

THE EFFECT OF GENES
ON A AND B CHROMOSOMES OF A NUMBER OF TRITICINAE
ON MEIOTIC BEHAVIOUR IN *TRITICUM AESTIVUM*
AND ITS HYBRIDS WITH RELATED SPECIES

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Wanda S. Viegas

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ON MEIOTIC BEHAVIOUR IN *TRITICUM AESTIVUM*
AND ITS HYBRIDS WITH RELATED SPECIES

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CONTENTS

Introduction	1
Articles:	
I. Viegas, W.S. - The effect of B-chromosomes of rye on chromosome association in F_1 hybrids <i>Triticum aestivum</i> X <i>Secale cereale</i> , in the absence of chromosome 5B or 5D, respectively. In press in Theor. Appl. Genet.	4
II. Viegas, W.S. - The effect of B-chromosomes of rye on chiasma frequency in <i>Triticum aestivum</i> in the presence and absence of chromosome 5D. In press in Genetica.	17
III. Neijing, M.A. and Viegas, W.S. - The effect of rye B-chromosomes on meiotic stability of rye-wheat hybrids in normal, nulli 5B and nulli 5D background. In press in Genetica.	30
IV. Viegas, W.S., Feldman, M. and Mello-Sampayo, T. - A suppressor for chromosome association in a mutant isochromosome 5D ^L of <i>Triticum aestivum</i> . Sent for publication in Canad. J. Genet. Cytol.	41
V. Viegas, W.S. - Genes in diploid Triticinae, compensating for the absence of the temperature regulating gene <i>Ltp</i> in chromosome 5D of <i>Triticum aestivum</i> . In press in Theor. Appl. Genet.	51
VI. Viegas, W.S. - Modulation of rRNA gene content by chromosome 5D in wheat. In press in Genetica.	65
Summary and conclusions	79
Acknowledgements	83
Curriculum Vitae	84

ADM. TIJDSCR. AGR.

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STELLINGEN

I

With few exceptions, genes on B-chromosomes interact with genes on A-chromosomes in the regulation of chromosome association in meiosis.

Müntzing, A., Ann. Rev. Genet.,
8, 243-266, 1974.

II

The interaction between genes on A and B chromosomes in the regulation of meiotic chromosome association is often apparent only in situations of unbalance. For instance, in hybrids *Triticum aestivum* X *Secale cereale*, B-chromosomes of the latter species increase chromosome association only in the absence of chromosome 5D of the former.

This thesis.

III

Increase in the number of rDNA copies in *T. aestivum* can considerably increase the protein content of the seed. The presence of a segment of chromosome 5D in *T. durum* increased the number of rDNA cistrons in 25% and the protein content from 12% to 19%.

Viegas, W.S.; unpublished.

IV

Spontaneous mutations occur that can impair chromosome association in hexaploid wheat. It may be that the *Ph* gene appeared in this way.

V

Only *in situ* DNA/rRNA hybridization studies can resolve difficulties found in the interpretation of *in vitro* hybridizations.

VI

The enormous and valuable gene pool existent in wild species of wheat and other species of the grass family contains potential sources for the improvement of wheat.

Sears, E. R.; Ann. Rev. Genet.,
10, 31-51 1976.

VII

When using multiple *in vitro* fertilization of Chinese hamster oocytes by human sperm for sperm karyotyping, it is necessary to include all late dividing nuclei unless it has been demonstrated that the genotype of the sperm nucleus has no effect on its own division. Late dividing nuclei may well overrepresent abnormal types.

Gulcan and Sybenga, J.; *Genetica*,
38, 163-170, 1967.

VIII

There is no more fascinating problem than the study of the kind of people that do problem finding since they undoubtedly form a scientific bottleneck.

Mackworth, N.H.; The 11th Vandyke
Bingham Mem. Lec., Harvard Univ.,
1974.

IX

In the nature-nurture controversy women identify more easily with nurture. Besides, they discovered agriculture.

Wanda S. Viegas

Wageningen, 27th June 1979.

INTRODUCTION

Chromosome pairing is the first prerequisite for the recombinational transfer of genes. The identification of the genes and processes regulating chromosome behaviour in general and chromosome pairing in particular is important for the understanding of the fundamental mechanisms involved in, and ultimately determining the results of plant breeding.

In our studies, species of the sub-tribe of the Triticinae have been used. This sub-tribe includes the genera *Triticum*, *Aegilops* (many species of which are presently often considered to belong to *Triticum*), *Agropyron*, *Eremopyron*, *Haynaldia* and *Secale*. Between these genera hybridization is possible. Wheat (hexaploid and diploid species of the genus *Triticum*) is the most important crop plant in the world, and its improvement is of great consequence. In this improvement, the related genera of the Triticinae, which are a considerable repository of desired characters (Sears, 1976), can play an important role.

The main obstacle to the transfer to wheat of genes from its relatives has been the low degree of pairing in the F_1 hybrids and their derivatives. The identification in the long arm of chromosome 5B of polyploid wheats of a major suppressor gene (*Ph*) preventing pairing between homoeologous chromosomes (Okamoto, 1957; Riley and Chapman, 1958), and the subsequent possibility of its elimination allowed the transfer of alien genes by recombination to wheat. Several other genes either suppressing or promoting homologous and homoeologous chromosome pairing at normal or low temperatures in wheat were discovered afterwards (for a review see Sears, 1976). The complexity of the genetic system regulating chromosome association appeared to be even greater than previously suspected when genes carried by *T. speltoides* and *T. tripsacoides* were found to inhibit the action of *Ph* in F_1 hybrids. Moreover, genes in B-chromosomes of these species were found to have a suppressive effect on chromosome pairing.

Chromosome 5D of wheat is of special interest. Besides having genes which control chromosome association at normal (in 5D^S) and low tem-

perature (in 5D^L), it carries a weak nucleolar organizer, one of the three existing in *T. aestivum*, hexaploid bread wheat. The effect of chromosome 5D on the total number of rDNA copies has been suggested to be higher than may be expected from the sole effect of the nucleolar organizing region (NOR) it carries.

Throughout this thesis I have emphasized the role of chromosome 5D (and to a lesser extent that of 5B), and its interaction with the genomes of diploid Triticinae, including the B-chromosomes of *S. cereale* (rye), in the control of meiosis.

The first three articles bear special relevance to the interaction between genes on B-chromosomes of rye and on chromosomes 5B and 5D of *T. aestivum*. The first concerns chromosome association in the F₁ hybrid *T. aestivum* X *S. cereale*, the second in *T. aestivum* itself, whereas the third is on meiotic regularity in general, again in the hybrid. In this paper, the analysis of the effect of rye B-chromosomes in the euploid and nulli-5B is by M. Neijzing.

In the fourth paper, a spontaneous mutation in the long arm of 5D, inhibiting homoeologous pairing in F₁ hybrids between wheat and closely related diploids is studied. The mutant was discovered by T. Mello-Sampayo.

The fifth deals with the detection in diploid triticinae of genes compensating for the absence of the *Ltp* (low temperature pairing) gene in chromosome 5D.

The sixth is the start of a new approach to the analysis of pairing regulation: by surveying the role of genes and nucleolar organizers in chromosome 5D in the control of rDNA multiplicity, it permits a first exploration of a possible effect of rDNA multiplicity on meiotic behaviour.

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RILEY, R. and CHAPMAN, V.: Genetic control of the cytologically diploid behaviour in hexaploid wheat. *Nature* (Lond.) 182, 713-715 (1958).

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THE EFFECT OF B-CHROMOSOMES OF RYE
ON CHROMOSOME ASSOCIATION IN F₁ HYBRIDS
TRITICUM AESTIVUM X *SECALE CEREALE*
IN THE ABSENCE OF CHROMOSOME 5B OR 5D, RESPECTIVELY

WANDA S. VIEGAS

Instituto Gulbenkian de Ciência, Oeiras, Portugal*

SUMMARY

T. aestivum var. Chinese Spring (monosomic 5B and 5D respectively) was crossed with *S. cereale* (with and without B-chromosomes). The resulting nullisomic 5B hybrids exhibited a high degree of chromosome association both at 20°C and 10°C. The presence of B-chromosomes slightly reduced association, whether 5B is present or not.

In nullisomic 5D hybrids B-chromosomes of rye raise chromosome association at 20°C compared to hybrids with 5D, with as well as without B's. At 10°C, due to the absence of the *Ltp* gene on 5D, chromosome association in nullisomic 5D hybrids is low, and no effect of rye B-chromosomes is detectable.

The hypothesis that B-chromosomes of rye carry (an) asynaptic gene(s) decreasing effective pairing, and (an) independent post-synaptic gene(s) increasing chiasma frequency on effective pairing sites, is presented.

* The work was supported by a fellowship of the Gulbenkian Foundation and partly carried out while the author was at the Department of Genetics, Agricultural University, Wageningen, The Netherlands.

INTRODUCTION

In common wheat *Triticum aestivum* var. Chinese spring ($2n=6x=42$) meiotic chromosome association is controlled by a number of suppressor and promoter genes which are located in several chromosomes of the complement.

In normal plants, the overall effect of this regulatory system is to create a balanced situation in which pairing occurs only between homologous chromosomes, and at a sufficient level. Pairing between homoeologous chromosomes, i.e., those of different related genomes is suppressed mainly by a strong "dominant" suppressor gene, *Ph*, in the long arm of chromosome 5B (Okamoto, 1957; Sears and Okamoto, 1958; Riley and Chapman, 1958; Wall et al., 1971). Minor homoeologous and general pairing suppressors were also found in $3D^L$, $3A^S$ and 4D (Mello-Sampayo and Carnas, 1973; Driscoll, 1973). Dominant alleles of promoters of homoeologous pairing have been referred to be carried by chromosomes $5D^L$, $5A^L$ and $5B^S$ (Feldman, 1966; Riley et al., 1966; Feldman and Mello-Sampayo, 1967; Riley and Chapman, 1967) and in chromosomes $5D^S$ (Feldman, 1968) and $5A_S$ (Dvorak, 1976).

Environmental factors influence the quantitative expression of the genetic component of chromosome pairing (Elliot, 1955; Jain, 1957; Dowrick, 1957). Riley (1966) was able to demonstrate a significant decrease in chromosome association at low temperature in the absence of chromosome 5D. A low temperature gene (*Ltp*) on the long arm of chromosome 5D of *T. aestivum* var. Chinese spring was found to stabilise meiotic pairing under low temperatures (Hayter, 1969). An equivalent to the *Ltp* locus was presumed to exist on chromosome 5A of tetraploid wheats (Hayter and Riley, 1967).

Two diploid species of *Triticum*, often considered to belong to the related genus *Aegilops*, *Triticum speltoides* (= *Aegilops speltoides*) and *Triticum tripsacoides* (= *Aegilops mutica*) have accessory chromosomes which have a suppressive effect on homoeologous pairing, in hybrids with *Triticum aestivum*. This effect was found to be very similar to that of *Ph* of chromosome 5B (Dover and Riley, 1972). Dover (1973) observed, however, that B-chromosomes of *T. tripsacoides* induced asynapsis in F_1 hybrids *T. aestivum* X *T. tripsacoides* at low temperatures. It is known that the

presence of B-chromosomes in rye increases the variability of the frequency of chiasmata (Jones and Rees, 1967). They do not compensate for the absence of *Ph* of 5B in wheat (Roothaan and Sybenga, 1976).

The present paper deals with the study of hybrids between *T. aestivum* (normal and monosomic for chromosome 5B or 5D) and inbred lines of *S. cereale* with and without B-chromosomes, at two temperatures.

MATERIAL AND METHODS

All wheat genotypes used were derived from stocks originally obtained by E. Sears (University of Missouri) of *Triticum aestivum* variety Chinese spring ($2n=6x=42$). They were either monosomic 5D, monosomic 5B or nullisomic 5B-tetrasomic 5A.

Secale cereale plants with standard B-chromosomes were from an original Transbaikal accession obtained from A. Müntzing at the University of Lund. These B-chromosomes had been transferred earlier to rye material of the Genetics Department, University of Wageningen. This was done by crossing inbred lines of rye without B-chromosomes with the original Transbaikal accessions carrying B-chromosomes. Plants from subsequent generations were crossed and backcrossed six times with different inbred lines.

Hybrids were obtained by crossing *Triticum aestivum* (monosomic 5D, monosomic 5B or nullisomic 5B-tetrasomic 5A), as the female parent, and *Secale cereale* (with or without B-chromosomes).

The hybrids were cytologically selected from the segregating F_1 generation. Chromosome numbers in all the tested plants were checked in the root-tips of germinating seedlings after pre-treatment with 1-bromonaphthalene and fixation in acetocarmine. The hybrids studied were either normal or devoid of chromosomes 5B or 5D, or disomic for 5A, with or without B-chromosomes of rye.

The plants were grown in winter in Portugal in the greenhouse (normal temperature $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) until they were one month old, well before the differentiation of meiotic cells. Then, they were sectioned in two halves. One of these was kept in the greenhouse and the other transferred

to a growth chamber at 10°C temperature and continuous light.

To estimate the chiasma frequency, anthers at first metaphase of meiosis were fixed in acetic-alcohol (1:3) and acetocarmine squash preparations were made. For each hybrid samples of 50-100 pollen mother cells (PMC's) were studied in three to five plants of each type. In the statistical analysis t-tests were used throughout.

At first metaphase of meiosis, the observation of chiasmata implicates that pairing occurred and that it was followed by crossing-over between synapsed regions of chromosomes. Metaphase association of chromosome arms looked convincingly like chiasmata and were considered as such. Since the presence of more than one chiasma per association was very rare, for convenience all associations were considered equivalent to one chiasma.

RESULTS

Chromosome association at 20°C and 10°C in F_1 hybrids *Triticum aestivum* (normal and monosomic 5D) X *Secale cereale* (with and without B-chromosomes) is summarized in Table 1 (10°C) and Figure 1 (20°C). It was not considered necessary to present tables and distributions (figures) for both temperatures.

In plants grown at 10°C no significant differences in chromosome association, which was notably low, were detected. In the absence of B-chromosomes of rye no difference could be observed between plants with or without chromosome 5D at both temperatures. A significant discrepancy becomes apparent at 20°C when chromosome association of plants carrying rye B-chromosomes was compared. A rise in pairing was found in absence of chromosome 5D, compared to that in the presence of this chromosome. The increase in chiasma frequency observed was from 0.58 in 28+2B plants to 2.28 in 27+2B, which is statistically significant at $P = 0.001$. Three B-chromosomes or one B-chromosome with a long-arm iso-chromosome have a very similar effect. The low chiasma frequency of 0.80 at 10°C (in the presence of rye B-chromosomes) in the absence of 5D must be concluded

to be due to the destabilizing effect of absence of *Ltp* at low temperature. The difference with 2.28 at 20°C is significant ($P = 0.001$).

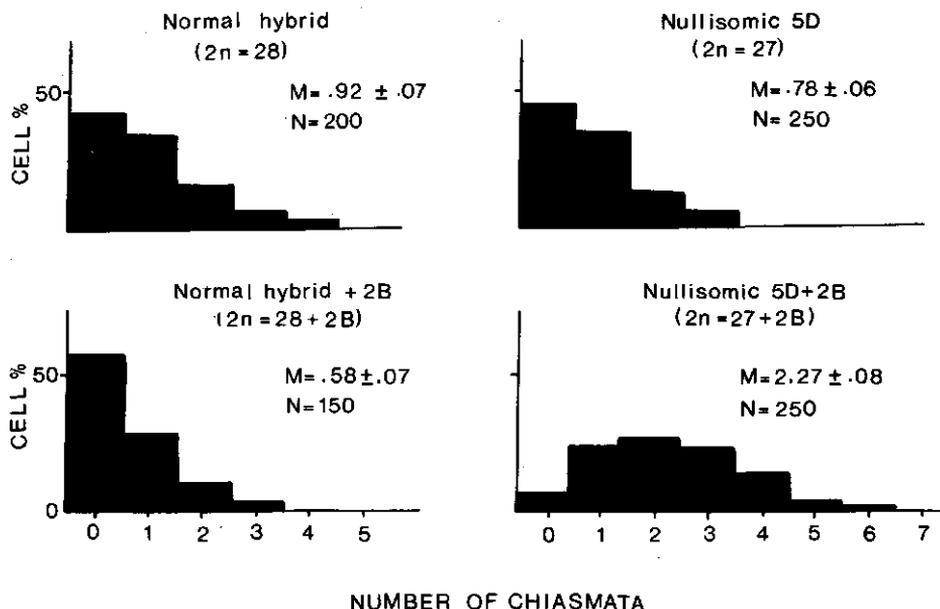


Figure 1. Distribution of chiasma frequencies per cell in hybrids *T. aestivum* X *S. cereale* at 20°C in the presence and absence of chromosome 5D of wheat and of B-chromosomes of rye.

Low means of 0.74 and 0.56 chiasmata per cell were observed at first metaphase in F_1 hybrids of normal *T. aestivum* X *Secale cereale* at 20°C and 10°C, respectively (Table 1). At this level an effect of absence of 5D cannot be expressed.

Table 1 - Mean chromosome association at low temperature ($10^{\circ}\text{C} \pm 2$) in taphase of the first division of meiosis in F1 hybrids *Triticum aestivum* ♀ (normal end monosom 5D) X *Secale cereale* ♂ (with and without B-chromosomes). (50 cells per plant).

