomes $H^{ch}H^{ch}$) and *Triticum aestivum* (2n = 6x = 42, and genomes *AA BB DD*) were obtained by Martin and Chapman (1977) at the Plant Breeding Institute (Cambridge, U. K.). Hybrid plants were treated with colchicine and fertile amphiploids (2n = 8x = 56, and genomes *AA BB DD* $H^{ch}H^{ch}$) that grew vigorously were developed (Chapman and Miller 1978).

The interspecific hybridization of *Hordeum chilense* with *T. turgidum* (2n = 4x = 28, and genomes *AA BB*) was obtained by Martin and Sánchez-Monge Laguna (1980) and fertile amphiploids were obtained by chromosome doubling with colchicine (Martin and Sánchez-Monge Laguna 1982).

This synthetic amphiploid was named Tritordeum. It has a genome formula of *AA BB* $H^{ch}H^{ch}$ and its fertility, agronomic performances, chromosome stability and grain quality offer a special value for plant breeding (Martin and Sánchez-Monge Laguna 1982).

The C-banding patterns of the somatic chromosomes of the genome H^{ch} of *Hordeum chilense* and *A, B,* and H^{ch} in one line of the amphiploid Tritordeum have been described by Fernández and Jouve (1984).

Giemsa staining techniques to analyse meiotic behaviour have been previously carried out in such different plant species as in *Allium flavum* (Loidl 1979), *Secale cereale* (Singh and Lelley 1975; Lelley and Gustafson 1979; Giráldez and Orellana 1979; Orellana and Giráldez 1981, 1983; Naranjo and Palla 1982; Santos et al. 1983), *Triticum timopheevi* (Hutchinson et al. 1982), *Triticum aestivum* (Jewell 1979; Jouve et al. 1982a; Ferrer et al. 1984), F_1 hybrids of 6x-Triticale × *Secale cereale* L. or its wheat-rye derivatives (Naranjo and Lacadena 1979, 1980; Walepa and Pilch 1979; Jouve et al. 1980a; Soler et al. 1980), or hybrids 6x-Triticale × *Triticum aestivum* (Jouve et al. 1980b, 1982b; Soler et al. 1980, 1982).

This paper describes the meiotic behaviour of the new amphiploid *Hordeum chilense* × *Triticum turgidum* con. *durum* using the Giemsa C-banding staining method to facilitate the assignation to genomes and/or identification of chromosomes.

Materials and methods

The line of tritordeum used in the study was kindly made available by Dr. A. Martín (E.T.S.I. Agrónomos, Cordoba, Spain). This line was named 'CHMA' and was obtained by chromosome doubling with colchicine from the hybrid *Hordeum chilense* × *Triticum turgidum* var. 'durum' Desf. (2n = 4x = 28), selection of cultivars 'Mexican 248' × 'Andalucía 344'.

Meiotic chromosome studies were made on anthers fixed in Carnoy's and stained by the Giemsa C-banding procedure previously reported by Jouve et al. (1980b). Pair-to-pair meiotic observations were made in 91 PMCs. The identification of C-banded characterized chromosomes was made following previous descriptions of *T. aestivum* meiotic chromo-

