Theor. Appl. Genet. 57, 75-79 (1980)

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C-Banding in 6x-Triticale X *Secale cereale* L. Hybrid Cytogenetics

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Summary. The meiotic behaviour of F_1 hybrids of hexaploid Triticale that differed in their genotypic or chromosomic constitution, and diploid rye, was investigated. Meiotic analysis were done by Feulgen and C-banding staining methods. A differential desynaptic effect in the hybrids was detected and explained in terms of genetic differences in pairing regulators. The high homoeologous pairing (A-B wheat chromosomes and wheat-rye chromosomes) observed in the hybrids can be explained in terms of an inhibition of the effect of a single dose of the *Ph* allele of the 5B chromosome produced by two doses of the 5R chromosome. The higher homoeologous pairing detected in the hybrid 188 x 'Canaleja' could be the overall result of the balance between the *Ph* diploidizing system (1 dose), the pairing promoter of the 5R chromosome (2 doses) and that of the 3D chromosome (1 dose coming from the parental line Triticale with the substitution 3R by 3D).

Key words: C-banding – Hybrid cytogenetics – 6x-Triticale

Introduction

Giemsa banding techniques have been extensively used in the study of chromosomes banding pattern in plant cytology. Sarma and Natarajan (1973) and Merker (1973) were the first to reveal the C-banding pattern of the chromosomes of rye and Triticale. The study was extended subsequently to several varieties of the same or different species of rye (Gill and Kimber 1974a; Verma and Rees 1974; Vosa 1974; Weimarck 1975; Singh and Röbbelen 1975; Nakata et al. 1977) and wheat (Gill and Kimber 1974b, 1977). Giemsa C-banding techniques have also been used to study wheat-rye homoeologous affinity (Gill and Kimber 1974b; Darvey and Gustafson 1975; Gustafson et al. 1976; de Vries and Sybenga 1976; Zeller et al. 1977), to detect chromosome segment deletions or translocations (Singh and Röbbelen 1976; Gill and Kimber 1977), to investigate the origin of B genome of wheat (Hadlaczky and Belea 1975), to reveal chromosome additions and substitutions in wheat and Triticale (Darvey and Gustafson 1975; Zeller 1973) and to identify chromosomes present in 4x-Triticale (Gustafson and Krolow 1978). Terminal and centromeric dark bands in rye chromosomes and intercalary faint bands in wheat chromosomes have been used as identifying features in all the above-mentioned studies.

Studies of meiotic behaviour in hybrids between Triticale and rye have been carried out by several authors (Tarkowski 1969; Kiss and Videki 1970; Krolow 1973; Bernard and Bernard 1978; Jouve and Montalvo 1978). However, meiotic analysis were done by techniques that do not permit individual identification of the chromosomes of rye and wheat in the hybrids.

The present study was undertaken in order to investigate reciprocal influences between wheat and rye genomes and its particular contribution at the meiotic pairing in F_1 hybrids of hexaploid Triticale and diploid rye, by means of Feulgen and C-banding staining methods.

Materials and Methods

A series of plants including seven 6x-Triticale by 2x-rye hybrids were cytologically investigated. Female parents used in the obtention of the hybrids were plants of the following lines of 6x-Triticale: Cachirulo, 188, 192, 477, JM 78, JM 135 and *Dicoccoides*. Male parents were plants of a diploid line of *Secale cereale* L. known as Canaleja.

Hybrids were cytologically analyzed and meiotic observations for irregularities at first metaphase were made (a minimal number of 30 PMC's per plant at different meiotic stages were analysed). Anthers for meiotic analysis of PMC's were fixed in Carnoy's fixative and stained by the Feulgen and C-banding methods. In order to detect chromosome substitutions in Triticale parents, karyo-

0040-5752/80/0057/0075/$ 1.00

typic analysis in root tips of these materials using C-banding staining were carried out.