PLANT N°	CHROMOSOME N°	CHROM 5D	A - C H R O M O S O M E S					B - C H R O M O S O M E S						
			UNIVA	BIVALENTS			CHIASM P/CELL	UNIVA	BIVALENTS			CHIASM P/CELL		
				RODS	RINGS	TOTAL			RODS	RINGS	TOTAL			
73	28	present	27.08	0.44	0.02	0.46	0.48							
90	28	present	26.84	0.56	--	0.58	0.58							
93	28	present	27.20	0.40	--	0.40	0.40							
197	28	present	26.20	0.90	--	0.90	0.90							
75	28 + 2B	present	26.88	0.56	--	0.56	0.56	0.48	0.76	--	0.76	0.76	0.76	0.76
87	28 + 2B	present	26.60	0.70	--	0.70	0.70	0.20	0.62	0.08	0.90	0.98	0.98	0.98
198	28 + 2B	present	27.28	0.34	0.02	0.36	0.38	0.16	0.88	0.04	0.92	0.96	0.96	0.96
89	27	absent	26.00	0.48	0.02	0.50	0.52							
90	27	absent	26.32	0.34	--	0.34	0.34							
91	27	absent	25.36	0.82	--	0.82	0.82							
100	27	absent	26.44	0.28	--	0.28	0.28							
101	27	absent	25.24	0.88	--	0.88	0.88							
86	27 + 2B	absent	25.52	0.74	--	0.74	0.74	0.12	0.80	0.14	0.94	1.08	1.08	1.08
152	27 + 2B	absent	25.12	0.94	--	0.94	0.94	0.32	0.84	--	0.84	0.84	0.84	0.84
155	27 + 2B	absent	25.08	0.88	0.08	0.96	1.04	0.24	0.78	0.10	0.88	0.98	0.98	0.98
156	27 + 2B	absent	26.20	0.40	--	0.40	0.40	0.44	0.70	0.08	0.78	0.86	0.86	0.86
161	27 + 2B	absent	25.20	0.90	--	0.90	0.90	0.48	0.76	--	0.76	0.76	0.76	0.76
154	27 + 3B	absent	25.24	0.88	--	0.88	0.88	1.28	0.86	--	0.86	0.86	0.86	0.86
157	27 + 3B	absent	25.16	0.88	0.04	0.92	0.96	1.20	0.86	0.04	0.90	0.94	0.94	0.94
158	27 + 3B	absent	25.36	0.80	0.02	0.82	0.84	1.38	1.36	--	1.36	1.36	1.36	1.36
96	27+1B+isoB	absent	25.72	0.64	--	0.64	0.64	2.00	--	--	--	0.00	0.00	0.00
99	27+1B+isoB	absent	25.44	0.76	0.02	0.78	0.80	2.00	--	--	--	0.00	0.00	0.00

Hybrids deficient for chromosome 5B showed higher pairing at both temperatures [Table 2, Figure 2]. At 20°C, it was observed that the presence of two or four B-chromosomes of rye have a slight but not significant suppressive effect on association, whether chromosome 5B is present or not ($P = 0.09$).

Hybrids nullisomic for 5B showed no effect of rye B-chromosomes (Roothaan and Sybenga, 1976), but, as expected, homoeologous association was greatly increased. Addition of an extra chromosome 5A (disomic in the hybrid) resulted in a further rise in number of chiasmata per cell, both at 10°C and at 20°C. At 20°C the average chiasma frequency in nulli 5B, disomic 5A was 8.59, in nulli 5B 6.71, which is statistically significant even when the extra bivalent due to disomy of 5A is taken into account ($P = 0.05$) (Figure 2). B-chromosomes of rye reduced chiasma frequencies slightly and not significantly. They increased the variability.

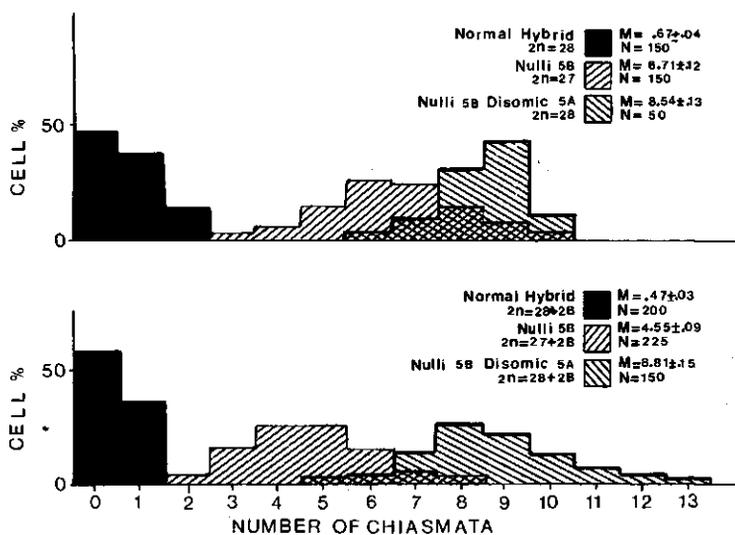


Figure 2. Distribution of chiasma frequencies per cell in F_1 hybrids *T. aestivum* X *S. cereale* at 20°C in the presence and absence of chromosome 5B of wheat and of B-chromosomes of rye, with chromosome 5A of wheat in monosomic and disomic condition.

Table 2 - Mean chromosome association at low temperature ($10^{\circ}\text{C} \pm 2$) in metaphase of the first division of meiosis in F_1 hybrids *Triticum aestivum* ♀ (normal, monosomic 5B and nullisomic 5B-tetrasomic 5A) X *Secale cereale* ♂ (with and without B-chromosomes). (50 cells per plant).

PLANT N°	CHROMOSOME N°	CHROM SB	A - CHROMOSOMES					B - CHROMOSOMES						
			UNIVA	B I V A L E N T S		TRIV	CHIASM P/CELL	UNIVA	B I V A L E N T S		CHIASM P/CELL			
				RODS	RINGS				TOTAL	RODS		RINGS	TOTAL	
82	28	present	26.96	0.52	--	0.52	--	0.52	--	0.52	0.12	0.86	0.94	1.02
83	28	present	26.76	0.62	--	0.62	--	0.62	--	0.62	0.12	0.80	0.94	1.08
196	28	present	26.92	0.54	--	0.54	--	0.54	--	0.54	0.06	0.90	0.86	1.02
84	28 + 2B	present	26.80	0.56	--	0.56	--	0.56	--	0.56	0.40	0.80	0.80	0.80
85	28 + 2B	present	26.92	0.54	--	0.54	--	0.54	--	0.54	0.12	0.80	0.94	1.08
172	28 + 2B	present	26.96	0.52	--	0.52	--	0.52	--	0.52	0.06	0.90	0.86	1.02
199	28 + 2B	present	26.48	0.76	--	0.76	--	0.76	--	0.76	0.40	0.80	0.80	0.80
81	27	absent	17.58	4.12	0.38	4.50	0.14	5.16						
213	27	absent	17.28	4.52	0.34	4.66	--	5.20						
214	27	absent	18.94	3.02	0.80	3.82	0.14	4.90						
168	27 + 2B	absent	18.42	3.76	0.50	4.26	0.02	4.80			0.28	0.74	0.86	0.98
170	27 + 2B	absent	17.80	4.38	0.22	4.60	--	4.82			0.04	0.82	0.98	1.14
171	27 + 2B	absent	19.52	3.74	--	3.74	--	3.74			0.12	0.94	0.94	0.94
212	27 + 2B	absent	13.32	6.28	0.56	6.84	--	7.40			0.32	0.84	0.84	0.84
215	27 + 2B	absent	18.68	4.14	0.02	4.16	--	4.18			0.28	0.82	0.86	0.90
169	27 + 4B	absent	17.56	4.30	0.42	4.72	--	5.14			1.20	1.40	1.40	1.40
216	27 + 4B	absent	17.20	4.02	0.58	4.60	0.20	5.58			0.12	1.74	1.94	2.14
162	28 + d15A	absent	12.58	5.84	1.44	7.38	0.22	9.26						
165	28+d15A+2B	absent	13.16	5.68	1.68	7.36	0.04	9.12			0.04	0.96	0.98	1.00
166	28+d15A+2B	absent	14.32	4.96	1.58	6.54	0.20	8.52			0.12	0.82	0.94	1.06
190	28+d15A+2B	absent	12.44	6.48	1.00	7.48	0.20	8.68			0.24	0.78	0.88	0.98

DISCUSSION

Hybrids between *Triticum aestivum* and either *T. speltoides* (*Aegilops speltoides*) or *T. tripsacoides* (*Aegilops mutica*) show that a digenic system existing in the last two diploid species is epistatic to the suppressor gene, *Ph*, carried by chromosome 5B of *T. aestivum* (Riley et al., 1961; Riley and Law, 1965). On the other hand, hybrids deficient for chromosome 5B have shown low homoeologous pairing, if accessory chromosomes from *T. speltoides* or *T. tripsacoides* are present (Dover and Riley, 1972).

The genome of *Secale cereale* has no apparent effect in raising homoeologous pairing in normal hybrids with *T. aestivum*. In the absence of chromosome 5B, the hybrids, as expected, display higher chromosome association at 10°C and 20°C (Figure 2). An increased dosage of chromosome 5A, significantly raises chromosome association in nullisomic 5B hybrids even further. Therefore, the effect of the pairing promoter carried by chromosome 5A (Feldman, 1966) is additive to the effect of the absence of 5B. The presence of accessory chromosomes of rye in F₁ hybrids *T. aestivum* X *S. cereale* seem to have a slight suppressive effect on chromosome association whether chromosome 5B is present or not, at 20°C (Figure 2).

In these hybrids chromosome association is usually very low and the variability is always very high, both between P.M.C.'s and between plants of the same genotype. Sometimes chromosome associations are observed in rye as a consequence of chromosome stickiness and the existence of large heterochromatic segments at the end of the chromosomes (Lima-de-Faria, 1952; Sarma and Natarajan, 1973). This non-homologous association is a source of error in the interpretation of meiotic configurations (John and Lewis, 1965). These problems make it sometimes difficult to detect differences in chromosome association induced for instance by B-chromosomes of rye. This may be the reason why the differences in chromosome pairing were not statistically significant (9% level of confidence).

No differences in chromosome association were detected in hybrids with or without chromosome 5D, either at low or normal temperatures. Chromosome 5D is referred as a carrier of a temperature stabilizer [*Ltp*]

gene of chromosome pairing (Riley, 1966). It was expected that the absence of this chromosome in the hybrids would produce a slight decrease in association at low temperature. That this difference was not detected in the present experiments, probably is a consequence of the low level of association in *T. aestivum* X *S. cereale* hybrids.

Plants deficient for chromosome 5D and simultaneously carrying two or three B-chromosomes, at 20°C show a significant rise in chromosome pairing in relation to the hybrids carrying the same dosage of B-chromosomes but not deficient for chromosome 5D (Figure 1).

Jones and Rees (1967) have found that B-chromosomes of rye increased variability in chiasma frequencies among P.M.C.'s and an intra-bivalent asymmetry in chromosome arm association and chiasmata distribution. Taking into consideration the overall constancy of mean number of chiasmata, which they found in all combinations (with or without B-chromosomes) it may be concluded that B-chromosomes increase the density of chiasma in each pairing chromosome arm (segment). In our hybrids the increase in chiasma frequency might be similarly explained by an effect of B-chromosomes on chiasma density, in the absence of chromosome 5D.

Homoeologous chromosome association between wheat and rye is always low. Besides the presynaptic effect of genes affecting the proper alignment of chromosomes (Feldman, 1966) there may exist in those regions where homoeologous chromosomes associate at pachynema, a discontinuous succession of homologous segments for legitimate synapsis. Those segments are potential crossover regions. Increased local density of chiasmata would correspond to a higher probability of crossing-over per unit length of paired segments. This would result in a higher probability of crossing-over at metaphase I per paired homologous segment.

If rye B-chromosomes have an effect on chromosome behaviour in absence of 5D irrespective of temperature (although not detectable at low temperatures) which they do not have in the presence of 5D, necessarily 5D must have meiotic effects in addition to *Ltp*. Also, rye B-chromosomes must have several effects. Perhaps, like some genotypes of *Ae. speltoides* but unlike their B-chromosomes, rye B's might slightly overrule *Ph* in 5B and permit some homoeologous pairing unless counteracted by a general

pairing restricting gene in 5D. Thus, two effects should be attributed to 5D (1. *Ltp*; 2. slight asynapsis) and three to rye B-chromosomes (1. homoeologous pairing in the presence of 5B; 2. general pairing restriction; 3. increased chiasma formation on effective pairing sites). Under normal conditions effect 3 balances 2, together causing increased variation. In absence of 5D, effect 1 is sufficiently strong in combination with 3, to counteract 2, but at low temperatures there simply is not enough pairing for 1 and 3 to have a detectable effect. In the presence of 5D, the combined pairing restricting effects of 5D and effect 2 are strong enough to balance 1 and 3.

This explanation is, of course, speculative. It is not clear for instance, why increased chiasma formation at effective pairing sites does not greatly increase chiasma frequency in combinations of rye B-chromosomes and a double dose of 5A, which carries a pairing promotor.

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THE EFFECT OF B-CHROMOSOMES OF RYE
ON CHIASMA FREQUENCY IN *TRITICUM AESTIVUM*

WANDA S. VIEGAS

Instituto Gulbenkian de Ciência, Deiras, Portugal*

SUMMARY

The influence of B-chromosomes of *Secale cereale* on chromosome association at metaphase of the first division of meiosis in an inter-varietal *T. aestivum* hybrid Chinese spring X Lindström (carrying rye B-chromosomes) was studied in the presence and absence of chromosome 5D of wheat.

The presence of rye B-chromosomes did not change the normal pattern of chromosome association in disomic and significantly though slightly increased chiasma frequency in monosomic 5D plants at 20°C. When chromosome 5D was absent, this increase was more pronounced, especially in respect to the number of ring bivalents. It is suggested that this increase is a consequence of an additive effect of postsynaptic gene(s) in rye B-chromosomes which locally increased chiasma frequency in A-chromosomes, and of the absence of (a) desynaptic gene(s) of chromosome 5D. Even in nullisomic 5D plants, at 10°C, where a high degree of asynapsis was observed, the addition of B-chromosomes increased chromosome association, but then there was no observable increase in the monosomics, which at 10°C did only show a slight reduction in pairing.

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INTRODUCTION

The chromosomes of the three constituent genomes of hexaploid wheat, *Triticum aestivum* ($2n=6x=42$) although closely related, do not pair at meiosis (Sears, 1952). The mechanism which prevents homoeologous pairing is complex and believed to be based on a delicate balance between suppressor and promoter genes for pre-meiotic and meiotic association (Feldman, Mello-Sampayo and Sears, 1966). Okamoto (1957) and Riley and Chapman (1958) found that the main suppressor gene(s) for homoeologous pairing (*Ph*) was (were) located on chromosome 5B. Other suppressor genes have been detected on the short arm of chromosomes 3D and 3A (Mello-Sampayo and Canas, 1973) and on chromosome 4D (Driscoll, 1973). Several pairing promoting genes have been identified in both arms of chromosomes 5D (Feldman, 1961, 1968) and 5A (Feldman, 1968; Dvorak, 1976) and on the short arm of chromosome 5B (Riley and Chapman, 1967; Feldman and Mello-Sampayo, 1967). The long arm of chromosomes 3D and 3B and the short arm of chromosome 2A also carry other promoter genes (Driscoll, 1972; Sears, 1954).

A low temperature stabilizer gene (*Ltp*), the absence of which results in a high degree of asynapsis at 15°C and lower, has been identified in chromosome 5D of *T. aestivum* (Riley, 1966). An equivalent to *Ltp* was presumed to exist on chromosome 5A of tetraploid wheats (Hayter and Riley, 1967). The presence in related diploid species of genes comparable to *Ltp* was demonstrated by good chromosome association at low temperature in nullisomic 5D hybrids *T. aestivum* X *T. tripsacoides* (= *Aegilops mutica*) (Vardi and Dover, 1972), *T. aestivum* X *T. speltoides* (= *Ae. speltoides*) (Attia et al., 1977; Viegas, 1979b) and *T. aestivum* X *T. longissimum* (= *Ae. longissima*) (Attia, 1977). Viegas (1979a), however, found no compensation for the *Ltp* gene in hybrids *T. aestivum* X *Secale cereale*, in the absence of chromosome 5D. B-chromosomes of *T. tripsacoides* induce asynapsis at low temperature in F_1 hybrids *T. aestivum* X *T. tripsacoides* (Vardi and Dover, 1972), apparently counteracting a *Ltp* gene elsewhere in the genome. B-chromosomes of rye (*S. cereale*) in nullisomic 5D hybrids *T. aestivum* X *S. cereale* increase chromosome association at 20°C but hardly at 10°C (Viegas, 1979a).

The present work is concerned with the study of the effect of B-chromosomes of *S. cereale* on chromosome association in an inter-varietal *T. aestivum* hybrid Chinese spring X Lindström (disomic, monosomic and nullisomic 5D) at normal (20°C) and low temperature (10°C), with rye B-chromosomes from Lindström.

MATERIAL AND METHODS

Plants of *T. aestivum* var. Chinese spring (euploid and monosomic 5D) were grown from seed stocks originally obtained by Dr. E. Sears, University of Missouri; seeds of Lindström strain of *T. aestivum* were obtained from Dr. A. Müntzing, Institute of Genetics, University of Lund, Sweden. The Lindström strain is a spring wheat carrying accessory chromosomes which were transferred by crossing a variety from Nepal with a spring rye variety from Transbaikal, Siberia, followed by spontaneous chromosome doubling and backcrossing to a wheat variety. Inter-varietal hybrids Chinese spring X Lindström were made using the variety Chinese spring as the female parent, either as normal disomic or as a monosomic 5D. The control (without B-chromosomes) was also obtained from the same crosses.

On the basis of analysis of root-tip mitoses (pre-treatment in 1-bromonaphthalene, fixation and staining in acetocarmine) the desired F_1 hybrids were selected (disomic and monosomic 5D both with and without B-chromosomes of rye), grown under normal greenhouse conditions, and selfed. Disomic, monosomic and nullisomic 5D hybrids with or without B-chromosomes were subsequently selected from the segregating F_2 generation, again using root-tip mitoses of germinating seedlings.

The F_2 hybrids were grown in the greenhouse in winter time. Plants were longitudinally split after one month of growth, so that two independent, genetically identical, plants were obtained. One of these was kept at 20°C, other at 10°C, both under continuous light.

To estimate chiasma frequency, anthers at metaphase of the first division of meiosis were fixed in acetic alcohol (1:3) and permanent squash preparations were made with Euparal after acetocarmine staining.

