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CYTOGENETIC STABILITY OF WHEAT LINES (*TRITICUM AESTIVUM* L.) WITH ADDED AND SUBSTITUTED CHROMOSOMES OF RYE (*SECALE CEREALE* L.)

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Transmission of added and substituted rye chromosomes through generations and behavior of chromosomes at meiosis was analyzed. Among the addition lines, chromosome 7R was most often transmitted and 6R most rarely. In substitution line 1B/1R, no elimination of rye chromosomes was observed in any generation. The tested lines differed from wheat in the behavior of chromosomes at meiosis. The rye and wheat chromosomes interacted during meiosis. The added rye chromosomes influenced bivalent shape and univalent frequency at metaphase I, the number of delayed chromosomes and chromatid bridges at anaphase I, and the occurrence of micronuclei in tetrads. In the majority of lines the frequency of open bivalents and univalents was higher than in 'Grana' wheat and 'Dańkowskie Złote' rye. Rye chromosomes occurred more frequently as univalents than wheat chromosomes did. Neither multivalent associations nor homoeologous pairing were observed. The studied lines had good cytogenetic stability.

Key words: Mitosis, meiosis, cytogenetic stability, rye, wheat, addition lines, substitution line 1B/1R.

INTRODUCTION

Attempts to create wheat addition lines have been undertaken many times worldwide. Poor transmission of added rye chromosomes to the following generations, disturbances at meiosis and preferential transmission of some chromosomes have been the main barriers hindering or even preventing the achievement of complete series. Addition lines are employed to localize genes on chromosomes and to create substitution forms. These lines make it possible to study the interaction between wheat and rye chromosomes. They can also lead to the identification of particular gene markers, helpful during the creation of new rye, wheat and triticale genotypes used in breeding new varieties. Two complete wheat addition line series were created on the basis of the cytogenetically well-known cv. Chinese Spring (Miller, 1984). Addition lines using Polish wheat and rye varieties have been created at the Institute of Genetics and Plant Breeding of the University of Agriculture in Lublin. A full series of wheat cv. Grana – rye cv. Dańkowskie Złote addition lines has been achieved (Miazga and Chrząstek, 1987; Chrząstek and Miazga, 1988). The substitution line 1B/1R was obtained by crossing addition line 1R with 'Grana' wheat monosomic line 1B. Creating series containing new varieties extends our knowledge of the interrelations between wheat and rye chromosomes. Using the new series, we can localize genes and evaluate their expression.

This paper evaluates the cytogenetic stability of addition lines of wheat and substitution line 1B/1R. Transmission of added and substituted rye chromosomes through generations was studied, and the behavior of chromosomes at meiosis was analyzed.

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MATERIALS AND METHODS

PLANT MATERIAL

The study material was the series of lines of wheat (*Triticum aestivum* L.) cv. Grana with added pairs of complete or telocentric chromosomes of rye (*Secale cereale* L.) cv. Dańkowskie Złote: 1R, 2R, 3R, 3RS, 4R, 5R, 6R, 6RL, 7R, and substitution line 1B/1R. Initial forms (rye, wheat and octoploid triticale from their crossing) were also analyzed.

CYTOLOGICAL TECHNIQUES

The chromosome number in each studied line was calculated every year from 1997 to 1999 before sowing. Seeds were germinated on moist filter paper in Petri dishes. Root tips were pretreated with α-bromonaphthalene for 4–5 h and fixed in acetic alcohol (1:3). Slides were made in a drop of 2% aceto-orcein. Chromosomes were identified on the basis of morphology, number, size, and arrangement of heterochromatin bands stained by Giemsa C-banding technique according to Darvey and Gustafson (1975).

Material for studies of meiosis was collected during vegetation in 1999. Young spikes were fixed with acetic alcohol (1:3) and a day later transferred to 75% ethanol. Slides in 2% acetocarmine with 1% ferrous chloride were made. Two hundred pollen mother cells (PMCs) were analyzed for chromosome configurations at metaphase I in all lines and initial forms. The number of rod- and ring-shaped bivalents and the presence of univalents was estimated. Chiasma frequency was analyzed in 50 cells of each line. The frequency of delayed chromosomes and chromatid bridges was observed at anaphase I in 100 cells of each form. The frequency of micronuclei was studied in ~3000 tetrads in every line and all initial forms. Chromosome configurations at metaphase I were analyzed by differential chromosome staining according to Jouve et al. (1980).

The results were analyzed statistically with the F-Snedecore test, and Tukey's semisections were applied to find significant differences. The studied lines were compared with all initial forms.

RESULTS AND DISCUSSION

CHROMOSOME NUMBER IN SOMATIC CELLS

In obtaining and then maintaining the addition and substitution lines, rye chromosome elimination was monitored annually. Stability, estimated on the basis of transmission of added rye chromosomes through the generations, differed between addition lines (Tab. 1). For the three successive years, line 7R appeared to be the most stable and line 6R the least. Plants with 42 chromosomes always occurred in the progeny of the analyzed 1B/1R substitution line. Miller (1984) analyzed rye chromosome transmission in three addition line series. He found that cytogenetic stability depended mainly on the line and to a lesser extent on the series. In the series 'Holdfast'–'King II,' rye chromosome transmission amounted to 85.7%, in 'Chinese Spring'–'Imperial' 86.8% and in 'Chinese Spring'–'King II' 83.0%, on average. Within the series, transmission of particular chromosomes ranged from 60% to 92%. We analyzed the karyotypes of the studied addition and substitution lines using differential chromosome staining (C-banding). In rye chromosomes, heterochromatin occurred mainly in the form of large blocks at the ends of the arms and weak interstitial and centromeric bands. In wheat, heterochromatin was dispersed in most of the chromosomes, and a few telomeric bands were smaller than in rye (Fig. 1a–f).

