Theor Appl Genet (1986) 72:816–821

Analysis of the effect of rye chromosomes 4R, 6R and telomeric heterochromatin on 7R on homologous chromosome pairing in pentaploid hybrids (AABBR, AABBD)*

P. K. Gupta [1] and G. Fedak [2]

[1] Department of Agricultural Botany, Meerut University, Meerut, India
[2] Cytogenetics Section, Ottawa Research Station, Research Branch, Agriculture Canada, Ottawa, Ontario K1A OC6, Canada

Received February 5, 1986; Accepted April 14, 1986
Communicated by G. S. Khush

Summary. Meiotic pairing in *Triticum turgidum* cv. Ma (4x) with a mean chiasmata frequency of 27.16 per cell was compared with chiasmata frequencies in its hybrids with several triticale strains, 'Chinese Spring' wheat and its addition lines for 'Imperial' rye chromosomes 4R and 6R. In hybrids between 'Ma' and ×*Triticosecale* cv. 'Rosner' the chiasmata frequency was marginally reduced by an average of 1.25%, by 8.8% in hybrids with ×*Triticosecale* cv. 'DRIRA' HH and by 6.7% with 'DRIRA' EE (lacking 90% telomeric heterochromatin from chromosome arm 7RL). In pentaploid hybrids between 'Ma' and *T. aestivum* cv. 'Chinese Spring' the reduction was an average of 10.30%, while addition lines with rye chromosome 6R reduced chiasmata frequencies by an average of 7.4% and rye addition line for 4R showed the greatest depression in chiasmata frequency in hybrids by a 25.04% reduction. An interchange difference involving long chromosome segments was observed between 'Ma' and 'Rosner'.

Key words: *Triticum* – ×*Triticosecale* – *Secale* – Rye – Meiosis – Chromosome pairing – Heterochromatin

Introduction

Genomes of rye (*Secale cereale*) and other species of *Secale* have been shown to promote homoeologous chromosome pairing in hybrids with *Triticum aestivum* (Lelley 1976; Dvorak 1977).

Similar effects were also observed in hybrids with different polyploid species of *Hordeum* and in those with 6x *Aegilops crassa* (Gupta and Fedak 1985a, b). In these earlier studies, the genetic system inducing this pairing has been suggested to be polygenic involving more than one rye chromosome. The degree of meiotic pairing in some of the hybrids has been attributed to the relative amounts of heterochromatin possessed by genomes of different species of *Secale* (Gupta and Fedak 1985b).

In view of the above, this study was initiated to study firstly the effect of individual rye chromosomes on meiotic pairing in intergeneric hybrids and secondly, to study the effect of loss of specific heterochromatin blocks on meiotic pairing in these hybrids. The first objective could be achieved by using individual rye chromosome addition lines on bread wheat and the second by using the isogenic lines of triticale cultivars 'Rosner' $6R^{++}$ and $6R^{--}$ (Roupakias and Kaltsikes 1977) and 'DRIRA' HH and EE (Merker 1976) differing in specific blocks of telomeric heterochromatin.

In order to achieve the above objective, a comparative study of meiosis was undertaken in the following parental and hybrid materials: (a) tetraploid wheat; (b) pentaploid hybrids (4x wheat×6x triticale 'Rosner' $6R^{++}$); (c) pentaploid hybrids (4x wheat×6x triticale 'DRIRA' EE); (e) pentaploid hybrids (4x wheat×6x wheat 'Chinese Spring'; (f) pentaploid hybrids with 2n=36 (4x wheat×'Imperial' rye additions for 4R and 6R in 6x wheat 'Chinese Spring'). Unfortunately, our efforts to produce the 4x wheat×6x triticale 'Rosner' $6R^{--}$ hybrids did not succeed. However, hybrids of triticale 'Rosner' $6R^{++}$ and 'Rosner' $6R^{--}$ with 6x *H. parodii* and those of triticale 'DRIRA' HH and 'DRIRA' EE with 4x *H. jubatum*, were also produced which is a part of a separate study. The results of meiosis in these hybrids with *H. parodii* and *H. jubatum* are being published separately but will be utilized for a relevant discussion in this communication.