The procedure for C-Banding was standardized as follows: fixed material in spread and squashed on glass slides with a drop of 45% acetic acid. The cover is removed after CO_2-freezing and the material bound to slides is dehydrated with absolute alcohol (98%), during 24 hours. The slides are air-dried and then immersed for 3 minutes in 0.2 N HCl in a 60°C water bath. After hydrolisis the slides are washed in three changes of distilled water for a total of one minute. Denaturation is carried out in a fresh saturated solution of barium hydroxide (5 g. $Ba(OH)_2 \cdot 8H_2O$ in 100 ml. distilled water). The slides are rinsed in distilled water for ten minutes, changing the water three times. Renaturation is obtained by means of hydrolisis in hot (60°C) 2xSSC for one hour (to prepare 20xSSC: disolve 35.06 g. NaCl and 17.64 g. of $Na_3C_6H_5O_7 \cdot 2H_2O$ in 200 ml. of distilled water; working solution is obtained by dilution with distilled water; pH will be 7.67). Slides are stained by immersion in Giemsa solution (3 ml. stock solution Gurr's improved R66 in 60 ml. Sorensen phosphate büffer, pH 6.9: 30 ml. KH_2PO_4 and 30 ml. $NaHPO_4$), during 3 or more minutes. The staining is checked at intervals of one minute to avoid overstaining. Slides with appropriate staining are quickly rinsed in distilled water, dryed in air, and stored in Xylol overnight. Preparations are made permanent using Depe-X mounting media.

Results

Chromosome Substitutions in Female Parents

Giemsa C-banding technique has been used to investigate the somatic chromosome constitution of hexaploid Triticales used in the development of the hybrids. No chromosome substitutions were observed in the lines Cachirulo, 192, 477, JM 78, JM 135 and *Dicoccoides*, all showing 7 pairs of rye chromosomes in all root tip meristem cells analyzed. On the other hand strain 188 showed only 6 pairs of rye chromosomes, which means that it is a substitution line. Strain 188 is a secondary line derived by selfing from an hybrid between the Spanish hexaploid Triticale Cachirulo and a dwarf mutant of the common wheat Marfed. Results of individual analysis of several plant of this strain lead to the assumption that rye chromosome 3R has been replaced by wheat chromosome 3D.

Pairing at Diakinesis

Data on chromosome associations at diakinesis detected in three hybrids are given in Table 1. The average of bivalents in the hybrids was close to 13. A minimum of 2 bivalents was detected in all plants analyzed (Fig. 1).

Pairing at Metaphase I: Feulgen Staining Method

Results of meiotic pairing at metaphase I in the hybrids are registered in Table 2. It can be seen that pairing was variable, the total number of bivalents being always less than 10 (Fig. 2).

Pairing at Metaphase I: C-banding Staining Method

Data on meiotic behaviour presented as mean number of chromosome associations are given in Table 3. It is perhaps of interest to note: (1) the higher number of uni-

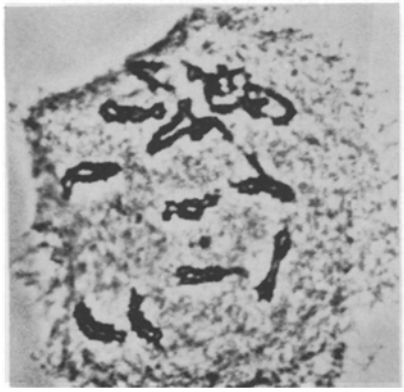

Fig. 1.

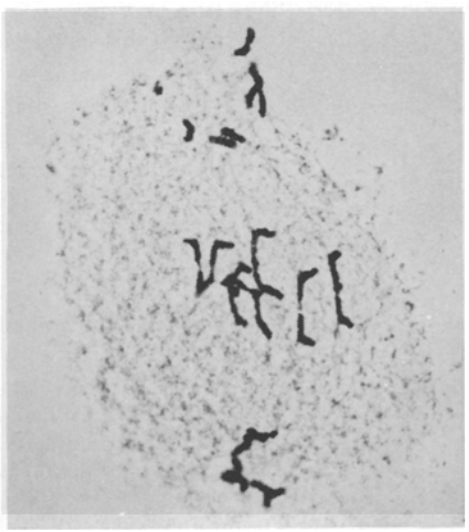

Fig. 2.

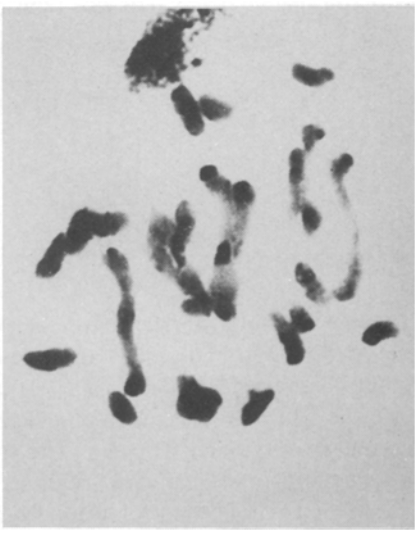

Fig. 3.