MEIOTIC BEHAVIOR OF RYE AND WHEAT CHROMOSOMES

Chromosome configurations at metaphase I

Metaphase I was analyzed in lines with stable chromosome number. They differed between lines and from the initial forms in the number and shape of bivalents formed, and in the frequency of occurrence of univalents (Tab. 2). Bivalents and univalents were observed in metaphase plates (Fig. 2c,e). No multivalent associations were found. Most bivalents were ring-shaped in lines of 'Grana' wheat with 'Dańkowskie Złote' rye chromosomes added. Rod-shaped bivalents ranged from 7.10% in line 5R to 13.89% in line 3R. In all addition lines and the 1B/1R substitution line, the percentage of open bivalents (rods) was higher than in 'Grana' wheat (5.43%). Only in addition line 5R and substitution line 1B/1R were the differences nonsignificant. Similar trends have been observed in addition lines of the 'Kharkov'–'Dakold' series (Lee et al., 1970; Orellana et al., 1984), 'Chinese Spring'–'Imperial' (Orellana et al. 1984) and 'Holdfast'–'King II' (Schlegel 1978). Schlegel (1978) found different results for the 'Chinese Spring'–'Imperial' series: in most lines of this series there were fewer rod-shaped bivalents than in wheat cv. Chinese Spring. In our study, chromosomes formed fewer open bivalents than 'Dańkow-

TABLE 1. Chromosome number in somatic cells of 'Grana' wheat – 'Dańkowskie Złote' rye addition lines and 1B/1R substitution line in three successive generations

Line	Year	Number of tested plants	Plants with established chromosome number	Frequency of plants with different chromosome numbers							
				44		43		42		42 + 2t	
				no.	%	no.	%	no.	%	no.	%
Addition lines											
1R	1997	61	38	39		1		2		0	
	1998	76	54	45		2		7		0	
	1999	80	61	54		0		7		0	
	total	217	154	135	87.67	3	1.95	16	10.38	0	0.00
2R	1997	54	30	22		0		8		0	
	1998	83	61	51		0		10		0	
	1999	73	48	42		0		6		0	
	total	210	139	115	82.73	0	0.00	24	17.27	0	0.00
3R	1997	70	46	40		1		5		0	
	1998	69	45	39		2		4		0	
	1999	63	57	52		0		5		0	
	total	202	148	131	88.51	3	2.03	14	9.46	0	0.00
3RS	1997	58	40	0		0		8		32	
	1998	77	53	0		0		15		38	
	1999	64	52	0		3		13		36	
	total	199	145	0	0.00	3	2.07	36	24.83	106	73.10
4R	1997	86	54	46		0		8		0	
	1998	90	62	52		0		10		0	
	1999	57	40	34		0		6		0	
	total	233	156	132	84.61	0	0.00	24	15.39	0	0.00
5R	1997	60	27	20		2		5		0	
	1998	82	58	52		0		6		0	
	1999	64	49	44		0		5		0	
	total	206	134	116	86.57	2	1.49	16	11.94	0	0.00
6R	1997	81	50	40		3		7		0	
	1998	75	47	38		0		9		0	
	1999	84	58	47		0		11		0	
	total	240	155	125	80.64	3	1.94	27	17.42	0	0.00
6RL	1997	49	39	0		0		11		28	
	1998	75	58	0		0		6		52	
	1999	98	48	0		0		13		35	
	total	222	145	0	0.00	0	0.00	30	20.69	115	79.31
7R	1997	70	65	62		0		3		0	
	1998	71	58	58		0		0		0	
	1999	53	44	41		0		3		0	
	total	194	167	161	96.41	0	0.00	6	3.59	0	0.00
Substitution line											
1B/1R	1997	71	38	0		0		38		0	
	1998	59	58	0		0		58		0	
	1999	83	66	0		0		66		0	
	total	213	163	0	0.00	0	0.00	163	100.00	0	0.00

t – telocentric chromosome.