* Contribution No. 819 Ottawa Research Station

Table 1. Different species/cultivars/stocks used in hybridization

Species/cultivar	Ploidy level	Chromosome no. (2 *n*)	Source
1. *T. turgidum*			
cv. 'Ma'	4x	28	A. Martin, Cordoba, Spain
2. × *Triticosecale* Wittmack			
(a) cv. 'Rosner' ($6R^{++}$, $6R^{--}$)	6x	42	University of Manitoba
(b) cv. 'Drira' (HH, EE)	6x	42	University of Manitoba
3. *T. aestivum*			
(a) cv. 'Chinese Spring'	6x	42	University of Missouri
(b) Alien addition lines (4R, 6R)	6x	44	University of Missouri

Materials and methods

Plant material

The details of species and stocks used as parents with their sources and other details are given in Table 1. Hexaploid triticale 'Rosner' $6R^{++}$ and 'Rosner' $6R^{--}$ were isolated by Roupakias and Kaltsikes (1977) and 'DRIRA' HH and 'DRIRA' EE were isolated by Merker (1976). 'Rosner' $6R^{--}$ differs from $6R^{++}$ in having lost 90% of the telomeric heterochromatin of the short arm of 6R. Similarly, 'DRIRA' EE differs from 'DRIRA' HH in having lost telomeric heterochromatin (90%) on the long arm of 7R. Seeds of the rye addition lines were supplied by Dr. E. R. Sears, University of Missouri, Columbia.

Plant culture

The plants were grown from seed in a controlled environment cabinet at a temperature of 20°/15 °C day/night and 16 h days provided by a combination of fluorescent and incandescent lamps supplying illumination at the intensity of 800 μE m^{-2} s^{-1}. The plants were vernalized where required as seedlings for 8 weeks at 4 °C and 8 h days prior to planting in a growth cabinet.

Crossing

Triticum turgidum cv. 'Ma' was always used as the female parent, and its spikes were emasculated two days prior to anthesis and bagged with dialysis tubing. Pollen from the other parent in each case was collected and applied to emasculated spikes by means of a small brush. At 15 days after pollination, the hybrid embryos were excised and plated on a B_5 medium modified by the exclusion of 2,4-dichlorophenoxyacetic acid (2,4-D). When shoots appeared, seedlings were moved into diffuse and eventually into strong light and were raised in the same manner as the parental material.

Cytological methods

Spikes of hybrid plants were fixed in Carnoy's solution (6 parts ethanol : 3 parts chloroform : 1 part glacial acetic acid) for at least 24 h and squashed in acetocarmine. For better staining, anthers of appropriate meiotic stages were immersed in Snow's solution (Snow 1963) overnight before they were squashed.

Results

Production of hybrids

Data on seed set, embryo yield and plantlets raised through embryo culture technique are given in Table 2. All crosses were characterized by high seed set, however, a corresponding high yield of plantlets was not obtained because of poorly differentiated embryos in the seeds.

Meiosis in hybrids

The results of chromosome pairing in different hybrids are given in Table 3 and characteristic meiotic configurations are shown in Figs. 1–3. The chiasmata frequency was reduced to different degrees in all hybrids listed in Table 3 relative to that observed in Ma. The reduction in chiasmata frequency was only negligible in hybrids with ×*Triticosecale* cv. 'Rosner' $6R^{++}$ at an average of 1.25%. The chiasmata frequencies in hybrids with ×*Triticosecale* cv. 'DRIRA' were all reduced relative to 'Ma'. The frequencies in the two hybrids with 'DRIRA' HH at 23.66 and 25.90 overlapped that with the 'DRIRA' EE hybrid at 25.35 thus no conclusive effect on chiasmata frequency can be attributed to the telomeric heterochromatin on rye chromosome arm 7RL. The suppression of chiasmata frequency in the hybrid carrying the genome of 'Chinese Spring' plus 6R was not as great as that in the pentaploid hybrid lacking 6R thus implying no chiasmata inducing properties associated with 6R. Rye chromosome 4R had the most pronounced effect on suppression of chiasmata frequency in the 36 chromosome hybrid at 25.04%.