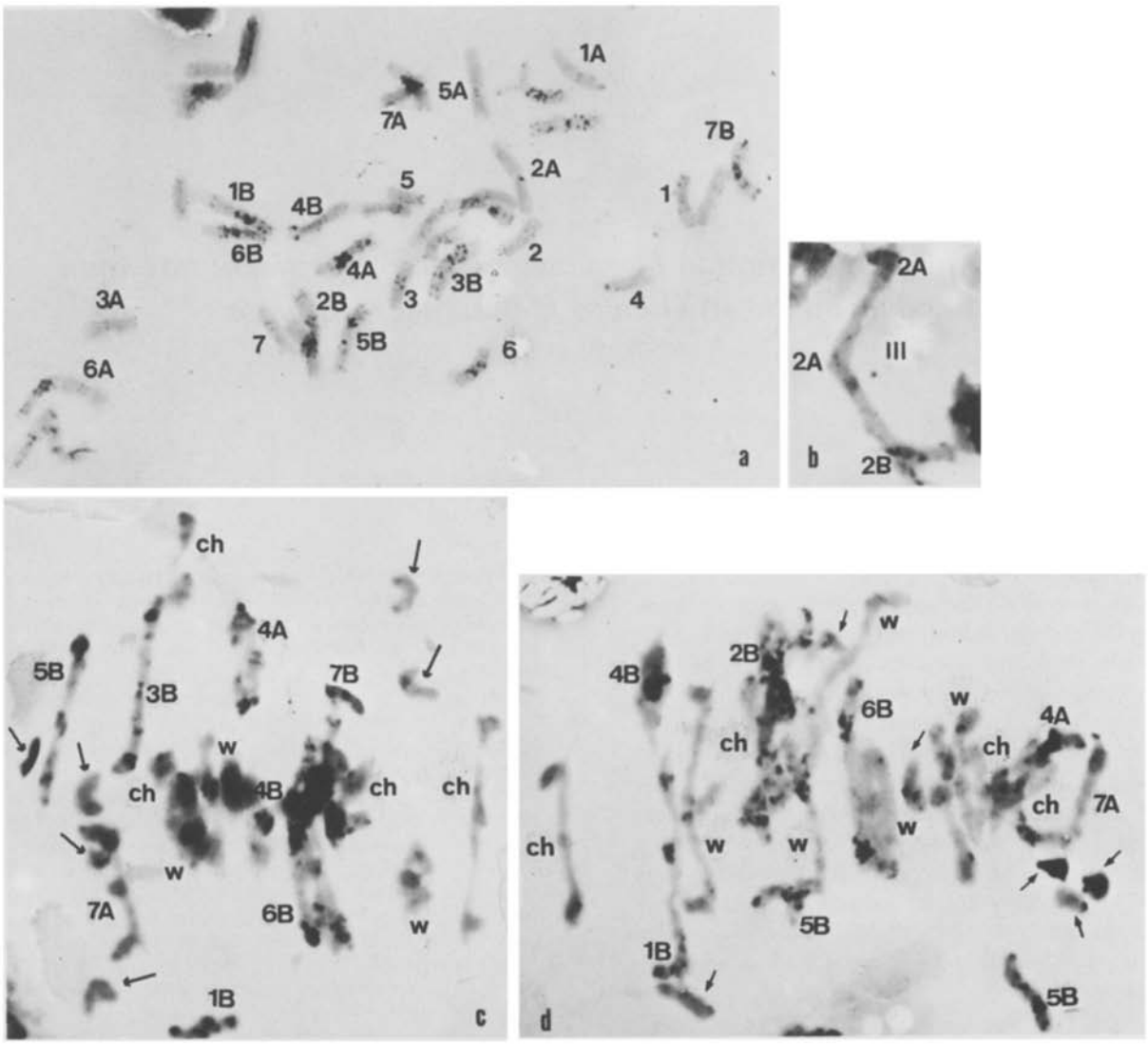

Fig. 1 a–d. C-banding microphotographs on cytological characteristics of the amphiploid *H. chilense* × *T. turgidum.* **a** Somatic metaphase. The chromosomes of *turgidum* (*1A* to *7B*) and *chilense* (*1–7*) are indicated; **b** Association of three chormosomes of wheat corresponding to one cell at first metaphase; **c** and **d** Metaphase I chromosomes. *Arrows* indicate univalent positions of chilense chromosomes. *ch* = chilense bivalents

Table 1. Mean number of chromosome pairing range at metaphase I in the amphiploid *H. chilense* × *T. turgidum* conv. *durum* analyzed by the C-banding technique

Genomes	Univalents	Open biv.	Ring biv.	Total biv.	Multivalents		Xata/PMC
					Triv.	Quadr.	
AA BB (14 pairs)	3.45±2.3 (0–8)	3.49±1.7 (0–7)	8.81±1.7 (5–14)	12.3±1.7 (10–14)	0.01±0.1 (0–1)	0.01±0.1 (0–1)	21.11±2.2 (16–25)
$H^{ch}H^{ch}$ (7 pairs)	3.08±1.9 (0–8)	1.84±1.2 (0–5)	3.63±1.5 (0–7)	5.47±1.3 (3–7)	–	–	9.10±2.2 (4–14)
AA BB $H^{ch}H^{ch}$ (21 pairs)	6.35±2.4 (2–14)	5.33±2.3 (1–11)	12.44±2.4 (7–9)	17.77±2.3 (14–20)	0.01±0.1 (0–1)	0.01±0.1 (0–1)	29.99±3.2 (21–39)

somes of Ferrer et al. (1984) and according to the somatic chromosomes of *chilense* and *tritordeum* described by Fernández and Jouve (1984).