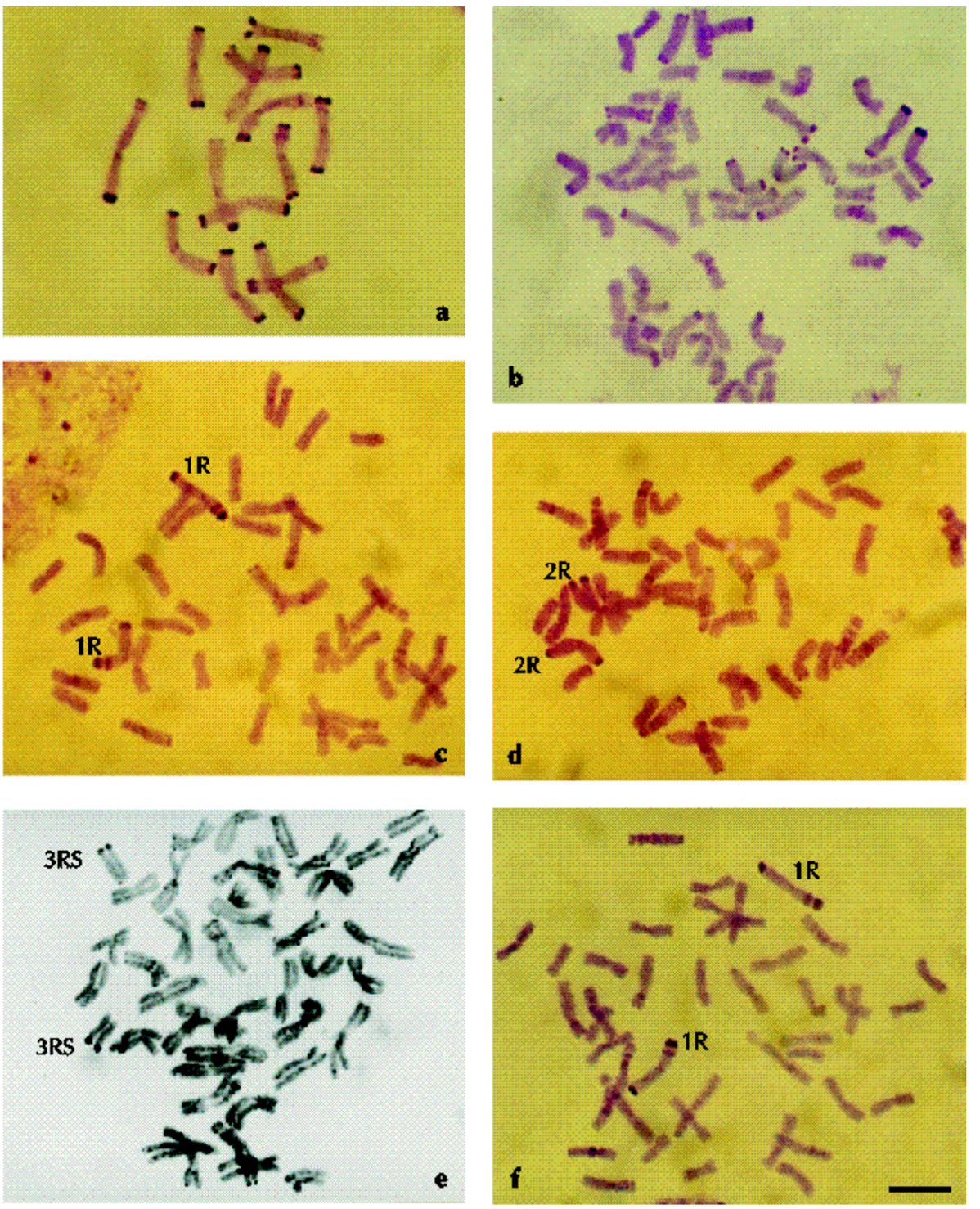

Fig. 1. Mitotic metaphases in addition and substitution lines and initial forms. (**a**) Rye (2n = 14), (**b**) Triticale (2n = 56), (**c–e**) Addition lines: (**c**) 1R (2n = 44), (**d**) 2R (2n = 44), (**e**) 3RS (2n = 42 + 2t), (**f**) Substitution line 1B/1R (2n = 42). Bar = 10 µm.

TABLE 2. Chromosome configurations at metaphase I in wheat – rye addition lines, 1B/1R substitution line and initial forms

Lines and initial forms	Chromosome number	Chromosome configurations					Mean chiasma number	
		Bivalents				Univalents per cell	per cell	per bivalent
		Rods per cell		Rings per cell				
		Number	%	Number	%			
Addition lines								
1R	44	1.95	8.89^{xz}	19.99	91.11^{xz}	0.12	45.62	2.07
2R	44	2.21	10.23^{xz}	19.73	89.77^{xz}	0.11	43.44	1.98
3R	44	3.04	13.89^{xyz}	18.85	85.47^{xyz}	0.22	43.01	1.96
3RS	42+2t	1.90	8.66^{xz}	20.00	86.11^{xz}	0.13	43.58	1.99
4R	44	2.36	10.71^{xz}	19.63	89.29^{xz}	0.02	46.12	2.10^{z}
5R	44	1.56	7.10^{z}	20.42	92.90^{z}	0.00	45.46	2.07
6R	44	2.82	12.59^{xz}	19.14	87.41^{xyz}	0.08	44.10	2.01
6RL	42+2t	2.81	13.00^{xz}	18.84	87.00^{xyz}	0.70^{xy}	42.36	1.94
7R	44	2.35	10.73^{xz}	19.60	89.27^{xz}	0.10	42.70	1.95
Substitution line								
1B/1R	42	1.61	7.65^{z}	19.36	92.35	0.06	46.64	1.94
Initial forms								
'Grana'	42	1.14	5.43	19.84	94.57	0.04	42.30	1.99
'Dańkowskie Złote'	14	0.58	8.28	6.42	91.72	0.00	13.98	2.01
'Grana' × 'Dańkowskie Złote'	56	5.56	20.18	21.99	79.82	0.90	53.24	1.94

x – differences with wheat significant at P = 0.05.
y – differences with rye significant at P = 0.05.
z – differences with 8x triticale significant at P = 0.05.
t – telocentric chromosome.

skie Złote' rye only in addition line 5R and substitution line 1B/1R. In octoploid triticale ('Grana' × 'Dańkowskie Złote') open bivalents were 20.18% (Fig. 2b). The percentages of rods in all studied lines as well as in rye and wheat were significantly lower than in triticale. Schlegel (1978) found that mainly genotype influenced the frequency of open bivalent formation.