From each hybrid combination, samples of 50-150 pollen mother cells (PMC's) were studied, and the number of chiasmata per cell and the frequency of different configurations, recorded. Since almost never more than one chiasma was observed in a chromosome arm, for convenience the number of associated arms at MI was considered equivalent to the number of chiasmata, and used in its place. To be able to compare chiasma frequencies of disomic plants, where 42 chromosomes are available for chiasma formation, with monosomic 5D and nullisomic 5D plants where only 40 can crossover, a numerical correction was used. The extra bivalent of disomic plants can be a ring or a rod, the probabilities of which were assumed to be identical to those seen in the other chromosomes. Comparisons between different combinations were made using t-tests. As only one nullisomic 5D plant was available, the t-test used, in this case, was based on the standard error of the cell population.

RESULTS

Chromosome association in inter-varietal *T. aestivum* hybrids Chinese spring X Lindström is summarized in Tables 1 and 2 for plants grown at 20°C and 10°C, respectively. These hybrids were either disomic, monosomic or nullisomic 5D, and with or without B-chromosomes of *S. cereale*.

20°C

In disomic 5D hybrids growing at 20°C ± 2°C no significant difference in chiasma frequency was observed between plants without or with B-chromosomes (36.42 ± .93 and 35.79 ± .56, respectively). In monosomic 5D hybrids the presence of B-chromosomes significantly increased (.05 > P > .02) the level of chromosome association (mean chiasmata per cell 31.70 ± ± 1.09 and 34.71 ± .32, respectively). Comparisons between disomic and monosomic 5D plants without B-chromosomes, did not, however, show any difference. No significant difference was observed between the same hybrids when B-chromosomes were present. The presence of B-chromosomes in nullisomic 5D plants significantly increased (P = 0.02) chromosome associa-

Table 1 - Mean chromosome pairing in *T. aestivum* at 20°C in first metaphase of meiosis in inter-varietal hybrids Chinese spring X Lindström (disomic, monosomic and nullisomic 5D) with or without B-chromosomes of *Secale cereale*.

PLANT N°	PMC's N°	CHROMO N°	CHROMOSOME 5D	A - C H R O M O S O M E S				B - C H R O M O S O M E S								
				B I V A L E N T S		CHIASM P/CELL	B I V A L E N T S		CHIASM PER TOTAL CHROMO	B I V A L E N T S		CHIASM PER TOTAL CHROMO				
				UNIV	RINGS TOTAL		TRIV	P/CELL		UNIV	RINGS TOTAL		UNIV	RINGS TOTAL		
33	50	42	disomic	0.00	4.65	16.35	21.00	--	37.25	--	--	--	--	--	--	--
27	100	42	disomic	0.14	6.35	14.55	20.90	0.02	35.49	--	--	--	--	--	--	--
111	50	41	monosomic	1.20	5.70	14.20	19.90	--	34.10	--	--	--	--	--	--	--
110	100	41	monosomic	1.00	8.10	11.90	20.00	--	31.90	--	--	--	--	--	--	--
108	50	41	monosomic	1.40	7.60	12.20	19.80	--	32.00	--	--	--	--	--	--	--
127	150	41	monosomic	1.80	10.37	9.20	19.57	0.02	28.84	--	--	--	--	--	--	--
117	150	40	nullisomic	0.20	4.80	15.10	19.90	--	35.00	--	--	--	--	--	--	--
11	50	42+6B	disomic	0.08	7.50	13.46	20.96	--	34.42	--	0.16	2.38	0.54	2.92	0.577	
9	50	42+6B	disomic	--	4.86	16.14	21.00	--	37.14	--	3.44	1.20	0.08	1.26	0.227	
10	100	42+3B	disomic	0.20	6.20	14.70	20.90	--	35.60	--	1.20	1.40	0.20	1.60	0.600	
130	50	42+2B	disomic	--	6.00	15.00	21.00	--	36.00	--	0.40	0.60	0.20	0.80	0.500	
8	100	41+5B	monosomic	1.12	4.30	15.64	19.94	--	35.58	--	1.36	1.42	0.42	1.84	0.452	
24	100	41+4B	monosomic	1.12	6.02	13.92	19.94	--	33.86	--	0.24	1.70	0.18	1.86	0.515	
112	50	41+4B	monosomic	1.12	5.04	14.90	19.94	--	34.64	--	1.16	1.28	0.12	1.40	0.360	
16	100	41+4B	monosomic	1.00	5.90	14.10	20.00	--	34.10	--	0.60	1.40	0.30	1.70	0.500	
129	50	41+2B	monosomic	1.04	4.78	15.20	19.98	--	35.18	--	0.64	0.64	0.04	0.68	0.360	
128	100	40+6B	nullisomic	0.04	1.30	18.65	19.95	--	38.60	--	1.90	1.55	0.30	1.85	0.358	
126	100	40+4B	nullisomic	0.14	3.92	15.98	19.90	0.02	35.92	--	1.30	1.00	0.30	1.30	0.400	
121	50	40+3B	nullisomic	0.20	3.10	16.80	19.90	--	36.70	--	1.84	0.42	0.16	0.58	0.247	

tion (mean chiasmata per cell in plants without B's $35.0 \pm .81$ and in plants with B's $37.07 \pm .79$). In the absence of B-chromosomes, values of chromosome association in nullisomic 5D hybrids were not different from the ones observed both in disomic and in monosomic 5D plants. When nullisomic 5D plants with B-chromosomes were, however, compared with either disomic 5D-with B's or monosomic 5D-with B's plants, a significant increase ($P < .02$) in chromosome association could be observed.

10°C

Table 2 summarizes mean chromosome association at 10°C for the same hybrid combinations described for 20°C. Chromosome association in disomic hybrids with or without B-chromosomes did not show any significant difference (mean chiasmata per cell $36.38 \pm .37$ and $36.17 \pm .33$, respectively). The absence of one chromosome 5D in these hybrids (monosomic 5D) induced a significant drop ($P < .01$) in chromosome association (mean chiasma per cell $30.02 \pm .38$ in the absence of B-chromosomes and $30.26 \pm .35$ in their presence), when compared with values found for disomics 5D. No difference was observed in monosomic 5D plants with or without B-chromosomes, however. Mean chiasmata per cell in nullisomic 5D hybrids was much lower in the absence of B-chromosomes ($3.75 \pm .87$) than in their presence (8.35 ± 2.65) the difference being highly significant ($P < .01$). Comparisons between nullisomic 5D plants without B-chromosomes and disomic or monosomic 5D plants showed a very significant decrease in chromosome association ($P < .001$). The same pattern was observed when B-chromosomes were present in all of these hybrids.

Comparisons between disomic 5D plants growing at 20°C and 10°C, with or without B-chromosomes, showed no differences in chromosome association. Monosomic 5D plants growing at 20°C consistently showed higher chromosome association, with or without B-chromosomes, as compared with the same plants at 10°C ($P < .02$). The same pattern was observed for nullisomic 5D plants but here the difference of the means showed an even higher reduction brought about by the 10°C treatment ($P < .001$).

It was observed throughout all these hybrids that the influence

Table 2 - Mean chromosome pairing in *T. aestivum* at 10°C in first metaphase of meiosis in the inter-varietal hybrids Chinese spring X Lindström (disomic, monosomic and nullisomic 5D) with or without B-chromosomes of *Secale cereale*.

PLANT N°	PMC's N°	CHROMO N°	CHROMOSOME 5D	A - CHROMOSOMES						B - CHROMOSOMES					
				B I V A L E N T S			TRIV	CHIASM P/CELL	UNIV	B I V A L E N T S		CHIASM PER CHROMO			
				UNIVA	RODS	RINGS				TOTAL	RODS		RINGS	TOTAL	
33	50	42	disomic	0.40	4.85	15.95	20.80	--	36.75	--	--	--	--	--	--
27	50	42	disomic	0.60	5.40	15.30	20.70	--	36.00	--	--	--	--	--	--
11	100	41	monosomic	2.50	7.75	11.50	19.25	--	30.75	--	--	--	--	--	--
110	50	41	monosomic	4.20	7.00	11.40	18.40	--	29.80	--	--	--	--	--	--
108	50	41	monosomic	2.50	9.00	10.25	19.25	--	29.50	--	--	--	--	--	--
117	100	40	nullisomic	32.50	3.75	---	3.75	--	3.75	--	--	--	--	--	--
11	100	42+6B	disomic	0.36	5.60	15.22	20.82	--	36.04	3.80	0.88	0.14	1.02	0.193	
9	50	42+6B	disomic	0.40	5.70	15.10	20.80	--	35.90	5.00	0.50	--	0.50	0.083	
10	50	42+3B	disomic	0.40	4.48	16.32	20.80	--	37.12	2.40	0.30	--	0.30	0.100	
130	100	42+2B	disomic	0.48	5.90	14.86	20.76	--	35.62	1.70	0.20	--	0.20	0.100	
8	50	41+5B	monosomic	4.20	6.50	11.90	18.40	--	30.30	4.60	0.20	--	0.20	0.040	
24	100	41+4B	monosomic	3.54	6.22	12.48	18.70	0.20	31.24	2.24	0.88	--	0.88	0.222	
112	50	41+4B	monosomic	2.68	8.38	10.78	19.16	--	28.94	2.64	0.58	--	0.58	0.145	
16	50	41+4B	monosomic	2.12	8.20	11.24	19.44	--	30.68	2.64	0.68	--	0.68	0.170	
129	100	41+2B	monosomic	5.10	6.75	11.20	17.95	--	29.15	1.80	0.10	--	0.10	0.050	
128	100	40+6B	nullisomic	29.00	5.30	0.20	5.50	--	5.70	4.80	0.56	0.04	0.60	0.107	
126	50	40+4B	nullisomic	19.60	9.40	0.80	10.20	--	11.00	4.00	--	--	--	--	

of the B-chromosomes was independent of their number.

DISCUSSION

In general the genetic interaction between B-chromosomes and A-chromosomes is complex. At least it seems that it can control both the level of chromosome pairing and the level of chiasma formation. In hybrids *T. aestivum* X *T. tripsacoides*, at low temperature, B-chromosomes of *T. tripsacoides* are able to induce a high degree of asynapsis due to pre-meiotic chromosome impairment, in spite of the presence of *Ltp* genes (Vardi and Dover, 1972). Moreover, B-chromosomes from *T. tripsacoides* and *T. speltoides* at normal temperature in hybrids *T. aestivum* X *T. tripsacoides*, X *T. speltoides*, have a similar effect to the *Ph* gene. This has been detected when chromosome 5B is absent, through a marked reduction in the extent of homoeologous pairing (Dover and Riley, 1972).

Comparisons between plants without B-chromosomes and plants with B-chromosomes showed that the presence of B-chromosomes enhances chromosome association in nullisomic 5D hybrids grown either at 10°C or at 20°C. This rise was mainly due to a larger count of ring bivalents. In monosomic 5D plants at 20°C, but not at 10°C, an increased chiasma frequency was also observed and was due to a shift among the bivalent configurations. A significant increase ($.05 > P > .2$) in the number of rings was observed in monosomic 5D plants with B-chromosomes ($14.75 \pm .33$) when compared with those without B-chromosomes (11.88 ± 1.03), the frequency of univalents in both combinations being very similar. This corresponds to a decrease in the number of rod bivalents (from $7.94 \pm .96$ in plants without B-chromosomes to $5.21 \pm .33$ in plants with B-chromosomes).

This increase in chiasma frequency in chromosomes already having one chiasma confirms the hypothesis previously put forward that the post-synaptic effect of B-chromosomes of rye on chromosome association in nullisomic 5D hybrids *T. aestivum* X *S. cereale* is mediated through an increase of crossing-over per unit length of paired chromosome (Viegas, 1979a). The absence of chromosome 5D seems to be fundamental to detect the

effect of B-chromosomes in these hybrids. In the absence of chromosome 5B no effect on chromosome association was induced by the presence of B-chromosomes (Roothaan and Sybenga, 1976). Jones and Rees (1967) also showed that in rye the presence of B-chromosomes enhances the appearance of A-chromosomes with an increased number of chiasmata, simultaneously with a greater variability of chromosome association among PMC's, being the overall mean number of chiasmata in all the combinations almost constant. The effect of B-chromosomes of rye in the inter-varietal hybrids of wheat studied showed no dosage dependence. Plants with 2 to 6 B's induced the same quantitative effect on chiasma frequency. This dosage independence was similar to that found in B-chromosomes of *T. tripsacoides* and *T. speltoides* (Vardi and Dover, 1972). Association of B-chromosomes at normal temperature is low and quite rare in plants growing at 10°C. The degree of pairing seems to be generally independent from their noticeable effect on the enhancement of chromosome association of A-chromosomes, in the absence of chromosome 5D.

Chromosome 5D of *T. aestivum* carries a low temperature stabilizer gene (*Ltp*) for chromosome association the absence of which induces a high degree of asynapsis, at 15°C (Riley, 1966). It has been demonstrated that *Ltp* of chromosome 5D controls the pre-meiotic chromosome pairing (Sayliss and Riley, 1972b). In the inter-varietal hybrids of wheat studied at 10°C, the effect of *Ltp* gene was confirmed, both for plants with B-chromosomes and without B-chromosomes. Nullisomic and monosomic 5D plants always show a decrease in chromosome association at 10°C, as compared with disomic plants.

At 20°C, different dosages of chromosome 5D, in the presence of B-chromosomes, showed that the density of crossing-over is modified when chromosome 5D is absent. Nullisomic 5D plants had higher chiasmata frequencies than monosomics or disomics. This increase was mainly due to a shift in the number of ring and rod bivalents, the univalent frequencies in all these combinations being very similar (not, of course, considering the single univalent in monosomics). The frequencies of rod bivalents from nullisomic 5D plants ($2.77 \pm .77$) showed a significant decrease ($.02 > P > .01$) when compared either with disomic 5D ($5.85 \pm .54$) or monosomic 5D

plants ($5.21 \pm .33$). This decrease in the number of rods was complementary to the corresponding increase ($.02 > P > .01$) observed in the frequencies of ring bivalents ($14.12 \pm .55$ in disomics; $14.75 \pm .33$ in monosomics and $17.14 \pm .79$ in nullisomic 5D plants). No significant change was, however, observed in the chiasma frequency per cell between disomic and monosomic 5D plants with B-chromosomes.

Bayliss and Riley (1972a) studied chromosome association of euploid and nullisomic 5D plants of *T. aestivum*, at 23.5°C. Their data show that in the absence of chromosome 5D an increase in the number of ring configurations with more than two chiasmata was observed, when compared with the euploid (average number of these rings 2.9 and 4.8, respectively). The average frequency of chiasmata per chromosome was not, however, significantly different between both combinations. Mello-Sampayo and Miller (1979) analysing mono-telosomic 5D^L, hetero-isosomic 5D^L and tetrasomic 5D plants of *T. aestivum*, at 20°C, found that an increasing dosage of 5D^L induced a corresponding increase in the number of rod bivalents, not changing the chiasma frequency, as well. The results obtained by Bayliss and Riley suggest that the increase in the density of chiasmata per chromosome paired in nullisomic 5D hybrids can be attributed to the existence of a desynaptic gene on that chromosome. Moreover, the data of Mello-Sampayo show that the effect of this desynaptic gene of 5D^L is correlated with increasing dosages of it.

In the presence of 5D, the effect of B-chromosomes of rye on the promotion of chiasmata in paired chromosomes of inter-varietal hybrids of wheat, cannot be observed. Inversely, a higher density of chiasmata per chromosome paired may be obtained in plants where 5D is absent and B-chromosomes present, by their additive effects.

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THE EFFECT OF RYE B-CHROMOSOMES
ON MEIOTIC STABILITY OF RYE-WHEAT HYBRIDS
IN NORMAL, NULLI 5B AND NULLI 5D BACKGROUND

M.G. NEIJZING and W.S. VIEGAS

Department of Genetics, Agricultural University, Wageningen
and
Instituto Gulbenkian de Ciência, Oeiras, Portugal

ABSTRACT

Meiotic synchrony and between-cell variation in chiasma frequency were analysed in nulli 5B and nulli 5D hybrids between *Triticum aestivum* (wheat) and *Secale cereale* (rye) with and without B-chromosomes of rye. As a measure of (a)synchrony the variance in apparent time elapsed since a fixed starting point (beginning zygotene) was used.

There was no effect of rye B-chromosomes nor of absence of chromosome 5B on meiotic synchrony and variation in chiasma frequency.

Absence of 5D appeared to cause a decrease of synchrony at 20°C and 15°C but genetic variation between plants also played an important role.

INTRODUCTION

In addition to genes which are responsible for their multiplication, usually acting at or just after meiosis, and which they need for their maintenance in absence of selective advantage, B-chromosomes in several species have been found to carry genes coding for effects not evi-

dently related to their own functioning. B-chromosomes of *Aegilops speltoides* and *Ae. mutica* carry genes which restrict homoeologous pairing between *Triticum aestivum* (wheat) chromosomes, much as the *Ph* gene in chromosome 5B does (Dover and Riley, 1972; Vardi and Dover, 1972). A similar effect have B-chromosomes of *Lolium perenne* in *L. perenne* X *L. temulentum* hybrids (Evans and Macefield, 1972). Viegas (1979a, 1979b) found an effect of B-chromosomes of *Secale cereale* (rye) on chromosome pairing in wheat in absence of chromosome 5D, which carries the low temperature pairing regulating gene *Ltp*. Rye B-chromosomes do not have an effect, like B-chromosomes of *Ae. speltoides* and *Ae. mutica*, on homoeologous pairing of wheat (Roothaan and Sybenga, 1976). In the course of the latter work it was observed that synchronisation and in general the regular course of meiosis were better (fewer chromatic inclusions, doubled nuclei, etc.) in the presence of rye B-chromosomes in tetraploid ($4x = 28 + B's$) rye-wheat hybrids than in their absence. This observation was not quite in line with expectation: in rye, B-chromosomes have been shown to have the tendency to increase variation between cells in respect to chiasma frequency (Jones and Rees, 1967). Synchrony is an important aspect of meiotic control in forms where it is a natural phenomenon: PMC's in anthers of several plant species, spermatocytes in cysts in the testes of several animals, etc. In anthers of many other plant species, however, and in EMC's in some cases, meiosis is sequential. Then, the regularity of the sequence could be subject to analysis. In rye and wheat, meiosis in anthers tends to show considerable synchrony, but in their hybrid, in addition to other indications of genetically conditioned meiotic unbalance (chromatic inclusions, degenerating cells, etc.) meiosis is somewhat less strictly synchronized. Since regular meiosis is of considerable interest for Triticale breeding, it was decided to conduct a more thorough experiment on the presumed regulatory effect of rye B-chromosomes.