Fig. 1. Diakinesis configuration of the hybrid 192 X Canaleja showing 14 bivalents (Feulgen staining). Fig. 2. Metaphase I configuration of the hybrid 188 X Canaleja showing 15 univalents + 5 bivalents + 1 trivalent (Feulgen staining). Fig. 3. Metaphase I configuration of the hybrid JM 78 X Canaleja showing 7 rye bivalents + 14 wheat univalents (C-banding staining)

Table 1. Chromosome pairing at diakinesis in hybrids Triticale × rye analyzed by Feulgen staining method

Material	Mean univalents per PMC (range)	Mean bivalents per PMC (range)		Mean chiasmata per PMC (range)
		Open	Closed	
188 × Canaleja	1.3 (0-4)	2.8 (1-4)	10.5 (2-12)	27.2 (24-32)
192 × Canaleja	1.4 (0-6)	3.1 (1-5)	10.2 (5-12)	25.6 (18-30)
JM 135 × Canaleja	2.5 (0-8)	3.3 (1-7)	9.4 (4-11)	32.9 (20-29)

valents of A and B wheat genomes than of R rye genomes, (2) the higher number of bivalents of rye chromosomes than of wheat chromosomes, (3) the presence of wheat-rye bivalents, (4) the variability of the relative ratios of homoeologous (A-B and A or B-R) to homologous pairing (R-R) in the hybrids. The high homoeologous detected in the hybrid 188 × Canaleja is to be remarked (Fig. 3).

Discussion

Our results on the variability of pairing among hybrids are in agreement with previous observations reported by Bernard and Bernard (1978). The desynaptic effect may be explained in terms of genetic differences in the system that interferes in part with chiasmata formation during pairing of homologous chromosomes from parental lines of Triticale. Lelley (1974) suggested a genetic system regulating the decrease in chiasmata rate in three hexaploid Triticale lines and their hybrids, and Jouve et al. (1977) pointed out that differences in meiotic behaviour in several lines of hexaploid Triticale (JM 78 and JM 135 included) could be explained by genetic differences in pairing regulators, indirectly acquired during selection processes of fertility improvement.

Assuming a strictly homologous pairing, a 14 univalents ($7'_A + 7'_B$) and 7 bivalents ($7''_{RR}$) pattern of pairing was to be expected. However, PMC's with more than 7

Table 2. Chromosome pairing at metaphase I in hybrids Triticale × rye analyzed by Feulgen staining method

Material	Mean univalents per PMC (range)	Mean bivalents per PMC (range)		Mean trivalents per PMC (range)	Mean chiasmata per PMC (range)
		Open	Closed		
Cachirulo × Canaleja	11.7 (6-20)	4.2 (0-9)	3.9 (1-5)		13.7 (7-21)
188 × Canaleja	11.3 (2-16)	3.9 (2-8)	4.4 (1-7)	0.04 (0-1)	13.1 (7-21)
192 × Canaleja	9.3 (2-18)	4.4 (0-10)	4.9 (0-8)		15.2 (8-21)
477 × Canaleja	15.2 (6-24)	4.9 (0-8)	1.4 (0-5)	0.05 (0-1)	7.8 (4-14)
JM 78 × Canaleja	19.7 (10-28)	3.2 (0-7)	0.9 (0-4)		5.3 (0-15)
JM 135 × Canaleja	9.5 (2-18)	5.1 (2-9)	4.1 (2-8)	0.03 (0-1)	13.1 (5-21)
Dicoccoides × Canaleja	19.6 (10-22)	2.7 (0-5)	1.4 (0-5)	0.05 (0-1)	9.1 (4-17)

Table 3. Chromosome pairing at metaphase I in hybrids Triticale × rye analisis by C-banding technique

Material	Mean univalents per PMC (range)		Mean open bivalents per PMC (range)			Mean closed bivalents per PMC					Mean trivalents per PMC (range)
						2-Xata (range)			3-Xata (range)		
	W	R	W	R	W-R	W	R	W-R	W	R	W-R-R
188 × Canaleja	4.3 (1-8)	2.2 (0-8)	3.2 (1-6)	1.7 (0-4)	1.1 (0-3)	1.8 (0-6)	2.4 (1-4)	0.1 (0-2)	0.2 (0-1)	0.2 (0-1)	0.07 (0-1)
477 × Canaleja	10.8 (8-14)	4.5 (2-8)	1.4 (0-2)	3.3 (2-5)		0.2 (0-2)	1.1 (0-3)		0.3 (0-1)		
JM 78 × Canaleja	11.8 (8-14)	5.4 (0-10)	0.7 (0-3)	2.6 (0-6)	0.2 (0-2)	0.2 (0-2)	1.5 (0-4)		0.1 (0-2)		
JM 135 × Canaleja	7.9 (2-14)	1.6 (0-6)	1.7 (0-6)	2.5 (0-3)	0.3 (0-3)	1.2 (0-3)	2.9 (0-6)		0.02 (0-1)	0.6 (0-2)	