A quadrivalent configuration was observed in 82–90% of PMCs in the hybrids between Ma and Rosner which was absent in meiocytes of all other hybrids. Forty percent of the ring quadrivalents were orientated as alternate arrangements of centromeres

Table 2. Frequencies of seed set, embryo and plantlet yield in seven different crosses involving *Triticum turgidum* cv. 'Ma'

Hybrid combination	Florets pollinated	Seeds obtained[a]	Embryos excised[a]	Plantlets obtained[a]
1. *T. turgidum* 'Ma' × *Triticosecale* 'Rosner' $6R^{++}$	32	25 (78.1)	21 (65.6)	3 (9.4)
2. *T. turgidum* 'Ma' × *Triticosecale* 'Rosner' $6R^{--}$	36	26 (72.2)	25 (69.4)	–
3. *T. turgidum* 'Ma' × *Triticosecale* 'DRIRA' HH	38	35 (92.1)	22 (57.9)	2 (5.3)
4. *T. turgidum* 'Ma' × *Triticosecale* 'DRIRA' EE	38	32 (84.2)	30 (78.9)	1 (2.6)
5. *T. turgidum* 'Ma' × *T. aestivum* 'Chinese Spring'	37	23 (62.2)	23 (62.2)	3 (8.1)
6. *T. turgidum* 'Ma' × *T. aestivum* (+4R)	35	11 (31.4)	11 (31.4)	2 (5.7)
7. *T. turgidum* 'Ma' × *T. aestivum* (+6R)	36	12 (33.3)	10 (27.8)	1 (2.8)

[a] Figures in parenthesis represent values percent florets pollinated

(Table 4) while the remainder were arranged in adjacent arrangements. A lower proportion of the quadrivalents were arranged as open chain configurations.

Discussion

In the present study, results of chiasmata frequencies observed in the maternal parent, i.e. 4x wheat (*T. turgidum* cv. 'Ma') have been compared with those in several pentaploid hybrids having an AABB tetraploid component derived from two parents. These hybrids different among themselves in several respects. First, these hybrids had either a D genome or an R genome, but not both. Second, the R genome from triticale cv. 'DRIRA' differed in the presence or absence of heterochromatin content on the long arm of rye chromosome 7R. (Efforts to produce a hybrid lacking heterochromatin on the short arm of 6R from 'Rosner' were not successful). Third, the D genome hybrids differed in the presence or absence of rye chromosome 6R or 4R (hybrids with other addition lines were not available for study). In view of the above, several comparisons were possible. It is obvious from the data presented in Table 3 that chiasmata frequencies in all hybrids were reduced relative to 'Ma'.

The chiasmata frequency in the 'Ma' × 'Rosner' hybrid was marginally reduced even with the presence of a translocation which resulted in a low frequency of multivalents per cell. 'Rosner' and 'DRIRA' HH are both complete triticales, so the differences in the meiotic behavior of their respective hybrids with 'Ma' could have been attributed to genotypic effects were it not for the translocations which enhanced the chiasmata frequency in hybrids involving 'Rosner'.

The chiasmata frequencies of the individual 'Ma' × 'DRIRA' HH hybrids overlapped those of the 'Ma' × 'DRIRA' EE hybrid and although the effect of the latter on chiasmata frequency appears to be marginally promotive the effect is minimal and the logical conclusion is that the telomeric heterochromatin on 7RL has a minimal effect on homologous chromosome pairing.