Whereas the frequency of doubled nuclei, chromatic inclusions, degenerating cells, anaphase buds, etc. can be screened relatively readily and quantitatively, a parameter like synchronization is much less easily expressed in a simple form, especially when fixed preparations at different stages of meiosis are scored.

In synchronous meiosis, there is little variation in stage between PMC's in an anther around the average. Since for each cell the stage can be determined, and for each consecutive stage its duration and thus for each stage the distance in time from the average per anther, the variation around the average, expressed as a variance, is a good measure of asynchrony. This total variance has two components: the between-stage variance σ_b^2 which can be estimated, and the within-stage variance σ_w^2 , which must be constructed in another way as within stages no reliable difference between cells can be observed. Thus $\sigma_{total}^2 = \sigma_b^2 + \sigma_w^2$.

For estimating the average we assume a starting point of meiosis at time 0. For each successive stage the mid-point is taken to represent the time progressed since the start of meiosis, for each cell in this stage. When doing so, and for estimating the within-stage variance we assume an even distribution of cells in a stage. This is not quite correct, but the best choice presently available. For the average stage a normal distribution may be more appropriate, but for stages earlier and later there must be a skewing towards the average. This means that with our approach the asynchrony will be somewhat overestimated, especially when stages of long duration are represented by a considerable number of cells but less than the modal number.

Besides meiotic asynchrony, several other abnormalities have been observed in hybrids: chromatic inclusions, degenerating cells, failure of cytokinesis, etc. Their occurrence was too infrequent and irregular to serve as a basis for analysis of meiotic regularity. A third parameter is between-cells variance of chiasma frequency. In normal hybrids between wheat and rye, there are very few chiasmata, but in nulli-5B hybrids, which lack the *Ph* (homoeologous pairing restricting) gene, each cell can have several. We have given the chiasma distribution in a few such cases.

MATERIAL AND METHODS

Heads of wheat (*Triticum aestivum*) var. (Chinese spring), mono

Table 1 - Duration of successive meiotic stages (hours) of several Triticeines. The variety Chinese spring (CS) of *T. aestivum* was used all the time, while the variety of *S. cereale* in some cases is unknown.

	Nº OF CHROM	LEPT	ZYG	PACH	DIPL	DIAK	MI	AI	TI	INT	MII	AII	III	TOTAL	TETRAD		
1	<i>S. cereale</i> "Petkus"	2x=14	20	11.4	8.0	1	0.6	2	1	1	2.5	1.7	1.0	1.0	51.2	8.5	
2	<i>S. cereale</i> "Svalöv"	4x=28	13	9	6.4	1	0.6	1.8	0.7	0.7	2.0	1.4	0.7	0.7	38	--	
3	<i>T. aestivum</i> X <i>S. cereale</i>	4x=28	--	--	--	--	--	--	--	--	--	--	--	--	35	--	
4	<i>T. aestivum</i> X <i>S. cereale</i>	4x=28	←	←	←	←	←	←	←	←	←	←	←	←	←	35.5	10
5	<i>T. aestivum</i> X <i>S. cereale</i> +(add. <i>Ae. mutica</i>)	4x=28	15	6.5	5	1	0.5	←	←	←	←	←	←	←	←	35.5	10
6	Triticale "Rosner"	6x=42	←	←	←	←	←	←	←	←	←	←	←	←	←	34	--
7	<i>T. aestivum</i> "CS"	6x=42	10.4	3.4	2.2	0.6	0.4	1.6	0.5	0.5	2.0	1.4	0.5	0.5	24	10	
8	Triticale (CS X King II)	6x=56	7.5	3.0	2.25	1.0	0.5	1.75	0.5	0.5	1.5	1.25	0.5	0.5	20.75	--	
9	Triticale (CS X Petkus)	6x=56	←	←	←	←	←	←	←	←	←	←	←	←	←	22	--
10	Triticale (CS X Petkus)	6x=56	7.5	3.0	2.2	1.0	0.5	1.8	0.5	0.5	1.5	1.2	0.5	0.5	20.7	7.3	
11	<i>T. aestivum</i> X <i>S. cereale</i>	4x=28	11.4	8.0	5.6	1.0	0.5	1.8	0.6	0.6	1.9	1.4	0.6	0.6	35	8.6	

References: 1, 7 and 10 - Bennett et al., 1971.

2, 6, 8 and 9 - Bennett & Smith, 1972.

3 - Bennett, 1973.

4 and 5 - Bennett et al., 1974.

11 - Extrapolated from literature.

5B and mono 5D were crossed with inbred lines of rye (*Secale cereale*) without B-chromosomes and with rye plants with B-chromosomes, backcrossed 3 to 4 generations to the inbreds mentioned above.

In the progeny, hybrid plants with and without 5B or 5D and with or without rye B-chromosomes were selected, using Feulgen staining after 1N-HCl fixation and hydrolysis at 59°C. The hybrids were grown in a cooled greenhouse during summer and in a climate chamber at 15°C. Florets were fixed approximately at meiotic first metaphase in 1:3 acetic alcohol. Squash preparations were made in 45% acetic acid after prolonged staining in acetocarmine. Preparations were made permanent in Euparal.

Estimates for the duration of the successive meiotic stages have been given in the literature for a number of species and hybrids of the Triticinae. No information, however, was available which could be used directly for our material. We have derived the most probable durations by extrapolation from the most relevant estimates published (Table 1). As the starting point we have chosen the beginning of zygotene, earlier stages not being present in the fixations. The histogram of Figure 1, which is an example of one of our analysis, gives a visual impression of the distribution of cells about the stages.

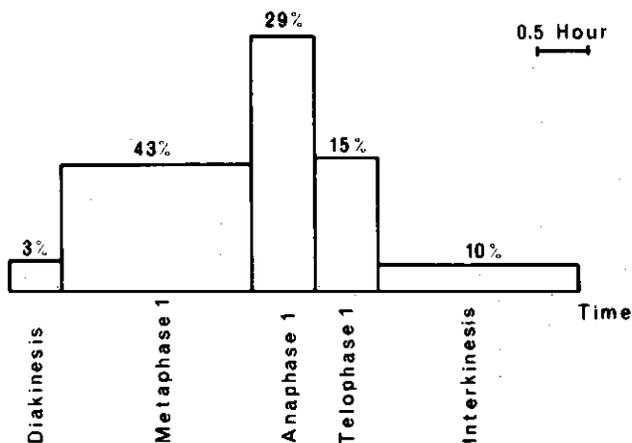


Figure 1. Example of the distribution of cells over meiotic stages in one anther of a wheat-rye hybrid, lacking chromosome 5B (plant 74791-5).

The average stage for an anther is obtained by multiplying for each stage the mid-point of that stage by the number of cells in it, adding the values for all stages and dividing by the number of cells.

The between stage

$$\sigma_b^2 = \frac{\sum n_i \mu_i^2 - \frac{1}{n} \left[\sum n_i \mu_i \right]^2}{n - 1}$$

and within stage

$$\sigma_w^2 = \frac{1}{12n} \cdot \sum_i n_i (b_i - a_i)^2$$

in which n_i is the number of cells observed in stage i ; μ the time at the midpoint; n the total number of cells observed in the anther; a_i the time at the beginning of stage i and b_i the time at the end.

As the variances may not be assumed to be normally distributed, non-parametric methods were used in the statistical analysis.

RESULTS

The results have been summarized in Tables 2 and 3. The variances of Table 2 appear to be very variable within classes. No between-class difference can be demonstrated using the Kruskal-Wallis test (Table 4). The means are very similar for all classes, and it is clear that neither rye B-chromosomes nor absence of 5B affect meiotic synchronization, separately or combined. Also the effect of B-chromosomes on the variance of chiasma frequencies is negligible, although there is a slight indication of reduced variance in the absence of 5B when rye B-chromosomes are present. This is in contradiction with the effect on rye, where B-chromosomes tend to increase the variance (Jones and Rees, 1967). As in the case of Roothaan and Sybenga (1976) there is a slight negative correlation between chiasmata in A and B chromosomes. This is expected, as it is the same material.

The data of Table 3 are different. There is a clear effect of absence of chromosome 5D ($P < 0.005$) slightly reinforced by rye B-chromosomes, which have no effect of themselves. This is true for 22°C and 15°C.

Table 2 - The mean stage (μ), total variances (σ^2), chiasma frequencies and its variances of PMC's at meiosis in F_1 hybrids of *T. aestivum* X *S. cereale*, in the presence and absence of 5B and rye B-chromosomes. (The average of PMC's observed for each anther ranged from 200 to 700).

PLANT N°	μ	σ^2_D	σ^2_W	$\sigma^2_{tot.}$	CHROMOSOMES			
					A		B	
					FREQ	VAR	FREQ	VAR
28 + 2B								
74786-16	17.03	1.48	0.21	1.69	0.28	0.32	1.10	0.93
74790-4	17.43	1.08	0.14	1.22	--	--	--	--
"	18.05	1.00	0.17	1.17	--	--	--	--
"	15.66	0.59	0.25	0.84	1.14	1.30	1.59	0.34
"	15.79	0.77	0.32	1.09	0.82	0.96	1.64	0.27
"	14.47	5.24	0.86	6.10	0.96	1.10	1.26	0.69
74800-1	17.09	1.39	0.19	1.58	0.46	0.46	1.36	0.25
"	16.14	0.17	0.24	0.41	0.70	0.59	1.30	0.43
74807-17	15.44	1.68	0.37	2.05	0.44	0.37	1.40	0.38
"	13.47	9.45	1.31	10.76	0.52	0.54	1.34	0.37
74807-18	15.99	0.06	0.26	0.32	0.82	1.41	1.40	0.49
27 + 2B								
74786-11	16.67	0.89	0.19	1.08	4.58	2.61	1.08	0.32
74786-15	15.35	2.04	0.41	2.45	5.12	5.53	1.12	0.17
74786-18	15.68	0.46	0.23	0.69	6.64	2.72	1.22	0.29
74790-1	16.45	0.80	0.23	1.03	4.16	4.79	1.00	0.08
"	16.19	0.20	0.23	0.43	4.96	3.50	1.06	0.18
"	15.54	1.49	0.36	1.85	4.92	2.80	1.02	0.14
"	15.70	0.87	0.30	1.17	5.58	3.63	1.18	0.27
"	16.01	0.76	0.15	0.91	4.60	2.42	1.02	0.10
74790-8	17.10	1.41	0.19	1.60	6.42	5.55	1.12	0.16
74803-1	17.13	1.48	0.17	1.65	7.34	3.64	0.94	0.72
74807-1	15.80	0.19	0.23	0.42	8.14	2.78	1.20	0.33
74808a-8	15.79	0.25	0.23	0.48	7.48	4.27	1.06	0.24
28								
74787-4	17.46	1.56	0.19	1.75	0.84	1.06	--	--
"	19.30	1.89	0.23	2.12	0.73	1.92	--	--
"	19.04	2.20	0.20	2.40	0.95	0.73	--	--
"	19.25	0.51	0.28	0.79	--	--	--	--
"	14.26	5.44	0.90	6.34	1.24	1.20	--	--
74791-8	16.62	0.67	0.18	0.85	0.66	0.58	--	--
"	17.93	2.16	0.19	2.35	0.72	0.36	--	--
"	13.40	4.69	1.09	5.78	0.60	0.55	--	--
"	14.95	2.85	0.49	3.34	1.42	1.59	--	--
74791-15	15.97	0.05	0.26	0.31	0.96	1.01	--	--
27								
74791-5	16.89	1.09	1.16	1.25	5.62	4.34	--	--
74791-7	16.00	0.00	0.27	0.27	7.02	8.28	--	--
"	15.83	0.53	0.22	0.75	7.12	3.70	--	--
"	15.22	2.84	0.52	3.36	6.98	7.31	--	--
74791-10	14.01	0.30	0.15	0.45	--	--	--	--
"	18.31	1.22	0.22	1.44	7.43	5.98	--	--
"	19.19	1.27	0.23	1.50	--	--	--	--
74791-12	17.00	2.04	0.25	2.29	7.10	5.17	--	--
74787-1	14.09	17.97	1.18	19.15	5.32	7.31	--	--
"	19.03	39.13	2.09	41.22	--	--	--	--

Table 3 - The mean stage (μ) and the total variances (σ^2) of PMC's at meiosis in F_1 hybrids of *T. aestivum* (normal or monosomic 5D) X *X. S. cereale* (with and without B-chromosomes), grown at 22°C and 15°C (200-500 PMC's per entry).

PLANT	22°C				15°C			
	μ	σ_b^2	σ_w^2	σ_{tot}^2	μ	σ_b^2	σ_w^2	σ_{tot}^2
<u>2B + 2B</u>								
46	16.07	0.081	0.256	0.337	16.05	0.070	0.260	0.330
20	16.05	0.063	0.260	0.323	16.10	0.109	0.250	0.359
27	16.00	0.000	0.270	0.270	16.23	0.268	0.228	0.496
28	16.00	0.000	0.270	0.270	--	--	--	--
<u>27 + 2B</u>								
44	17.15	8.614	0.545	9.159	18.13	8.316	0.547	8.863
23	17.20	8.496	0.463	8.959	16.36	6.525	0.358	6.883
21	17.07	4.478	0.268	4.746	16.66	6.080	0.200	6.280
19	16.37	6.163	0.374	6.537	18.55	10.256	0.707	10.963
<u>27+1B+isoB</u>								
43	16.30	0.274	0.211	0.485	16.17	0.182	0.236	0.418
43	16.30	0.299	0.213	0.512	16.03	1.646	0.115	1.761
<u>2B</u>								
5	16.03	0.049	0.262	0.311	15.98	0.025	0.265	0.290
11	15.97	0.000	0.264	0.264	16.01	0.009	0.268	0.277
30	15.98	0.036	0.263	0.299	16.00	0.000	0.270	0.270
31	16.00	0.000	0.270	0.270	16.03	0.032	0.264	0.296
<u>27</u>								
49	15.66	2.159	0.329	2.488	15.85	0.197	0.246	0.443
9	15.60	1.474	0.340	1.814	14.19	11.322	0.758	12.080
3	16.64	1.708	0.231	1.939	15.87	0.456	0.206	0.662
45	17.33	2.300	0.206	2.506	16.24	6.785	0.410	7.195
17	16.80	1.811	0.249	2.060	17.87	3.866	0.176	4.042

Table 4 - The influence of the rye B-chromosome and the 5B and 5D chromosomes of wheat on the synchrony of the meiosis of *Triticum X Secale* — hybrids tested with the Kruskal-Wallis test (Statistic H)

CONSTRAST	TABLE	H
With and without 2B's (22°C)	2	2.43 0.10 < P($\chi^2 = 2.43$) < 0.20
With and without 5B (22°C)	2	0.92 0.70 < P($\chi^2 = 0.92$) < 0.80
With and without 2B's (22°C)	3	0.67 0.70 < P($\chi^2 = 0.67$) < 0.80
With and without 5D (22°C)	3	13.20 P($\chi^2 = 13.20$) < 0.05*
With and without 2B's (15°C)	3	1.22 0.20 < P($\chi^2 = 1.22$) < 0.30
With and without 5D (15°C)	3	10.93 P($\chi^2 = 10.93$) < 0.05*
Without 5D at 15°C and 22°C	3	0.24 0.3 < P($\chi^2 = 0.24$) < 0.7
Without 5D at 15°C and 22°C	3	1.34 0.2 < P($\chi^2 = 1.34$) < 0.3

* H_0 is rejected

DISCUSSION

The contention that rye B-chromosomes might have a positive effect on meiotic regularity in wheat-rye hybrids is not born out by the present results. Nor is there an effect of the absence of chromosome 5B of wheat. Bennett (1973) found that absence of 5B increased the total duration of meiosis in wheat. If this is proportionally distributed about all stages, it should not affect the variation as studied by us.

An interesting new observation is the apparent strong disturbing effect of the absence of chromosome 5D of wheat. It is this chromosome which carries the low temperature pairing regulating gene. At 15°C the effect in meiotic synchrony seems to be more pronounced, but the variation is such that any conclusion would be premature. It is remarkable that in this material the variation is so much lower than that in Table 2. However, when the single plant without 5D and with one normal and one iso B of rye is considered, the variation becomes more pronounced. The data of Table 2 strongly suggest a considerable between-plant (= between genotype) variation in meiotic synchrony. Between preparations within plants and to some extent between fixations within plants this variation is much smaller. In fact, the entire range in synchrony observed between types in Table 3, is present within types in Table 2. Especially at 15°C the variation between (and consequently within some) plants seems to increase. This suggests some caution in the interpretation of the effect of wheat chromosome 5D on meiotic synchrony. It may be noted that Bennett (1973) found no effect of 5D on total duration of meiosis.

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A SUPPRESSOR FOR CHROMOSOME ASSOCIATION
IN A MUTANT ISOCHROMOSOME 5D^L OF *TRITICUM AESTIVUM*

WANDA S. VIEGAS, MOSHE FELDMAN* and T. MELLO-SAMPAYO

Instituto Gulbenkian de Ciência, Oeiras, Portugal

SUMMARY

Unusual asynapsis was detected in a plant of *Triticum aestivum* var. Chinese spring disomic for an isochromosome of the long arm of chromosome 5D. The two isochromosomes themselves also paired less frequently than those in di-isosomics of other chromosomes arms. Chromosome pairing in F₁ hybrids of this plant with *Secale cereale*, *T. longissimum* (= *Aegilops sharonensis*) and *T. longissimum* (= *Ae. longissima*, intermediate pairing line) showed that the isochromosome carried a suppressor of chromosome association, not normally present in 5D^L.

The possibility that the pairing suppressor in this isochromosome has been transferred from 5B^L through homoeologous chromosome pairing and recombination cannot be discarded. It seems more likely, however, that it rose by mutation or duplication from a pre-existing but otherwise undetectable gene. The isochromosome is therefore designated as 5D^L_M.