The percentages of ring-shaped bivalents in 'Grana' wheat lines with added rye chromosomes ranged from 87.0% (6RL) to 92.9% (5R), and was 92.35% in substitution line 1B/1R (Tab. 2). All tested lines formed fewer ring-shaped bivalents than 'Grana' wheat (94.57%). Only in addition line 5R and substitution line 1B/1R were the differences nonsignificant. Lines 3R, 6R and 6RL had significantly fewer rings than rye. All tested lines contained more rings than octoploid triticale. Telocentric chromosomes 3RS and 6RL most often paired with their homologues. No heteromorphic bivalents were observed. Univalents occurred in all lines except 5R, and varied in frequency. Generally they were few, ranging from 0.00 (5R) to 0.70 (6RL) per cell (Tab. 2). Only line 6RL significantly differed from the other lines and from the initial forms in the number of univalents per PMC. In this line, beyond the metaphase plate, mainly added rye telocentric chromosomes were observed. Differential staining technique revealed that both wheat and rye chromosomes occurred as univalents, but univalents were more often of rye origin. 'Grana' wheat contained 0.04 univalents on average; 'Dańkowskie Złote' rye contained no univalents at all (Fig. 2a). Except line 6RL, the differences between lines and between rye and wheat were not significant. In 'Holdfast'-'King II' addition lines tested by Riley (1960) univalents occurred in 15.5% of cells, and in the Holdfast wheat variety in 6% of cells. The problem of univalent formation in addition lines and in other wheat-rye combinations is still controversial. According to some authors, the presence of univalents can be a result of disturbances in zygotene and diplotene, as well as asynapsis or desynapsis (Riley, 1960; Lee et al., 1970).