The pentaploid hybrid, AA_tBB_tD between 'Ma' and 'Chinese Spring' was produced from a 'Chinese Spring'-rye chromosome addition line from which the rye chromosomes had been eliminated, therefore the pentaploid and hybrids with 4R and 6R as additions can be considered as isogenic lines. On the average, the hybrids with the 6R chromosome addition showed less of a reduction in chiasmata frequency than did the pentaploid hybrid although there were hybrids from each combination that had identical chiasmata frequencies. With chromosome synapsis being the end result of an oppositional system consisting of chromosomes with promotional and others with suppression effects; chromosome 6R has no effect on chiasmata frequency. This lack of effect on homologous pairing should, of course, be differentiated from homoeologous chromosome pairing induced between A, B and D genomes, in wheat × rye hybrids (ABDR), because while in the former (AABBR), suppression of the diploidizing system (5B system) may lead to multivalent formation; in the latter (ABDR) it will lead to only bivalent or trivalent formation. Furthermore, while in the former, homologous partners are available for pairing and

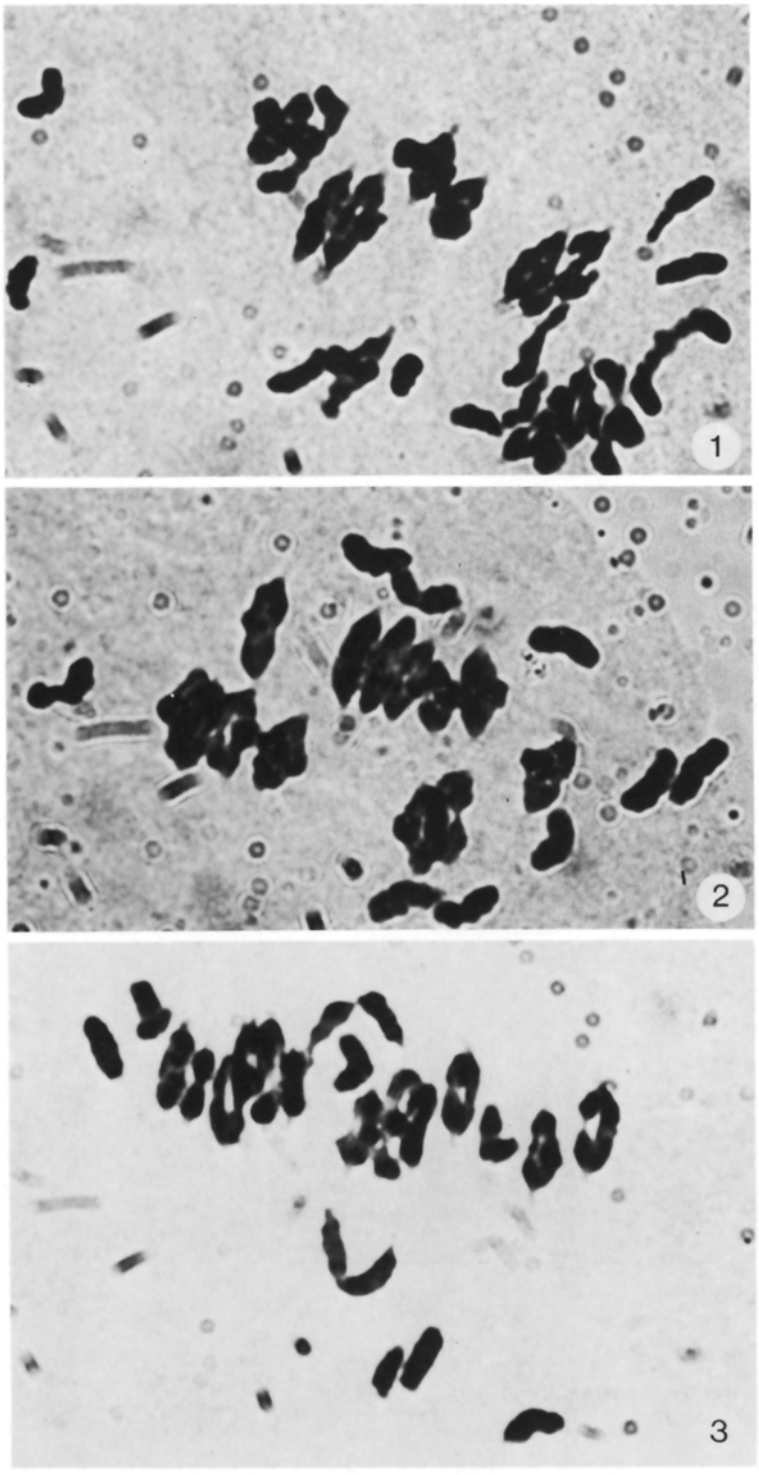

Fig. 1. Metaphase I showing $14^{II}+7^{I}$ in a pentaploid hybrid, AABBD

Fig. 2. Metaphase I showing $13^{II}+9^{I}$ in a pentaploid hybrid, AABBR

Fig. 3. Metaphase I showing $1^{IV}+12^{II}+7^{I}$ in the pentaploid hybrid AABBR between *T. turgidum* cv. 'Ma' and × *Triticosecale* cv. 'Rosner'. (Note a prominent interchange ring)

there are two doses of the 5B system to be overcome by the R genome, in the latter, the homologous partners are not available for pairing and there is only one dose of the 5B system to be overcome. Since no multivalents were observed except in hybrids with 'Rosner', it can be safely concluded that no homologous pairing is induced in these hybrids while in ABDR hybrids, there is