INTRODUCTION

Common wheat *Triticum aestivum* (2n=6x=42) carries three different genomes A, B and D donated by three independent but closely related

* Present address: Weizman Institute of Science, Israel.

diploid species from the Sub-Tribe Triticinae. Chromosomes of each genome have close genetic affinities to corresponding (homoeologous) chromosomes of the other two genomes, but homoeologous chromosomes do not normally associate at meiosis. A major suppressor of homoeologous chromosome association designated *Ph* and located in the long arm of chromosome 5B ($5B^L$) was found to be responsible for this effect (Okamoto, 1957; Riley and Chapman, 1958; Wall, Riley and Gale, 1971). Minor suppressors were found in the short arms of chromosome 3D ($3D^S$) and 3A ($3A^S$) (Mello-Sampayo, 1971; Driscoll, 1972). The long arm of chromosome 5D ($5D^L$) was shown to carry a stabilizer gene for chromosome association designated *Ltp* which prevents asynapsis at low temperature (Riley, 1966; Hayter and Riley, 1967).

A monosomic plant of *T. aestivum* cultivar Chinese spring carrying a single isochromosome $5D^L$ was grown from a seed sample obtained from Dr. E.R. Sears, University of Missouri. Mono-isosomic $5D^L$, di-isosomic $5D^L$ and nullisomic 5D individuals were recovered from selfing. Mono-isosomic and nullisomic plants showed regular meiosis. A relatively low degree of chromosome pairing was observed in di-isosomic $5D^L$ plants.

We want to report data showing that this abnormal or mutant isochromosome $5D^L$ (iso $5D^L_M$) carried a general suppressor of chromosome association.

MATERIAL AND METHODS

F_1 hybrids *T. aestivum* X *S. cereale* and *T. aestivum* X *T. longissimum* (= *Ae. sharonensis*) show very low chromosome association at meiosis. In order to raise homoeologous chromosome association in such hybrids a deficiency for the short arm of chromosome 3D ($3D^S=3D\delta$) was included in the wheat parent. It had been demonstrated earlier that $3D^S$ carried a suppressor for chromosome association (Mello-Sampayo, 1971). Wheat plants were obtained which carried simultaneously an isochromosome $5D^L_M$ and a telocentric $3D^L$ (= $3D\alpha$) together with a normal chromosome of each kind (heteroiso $5D^L_M$ -heterotelo $3D^L$), so four types of gametes were expected from these plants: normal, telosomic $3D^L$, isosomic $5D^L_M$ and

telosomic $3D^L$ -isosomic $5D^L_M$. These double heterosomic wheat plants were crossed as female parents with both *S. cereale* cultivar Centeio do Alto, from Northern Portugal and with *T. longissimum* (= *Ae. sharonensis*) from a seed stock obtained from Dr. M. Tanaka, Kyoto University. All four F_1 hybrids which were expected from the four gametic variants were obtained in crosses with rye, whereas in those with *T. longissimum* (= *Ae. sharonensis*) it was not possible to recover the one carrying both telo $3D^L$ and iso $5D^L_M$. F_1 hybrids telosomic $3D^L$ and isosomic $5B^L$ were also obtained from crosses *T. aestivum* (heterotelo $3D^L$ -heteroiso $5B^L$) X *S. cereale*.

An accession of *T. longissimum* (= *Aegilops longissima*, intermediate pairing - I.P.) was found to induce an intermediate type of homoeologous association (Mello-Sampayo, 1971) in F_1 hybrids with *T. aestivum*. Plants of this accession were crossed with *T. aestivum* either normal or mono-isosomic $5D^L_M$, and from these crosses three types of hybrids were obtained: normal, nullisomic $5D$ and isosomic $5D^L_M$.

A search was made among different di-isosomic combinations (di-iso $2B^L$, di-iso $5B^L$, di-iso $5B^S$, di-iso $6B^S$, di-iso $5D^L$ and di-iso $5D^L_M$) of *T. aestivum* for rates of PMC's with paired vs. univalent isochromosomes. All these plants, except di-iso $5B^L$ and di-iso $5D^L_M$, were from stocks maintained at the Weizman Institute of Science. They concern plants in which asynapsis was never detected at meiosis. In di-iso $5B^L$, however, mosaic effects of asynapsis in PMC's such as in di-iso $5D^L_M$, were found.

A plant disomic for two telocentric $5D^L_M$ (ditelosomic $5D^L_M$) was obtained through misdivision of the isochromosome and subsequent doubling of the resulting telocentric. Crosses were performed between this ditelosomic plant and ditelosomic $5B^L$ individuals.

All plants were grown in greenhouse conditions at spring time. Acetocarmine squashes in anthers pre-fixed in Carnoy (6:3:1) were performed.

RESULTS

In di-isosomic $5D_M^L$ plants, limited areas of squashed PMC's showed a very high number of rod bivalents and univalents at the first division of meiosis (Figure 1 - 1).

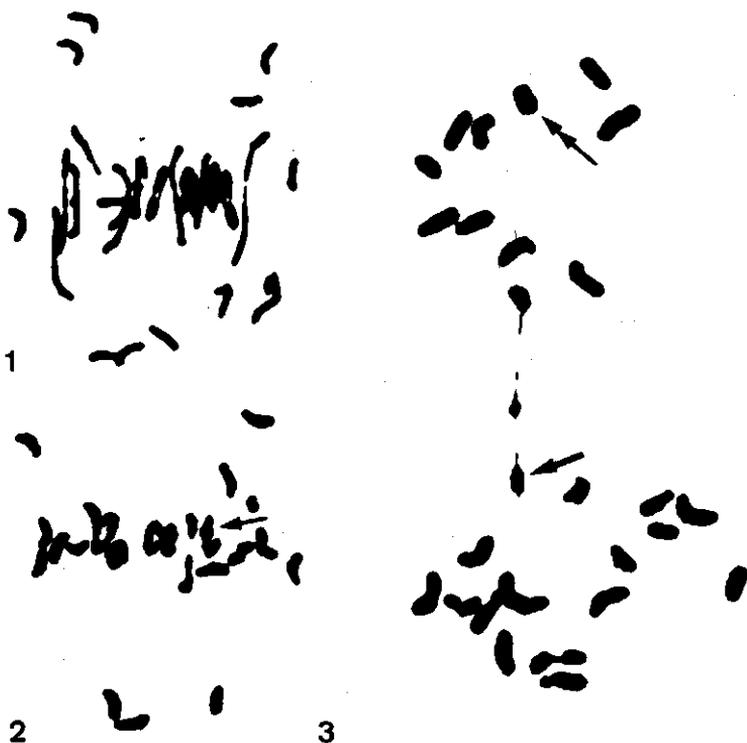


Figure 1 - Metaphases of the first division of meiosis. 1 - Asynaptic effect in di-isosomic $5D_M^L$ in *T. aestivum* var. Chinese spring. Note the unusual number of rod bivalents and univalents. 2 - A pronounced homoelogous chromosome association in a F_1 hybrid *T. aestivum* (ditelo $3D^L$) X *S. cereale* (arrow indicates heteromorphic bivalent involving $3D^L$). 3 - Reduced association in a F_1 hybrid *T. aestivum* (di-isosomic $5D_M^L$ -ditelosomic $3D^L$) X *S. cereale* (single arrow indicates $3D^L$ in a homoelogous heteromorphic bivalent, double arrow isochromosome $5D_M^L$). (Magnification between X 1,000 to X 1,500).

Table 1 shows meiotic data on F_1 hybrids *Triticum aestivum* X *Secale cereale*. Chromosome association as expressed by mean chiasmata per cell was very low in hybrids originated from normal gametes ($.345 \pm .04$). When hybrids were deficient for the short arm of chromosome 3D the homeologous chromosome pairing ($2.25 \pm .05$) rose very significantly ($P < .001$), as expected. In telosomic $3D^L$ -isosomic $5D_M^L$ hybrids, chromosome association fell drastically ($.865 \pm .14$) as compared with the previous ones ($P < .001$). An identical effect in decreasing ($P < .001$) chromosome association was observed in F_1 hybrids carrying telocentric $3D^L$ together with an isochromosome $5B^L$ ($.795 \pm .01$) which is known to carry the *Ph* suppressor of chromosome association (Figure 1 - 2, 3).

Table 1 - Chromosome association at MI (average number of chiasmata per cell) in F_1 ($2n=28$) of *Triticum aestivum* (heteroiso $5D_M^L$ -heterotelo $3D^L$ or heteroiso $5D_M^L$ -heterotelo $3D^L$) ♀ X *Secale cereale* ♂ (two plants of each type).

WHEAT GAMETE	CHROM 3D	CHROM 5D AND 5B	N ^o PMC's	UNIVALENT	BIVALENT	TRIV	CHIASMA.
Normal	Normal	Normal	100	27.12±.29	.30±.05	--	.30±.05
			100	27.22±.01	.39±.06	--	.39±.06
Telosomic $3D^L$	Telo $3D^L$	Normal	100	21.65±.28	3.02±.14	.16	3.48±.15
			100	21.37±.29	3.02±.14	.16	3.58±.17
Isosomic $5D_M^L$	Normal	Iso $5D_M^L$	100	27.92±.04	.04	--	.04
			100	27.88±.05	.06	--	.06
Telo $3D^L$ - -Iso $5D_M^L$	Telo $3D^L$	Iso $5D_M^L$	100	26.55±.16	.41±.08	.01	.73±.08
			100	26.04±.16	.98±.10	--	1.00±.10
Telo $3D^L$ - -Iso $5B^L$	Telo $3D^L$	Iso $5B^L$	100	26.47±.17	.74±.08	.02	.80±.09
			100	26.53±.17	.75±.09	.01	.79±.09

$5D_M^L$ - mutant long arm of chromosome 5D.

In F_1 hybrids *T. aestivum* X *T. longissimum* (= *Ae. sharonensis*) (table 2) a significant drop in chromosome association in hybrids carrying

an isochromosome $5D_M^L$ was also observed (chiasma frequency per cell in normal $.945 \pm .04$ and in the presence of iso $5D_M^L$ $.235 \pm .01$). The absence of chromosome 5D (in nullisomic 5D hybrids), however, did not sig-

Table 2 - Chromosome association at MI (average chiasmata per cell) in F_1 ($2n=28$) *Triticum aestivum* (heteroiso $5D_M^L$ -heterotelo $3D^L$) ♀ X *Triticum longissimum* (=Ae. *sharonensis*) ♂ (two plants of each type)

WHEAT GAMETE	CHROM N° (2n)	CHROM 3D	CHROM 5D	N° PMC's	UNIVALENT	BIVALENT	TRIV	CHIASMA.
Normal	28	Normal	Normal	100	25.94±.05	0.87±.04	0.02	0.91±.06
				100	26.11±.18	0.95±.03	0.01	0.98±.05
Isosomic $5D_M^L$	28	Normal	Iso $5D_M^L$	100	27.52±.17	0.24±.01	--	0.24±.01
				150	27.54±.15	0.23±.01	--	0.23±.01
Nulli- somic 5D	27	Normal	Absent	100	25.13±.21	0.95±.05	--	0.95±.06
				150	26.12±.16	1.02±.07	--	1.02±.05

$5D_M^L$ - mutant long arm of chromosome 5D.

nificantly change the mean chromosome association compared to that in normal hybrids (mean chiasma per cell $.985 \pm .04$ and $.945 \pm .04$, respectively). It may be stated that a suppressing effect in chromosome association was mediated through the mutant isochromosome $5D_M^L$, and this is evident for the high doses of $5D_M^L$ present.

A third element of proof of the asynaptic effect of isochromosome $5D_M^L$ comes from the data on F_1 hybrids *T. aestivum* X *T. longissimum* (= Ae. *longissima*, I.P.) (table 3). Again the inclusion of the isochromosome $5D_M^L$ in the genetic background of the hybrids decreased very significantly ($P < .001$) the chromosome association (from $5.385 \pm .4$ to $2.52 \pm .08$, respectively). This was not observed in hybrids that were nullisomic 5D, in which no change in chromosome association (mean chiasma per cell $5.25 \pm .4$) when compared with the normal hybrids was detected.

Double heteromorphous bivalents were observed at meiosis in F_1 heterotelosomic $5D_M^L$ -heterotelosomic $5B^L$ plants. This showed that the

Table 3 - Chromosome association at MI (average chiasmata per cell) in F_1 ($2n=28$) *Triticum aestivum* (heteroiso $5D_M^L$ -heterotelo $3D^L$) ♀ X *Triticum longissimum* (= *Ae. longissima*, intermediate pairing type) ♂

WHEAT GAMETE	CHROM N ^o (2n)	CHROM 5D	N ^o PMC's	UNIVALENT	BIVALENT	TRIV	QUAD	CHIASMA.
Normal	28	Normal	100	17.49±.32	4.57±.16	0.35	0.08	5.84±.18
			100	17.07±.40	4.92±.17	0.29	0.05	6.10±.23
			100	18.30±.32	4.41±.16	0.14	0.01	5.31±.13
			100	20.15±.37	3.71±.18	0.16	0.02	4.29±.21
Isosomic $5D_M^L$	28	Iso $5D_M^L$	100	22.99±.33	2.31±.14	0.11	--	2.59±.17
			100	23.42±.31	2.12±.14	0.11	--	2.36±.17
			100	23.00±.32	2.42±.15	0.08	--	2.62±.17
Nulli- 5D	27	Absent	100	16.93±.42	4.51±.20	0.24	0.03	5.62±.25
			100	18.24±.37	4.04±.18	0.25	0.01	4.89±.20

$5D_M^L$ - mutant long arm of chromosome 5D

telocentric $5D_M^L$ studied did not carry translocated segments of the long arm of chromosome 5B.

The data presented in table 4 give evidence of the asynaptic effect mediated both by di-iso $5D_M^L$ and by di-iso $5B^L$: Comparisons of iso-

Table 4 - MI chromosome configurations in PMC's in several di-isosomics of *Triticum aestivum* var. Chinese spring ($2n=42$). (S and L represent short and long arm, respectively).

TYPE OF PLANT	MEAN UNIVALENTS (excluding isochromosomes)	ISOCHROMOSOME CONFIGURATION		TOTAL CELLS
		UNIVAL	BIVAL	
Di-iso $2B^L$	0.93	13	11	24
Di-iso $5B^S$	1.56 ± .18	17	12	29
Di-iso $6B^S$	1.54 ± .28	21	18	39
Di-iso $5D^L$	1.14 ± .14	24	24	48
Di-iso $5D_M^L$	1.89 ± .23	54	27	81
Di-iso $5B^L$	3.58 ± .31	51	26	77

$5D^L$ and $5D_M^L$ normal and mutant long arm of chromosome 5D

chromosome configurations showed for both combinations the number of univalents to be consistently higher than in the remaining di-iso plants (di-iso $2B^L$, $5B^S$, $6B^S$ and $5D^L$). The ratio of univalent over bivalent configurations for $5D^L_M$ and $5B^L$ is 2:1, where for the remaining di-iso combinations is 1:1. Results concerning di-iso $5B^L$ fully agree with Feldman's (1972) previous observations of unusually higher rates of PMC's with univalent isochromosomes in di-isosomic plants carrying high dosages of *Ph* suppressor. The effect of iso $5D^L_M$ can be considered an overall suppression of association that is not restricted to homoeologous chromosomes.

DISCUSSION

The data presented give evidence of a suppressing effect on chromosome pairing of the mutant isochromosome $5D^L_M$. The pairing of homoeologous chromosomes was significantly lower in any hybrid combination carrying that isochromosome than in those, comparable, not carrying it. Particularly when mean chromosome association in telo $3D^L$ -iso $5D^L_M$ and telo $3D^L$ -iso $5B^L$ hybrids with rye was compared (Table 1) it was found that a similar degree of suppression of chromosome pairing was obtained.

The consistently higher number of PMC's with two univalent isochromosomes $5D^L_M$ in di-isosomic $5D^L_M$ plants as compared with that in di-isosomic $2B^L$, $5B^S$, $6B^S$ and $5D^L$ individuals, of non-arysaptic expression (Table 4), can be used in support of the conclusion that iso $5D^L_M$ carries a general suppressor of chromosome association. It was found that isochromosome $5B^L$ produces a similar effect on raising the number of PMC's with isochromosome $5B^L$ univalents, in di-isosomic $5B^L$ plants.

There is no means of telling, at the moment, how the mutant locus or loci appeared in $5D^L_M$. But some pertinent speculation about its origin can be made. First of all, it can be said that there is no evidence that the *Ph* suppressor was transferred from $5B^L$ to $5D^L$ through homoeologous pairing. Extensive search for a bivalent made out of the two

homoeologous telecentrics in double heterosomic $5B^L-5D_M^L$ plants was made without any success. It can not be excluded that a small segment carrying the critical element could be transferred without being detected through association at the first metaphase of meiosis.

Mutation of the antimorphic type has been considered a reasonable possibility for a promoter to change into a suppressor gene (Feldman, 1968; Sears, 1976).

A stabilizer of chromosome association (*Ltp*) was described in chromosome $5D^L$ (Hayter and Riley, 1967). Its role is to prevent synapsis at low temperature. A spontaneous allelic alternate might have arisen which could suppress chromosome association at normal temperature, and low temperatures would aggravate this effect.

A fourth hypothesis is that normal $5D^L$ carried a weak suppressor gene that was undetectable through the normal procedure of scoring chromosome pairing at metaphase of the first division of meiosis in di-isosomic plants. A duplication may have occurred raising the suppressing effect to a detectable level. A duplication of that kind has already been suggested for *Ph* suppressor on chromosome 5B (Mello-Sampayo, 1972).

Finally, a mutation of pleiotropic effect could have occurred, which induced asynapsis as well as other different effects within the cell. A higher nucleolar organizer activity has been found in root-tip cells of di-isosomic $5D_M^L$ plants. Higher transcriptional rates for rRNA in these cells have been measured (Viegas and Mello-Sampayo, 1975).