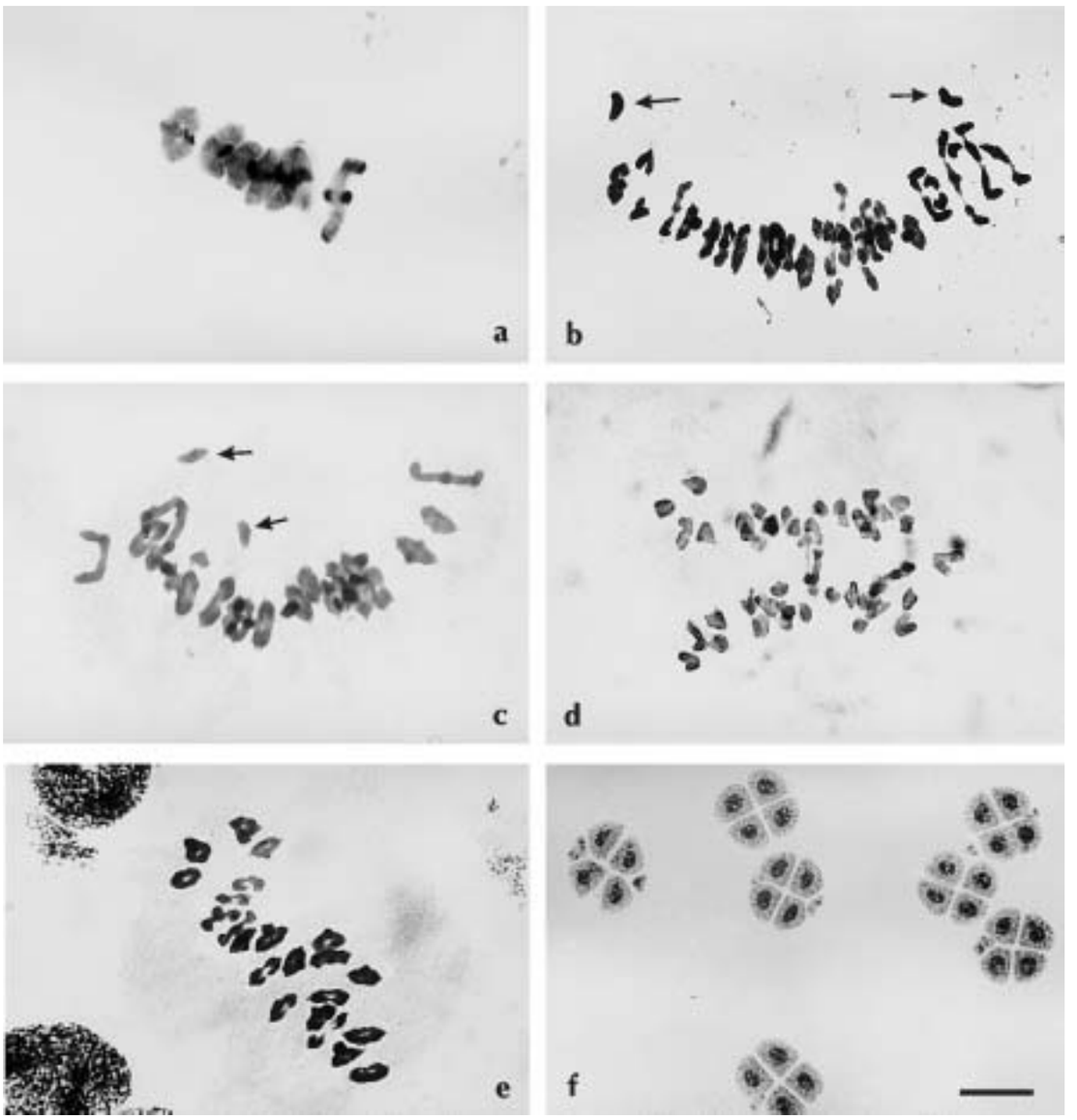

Fig. 2. Meiosis in initial forms and addition lines. (**a, b, c, e**) Metaphase I. (**a**) Rye – 7II, (**b**) Triticale (8x) – 27II + 2I (arrows), (**c**) Addition line 3R – 21II + 2I (arrows), (**e**) Addition line 4R – 22II, (**d**) Anaphase I in addition line 2R; visible chromatid bridges, (**f**) Disturbances in tetrads of addition line 6R. II – bivalent; I – univalent. Bar = 10 μm.

In wheat-rye hybrids, rye chromosomes can influence the pairing of wheat chromosomes and vice versa. Orellana et al. (1984) found disturbed chromosome pairing in 'Holdfast'–'King II' and 'Chinese Spring'–'Imperial' addition lines and in three substitution lines 6B/6R, 6B/mono-6R and 6D/6R. They observed more open bivalents and univalents in those metaphase plates than in wheat varieties. Schlegel (1978) observed similar behavior of rye chromosomes in addition lines. Naranjo et al. (1979) found that wheat chromosomes impaired rye chromosome pairing in hybrids between hexaploid triticale and rye. Some authors have observed disturbances in wheat chromosome pairing in wheat-rye combinations (Lee et al., 1970; Orellana et al., 1984). Chromosome 5R of 'Imperial' rye interacted the strongest and 3R the weakest with wheat chromosome pairing Orellana et al. (1984). We also found

interaction between rye and wheat chromosomes at meiosis in the 'Grana'–'Dańkowskie Złote' lines. More wheat and rye open bivalents and univalents were observed in metaphase plates in most analyzed addition lines than in 'Grana' wheat or 'Dańkowskie Złote' rye. The frequency of wheat univalents in the tested lines was lower than that of rye univalents, suggesting that added rye chromosomes disturbed wheat chromosome pairing less than vice versa.

Explanations of homoeologous pairing in wheat-rye combinations have been sought for many years (Naranjo et al., 1979; Schlegel et al., 1980; Naranjo, 1982). In wheat as well as in hybrids between wheat and related species, homoeologous pairing of chromosomes is inhibited by gene *Ph1* situated on the long arm of chromosome 5B. It is the oldest and best-known genetic system controlling pairing of wheat chromosomes with those of related species (Riley and Chapman, 1958; Sears and Okamoto, 1958). It was confirmed by studies using new cytogenetic methods including hybridization in situ (King et al., 1994; Miller et al., 1994; Mikhailova et al., 1998; Reader et al., 2000). Apart from the *Ph1* gene, other genes controlling homoeologous pairing have been identified mainly on chromosomes of homoeologous groups 3 and 5 (Feldman, 1966; Mello-Sampayo, 1971). Wheat chromosomes from genome B paired more often with rye chromosomes than those from genomes A or D (Naranjo and Fernandez-Rueda, 1996; Cuadrado et al., 1997).

According to some authors there is genetic chromosome pairing control in rye also. Genes responsible for pairing have been localized on the long arm of chromosome 5R (Bielig and Driscoll, 1970; Naranjo and Palla, 1982). Meiosis usually occurs regularly in heterozygotic generations. In inbred rye lines the number of univalents at metaphase I increases and the number of chiasmata in bivalents decreases. Control of chiasma formation in rye is of polygenic character (Rees and Thomson, 1956; Lelley, 1975). On the basis of meiosis analysis in hybrids achieved due to crossing 'Chinese Spring' wheat with three inbred rye lines, Lelley (1975, 1976) demonstrated that a similar chiasma control system is present in wheat-rye combinations. Our results indicate that chromosomes of 'Dańkowskie Złote' rye can affect chiasma formation in addition lines and substitution line 1B/1R. The highest number of chiasmata per bivalent (2.10) was observed in line 4R, differing significantly from lines 6RL and 7R as well as from substitution line 1B/1R (Tab. 2). The lowest number of chiasmata per bivalent was found in lines 6RL and 1B/1R (1.94). 'Grana' wheat contained 42.3 chiasmata per cell and 1.99 chiasmata per bivalent on average. In 6 of the lines the mean number of chiasmata per bivalent was the same or lower than in wheat. More chiasmata were observed in other lines, but the differences were not significant. Only three lines (1R, 4R, 5R) had higher mean numbers of chiasmata per bivalent than in 'Dańkowskie Złote' rye. Line 4R, with 2.10 chiasmata per bivalent, significantly exceeded octoploid triticale. The results on the mean number of chiasmata and univalents in wheat, rye and triticale are consistent with those of Stefanowska (1988). According to Lelley (1980), differences in the frequency of chiasmata in bivalents formed from wheat chromosomes in triticale greatly depend on the rye genotype. Analyzing 'Chinese Spring'–'Imperial' addition lines, Dörgemüller and Lelley (1984) found no evident influence of wheat genomes on chiasma formation in rye bivalents. They also rejected the hypothesis that the number of chiasmata formed in rye bivalents depends on the size of blocks of constitutive heterochromatin. However, they showed that the number of chiasmata in bivalents depends on chromosome arm length.