sufficient evidence of induction of homoeologous pairing due to R genome, leading to the formation of bivalents and trivalents (Nakajima 1952; Nakajima and Zennyozi 1966; Miller and Riley 1972; Mettin et al. 1976; Lelley 1976; Dvorak 1977). In an earlier study on several hybrids involving various combinations of A, B, D and R genomes, Miller and Riley (1972) concluded that a reduction in the dosage of wheat genomes induces homoeologous pairing between rye chromosomes and an increase in the dosage of rye genome does so between the

Table 3. Chromosome association at metaphase I in pentaploid hybrids (4x wheat × 6x triticale; 4x wheat × 6x wheat)

Parent/hybrid	2n	I	II			III	IV	c	Xta	(% redn.) over Ma	Cells
			Rings	Rods	Total						
1. *T. turgidum* cv. 'Ma'	28	7.0	13.19	0.81	14.00	–	–	0.97	27.19		26
(AABB)	28	7.0	13.12	0.88	14.00	–	–	0.97	27.12		28
2. *T. turgidum* cv. 'Ma' × × *Triticosecale* cv. 'Rosner' $6R^{++}$	35	7.03	11.14	1.03	11.17	0.03	0.89	0.96	26.84	1.18	35
(AA_1BB_1R)	35	7.09	11.36	0.82	12.18	0.09	0.82	0.96	26.77	1.43	22
	35	7.00	11.28	0.93	12.21	–	0.90	0.96	26.84	1.18	29
									26.82*	1.25*	
3. *T. turgidum* cv. 'Ma' × × *Triticosecale* cv. 'DRIRA' HH	35	8.00	10.18	3.30	13.48	0.01	–	0.85	23.66	9.2	69
(AA_1BB_1R)	35	7.08	11.94	2.02	13.96	–	–	0.93	25.90	4.6	48
									24.78*	8.8*	
4. *T. turgidum* cv. 'Ma' × × *Triticosecale* cv. 'DRIRA' EE (AA_1BB_1R)	35	7.26	11.48	2.39	13.87	–	–	0.91	25.35	6.7	93
5. *T. turgidum* cv. 'Ma' ×		7.10	10.90	3.05	13.95	–	–	0.89	24.85	8.5	20
T. aestivum cv. 'Chinese Spring'	35	7.10	10.65	2.60	13.25	–	–	0.85	23.90	12.0	20
									24.35*	10.3*	
(AA_1BB_1D)		8.09	10.86	2.59	13.45	–	–	0.87	24.31	10.5	22
6. *T. turgidum* cv. 'Ma' × *T. aestivum* cv. 'Chinese Spring' + 6R	36		10.88	3.09	13.97			0.89	24.85	8.5	20
									25.19*	7.4*	
			11.56	2.30	13.86	–	–	0.91	25.43	6.4	23
7. *T. turgidum* cv. 'Ma' × *T. aestivum* cv. 'Chinese Spring' + 4R	36		7.48	5.41	12.89	–	–	0.73	20.36	25.04	44