Results

Meiotic observations on pairing of chromosomes of the genomes *AA*, *BB* and $H^{ch}H^{ch}$ are illustrated in Fig. 1. Chromosome identification was possible for 4A, 7A and the seven of B genome. Moreover, recognition of the genome nature of the remaining wheat chromosomes (1A, 2A, 3A, 5A and 6A) and the seven *chilense* chromosomes was possible by studying their cytomorphological differences. Differences in size were mainly used (the chromosomes of the A and B genomes are larger, 8.3–11.1 μm, than the *chilense* ones, 6.6–7.7 μm; data from Fernández and Jouve 1984).

The Giemsa staining technique also provides good results for distinguishing between *A* and *B* genomes, the H^{ch} being less highly banded and showing similar patterns of distribution of constitutive heterochromatin (pericentromeric and intercalary C-bands) with respect to wheat chromosomes. These banding patterns are substantially in agreement with previously published karyotypes for tritordeum (Fernández and Jouve 1984) and what chromosomes (Ferrer et al. 1984). There are, however, important difficulties in making cell-by-cell individual meiotic observations for the *chilense* chromosomes because of the absence of clear differences at first Metaphase amongst them.

Data on the meiotic pairing of the tritordeum are summarized in Table 1. The number of bivalents per cell ranged from 10 to 14 for wheat chromosomes ($AA+BB$) and 2 out 91 cells showed multivalent configurations. The mean chromosome pairing per cell was 3.45 univalents + 12.3 bivalents for the *AA* and *BB* genomes and 3.08 univalents + 5.47 bivalents for the *chilense* one. Mean number of chiasmata per bivalent was 1.71 and 1.66, respectively, for wheat and *chilense* chromosomes.

Figure 2 illustrates the difference in PMC distribution for a range (0 to 7) of number of bivalents for three genomes (*A*, *B* and H^{ch}). The histogram of H^{ch} genomes gives a bimodal distribution of bivalents with the mean number around 5 being lower than the pairing values showed for the *A* and *B* genomes. Overall data on pairing of chromosomes of each wheat genome showed descending values from *A* (maximum number of cells having 7 bivalents) to *B* (maximum number of cells having 6 bivalents).

Meiotic pairing of the nine identifiable pairs of wheat (4A, 7A and the seven chromosomes of the B genome) was individually analyzed. These pairs have characteristic banding patterns making it possible to