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GENES IN DIPLOID TRITICINAE,
COMPENSATING FOR THE ABSENCE
OF THE TEMPERATURE REGULATING GENE *LTP*
IN CHROMOSOME 5D OF *TRITICUM AESTIVUM*

WANDA S. VIEGAS

Instituto Gulbenkian de Ciência, Oeiras, Portugal*

SUMMARY

Hybrids of *Triticum aestivum* (monosomic 5D or ditelosomic 5D^L) X *T. speltoides* (= *Aegilops speltoides*) showed that the genotype of *T. speltoides* carries (a) gene(s) which can partially compensate for the expected decrease in chromosome association at low temperatures (10°C), in the absence of chromosome 5D. In hybrids of *T. aestivum* (normal, ditelosomic 3D^L or ditelosomic 3D^L-monosomic 5D) X *T. longissimum* (= *Ae. sharonensis*), this compensation was not observed.

In normal F₁ hybrids of *T. durum* X *T. longissimum* partial chromosome association occurred at 10°C and this stabilizer effect may be explained by the presence of a *Ltp*-like gene on chromosome 5A. When a line of *T. durum* carrying a homozygous translocated 5B-5D chromosome was used in the crosses an even higher chromosome association was observed.

These results suggest either the existence of a promoter gene for chromosome association in the 5D translocated segment or the loss of a weak suppressor gene in the removed segment of 5B. It was concluded that the translocated 5D segment did not carry the *Ltp* stabilizer gene.

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INTRODUCTION

Triticum aestivum is an allohexaploid species ($2n=6x=42$) with a genomic constitution AABBDD. The three genomes, each derived from a different diploid species, are genetically related. Sears and Okamoto (1958) have shown that the corresponding chromosomes of those three genomes do indeed carry duplicate genes.

In meiosis of *T. aestivum* chromosome pairing normally takes place only between fully homologous partners, as a consequence of the balanced effect of several promoter and suppressor genes affecting the premeiotic alignment of chromosomes (Feldman, 1966). This results in an entirely bivalent-forming meiotic constitution.

In addition to genetic components there are also environmental factors that influence the quantitative expression of chromosome pairing in wheat. Low and high temperatures have been found to generally diminish chromosome pairing in wheat. However, temperature sensitivity is under genetic control, with a major factor in chromosome 5D (Riley, 1966). The long arm of this chromosome ($5D^L$) carries a gene (*Ltp*) that sustains such pairing (Hayter, 1969). Bayliss and Riley (1972a) showed that lowering of chiasma frequency at low temperatures in plants of *T. aestivum* deficient for chromosome 5D was correlated with failure of zygotene chromosome pairing. Moreover, they showed that the temperature-sensitive stage lies in premeiotic interphase before premeiotic DNA synthesis (Bayliss and Riley, 1972b). Chromosome 5A also exerts a weak stabilizing effect on chromosome association at low temperature (Riley, Chapman, Young and Belfield, 1966). Hayter and Riley (1967) demonstrated the presence of another *Ltp* gene in tetraploid wheat ($2n=4x=28$; genomic constitution AABB) which is epistatic to the *Ltp* gene carried by the D genome, and which sustains chiasma formation at low temperatures. Vardi and Dover (1972) found a gene in *Triticum tripsacoides* (= *Aegilops mutica*) which was able to compensate for the absence of *Ltp* in F_1 hybrids *T. aestivum* (monosomic 5D) X *T. tripsacoides*, lacking 5D. A genetic system which compensates for the absence of *Ltp* at different levels was also found in F_1 hybrids *T. aestivum* (monosomic 5D) X *T. speltoides* (= *Ae. speltoides*) (Attia,

Lelley and Röbbelen, 1977).

This paper reports the variation in chromosome pairing at low temperatures in F_1 hybrids between *T. aestivum* X *T. longissimum* (= *Ae. sharonensis*), *T. durum* X *T. longissimum* and *T. aestivum* X *T. speltoides*, in the presence or absence of chromosomes 5D and 3D. Low temperature effects were also tested in F_1 hybrids between *T. longissimum* and a tetraploid variant of wheat in which a considerable segment of the long arm of chromosome 5B was substituted for a corresponding segment of the long arm of chromosome 5D. It was hoped that results obtained in this translocated 5B-5D line would show if the replaced 5D segment did carry the *Ltp* gene.

MATERIAL AND METHODS

Plants of *T. aestivum* var. "Chinese spring" ($2n=42$) and *T. durum* variety "Ld 222" ($2n=28$) were grown at the Biology Center, Oeiras, from seed stocks originally received from Dr. E.R. Sears, University of Missouri. The hexaploid seeds were either normal, monosomic 5D, ditelosomic 5D^L or ditelosomic 3D^L (= 3D α).

A tetraploid wheat homozygous for a translocated 5B-5D chromosome, where a considerable segment of the long arm of chromosome 5D was substituted for the corresponding segment of the long arm of chromosome 5B, was isolated by Dr. Mello-Sampayo. The genetic background of the line is the same as *T. durum* variety "Ld 222" because several backcrosses were done using this variety. This line is usually denominated as "Resende".

Seeds of *T. longissimum* ($2n=14$) and *T. speltoides* ($2n=14$) were originally obtained from a stock kept at the National Institute of Genetics, Kyoto University.

Normal hybrids of *T. aestivum* X *T. longissimum* are nearly asynaptic. In order to detect any effect of low temperature on the frequency of chiasmata, it was necessary to increase the level of chromosome association in such hybrids. This was done through the use of a 3D^L telocentric line, which had lost the suppressor gene for chromosome association located on the short arm of chromosome 3D. Ditelosomic 3D^L and mono-

somic 5D plants were crossed to each other, and the F_1 hybrids were used as female parents in crosses with *T. longissimum*. The resulting seeds were classified at mitosis in order to select the desired combinations (normal euploid, telosomic $3D^L$ and telosomic $3D^L$ -nullisomic 5D).

T. durum variety "Ld 222" and tetraploid "Resende" were crossed with *T. longissimum*. Due to the low number of germinated seeds embryo cultures, using "Difco" orchid agar, were tried and in many cases embryos were obtained by this method. Triploid seeds were made using the tetraploid as the female parent and main endosperm donor.

Crosses were also made between *T. aestivum* (normal, monosomic 5D, ditelosomic $5D^L$) X *T. speltoides* using hexaploid wheat as the female parent. F_1 hybrids carried either an entire gametic complement of common wheat or they were independently deficient for chromosome 5D or for the short arm of the same chromosome.

All the F_1 hybrids were grown in winter at normal greenhouse conditions (temperature $20^\circ\text{C} \pm 2^\circ\text{C}$). Fixation of flowers in acetic acid-alcohol (1:3) was done first in the greenhouse for each F_1 hybrid. Plants were then transferred to a chamber with continuous light at $10^\circ\text{C} \pm 2^\circ\text{C}$, and before sampling for meiotic stages the plants were left there for at least a week, which ensured that sampled anthers had undergone their complete meiotic development at the controlled temperature (Bayliss and Riley, 1972a, b). Then meiocyte samples were again taken from each plant, after different periods. Some of these plants were returned to the original greenhouse conditions and further meiocyte samples were taken in order to study chromosome pairing recovery.

Chromosome association was studied at the metaphase of the first division of meiosis (metaphase I) in pollen mother cells stained with acetocarmine. Frequency of chiasmata per cell, as considered here, refers specifically to the frequency of chromosome association, which is an expression of the intensity of homoeologous chromosome pairing in each plant.

RESULTS

A gradual decrease in chromosome association was noticed in most F_1 hybrids studied, over the period of treatment at low temperatures, until a level at which it stabilizes. Reversely, chromosome association gradually returned to normal level when the plants were again exposed to greenhouse conditions.

F_1 hybrids *T. aestivum* X *T. longissimum* showed a very low degree of chromosome association, both at normal and low temperatures (chiasma frequency per cell 0.85). The absence of the short arm of chromosome 3D (= 3D δ) resulted in an increase in chromosome association (chiasma frequency per cell 7.18). At normal temperature, the absence of the short arm of chromosome 3D and of the whole chromosome 5D, resulted in levels of pairing similar to those observed in the absence of 3D δ alone (Figure 1).

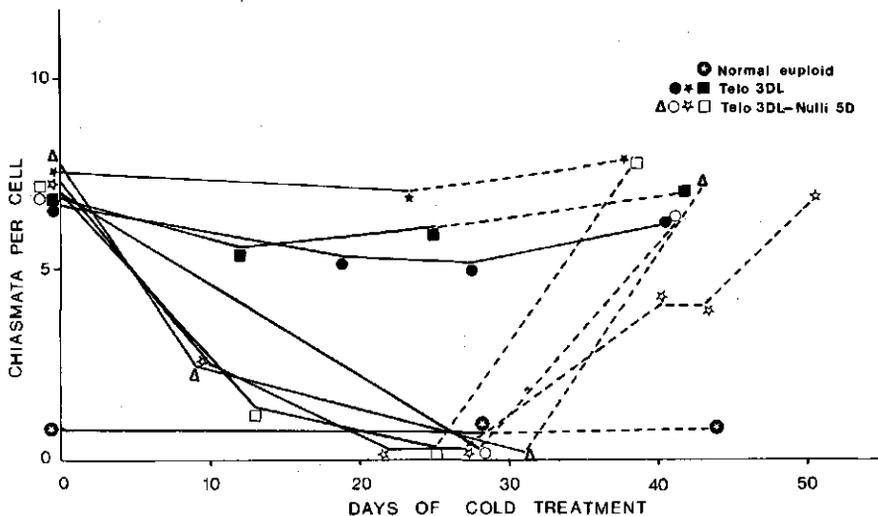


Figure 1 - Chiasmata per cell in F_1 hybrids *T. aestivum* X *T. longissimum* at metaphase I of meiosis. Straight lines: values obtained after different periods (0 - 40 days) at low temperature (10°C). Dotted lines: recovery to normal after transfer back to 20°C. (Each point represents the average of 50 PMC's).

Table 1 - Mean chromosome pairing at first metaphase of meiosis in F₁ hybrids *Triticum aestivum* ♀ (normal, ditelosomic 3D^L and ditelosomic 3D^L-monosomic 5D) X *Triticum longissimum* ♂

PLANT N°	CHROM N°	CHROM 3D	CHROM 5D	COLD TREAT (DAYS)	RECOVERY (DAYS)	UNIV	BIV	TRIV	QUADRIV AND OTHERS	CHIASM P/CELL	N° PMC's
127	28	present	present	--	--	26.20	0.90	--	--	0.90	50
127	28	present	present	9	--	26.34	0.83	--	--	0.83	50
127	28	present	present	28	--	26.44	0.78	--	--	0.78	50
127	28	present	present	--	15	25.66	0.87	0.20	--	0.91	100
1	28	telo	present	--	--	16.02	5.59	0.20	0.05	6.78	100
1	28	telo	present	19	--	17.98	4.70	0.18	0.02	5.38	100
1	28	telo	present	27	--	18.10	4.90	0.36	0.02	6.10	150
1	28	telo	present	40	--	16.98	4.50	0.22	--	5.18	50
8	28	telo	present	--	--	15.58	5.53	0.34	0.08	6.88	100
8	28	telo	present	12	--	19.17	4.08	0.17	0.04	5.62	100
8	28	telo	present	25	--	18.50	4.54	0.14	--	6.16	50
8	28	telo	present	--	17	15.70	5.32	0.30	0.19	7.05	100
125	28	telo	present	--	--	14.24	6.73	0.10	--	7.60	50
125	28	telo	present	23	--	14.48	6.50	0.12	0.04	7.18	50
125	28	telo	present	--	14	14.66	5.66	0.55	0.12	7.78	100
2	27	telo	absent	--	--	13.10	6.10	0.44	0.09	7.35	100
2	27	telo	absent	10	--	22.06	2.41	0.04	--	2.56	100
2	27	telo	absent	22	--	26.32	0.34	--	--	0.34	100
2	27	telo	absent	27	--	26.35	0.32	--	--	0.32	100
2	27	telo	absent	--	13	18.28	3.72	0.10	--	4.08	100
2	27	telo	absent	--	16	18.23	3.70	0.12	--	4.12	100
2	27	telo	absent	--	23	16.86	4.65	0.24	0.03	6.71	100
124	27	telo	absent	--	--	15.40	5.50	0.20	--	7.18	50
124	27	telo	absent	13	--	23.68	1.66	--	--	1.70	50
124	27	telo	absent	25	--	26.28	0.36	--	--	0.36	50
124	27	telo	absent	--	13	14.40	5.90	0.24	0.02	7.68	50
129	27	telo	absent	--	--	13.10	6.72	0.10	0.04	7.60	50
129	27	telo	absent	9	--	21.92	2.54	--	--	2.54	50
129	27	telo	absent	31	--	26.48	0.26	--	--	0.28	50
129	27	telo	absent	--	12	14.08	5.80	0.36	0.06	7.16	50
4	27	telo	absent	--	--	14.26	6.06	0.18	0.02	7.02	100
4	27	telo	absent	28	--	26.40	0.30	--	--	0.30	100
4	27	telo	absent	--	13	17.40	4.52	0.16	0.02	6.14	100

These hybrids showed, however, a very significant drop in the frequency of chiasmata (chiasma frequency per cell 0.30) at 10°C. Some of these plants were returned to 20°C and a gradual recovery to their normal frequencies of chromosome association was observed (Table 1).

Figure 2 shows the mean chromosome association at 20°C and 10°C in F₁ hybrids *T. durum* variety "Ld 222" X *T. longissimum* (chiasma frequency per cell 3.50) to be higher than that observed in euploid hybrids *T. aestivum* X *T. longissimum*. The presence of a translocated 5B-5D segment in "Resende" hybrids, significantly increased chromosome association (chiasma frequency per cell 4.72) in relation to that of normal triploid hybrids. At 10°C, hybrids of *T. longissimum* with "Ld 222" and those with "Resende" showed a significant drop in the frequency of chiasmata, which

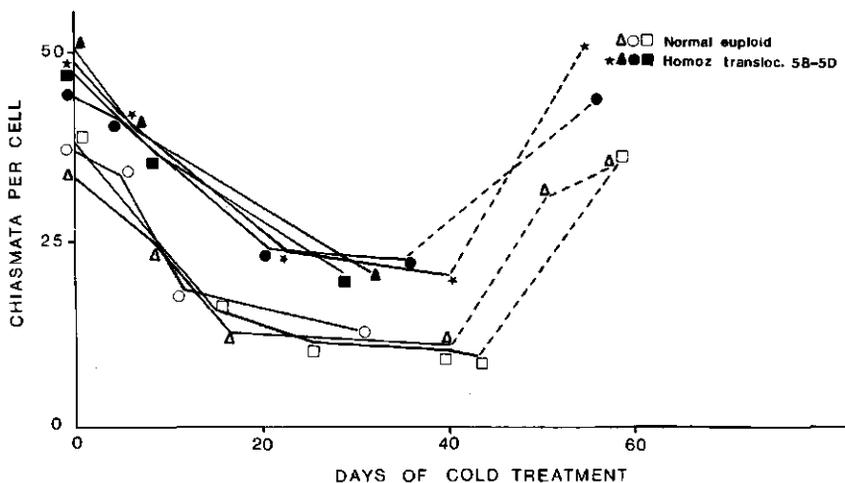


Figure 2 - Chiasmata per cell in F₁ hybrids *T. durum* X *T. longissimum* and in F₁ hybrids between a line of *T. durum* carrying a homozygous translocated 5B-5D chromosome X *T. longissimum* at metaphase I of meiosis. Straight lines: values obtained after different periods (0 - 43 days) at low temperatures (10°C). Dotted lines: recover to normal after transfer back to 20°C. (Each point represents the average of 50 PMC's).

Table 2 - Mean chromosome pairing at first metaphase of meiosis in F_1 hybrids
 (2n=21) *Triticum durum* ♀ (normal euploid and homozygous translocated
 5B-5D) X *Triticum longissimum* ♂. (50 cells per plant).

	COLD TREATMENT (DAYS)	RECOVERY (DAYS)	UNIVA	B I V A L E N T S			TRIV	CHIASM PER CELL
				RODS	RINGS	TOTAL		
Normal euploid								
1	--	--	14.24	2.76	0.22	2.98	0.26	3.72
1	15	--	17.98	1.36	0.06	1.42	0.06	1.60
1	25	--	18.68	1.16	--	1.16	--	1.16
1	40	--	18.96	1.02	--	1.02	--	1.02
1	43	--	19.08	0.96	--	0.96	--	0.96
1	--	15	14.96	2.34	0.38	2.72	0.20	3.50
2	--	--	14.90	2.50	0.18	2.68	0.28	3.42
2	8	--	16.18	2.14	0.12	2.26	0.10	2.58
2	17	--	16.48	1.18	0.02	1.20	0.04	1.30
2	40	--	19.02	0.88	0.08	0.96	0.02	1.08
2	--	10	15.26	2.56	0.10	2.66	0.14	3.04
2	--	17	14.66	2.62	0.18	2.80	0.24	3.46
3	--	--	14.20	2.82	0.16	2.98	0.28	3.70
3	5	--	14.70	2.84	0.22	3.06	0.06	3.40
3	12	--	17.48	1.56	0.08	1.64	0.08	1.88
3	30	--	16.48	1.16	0.04	1.20	0.04	1.32
Homz. transloc. 5D-5B								
1	--	--	13.68	2.52	0.60	3.12	0.36	4.44
1	5	--	13.54	3.18	0.28	3.46	0.18	4.10
1	21	--	16.42	2.12	0.08	2.20	0.06	2.40
1	35	--	16.74	1.90	0.08	1.98	0.10	2.26
1	--	20	13.40	3.04	0.34	3.38	0.28	4.28
2	--	--	12.58	3.18	0.58	3.76	0.30	4.94
2	6	--	13.54	3.08	0.26	3.34	0.26	4.12
2	23	--	16.58	1.98	0.14	2.12	0.06	2.38
2	40	--	17.42	1.48	0.22	1.70	0.06	2.04
2	--	14	12.18	3.42	0.36	3.78	0.42	4.98
4	--	--	12.24	3.64	0.26	3.90	0.32	4.80
4	9	--	15.18	2.04	0.66	2.70	0.14	3.64
4	29	--	17.02	1.80	0.10	1.90	0.06	2.12
5	--	--	12.12	3.52	0.44	3.96	0.32	5.04
5	7	--	14.40	2.50	0.56	3.06	0.16	3.94
5	32	--	17.06	1.82	0.06	1.88	0.06	2.06

was similar in both cases (Table 2).