* Mean values

Table 4. Chromosome associations among chromosomes involved in an interchange and the orientations of interchange quadrivalents in *Triticum turgidum* 'Ma' × × *Triticosecale* 'Rosner' pentaploid hybrids (AABBR)

No. of cells scored	No. of cells with chromosome associations					
	2^{II}	$1^{III}+1^{I}$	1^{IV}			
			Adjacent	Alternate	Chain	Total
86	8	3	39	26	10	75
Mean/PMC	0.09	0.03	0.46	0.30[a]	0.12	0.87

[a] 40% of total ring IV

wheat chromosomes. When AABBR and ABDR hybrids are compared the ratio of wheat : rye genomes is 4 : 1 in the former and 3 : 1 in the latter, suggesting that the relative proportion of wheat genome has increased and that of rye genome decreased in the present pentaploid hybrids when compared with tetraploid ABDR hybrids exhibiting homoeologous pairing. This may also partly explain the absence of homoeologous pairing in hybrids in the present study.

The above results do not mean that individual rye chromosomes do not influence homologous pairing among wheat chromosomes in hybrids. A comparison of the results of two hybrids involving addition lines of wheat for 6R and 4R and

those of the pentaploid hybrid (AABBD), having no rye chromosome, proves that at least rye chromosome 4R definitely influences homologous pairing. In another study, Orellana et al. (1984) studied meiosis in wheat-rye addition and substitution lines and suggested that rye and wheat chromosomes affect each other's homologous pairing. They also suggested that the effect of 5R in the diminution of wheat chromosome pairing was strongest and that of 3R the weakest. In the present study, although pentaploid hybrids with rye chromosome 5R (2n=36) were not available, those with 4R chromosome showed a definite effect in suppressing homologous chromosome pairing (reduction 25.04%). This effect is much higher than the one reported for 5R by Orellana et al. (1984), who actually examined the effect in hexaploid addition and substitution lines, while in the present study pentaploid hybrids were used.

Our results on the effect of 4R can be examined with another study conducted by us on the effect of three triticale cultivars on the homoeologous pairing in 6x *H. parodii* (Gupta and Fedak 1986). In these studies three cultivars of triticale ('Rosner', 'DRIRA' and 'Welsh') were used. The maximum pairing was observed in the hybrid with 'Welsh', having 2R/2D or 4R/4D or 7R/7D substitution and no pairing was observed in the 'Rosner' hybrid having a 2R/2D substitution or 2R/2D translocation. This led us to conclude earlier that 2R has a promotional effect and if 'Welsh' does not have 2D/2R or 7D/7R substitution then 4R may have a suppression effect on homoeologous pairing. If the difference in homoeologous and homologous pairing is simply that of degree of relatedness, the same mechanism may actually be involved in the two cases. We propose to conduct further studies on the effect of different rye chromosomes on homoeologous chromosome pairing in intergeneric hybrids of rye with several genera of Triticineae (*Aegilops, Agropyron, Hordeum* and *Triticum*), which should further elucidate the effect of individual rye chromosomes on homologous and homoeologous chromosome pairing in intergeneric hybrids.