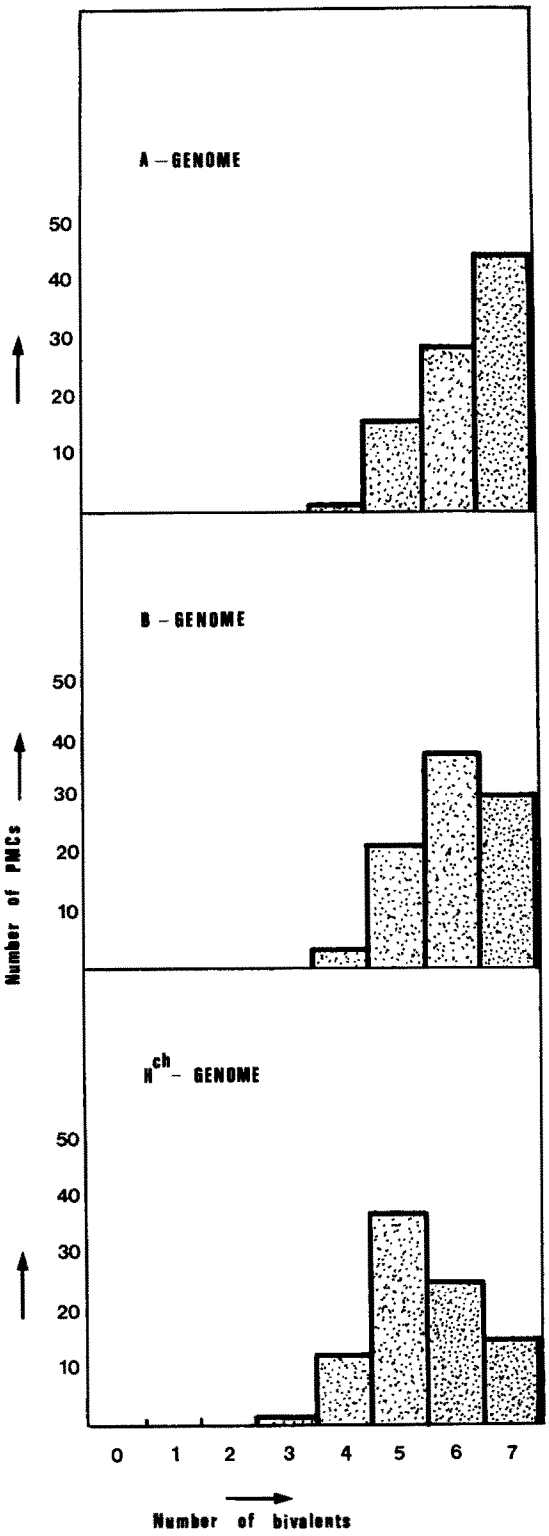

Fig. 2. Frequency distribution histograms of bivalent contribution to pairing for each genome