Many other authors have asked whether the presence of large blocks of constitutive heterochromatin in rye chromosome telomeres as well as in interstitial and centromeric regions of wheat chromosomes affects the behavior of chromosomes at meiosis. According to Rogalska (1983) and Schlegel (1980), the number of PMCs with disturbances is correlated with the content of telomeric heterochromatin. Numerous studies have revealed that heterochromatin negatively affects the formation and terminalization of chiasmata (Thomas and Kaltsikes, 1976; Roupakias and Kaltsikes, 1977; Schlegel, 1979; Naranjo and Lacadena, 1980; Attia and Lelley, 1987). Telomeric heterochromatin is one of many factors that can disturb the course of meiosis. According to Orellana et al. (1984), pairing of wheat and rye chromosomes in addition and substitution lines is a complex process in which such factors as genetic control, constitutive heterochromatin and interactions difficult to estimate can play an important role.

Chromosome segregation at anaphase I

Anaphase I was studied in 'Grana'-'Dańkowskie Złote' addition lines and substitution line 1B/1R (Tab. 3). The mean number of delayed chromosomes per cell ranged from 0.024 in line 5R up to 0.208 in line 1R, and was 0.05 in 'Grana' wheat. In 'Dańkowskie Złote' rye, 0.07 delayed chromosomes per cell were counted. The number of delayed chromosomes

TABLE 3. Chromosome segregation at anaphase I and number of micronuclei per tetrad in wheat – rye addition lines, 1B/1R substitution line and initial forms

Lines and initial forms	Chromosome number	Anaphase I		Frequency of micronuclei in tetrads						
		Mean no. of delayed chromosomes	Mean no. of chromatid bridges	No. of analyzed tetrads	% tetrads				Mean no. of micronuclei/ tetrad	No. of pentads
					0	1	2	3		
Addition lines										
1R	44	0.208^{xyz}	0.390^{xyz}	2109	96.46	2.46^{xy}	0.80^{z}	0.28^{z}	0.050^{xyz}	0
2R	44	0.050^{z}	0.384^{xyz}	2070	98.79	0.97^{z}	0.24^{z}	0.00^{z}	0.012^{z}	0
3R	44	0.080^{z}	0.172^{xyz}	2032	97.29	1.79^{z}	0.64^{z}	0.28^{z}	0.032^{xz}	2
3RS	42+2t	0.107^{z}	0.092^{z}	2501	98.04	1.02^{z}	0.74^{z}	0.20^{z}	0.030^{yz}	0
4R	44	0.190^{xyz}	0.310^{xy}	2120	98.39	1.29^{z}	0.28^{z}	0.04^{z}	0.016^{z}	5
5R	44	0.024^{z}	0.264^{xy}	2001	99.89	0.09^{z}	0.02^{z}	0.00^{z}	0.001^{z}	2
6R	44	0.079^{z}	0.082^{z}	2332	88.89^{xy}	5.88^{xyz}	4.53^{xyz}	0.70^{xyz}	0.162^{xyz}	20
6RL	42+2t	0.161^{xyz}	0.210^{xyz}	2349	93.38	3.75^{xy}	2.48^{xy}	0.39^{xyz}	0.100^{xyz}	1
7R	44	0.176^{xyz}	0.180^{xyz}	2149	97.26	1.92^{yz}	0.82	0.00^{z}	0.034^{xyz}	5
Substitution line										
1B/1R	42	0.111^{xz}	0.170^{xyz}	2212	98.29	0.74^{z}	0.72	0.25	0.028^{yz}	1
Initial forms										
'Grana'	42	0.050	0.052	2014	99.47	0.49	0.04	0.00	0.006	0
'Dańkowskie Złote'	14	0.070	0.062	2112	99.71	0.24	0.05	0.00	0.002	0
'Grana' × 'Dańkowskie Złote'	56	0.330	0.304	2085	91.99	3.83	2.88	1.30	0.130	10

x – differences with wheat significant at P = 0.05.
y – differences with rye significant at P = 0.05.
z – differences with 8x triticale significant at P = 0.05.
t – telocentric chromosome.

in addition lines 1R, 4R, 6RL and 7R was significantly higher than in wheat and rye. All addition lines had significantly fewer delayed chromosomes than octoploid triticale, which had 0.330 per cell. In substitution line 1B/1R the mean number of delayed chromosomes at anaphase I was 0.111 per cell. In this line, delayed chromosomes occurred significantly more often than in wheat, but were less numerous than in cells of octoploid triticale. No differences between the substitution line and 'Dańkowskie Złote' rye were observed. Rye chromosomes were the most delayed in addition lines and substitution line 1B/1R. This probably was due to asynchrony between the rye and wheat meiotic cycles. Rye meiosis takes longer than wheat meiosis. Another reason for rye chromosome delay at anaphase I could be a lack of genetic affinity between the wheat karyokinetic spindle and the rye chromosomes. The presence of delayed rye chromosomes may also be associated with the amount and distribution of heterochromatin.