A comparison of chiasmata frequencies in the hybrids with 'DRIRA' HH and 'DRIRA' EE should also be made to understand the effect of telomeric heterochromatin located on 7RL. There was hardly any difference in chiasmata frequencies, suggesting that this heterochromatin does influence the homologous chromosome pairing in pentaploid (AABBR) hybrids. Similar observations were made regarding the homoeologous pairing between the H_1 and H_2 genomes in the pentaploid hybrids obtained by crossing *H. jubatum* ($2n=4x=28=H_1H_1H_2H_2$) with 'DRIRA' HH and 'DRIRA' EE (Gupta and Fedak in preparation). However, the results in the case of hybrids of *H. parodii* (2n=6x=42) with 'Rosner' $6R^{++}$ and $6R^{--}$ were different such that the chiasmata frequency in hybrid with $6R^{--}$ was slightly higher although the increase is not really appreciable (0.58 in hybrid with $6R^{++}$ and 0.94 and 1.40 in two hybrids with $6R^{--}$). This increase, if attributed to loss of heterochromatin, may be due to different organization of repetitive sequences, because both 6RS and 7RL have all the four repeat sequence families, including 480 bp, 610 bp, 120 bp and 630 bp (Jones and Flavell 1982).

Another noticeable feature in the present study was the high frequency of cells with a quadrivalent observed only in the *T. turgidum*×'Rosner' hybrid. The frequency was very high, with a single quadrivalent observed in 82–90% of PMCs. It is thus apparent that the quadrivalent could not have resulted due to homoeologous pairing, but must be due to a difference for an interchange between two non-homologous chromosomes from among the chromosomes of A and B genomes in the two parents.

The data on chromosome associations presented in Table 4 clearly indicate that long segments of chromosomes are involved in the interchange. This is evident from the high frequency of cells with quadrivalents and trivalents (91% of cells) and, from the fact that the alternate orientation of the ring quadrivalent is achieved in 40% of the cells. Although orientation of the interchange quadrivalent may be genetically regulated, alternate orientation is facilitated by median centromeres and exchange of long segments. The chromosomes involved in the interchange can be identified by crossing 'Ma' as well as 'Rosner' with ditelocentrics available in 'Chinese Spring', followed by a study of meiosis in the F_1 hybrids.

References

Dvorak J (1977) Effect of rye on homoeologous chromosome pairing in wheat and rye hybrids. Can J Genet Cytol 19:549–556

Gupta PK, Fedak G (1985a) Variation in induction of homoeologous chromosome pairing in 6x *Aegilops crassa* by genomes of sox different species of *Secale*. Can J Genet Cytol 27:531–537

Gupta PK, Fedak G (1985b) Genetic control of meiotic chromosome pairing in polyploids in the genus *Hordeum*. Can J Genet Cytol 27:515–530

Gupta PK, Fedak G (1986) Variation in the induction of homoeologous pairing among chromosome of 6x *Hordeum parodii* as a result of three triticale (×*Triticosecale* Wittmack) cultivars. Can J Genet Cytol 28:420–425

Jones JDG, Flavell RB (1982) The mapping of highly repeated DNA families and their relationship to C-bands in chromosomes of *Secale cereale*. Chromosoma 86:595–612

Lelley T (1976) Induction of homoeologous pairing in wheat by genes of rye suppressing chromosome 5B effect. Can J Genet Cytol 18:485–489

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

Mettin von D, Schlegel R, Blüthner W-D, Weinrich M (1976) Giemsa-banding von MI-Chromosomen bei Weizen-Roggen-Bastarden. Biol Zentralbl 95:35–41

Miller TE, Riley R (1972) Meiotic chromosome pairing in wheat. Genet Iber 24:241–250

Nakajima G (1952) Cytological studies on intergeneric hybrids between *Triticum* and *Secale*, with special reference to the number of bivalents in meiosis PMCs. Cytologia 17:144–155

Nakajima G, Zennyozi A (1966) Cytogenetics of wheat and rye hybrids, Seiken Ziho 18:38–48

Orellana J, Cermeno MC, Lacadena JR (1984) Meiotic pairing in wheat-rye addition and substitution lines. Can J Genet Cytol 26:25–33

Roupakias DG, Kaltsikes PJ (1977) The effect of telomeric heterochromatin on chromosome pairing of hexaploid triticale. Can J Genet Cytol 19:543–548

Snow R (1963) Alcoholic hydrochloric acid carmine as a stain for chromosomes in squash preparations. Stain Technol 38:9–13