W = wheat chromosomes; R = rye chromosomes; W-R = wheat-rye bivalents; W-R-R = wheat-rye-rye trivalents

bivalents and with more than 14 univalents were detected in all hybrids. This fact can be explained in terms of an intensification of homoeologous pairing and a reduction of homologous pairing due to mutual interactions between wheat and rye genetic systems of meiotic regulation in the hybrids.

An increase of homoeologous pairing in the haploid complement of common wheat chromosomes by addition of increasing doses of rye genome (0 to 3) was reported by Miller and Riley (1972) and Riley et al. (1973). The effect seems to depend on the balance between the 5B chromosome of wheat (one dose), which shows a diploidizing genetic system *Ph* located on the long arm (Wall et al. 1971a and b), and the number of doses of chromosome 5R from rye, which has a pairing promoter on the short arm, which enhances homoeologous pairing of the chromosomes from wheat. The high homoeologous pairing observed in the hybrids studied here can be explained in terms of an inhibition of the effect of a single dose of the *Ph* allele of the 5B chromosome produced by two doses of the 5R chromosome. The higher homoeologous pairing detected in the hybrid 188 × Canaleja could be the overall result of the new balance between the *Ph* diploidizing system of the 5B chromosome (1 dose) the pairing promoter of the 5R chromosome (2 doses) and that of 3D chromosome (1 dose) which has also a pairing regulator system (Mello-Sampayo 1971; Mello-Sampayo and Canas 1973; Sears 1976).

Pairing of wheat and rye chromosomes has been reported in other materials (Mettin et al. 1976; Schlegel 1977; Dhaliwal et al. 1977; Pohler and Kistner 1977). This homoeologous pairing seems to be increased by the presence of chromosome 3D. Our results about individual chromosome associations suggest that the increase of wheat-rye pairing in the hybrid 188 × Canaleja may not be solely the result of specific effect of chromosome 3D, and that an increase in general homoeologous pairing because of the new interaction of genetic system of pairing should be also taken into account.

It is hoped that further study of genome influences will permit new recombinations involving Triticale, in order to increase germplasm stores in this synthetic crop. The inconvenience of self-sterility in Triticale × rye F_1 hybrids would not be important if these hybrids were used as female parents in the first generation in successive substitution backcrosses using Triticale as the recurrent parent.

Literature

Bernard, M.; Bernard, S. (1978): Methods of gene transfer from breed wheat and rye to hexaploid triticale. Proc. 8th Congr. Eucarpia, 181-189

Darvey, N.L.; Gustafson, J.P. (1975): Identification of rye chromosomes in wheat-rye addition lines in Triticale by heterochromatin bands. Crop Sci. **15**, 239-243

De Vries, J.M.; Sybenga, J.M. (1976): Identification of rye chromosomes: the Giemsa banding pattern and the translocation tester set Theor. Appl. Genet. **48**, 35-43

Dhaliwal, H.S.; Gill, B.S.; Waines, J.G. (1977): Analysis of induced homoeologous pairing in a Ph mutant wheat × rye hybrid. J. Hered. **68**, 206-209

Gill, B.S.; Kimber, G. (1974a): The Giemsa C-banded karyotype of rye. Proc. Nat. Acad. Sci. (Wash.) **71**, 1247-1249

Gill, B.S.; Kimber, G. (1974b): Giemsa C-banding and the evolution of wheat. Proc. Nat. Acad. Sci. (Wash.) **71**, 4086-4090

Gill, B.S.; Kimber, G. (1977): Recognition of translocation and alien chromosome transfers in wheat by the Giemsa-C-banding technique. Crop Sci. **17**, 264-266

Gustafson, J.P.; Evans L.E.; Josifek, K.K. (1976): Identification of chromosomes in *Secale montanum* and individual *S. montanum* chromosome additions to 'Kharkov' wheat by heterochromatin bands chromosome morphology. Can. J. Genet. Cytol. **18**, 339-343

Gustafson, J.P.; Krolow, K.D. (1978): A tentative identification of chromosomes present in tetraploid Triticale based on heterochromatin banding patterns. Can. J. Genet. Cytol. **20**, 199-204

Hadlaczky, G.Y.; Belea, A. (1975): C-banding in wheat evolutionary cytogenetics. Plant Sci. Letters **4**, 85-88