Few chromatid bridges were observed at anaphase I in all studied forms (Tab. 3; Fig. 2d). The mean number of bridges per cell varied and depended on the line. The highest number of bridges was found in line 1R (0.390), and the least in line 3RS (0.092). The majority of addition lines had significantly more chromatid bridges per cell than wheat and rye. Only lines 3RS and 6R had numbers of bridges similar to those two initial forms. There were 0.304 chromatid bridges per cell in octoploid triticale. Lines 1R and 2R had significantly higher frequencies of bridges than triticale, and lines 4R and 5R were similar to triticale in this trait. Far fewer chromatid bridges were observed in the other lines. Substitution line 1B/1R had significantly more bridges per cell than in wheat and rye, and less than in octoploid triticale. In the studied lines, both wheat and rye chromosomes formed chromatid bridges.

ANALYSIS OF MICRONUCLEI IN TETRADS

Besides chromosome pairing at metaphase I and segregation at anaphase I, the frequency of occurrence of micronuclei in tetrads indicates the stability of tested forms. In general, few micronuclei were found in the analyzed material (Tab. 3). Most often properly formed tetrads, rarely with one and sporadically with two or three micronuclei, were observed. Wheat lines with added rye chromosomes differed in this trait between lines and from the initial forms. The number of micronuclei per tetrad in lines ranged from 0.001 (5R) to 0.162 (6R). 'Grana' wheat contained 99.47% properly developed tetrads, and the frequency of micronuclei was only 0.006. Addition lines 1R, 3R, 6R, 6RL and 7R, had significantly more micronuclei than wheat, and the majority of lines had them significantly more than 'Dańkowskie Złote' rye. Relatively many micronuclei were observed in tetrads of octoploid triticale. Only line 6R had significantly more micronuclei than triticale; other lines contained much fewer. Rogalska (1981) found from 14.7% (Beeagle CIMCIANO) to 34.3% (Beaver) tetrads with micronuclei in some strains of hexaploid triticale. In that study the number of micronuclei per tetrad ranged from 0.5 to 0.8 depending on the strain. According to Gruszecka (1983/1984) the number of micronuclei in tetrads was higher in aneuploid lines than in strains and lines of hexaploid triticale. The mean number of micronuclei in tetrads for three strains (Nakajima, Kiskun and CZR 45) was 1.30, and for aneuploid forms it ranged from 2.25 to 2.72. Pentads were rarely observed in our tested lines (Fig. 2f). This means that added or substituted rye chromosomes did not disturb the formation of the karyokinetic spindle. The highest number of pentads was observed in line 6R (20 cases) and in octoploid triticale (10 cases).

The regular course of reduction divisions and the quite good level of rye chromosome transmission through the generations confirms the good cytogenetic stability of the 'Grana' wheat line with added and substituted chromosomes of 'Dańkowskie Złote' rye.

REFERENCES

ATTIA T, and LELEY T. 1987. Effects of constitutive heterochromatin and genotype on frequency and distribution of chiasmata in the seven individual rye bivalents. *Theoretical Applied Genetics* 74: 527–530.

BIELIG LM, and DRISCOLL CJ. 1970. Substitution of rye chromosome 5RL for chromosome 5B of wheat and its effects on chromosome pairing. *Genetics* 65: 241–247.

CHRZĄSTEK M, and MIAZGA D. 1988. Studies on the cv. Grana wheat (*Triticum aestivum* L.) lines with the cv. Dańkowskie Złote rye (*Secale cereale* L.) chromosome additions. II. An estimate of important quantitative traits. *Genetica Polonica* 29: 21–26.

CUADRADO A, VITELLOZZI F, JOUVE N, and CEOLONI C. 1997. Fluorescence in situ hybridization with multiple repeated DNA probes applied to the analysis of wheat-rye chromosome pairing. *Theoretical and Applied Genetics* 94: 347–355.

DARVEY NL, and GUSTAFSON JP. 1975. Identification of rye chromosomes in wheat-rye addition lines and triticale by heterochromatin bands. *Crop Science* 15: 239–243.

DRÖGEMÜLLER EM, and LELLEY T. 1984. Chiasma distribution in rye chromosomes of diploid rye and in wheat-rye addition lines in relation to C-heterochromatin. *Theoretical and Applied Genetics* 67: 457–461.

FELDMAN M. 1966. The effect on chromosomes 5B, 5D and 5A on chromosomal pairing in *Triticum aestivum*. *Proceedings of National Academy of Science U.S.A.* 55: 1447–1453.

GRUSZECKA D. 1983/84. Cytogenetyka aneuploidalnych roślin Triticale 6x. Cz. III. Mejoza, żywotność i wielkość ziarn pyłku oraz analiza liczby ziarniaków i płodności roślin. *Annales Universitatis Mariae Curie-Skłodowska* 25 Sectio E: 281–296 .

JOUVE N, DIÉZ N, and RODRIGUEZ M. 1980. C-Banding in 6x-Triticale × *Secale cereale* L. Hybrid cytogenetics. *Theoretical and Applied Genetics* 57: 75–79.

KING IP, READER SM, PURDIE KA, OXFORD SE, and MILLER TE. 1994. A study of the effect homoeologous pairing promoter on chromosome pairing in wheat/rye hybrids using *in situ* hybridization. *Heredity* 74: 318–321.

LEE YH, LARTER EN, and EVANS LE. 1970. Meiotic behaviour of wheat rye addition and substitution lines. *Crop Science* 10: 144–145.