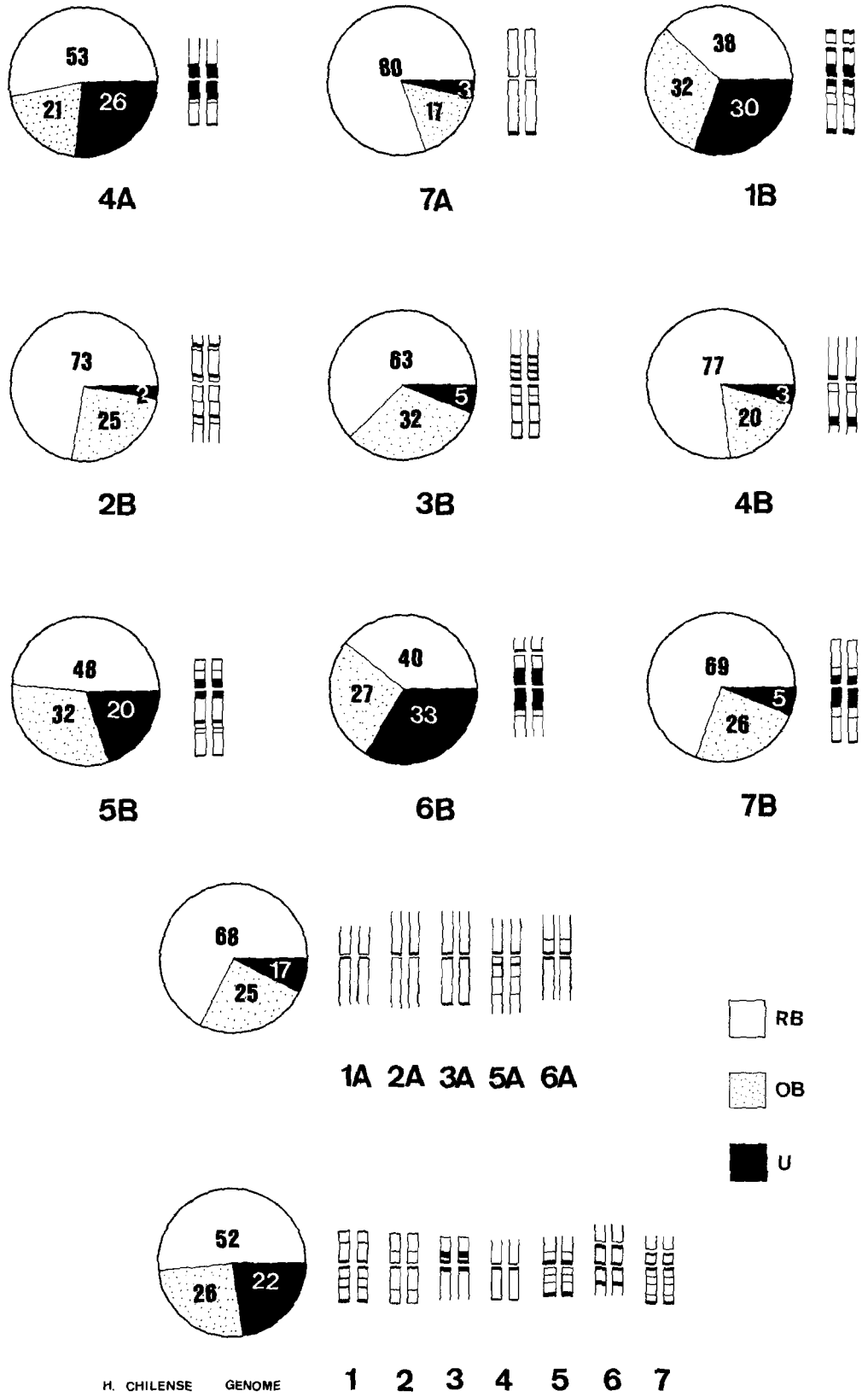

Fig. 3. Meiotic behaviour showed by the nine identified chromosomes of wheat (*4A*, *7A*, and the seven of the *B* genomes) and overall of the remaining of the *A* and H^{ch} genomes. Idiogram for somatic chromosomes near to each cyclogram correspond to Giemsa C-banding studies for the chromosomes of *Hordeum chilense* and its amphiploid × *Triticum turgidum* (see text for reference)

identify them clearly as ring bivalents, rod bivalents and univalents such as have been reported in a previous paper for wheat chromosomes (Ferrer et al. 1984). Overall observations of the remaining chromosomes of the *A* and H^{ch} genomes have also been considered.

Figure 3 shows the frequences of the meiotic configurations for the nine identified pairs and summarizes data for the remaining *A* and H^{ch} genomes. C-banding pattern of each one of the 21 pairs of chromosomes are graphically represented near the corresponding cyclogram.

From the analysis of this figure it can be deduced that:

(i) chromosomes 4A, 1B and 6B, carrying the largest amount of heterochromatin (around the centromere), also show the highest frequency of univalents.

(ii) chromosomes 2B and 4B, that exhibit subtelomeric bands, show a higher level of pairing than the others which lack these heterochromatic characteristics.

(iii) chromosome 7A, that only shows one dark telomeric band in its large arm, gives the highest frequency of ring bivalents and the lowest frequency of open bivalents amongst distinguishable chromosomes.

(iv) nearly 50% of the H^{ch} pairs observed as ring bivalents comprised approximately 25% of the observations as open bivalents and univalents for this genome.

When the meiotic behaviour of nine identified wheat chromosomes were compared by means of a contingency chi-square test, highly significant differences were found ($\chi^2 = 107.4$; d.f. = 16; $P < 0.001$). Moreover, comparisons made by contingency chi-square test between genomes were also found to be significant: $AA \equiv H^{ch}H^{ch}$ ($\chi^2 = 46.9$; d.f. = 2; $P < 0.001$), $BB \equiv H^{ch}H^{ch}$ ($\chi^2 = 14.04$; d.f. = 2; $P < 0.001$); $AA \equiv BB$ ($\chi^2 = 12.7$; d.f. = 2; $P < 0.001$).

Discussion

Martín and Sánchez-Monge Laguna (1980) showed that there is no meiotic pairing between *chilense* and wheat chromosomes in the amphiploid derived from *H. chilense* × *T. aestivum* hybrids. This assumption was later extended to the amphiploid *H. chilense* × *T. turgidum* conv. *durum* (Martín and Sánchez-Monge Laguna 1982). Our observations are in agreement with that finding. Only two multivalents (one trivalent and one quadrivalent, see Fig. 1 b) were detected in the analysis of 91 PMCs of the amphiploid. These multivalents involved only wheat chromosomes, as was deduced by the heterochromatic pattern of bands of the associated chromosomes. According to the above-mentioned authors the lack of meiotic pairing between the wild barley and wheat chromosomes will be a handicap in trying to interchange genetic information between both species, but it will bring about better meiotic regularity in the amphiploid, resulting in diploid-like behaviour.

The wheat multivalents observed could be explained in terms of homoeologous pairing ($AA \equiv BB$) and a minor effect could be assumed of some genetic system of *chilense* chromosomes present in the amphiploid promoting non-homologous pairing of wheat chromosomes. However, this meiotic disturbance is remarkably low (total mean number of multivalents = 0.02) and could not have an effect on fertility.