Jouve, N.; Montalvo, D. (1978): Meiotic behaviour in hybrids between 6x-Triticale and *Secale cereale*. Proc. 8th Congr. Eucarpia, 191-197

Jouve, N.; Soler, C.; Saiz, G. (1977): Cytoplasmic effect on the meiosis of 6x-Triticale. Z. Pflanzenzücht. **78**, 124-134

Kiss, A.; Videki, L. (1971): Development of secondary hexaploid Triticales by crossing triticale by rye. Wheat Inf. Serv. **32**, 17-20

Krolow, K.D. (1973): 4x-Triticale, production and use in triticale breeding. Proc. 4th Int. Wheat Genet. Symp. 237-244

Lelley, T. (1974): Desynapsis as a possible source of univalents in metaphase I of triticale. Z. Pflanzenzücht. **73**, 249-258

Mello-Sampayo, T. (1971): Genic regulation on meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. Nature (London) New Biol. **230**, 22-23

Mello-Sampayo, T.; Canas, A.P. (1973): Suppressors of meiotic chromosome pairing on common wheat. Proc. 4th Int. Wheat Genet. Symp. 709-713

Merker, A. (1973): A Giemsa technique for rapid identification of chromosomes in Triticale. Hereditas **75**, 280-282

Mettin, D.; Schlegel, R.; Blüthner, W.D.; Weinrich, M. (1976): Giemsa-Banding von MI-Chromosomes bei Weizen-Roggen-Bastarden. Biol. Zbl. **95**, 35-41

Miller, T.E.; Riley, R. (1972): Meiotic chromosome pairing in wheat-rye combinations. Genet. Iberica **24**, 241-250

Nakata, N.; Yasumuro, Y.; Sasaki, M. (1977): An acetocarmine-Giemsa staining of rye chromosomes. Jap. J. Genet. **52**, 315-318

Pohler, W.; Kistner, G. (1977): Meioseuntersuchungen an Triticale. III. Chromosomenanordnung und Chromosomen Paarung bei polyhaploiden Weizen-Roggen-Bastarden. Biol. Zbl. **96**, 679-691

Riley, R.; Chapman, V.; Miller, T.E. (1973): The determination of meiosis chromosome pairing. Proc. 4th Int. Wheat Genet. Symp. 731-738

Sarma, N.P.; Natarajan, A.T. (1973): Identification of heterochromatic regions in the chromosomes of rye. Hereditas **74**, 233-238

Schlegel, R. (1977): Intergeneric wheat-rye chromosome pairing and karyotype evolution in the genera *Secale*. Helsinki Chromosome Conf.

Sears, E.R. (1976): Genetic control of chromosome pairing in wheat. Ann. Rev. Genet. **10**, 31-51

Singh, R.J.; Röbbelen, G. (1975): Comparison of somatic Giemsa banding pattern in several species of rye. Z. Pflanzenzücht. **75**, 270-285

Singh, R.J.; Röbbelen, G. (1976): Giemsa banding techniques reveals deletions within rye chromosomes in addition lines. Z. Pflanzenzücht. **76**, 11-18

Tarkowski, Cz. (1969): Cytogenetics of hexaploid triticale hybrids with wheat and rye. Genet. Polonica **10**, 85-86

Verma, S.C.; Rees, H. (1974): Giemsa staining and the distribution of heterochromatin in rye chromosomes. Heredity **32**, 118-122

Vosa, C.G. (1974): The basis karyotype of rye analyzed with Giemsa and fluorescence methods. Heredity **33**, 403-408

Wall, A.M.; Riley, R.; Gale, M.D. (1971a): The position of a locus on chromosome 5B of *Triticum aestivum* affecting homoeologous meiotic pairing. Genet. Rev. **18**, 329-339

Wall, A.M.; Riley, R.; Chapman, V. (1971b): Wheat mutants permitting homoeologous meiotic chromosome pairing. Genet. Rev. **18**, 311-328

Weimark, A. (1975): Heterochromatin polymorphism in the rye karyotype as detected by the Giemsa C-banding technique. Hereditas **79**, 293-300

Zeller, F.J. (1973): 1B/1R wheat-rye chromosome substitutions and translocations. Proc. 4th Int. Wheat Genet. Symp. 209-221

Zeller, F.J.; Kimber, G.; Gill, B.S. (1977): The identification of rye trisomics by translocations and Giemsa staining. Chromosoma **62**, 279-289

Received October 3, 1979
Communicated by R. Riley

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