F₁ hybrids of *T. aestivum* X *T. speltoides* at 20°C had very high levels of chromosome association (chiasma frequency per cell 15.20). The absence of the short arm of chromosome 5D or the entire chromosome 5D in these hybrids did not modify the pattern of chromosome association at 20°C (Figure 3). At low temperature, however, a significant drop in chro-

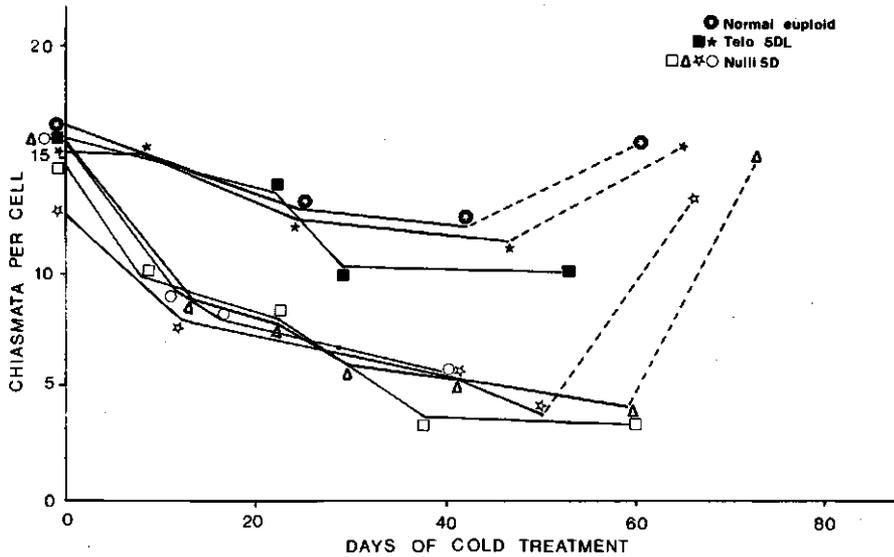


Figure 3 - Chiasmata per cell in F₁ hybrids of *T. aestivum* X *T. speltoides* at metaphase I of meiosis. Straight lines: values obtained after different periods (0 - - 59 days) at low temperature (10°C). Dotted lines: recovery to normal after transfer back to 20°C. (Each point represents the average of 50 PMC's).

mosome association was observed in nullisomic 5D hybrids, with a subsequent recovery after a few days at 20°C. (Table 3).

Table 3 - Mean chromosome pairing at first metaphase of meiosis in F_1 hybrids
Triticum aestivum ♀ (normal, monosomic 5D and ditelosomic 5D^L) X *Triticum longissimum* ♂. (50 cells per plant).

PLANT N ^o	CHROM N ^o	CHROM. 5D	COLD TREAT (DAYS)	RECOVERY (DAYS)	UNIVA	BIV	TRIV	QUADRV	CHIASMATA PER CELL
2	28	present	--	--	5.72	5.52	1.46	1.66	16.52
2	28	present	24	--	7.66	8.30	0.74	0.32	13.26
2	28	present	42	--	7.88	8.50	0.64	0.26	12.22
2	28	present	--	17	5.22	7.36	1.34	0.94	15.44
3	27	absent	--	--	5.38	5.54	1.36	1.52	15.82
3	27	absent	13	--	12.20	4.90	0.84	0.62	8.90
3	27	absent	23	--	14.34	4.70	0.64	0.28	7.62
3	27	absent	30	--	16.86	4.42	0.38	0.04	5.94
3	27	absent	59	--	19.38	3.40	0.22	0.04	4.14
3	27	absent	--	13	7.02	5.82	1.08	1.24	14.06
1	27	absent	--	--	5.50	5.30	1.64	1.46	14.82
1	27	absent	8	--	10.96	6.00	0.88	0.32	9.82
1	27	absent	23	--	13.08	6.12	0.48	0.06	7.80
1	27	absent	38	--	20.12	2.94	0.28	0.04	3.68
1	27	absent	59	--	20.54	3.08	0.10	--	3.36
7	27	absent	--	--	5.36	6.06	1.10	1.54	15.82
7	27	absent	12	--	12.04	6.14	0.76	0.10	9.12
7	27	absent	16	--	13.80	5.18	0.84	0.08	7.86
7	27	absent	40	--	17.30	4.02	0.42	0.10	5.60
6	27	absent	--	--	9.50	6.64	1.22	0.14	12.50
6	27	absent	12	--	14.06	5.36	0.56	0.12	7.90
6	27	absent	41	--	17.77	3.93	0.35	0.08	5.27
6	27	absent	50	--	20.34	2.94	0.26	--	3.70
6	27	absent	--	15	7.86	5.26	1.46	1.06	12.86
10	28	telo 5D ^L	--	--	4.16	5.98	1.66	1.70	15.38
10	28	telo 5D ^L	8	--	6.58	6.76	1.54	0.78	15.16
10	28	telo 5D ^L	24	--	8.88	7.08	1.26	0.26	12.48
10	28	telo 5D ^L	46	--	9.56	7.06	0.84	0.40	11.40
10	28	telo 5D ^L	--	18	5.14	5.54	1.74	1.60	15.56
11	28	telo 5D ^L	--	--	4.88	6.54	1.36	1.40	15.86
11	28	telo 5D ^L	22	--	8.00	5.96	1.64	0.79	13.43
11	28	telo 5D ^L	29	--	11.50	5.64	1.26	0.36	10.32
11	28	telo 5D ^L	52	--	11.38	5.82	1.30	0.26	10.05

DISCUSSION

The chromosomes of species belonging to the *Sitopsis* section of *Triticum* are closely related to those of the B genome of *Triticum* (Sears, 1956; Riley et al., 1958; Feldman, 1978). This close relationship reveals itself in the relatively consistent chromosome pairing shown in F_1 hybrids *T. durum* X *T. longissimum* (chiasma frequency per cell 3.50). The presence of a D genome in F_1 hybrids *T. aestivum* X *T. longissimum* induces asynapsis (chiasma frequency per cell 0.85). The most plausible explanation derives from the presence of a chromosome pairing suppressor gene located on the short arm of chromosome 3D (Mello-Sampayo, 1971). Evidence for this hypothesis is primarily based on the higher chromosome association shown by telosomic $3D^L$ plants (chiasma frequency per cell 7.18). A very significant drop in chromosome association (chiasma frequency per cell 0.30) was found in F_1 hybrids *T. aestivum* X *T. longissimum* at low temperature, when chromosome 5D and $3D^S$ were simultaneously absent. This result agrees with what was expected, since *Ltp* gene was missing.

A very different pattern was seen in hybrids with tetraploid wheat. The hybrids "Ld 222" X *T. longissimum* and "Resende" X *T. longissimum* showed similarly shaped curves of chromosome association (expressed in frequency of chiasmata per cell versus number of days at low temperature) which indicates that a "*Ltp*-like" gene is acting in both hybrids. Hayter (1969) has suggested that *T. durum* carried a *Ltp* gene allele, since the frequency of chiasmata remains at a constant level with lower temperatures. A single dosage of this gene in F_1 hybrids should be enough to stabilize chromosome association to some degree, even if lower than that of *T. durum* in the same situation.

The results obtained in "Resende" X *T. longissimum* show a constant higher value of chromosome association, with increasing number of days at low temperature, as compared with "Ld 222" X *T. longissimum*. It is possible that the translocated 5B-5D chromosome of "Resende" is responsible for this different level which is maintained either at normal or at low temperatures.

It has been suggested (Mello-Sampayo, 1972; Mello-Sampayo and

Viegas, 1973) that the intermediate type of pairing shown in hybrids carrying such translocated 5B-5D chromosome is due either to the addition of a promoter gene carried by the segment of 5D^L or to loss of a weak suppressor gene carried in the distal segment of 5B^L. The pattern obtained for chromosome association in both hybrids (parallel curves) indicates that no *Ltp* gene was carried by the translocated segment of 5D. Moreover, the observed pattern (a constant difference for chromosome association between both hybrids, over days of cold treatment), suggests that the effect of the stabilizer gene for low temperature is the same in both curves. It seems, then, that their difference may be explained just by the different starting levels of chromosome association.

Hybrids of *T. aestivum* with *T. speltoides* yielded two different types of curves: when hybrids had a normal 5D or telosomic 5D^L, a high chromosome association at low temperature was observed; when the hybrids were deficient for chromosome 5D a drastic reduction in chromosome association occurred, at 10°C. Both results confirm that the *Ltp* gene is located in the long arm of chromosome 5D. This is seen in Figure 3, which also shows that chromosome pairing stabilizes at a low but significant level. This indicates that *T. speltoides* carries a low temperature stabilizer gene, which appears to have a weaker effect than the one carried by chromosome 5D (Attia, et al., 1977).

The availability and the interrelationship of pairing stabilizer genes for low temperature in Triticinae species must be related to their geographical distribution. Chromosome engineering designed to increase their content in the genome of useful varieties, would enhance stability for a wider temperature range.

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MODULATION OF rRNA GENE CONTENT
BY CHROMOSOME 5D IN WHEAT

WANDA S. VIEGAS

Instituto Gulbenkian de Ciência, Oeiras, Portugal*

SUMMARY

The number of DNA genes coding for ribosomal RNA in a number of species and karyotypes of the sub-tribe Triticinae was determined through rRNA/DNA *in vitro* hybridization. One of the Nucleolar Organizing Regions in wheat is carried by the short arm of chromosome 5D. The combinations studied included aneuploid lines with different dosages of chromosome 5D or of its long arm (5D^L). At the hexaploid level, the deletion of the short arm of this chromosome induced different alterations in total rDNA depending on the morphological structure of the long arm (ditelosomic 5D^L, 68%; mono-isosomic 5D^L, 95%; di-isosomic 5D^L, 84%). In tetrasomic 5D plants rRNA gene number was decreased by 30% in contrast with nullisomic 5D-tetrasomic 5A or 5B which only showed a slight reduction (10%). At the tetraploid level, the presence of a segment of 5D^L in the genotype of *T. durum* or the addition of two chromosomes 5D to the genotype of *T. dicoccum* increased by 19 and 33%, respectively the number of rRNA genes.

These results show not only that the short arm of chromosome 5D carries very few rRNA genes in its NOR, but that there is no correlation between the number of rRNA genes and of NOR's in 5D. The existence of a regulatory mechanism, located in 5D^L, which modulates the number of rRNA genes present in the NOR's of wheat chromosomes, is suggested.

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INTRODUCTION

The chromosome complement of eukaryotic organisms carries specific sites (Nucleolar Organizer Regions, NOR) of DNA coding for ribosomal RNA (Birnstiel, et al., 1971). These regions are organized in reiterated sequences that code for 18S, for 25S and some 5S ribosomal RNA (rRNA). A considerable variation in the level of rDNA gene redundancy among species has been reported. In some species like *Drosophila melanogaster*, *Xenopus laevis*, *Zea mays*, *Datura innoxia* and *Nicotiana* species, it was observed that the number of rRNA genes was proportional to the number of NOR's (Ritossa and Spiegelman, 1965; Wallace and Birnstiel, 1966; Phillips et al., 1971; Cullis and Davies, 1974; Cullis, 1975). In several other species such proportional relationship was not observed, under certain genetic or developmental conditions (Gall, 1969; Tartoff, 1971; Spear and Gall, 1973). The gene redundancy for each NOR in higher plants was found to be variable in several studies on the rDNA content of aneuploids and species of differing ploidy (Timmis, Sinclair and Ingle, 1972; Timmis and Ingle, 1973; Siegel et al., 1973; Maher and Fox, 1973).

Nucleolar organizers have been under extensive study in hexaploid wheat ($2n=6x=42$) *Triticum aestivum*. In the variety Chinese spring, Crosby (1957) observed four active nucleolar organizers in microspore cells, which were assumed to be located in the short arms of chromosomes 1A, 1B, 6B and 5D. In aneuploid plants of the same variety Mohan and Flavell (1974) and Liang et al. (1977), found shifts in the number of rRNA genes which were not always proportional to the number of existing NOR's. Changes in the number of NOR's carried either by chromosome 1B or by chromosome 6B induced proportional alterations in the number of rRNA genes. The absence of the short arm of chromosome 1A was found to be responsible for a high decrease in rDNA content, although the presence of four doses of this chromosome did not alter the normal rDNA amount. An even more complex situation could be observed in relation to chromosome 5D since plants lacking the short arm of this chromosome (ditelosomic 5D^L) showed a significant decrease in rRNA gene number, and a similar effect was also observed with an increased dosage of that chromosome, as in tetrasomic 5D

plants (Flavell and O'Dell, 1976). The complexity of these somehow unexpected results, prompted us to further investigate this problem. Several hexaploid and tetraploid wheat genotypes (5D chromosome variants) were available for study using *in vitro* hybridization DNA/rRNA techniques. Experimental data concerning variation in the number of rRNA genes for these aneuploid wheats is presented and their relevance on the genetic control of rRNA gene multiplicity discussed.

MATERIAL AND METHODS

Seeds of *T. aestivum* var. Chinese spring, *T. spelta* synthetic, *T. durum* var. Ld 222, *T. dicoccum* and *T. tauschii* [= *Aegilops squarrosa*] were all originally obtained from Dr. E.R. Sears.

In *T. aestivum* ditelosomic 5D^L plants the short arm of chromosome 5D is absent. The same kind of deficiency exists in mono-isosomic 5D^L plants in which a single isochromosome with a duplicate long arm of chromosome 5D is present. Di-isosomic 5D^L plants were derived directly from the selfing of mono-isosomic 5D^L. In tetrasomic 5D plants, four chromosomes 5D are present. Disomic 5D plants which were derived from crossing monosomic 5D X tetrasomic 5D are referred to in this paper as disomic 5D "recovered". *T. spelta* synthetic was derived from the crossing of *T. dicoccum* X *T. tauschii* with subsequent duplication of the haploid genome by colchicine (McFadden and Sears, 1946). It is thought that *T. tauschii* is the donor of the D genome of *T. aestivum* (Kihara, 1944).

"Resende" is a stable line of *T. durum* var. Ld 222 in which a large distal segment of the long arm of chromosome 5D was homozygously substituted for an homoeologous segment of the long arm of chromosome 5B. This line was originated through initial crossing of *T. aestivum* "Chinese spring" (nulli 5B-tetra 5A) X *T. durum* var. Ld 222, followed by five backcrosses with Ld 222 (Mello-Sampayo and Viegas, 1973). "AAL9" and "AAL1" are disomic alien chromosome addition lines in which chromosomes 5D and 4D of *T. tauschii* were, respectively added to *T. dicoccum*. These lines were obtained by Dr. Noronha-Wagner (1969) from *T. spelta* synthetic X *T. tauschii*.

chii and successive backcrosses to *T. dicoccum* for elimination of all but one *T. tauschii* chromosome and final selfing for recovery of disomic alien chromosome condition. The added *T. tauschii* chromosome was identified as 5D (in AAL9) and 4D (in AAL1) through test crosses to the corresponding ditelosomic lines of *T. aestivum*.

In all the genotypes studied, root-tips of germinating seedlings were used to cytologically check the chromosome number and the maximum number of nucleoli per cell. Metaphase plates were studied after pre-treatment of the root-tips with 1-bromonaphtalene and fixation in aceto-carmine. The silver impregnation technique used to stain the nucleoli followed the method described by Fernandez-Gomez et al. (1969).

Plants from all the genotypes were grown at 20°C under continuous light and leaves were harvested after 2 months and they were stored at -20°C, for DNA extraction.

DNA was extracted and purified according to the procedure described by Flavell and Smith (1974). DNA samples were finally purified by CsCl gradient centrifugation as described by Flamm, Bond and Burr (1966) and recovered by ethanol precipitation from the diluted caesium chloride gradient.

³H-labelled rRNA was extracted from wheat embryos of *T. aestivum* var. Chinese spring which were incubated in a medium with ³H-uridine during 16 hours. Extraction and purification of ³H-labelled rRNA was made according to the procedure described by Payne and Dyer (1971). The ³H-rRNA which was obtained had a specific activity of 39,474 cpm/μg.

Hybridization between DNA and H³-rRNA was made following methods previously described by Mohan and Flavell (1974). The percentage of hybridization between rRNA and DNA was calculated from the specific activity of the rRNA and the DNA kept in the filter after hybridization. DNA was calculated after acid hydrolysis by the method of Brown and Weber (1968).

All measurements were corrected for the values obtained in filters without DNA. In each experiment six duplicate filters for each DNA were incubated as well as six filters of DNA of Chinese spring (control) and in many cases, duplicate DNA preparations were also used.

Statistical analysis was carried out to compare the mean percentage of each DNA which hybridized to rRNA using t-tests.

The number of genes per 2C genome was calculated with the values obtained for rRNA/DNA hybridization and the DNA content per nucleus for each genotype, assuming a molecular weight of 2×10^6 daltons for the two rRNA types combined. The DNA amount per nucleus was assumed to be proportional to the total chromosome length. The values used for chromosome arm lengths were taken from results published by Sears (1954).

RESULTS

Table 1 shows the different hexaploid and tetraploid wheat genotypes used in this work. The maximum number of nucleoli present per cell is also shown as well as the assumed number of nucleolar organizers (NOR) in each genotype studied (calculated in accordance with Crosby, 1957). Although it has been suggested that there are eight NOR's in euploid *T. aestivum*, the maximum number of nucleoli present has always been six, in our material.

Ditelosomic 5D^L, mono-isosomic 5D^L, di-isosomic 5D^L, nullisomic 5D - tetrasomic 5A and nullisomic 5D - tetrasomic 5B, all have shown a decrease from six to four in the maximum number of nucleoli, since chromosome 5D carries in its short arm a nucleolar organizer. In tetrasomic 5D plants the presence of two extra nucleolar organizers increased the number of nucleoli to eight.

Tetraploid wheats (*T. durum* and *T. dicoccum*) exhibit a maximum of four nucleoli. The existence of a translocated segment 5B-5D in "Re-sende" did not change this number of nucleoli. In *T. dicoccum*, however, the addition of two chromosomes 5D (AAL9) changed the number of nucleoli from 4 to 6. Cells of the diploid *T. tauschii* never showed more than two nucleoli.