A number of amphiploids have been artificially synthesized in the tribe *Triticeae* and the most promising recognized by many breeders has been the synthetic species triticale (× *Triticosecale* Whittmack). Because of its similar cytogenetic origin comparisons between meiotic characteristics of hexaploid triticale (2n = 42; *AA BB RR*) and the hexaploid tritordeum (2n = 42; $AA\ BB\ H^{ch}H^{ch}$) could be of interest.

Univalents at first metaphase of meiosis are one of the several limitations to the commercial utilization of triticale. The mean frequency of univalents observed in hexaploid triticale was near to 2 (range 0–10) (Sánchez-Monge 1958). This figure has been subsequently confirmed in other triticale lines (for review, see Gupta and Priyadarshan 1982).

The possibilities of using the amphiploid tritordeum in cereal breeding as a new crop were discussed by Martín and Cubero (1981) and Martín and Sánchez-Monge Laguna (1982). The pairing observed in the amphiploid now studied (total mean number of univalents/PMC = 6.35) is lower than observed by Martín and Sánchez-Monge Laguna (1982) in a study on meiotic stability in the same line of tritordeum using conventional staining techniques (total mean number of univalents/PMC = 2.68). This could be explained because of environmental differences of growth since our material was maintained under greenhouse conditions, whereas the line of tritordeum studied by the above-mentioned authors grew in the field.

Giemsa staining techniques have permited recognition that univalents are mainly rye chromosomes in hexaploid triticale (Lelley 1975; Thomas and Kaltsikes 1974, 1976; Merker 1976; Roupakias and Kaltsikes 1977; Naranjo and Lacadena 1980, 1982). A decreasing effect of large telomeric heterochromatin bands of rye chromosomes has often been discussed in all these papers.

The patterns of distribution of the constitutive heterochromatin in *chilense* chromosomes are similar to those in *A* and *B* genomes of wheat, all exhibiting relatively short C-bands proximal to the centromere together with usually thin bands in intercalary positions and only occasionally faint telomeric bands. Naranjo and Lacadena (1982) using C banding observed that five identified pairs of rye chromosomes (1R, 2R, 3R, 6R and 7R/4R) give a total of 42 univalents and 718 bivalents in a analysis of 152 PMCs at first metaphase of hexaploid triticale 'Cachirulo'. The remaining unidentified rye chromosomes (4R/7R and 5R) and the 14 pairs of wheat chromosomes (A and B genomes) give a total number of 21 univalent pairs and 2411 bivalents. Thus, only the five identified rye chromosomes showed a relatively high contribution to univalent formation

(42/3192 = 0.013) with respect to the total frequency of univalents (63/3192 = 0.019).

The frequency of univalents in the line of tritordeum now analyzed was relatively higher for *chilense* (140/637 = 0.219) than for *A* and *B* genomes (148/1274 = 0.116). However, tritordeum shows similar figure for the absolute contribution to univalency from chromosomes of both parental species, this being in disagreement with the results on meiotic instability in triticale mainly attributed to rye genome.

The differences in the pairing level found for the nine identified chromosomes of wheat clearly suggest an influence of heterochromatin on meiotic pairing together with a possible effect of chromosome length.

The increased level of pairing that chromosomes 2B and 4B exhibit could be explained in terms of intercalary heterochromatin interfering with the process to terminalisation of chiasmata. A similar effect of C-bands on maintainance of chiasmata was observed by Santos and Giráldez (1978) in *Chorthippus biguttulus* L. (Acrididae, Orthoptera), and by Loidl (1979) in *Allium flavum*.

Moreover, Sallee and Kimber (1979) assumed that different pairing level showed by chromosomes of different genomes in wheat could be related with its differences in length. Our results are in agreement with this general assumption. Thus, H^{ch} genome chromosomes, having a middle size of 6.84 μm per chromosome, show a relatively lower number of ring bivalents then *A* and *B* genome chromosomes, that have a middle size of 9.94 μm and 10.57 μm, respectively (data from Fernández and Jouve 1984). Other characteristic cytomorphological circumstances, such as the presence of satellites, relative arm length, arm-to-arm differences in the amount and distribution of heterochromatin could act to influence the variation in pairing behaviour observed for each pair of chromosomes.

The meiotic stability of different genomes put together in tritordeum, and its good agronomic characters reported by Martín and Sánchez-Monge Laguna (1982) permits to assume that this new synthetic crop can be considered as a good starting point for direct or indirect utilization in breeding programmes for cereals.

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