Estimates of the DNA contents of 2C nuclei in the different combinations were made taking into consideration the alterations in total chromosome length which are thought to have occurred for each aneu-

Table 1 - Number of NDR's and DNA content in diploid nuclei of some Triticinae species.

	$2n$ CHROMOSOME NUMBER	MAXIMUM OBSERVED NUMBER OF NUCLEOLI PER CELL	ESTIMATED NUMBER OF NUCLEAR ORGANIZERS	10^{-12} g/2C NUCLEUS DNA CONTENT
<i>T. aestivum</i> (AABBDD)	Euploid	42	6	36.20
	Ditelosomic 5D ^L	40 + 2 telocentrics	4	35.80
	Mono-isosomic 5D ^L	40 + 1 isochromosome	4	35.80
	D1-isosomic 5D ^L	40 + 2 isochromosomes	4	36.55
	Nulli 5D-Tetra 5A	42	4	37.04
Nulli 5D-Tetra 5B Tetrasomic 5D	42	4	37.35	
	44	8	37.40	
			10	
<i>T. spelta</i> (AABBDD)	Synthetic	42	6	35.20
				8
<i>T. durum</i> (AABB)	Euploid	28	4	26.28
	Homoz. trans. (Resende)	28	4	26.28
	5B-5D			
<i>T. dicoccum</i> (AABB)	Euploid	28	4	26.28
	Allen add. 5D (AAL9)	30	6	27.45
	Allen add. 4D (AAL1)	30	4	27.57
		14	2	9.94
<i>T. tauschii</i> (DD)				

ploid plant.

The estimation, by rRNA/DNA hybridization, of the multiplicity of rRNA genes, in different wheat genotypes is summarized in Table 2. The values shown for "percent DNA hybridized to rRNA" represent means of at least six replications. Corrections of these values for normal hexaploid DNA content have been carried out.

Ditelosomic 5D^L plants showed a significant decrease ($P < 0.001$) in the number of rRNA genes when compared with the euploid (7192 ± 72 and 10518 ± 210 , respectively). In mono-isosomic 5D^L plants, where the same deletion of the short arm has occurred, no significant alteration in the rRNA number (9959 ± 199) was, however, observed. In di-isosomic 5D^L plants a decrease in rRNA was detected (8812 ± 88 , significant at $P < 0.001$). An even higher reduction (7318 ± 146) in rRNA genes was found in tetrasomic 5D plants ($P < .001$). The study of a disomic 5D "recovered" plant showed that this reduction is reversible since this plant reverted to the normal euploid level (10182 ± 204). Nullisomic 5D-tetrasomic 5A and nullisomic 5D-tetrasomic 5B plants showed only a slight decrease in rRNA gene content when compared with the euploid, even though neither 5A nor 5B carry NOR's.

Significant differences in the number of rRNA genes can be observed in species with the same level of ploidy. This is the situation seen when *T. spelta* synthetic (11637 ± 233) is compared with *T. aestivum* and when *T. durum* var. Ld 222 (7717 ± 309) is compared with *T. dicoccum* (6011 ± 240). "Resende" showed a significant increase ($P < 0.001$) in the rRNA amount (9666 ± 290), when compared with Ld 222. A similar effect was observed in "AAL9" (9502 ± 570) due to the addition to *T. dicoccum* of two chromosomes 5D. No alteration was detected, however, by the addition of chromosome 4D (AAL1) to the same "dicoccum" background. *T. tauschii*, a diploid species, presented an average number of rRNA genes per 2C nucleus equal to 4086 ± 82 .

Global comparisons of all the genotypes studied in reference to *T. aestivum* are presented in Fig. 1 and in the next section.

Table 2 - Hybridization rRNA/DNA in several species of the sub-tribe Triticeinae. Anu-
ploids of polyploid wheat carrying different dosages of chromosome 5D are in-
cluded.

GENOTYPE OF DNA	PERCENT HYBRIDIZATION (µg rRNA/100 µg DNA)	PERCENT HYBRIDIZATION CORRECTED TO NORMAL HEXAPLOID DNA CONTENT	rRNA GENE NUMBER/2C NUCLEUS
<i>T. aestivum</i>			
Euploid	0.094 ± .002	0.094 ± .002	10518 ± 210
Ditelosomic 5D ^L	0.085 ± .001	0.064 ± .001	7192 ± 72
Mono-isosomic 5D ^L	0.090 ± .002	0.069 ± .002	9959 ± 199
Di-isosomic 5D	0.078 ± .001	0.079 ± .001	8812 ± 88
Nulli 5D-Tetra 5A	0.082 ± .002	0.084 ± .002	9388 ± 188
Nulli 5D-Tetra 5B	0.085 ± .002	0.088 ± .002	9813 ± 196
Tetrasomic 5D	0.063 ± .002	0.065 ± .002	7318 ± 146
Disomic 5D "Recovered"	0.091 ± .002	0.091 ± .002	10182 ± 204
<i>T. speitza</i>			
Synthetic	0.104 ± .002	0.104 ± .002	11637 ± 233
<i>T. durum</i>			
Euploid	0.095 ± .004	0.089 ± .004	7717 ± 309
Homoz. trans. 5B-5D (Resende)	0.119 ± .003	0.086 ± .003	9666 ± 290
<i>T. dicoccum</i>			
Euploid	0.074 ± .004	0.054 ± .004	6011 ± 240
Alien add. 5D (AAL9)	0.112 ± .006	0.085 ± .006	9502 ± 570
Alien add. 4D (AAL1)	0.078 ± .001	0.060 ± .001	6671 ± 67
<i>T. tauschii</i>	0.133 ± .002	0.036 ± .002	4086 ± 82

DISCUSSION

Within the available data comparisons between the number of rRNA genes in the different karyotypes and the number of nucleolar organizers (NOR's) present, do not disclose clear correlations between those parameters. It is as if the chromosomal level of rDNA is subjected to quantitative regulation by gene(s) besides those located in the NOR's.

Fig. 1 shows a more global view of the results. The number of rRNA genes for each genotype is expressed as the percentage of the number of the genes determined in euploid *T. aestivum*. Combinations where the

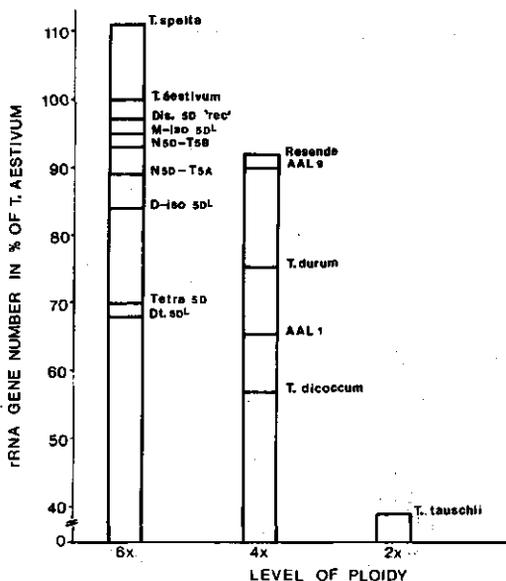


Figure 1 - Relative values of rRNA/DNA hybridization in euploid and aneuploid plants of *Triticum* spp. at different levels of ploidy.

short arm of chromosome 5D was absent showed unexpected differences. The number of rRNA genes in ditelosomic 5D^L plants appeared to be reduced by 32%, when compared with the euploid. In mono-isosomic 5D^L plants, no significant reduction was, however, detected. The presence of two isochromo-

somes in di-isosomic 5D^L plants decreased the number of rRNA genes by 16%.

Chromosome 5D has been referred to as carrying a nucleolar organizer in its short arm (Crosby, 1957). The different alterations in the rRNA gene number induced by the deletion of the short arm of chromosome 5D, indicates that the role of this chromosome in the control of rRNA content is very complex. Comparisons between results obtained for ditelosomic and mono-isosomic 5D^L plants suggest the possible relevance on the modulation of rRNA gene amount by the two different genotypes of the long arms of chromosome 5D. The increase in the total dosage of chromosome 5D (in tetrasomic 5D plants) substantially reduced (-30%) the number of rRNA genes. This seems to suggest the existence of a gene(s) in the long arm of chromosome 5D which can suppress rRNA genes. The disomic 5D "recovered" plant showed the normal euploid content of rRNA. This quantitative reversibility in monosomic 5D X tetrasomic 5D F₁ plants, suggests a dose dependent regulatory mechanism located in 5D^L. It may be that a suppressor gene(s) as mentioned above, located in the long arm of chromosome 5D, becomes noticeable specially when present in high numbers. In the total absence of chromosome 5D as in nullisomic 5D-tetrasomic 5A or 5B only a slight reduction in the rRNA gene amount was detected and this may correspond to the lack of the respective NOR's, combined with loss of the suppressor.

At the tetraploid level, high values of rRNA genes were measured, either when a segment of the long arm of chromosome 5D was substituted for the corresponding homoeologous segment of 5B^L in the genome of *T. durum* (as in "Resende", +19%) or when two entire chromosomes 5D were added to *T. dicoccum* (as in "AAL9", +33%). The high values measured in total rRNA amount in "Resende" may be due to the existence of a gene(s) that amplifies the total number of rRNA genes.

Our results and those from Flavell and O'Dell (1976) showed a significant decrease in rDNA amount in tetrasomic 5D plants, when compared with the euploid, which was not detected in the plants measured by Liang et al. (1977). A genetic divergence of the stocks analysed or differences in the techniques used may be a possible explanation for the discrepant results obtained. All the comparative results described are, however, con-

sistent in what concerns the absence of correlation between the number of nucleolar organizers, from chromosome 5D of *T. aestivum*, and the number of rRNA genes. Moreover, Flavell and D'Dell (1976), working on chromosomally balanced substitution lines, where substitution of chromosome 5D was achieved in a constant background, found evidence that the number of rRNA genes carried by chromosome 5D must be a very small proportion of the total amount. This again reinforces the hypothesis that the evident alterations observed in the total rRNA amount in the different combinations with chromosome 5D are not due to alteration in the number of rRNA genes from this chromosome but to the existence of a regulatory mechanism coded by genes located in its long arm.

In plants, very little is known about the amplification of rRNA genes during development. Some authors have measured the number of rRNA genes during the development of the wheat embryo, but the results obtained until now are quite diverse (Chen and Osborne, 1970; Ingle and Sinclair, 1972). The complexity of the situation described in this paper, did not allow the construction of a global model to integrate all the data. It may be that this has to wait until further experiments have been performed. We put forward, nevertheless, the need for the existence of two genes in the long arm of chromosome 5D, with opposite effects, in the control of rRNA gene number.

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SUMMARY AND CONCLUSIONS

Results obtained and hypotheses that have been put forward to integrate them, are summarized in this section.

1. Studies of chromosome association in F_1 hybrids *Triticum aestivum* X *Secale cereale* with and without chromosome 5B and in the presence or in the absence of B-chromosomes of rye, showed, as expected, that in the absence of chromosome 5B a high degree of pairing was observed. The presence of B-chromosomes only slightly reduced chromosome association, whether chromosome 5B was present or not.

2. In F_1 *T. aestivum* X *S. cereale* hybrids, at 20°C, a significant increase in the mean chiasma frequency per cell in nulli 5D + B's hybrids was observed. This interaction of genes carried by B-chromosomes of rye with genes carried by A-chromosomes was only detected in the absence of chromosome 5D of wheat.

3. A substantial rise in chromosome association was also observed in an inter-varietal *T. aestivum* hybrid Chinese spring X Lindström when chromosome 5D of wheat was absent and B-chromosomes of rye were present, both at 20°C and at 10°C. This was mainly due to a shift in the relative numbers of rod and ring bivalents, since the number of univalents was very low and similar. No change in chromosome association due to B-chromosomes was detected when 5D was present.

4. Bayliss and Riley (1972) found in nullisomic 5D plants of *T. aestivum* a higher frequency of ring bivalents (with more than two chiasmata) than in disomic 5D plants, being however, the values of mean chiasmata per cell very similar in both plants. Mello-Sampayo and Miller (1979) detected a reduction in the number of ring bivalents with an increasing dosage of the long arm of chromosome 5D. These data together with our results led us to suggest the existence of a desynaptic gene in chromosome 5D which directly interacts with gene(s) carried by B-chromosomes of rye.

5. It was also found that in F_1 hybrids *T. aestivum* X *S. cereale*, in the absence of chromosome 5D, synchronization within PMC's was reduced, which was not affected by the presence of B-chromosomes neither by

differences in temperature (22°C and 15°C). In the absence of chromosome 5B no alteration in the synchronization of meiosis was detected, but the variation between plants covered the entire range observed between types in the experiments with nulli 5D.

6. Asynapsis at low temperature is prevented by a stabilizer gene of chromosome association (*Ltp*) located in the long arm of chromosome 5D of *T. aestivum*. Studies in the detection of compensatory genes, at 10°C, for *Ltp* in diploid relative species of wheat were performed using F₁ hybrids *T. aestivum* X *S. cereale*, *T. longissimum* (= *Ae. sharonensis*) and *T. speltoides*, as well as *T. durum* X *T. longissimum* (= *Ae. sharonensis*) in which chromosome 5D was absent (nullisomic 5D). Results obtained demonstrated the existence of a *Ltp*-like gene in *T. speltoides* and *T. durum*, which compensates to a certain degree the absence of chromosome 5D. No compensation for the absence of *Ltp* was, however, found in *S. cereale* and in *T. longissimum* (= *Ae. sharonensis*).

7. Asynapsis has also been detected in plants of *T. aestivum* which were disomic for a special isochromosome of the long arm of chromosome 5D (iso 5D_M^L). This suppressive effect in chromosome association was even more detectable in F₁ hybrids *T. aestivum* (di-isosomic 5D_M^L) X *S. cereale*, *T. longissimum* (= *Aegilops sharonensis*) and *T. longissimum* (= *Ae. longissima*, intermediate pairing line).

8. Meiotic isochromosome configurations in several lines of *T. aestivum* which were disomic for different isochromosomes, were studied. A higher number of univalent isochromosomes was observed in di-isosomic 5D_M^L and 5B^L plants than in other di-isosomic combinations. The results demonstrated that (a) gene(s) of 5D_M^L affecting chromosome pairing can be considered as (a) general suppressor(s) of chromosome association rather than a specific inhibitor of homoeologous pairing.

9. Several hypotheses were put forward to explain the appearance of this suppressor gene(s) in isochromosome 5D_M^L. Although we cannot discard the hypothesis that it derives from a translocation with 5B^L, it seems, however, more likely that it arose by mutation or duplication of a pre-existing but otherwise undetectable gene.

10. Besides the described effects dependent on chromosome 5D of

T. aestivum, it is also known that this chromosome carries in its short arm a nucleolar organizer. In ditelosomic $5D^L$ plants the total number of rDNA copies decreases substantially (-32%) and this was also observed in plants carrying four doses of chromosome 5D (tetrasomic 5D, -30%). In a disomic 5D plant which had been derived from a tetrasomic 5D the number of rDNA copies was, however, similar to that observed in euploids. Mono-isosomic $5D^L$ and di-isosomic $5D^L$ plants showed a reduction of only 5% and 16%, respectively, in the total number of rDNA copies.

11. In tetraploid wheats it was also observed that the introduction of a segment of the long arm of chromosome 5D in the complement (as in "Resende") or the addition of two entire chromosomes 5D (as in "AAL9") increased in 19% and 33%, respectively, the number of rRNA genes.

12. These results show that chromosome 5D plays an important role on the control of the total number of rRNA genes although only a few rDNA genes are carried by its nucleolar organizer. The existence of a regulatory mechanism located in $5D^L$ which modulates the number of rRNA genes is suggested.

13. A higher transcriptional rate for rRNA in root-tip cells of di-isosomic $5D^L_M$ plants has been measured (Viegas and Mello-Sampayo, 1975). In these same plants a high degree of asynapsis was detected both in hexaploid wheat and F_1 hybrids with diploid relative species. These observations taken together may mean a functional correlation between rDNA multiplicity and the control of meiotic behaviour.

The results obtained throughout this work contributed especially to a better understanding of the role of chromosome 5D, since several new aspects of this chromosome in different cellular mechanisms, were detected. It was suggested that a new desynaptic gene and a gene to control synchronization within PMC's in meiosis should exist in 5D. Moreover, the long arm of chromosome 5D should carry a suppressor of pairing (in a mutated isochromosome $5D^L$) and gene(s) regulating the total number of rDNA copies. Also the existence of the *Ltp* gene in this arm has been confirmed.

The way in which genes carried by B-chromosomes interact with genes carried by A-chromosomes has also been a goal of this work. When an

A-chromosome carrying a promoter of pairing is absent from the genetic complement, the addition of B-chromosomes of rye increased chromosome association. On the contrary, in the absence of a suppressor gene, B-chromosomes decreased chromosome association. This seems to suggest a regulating role for the genes carried by B-chromosomes of rye in what concerns the control of chromosome association.

To construct a more discriminative map of gene location for chromosome 5D, subsequent crosses between ditelosomic $5D^L$, $5D^S$ plants and the diploid relative species would be performed. In the same way, the future study of chromosome association in plants carrying simultaneously the isochromosome $5D^L$ and $5D^L_M$ would give more information about the suppressor gene existing in $5D^L_M$. It would also be possible to evaluate if the parallel made between the effect of this gene and *Ph* gene carried by $5D^L$ is legitimate by the study of nulli 5B-iso $5D^L_M$ plants. A better understanding of the control of rDNA copies in *T. aestivum*, will be achievable through the future use of *in situ* hybridization DNA/rRNA techniques, complementary to the results already obtained by *in vitro* techniques.

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CURRICULUM VITAE

MARIA WANDA SARUJINE VIEGAS was born in Mozambique on the 7th of March 1950. In 1975 she took the degree of Eng. Agr. at the Agricultural University of Lisbon. Since 1973 she has been working at the Cytogenetics Department of the Gulbenkian Institute of Science and for a time at the Plant Breeding Institute, Cambridge. In November 1975 she has started to work with Dr. Ir. J. Sybenga at the Genetics Department, Agricultural University of Wageningen.