LELLEY T. 1975. Genetic control of pairing of rye chromosomes in Triticale. *Zeitschrift für Pflanzenzüchtung* 75: 24–29.

LELLEY T. 1976. Effect of nulli/tetrasomic combinations of wheat chromosomes on the pairing of rye chromosomes in triticale. *Zeitschrift für Pflanzenzüchtung* 77: 89–99.

LELLEY T. 1980. The influence of the rye genome on chiasma localization in the wheat chromosomes of Triticale. *Hodowla Roślin Aklimatyzacja i Nasiennictwo* 242: 315–321.

MELLO-SAMPAYO T. 1971. Genetic regulation of meiotic chromosome pairing by chromosome 3D of *Triticum aestivum*. *Nature New Biology* 230: 22–23.

MIAZGA D, and CHRZĄSTEK M. 1987. Studies on the cv. Grana wheat (*Triticum aestivum* L.) lines with the cv. Dańkowskie Złote rye (*Secale cereale* L.) chromosome additions. I. Chromosome configurations at metaphase I. *Genetica Polonica* 28: 327–331.

MIKHAILOVA EI, NARANJO T, SHEPHERD K, EDEN J, HEYTING C, and JANG JH. 1998. The effect of the wheat *Ph1* locus on chromatin organization and meiotic chromosome pairing analysed by genome painting. *Chromosoma* 107: 339–350.

MILLER TE. 1984. The homoeologous relationship between the chromosomes of rye and wheat. Current status. *Canadian Journal of Genetics and Cytology* 26: 578–589.

MILLER TE, READER SM, PURDIE KA, and KING IP. 1994. Determinations of the frequency of wheat-rye chromosome pairing in wheat-rye hybrids with and without chromosome 5B. *Theoretical and Applied Genetics* 89: 255–258.

NARANJO T. 1982. Preferential occurrence of wheat-rye meiotic pairing between chromosomes of homoeologous group 1. *Theoretical and Applied Genetics* 63: 219–225.

NARANJO T, and FERNANDEZ-RUEDA P. 1996. Pairing and recombination between individual chromosomes of wheat and rye in hybrids carrying the ph1b mutation. *Theoretical and Applied Genetics* 93: 242–248.

NARANJO T, and LACADENA JR. 1980. Interaction between wheat chromosomes and rye telomeric heterochromatin on meiotic pairing of chromosome pair 1R of rye in wheat-rye derivatives. *Chromosoma* 81: 249–261.

NARANJO T, and PALLA O. 1982. Genetic control of meiotic pairing in rye. *Heredity* 48: 57–62.

NARANJO T, LACADENA JR, and GIRALDEZ R. 1979. Interaction between wheat and rye genomes on homologous and homoeologous pairing. *Zeitschrift für Pflanzenzüchtung* 82: 289–305.

ORELLANA J, CERMEÑO MC, and LACADENA JR. 1984. Meiotic pairing in wheat-rye addition and substitution lines. *Canadian Journal of Genetics and Cytology* 26: 25–33.

READER SM, MARTINEZ-PEREZ E, GRIFFITHS S, ROBERTS MA, FOOTE TN, DALGLEISH C, SHAW PJ, ARAGÓN-ALCAIDE L, MILLER TE, POWELL W, and MOORE G. 2000. The *Ph1* locus of wheat: characterisation and localisation via molecular and cytological analyses. *EWAC Newsletter* 42–44.

REES H, and THOMPSON JB. 1956. Genotypic control of chromosome behaviour in rye. III. Chiasma frequency in homozygotes and heterozygotes. *Heredity* 10: 409–424.

RILEY R. 1960. The meiotic behaviour, fertility and stability of wheat-rye chromosome addition lines. *Heredity* 14: 89–100.

RILEY R, and CHAPMAN V. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* 182: 713–715.

ROGALSKA S. 1981. Przebieg mejozy u Triticale (2n=42) w zależności od rodzaju tworzonych jąderek i zawartości heterochromatyny telomerycznej w chromosomach żyta. *Roczniki Naukowe Akademii Rolniczej w Poznaniu* 113: 1–35.

ROGALSKA S. 1983. The influence of the amount of telomeric heterochromatin on the course of meiosis in PMCs of hexaploid triticale. *Genetica Polonica* 24: 227–237.

ROUPAKIAS DG, and KALTSIKES PJ. 1977. The effect of telomeric heterochromatin on chromosome pairing of hexaploid triticale. *Canadian Journal of Genetics and Cytology* 19: 543–548.

SCHLEGEL R. 1978. Koniugacija chromosom w pszeniczno-rżanych dopołniennych liniach. *Genetika* 14: 1365–1375.

SCHLEGEL R. 1979. The effect of heterochromatin on chromosome pairing in different amphidiploid wheat-rye hybrids. *Cereal Research Communications* 7: 319–327.

SCHLEGEL R. 1980. Amphiploid hybrids from crosses of hexaploid wheat with several species of rye and the relationship between the amount of heterochromatin and meiotic chromosome pairing. *Hodowla Roślin Aklimatyzacja i Nasiennictwo* 24: 307–314 .

SCHLEGEL R, ZARIPOVA Z, and SHCHAPOVA AI. 1980. Further evidence on wheat-rye chromosome pairing in F_1 triticale × wheat hybrids. *Biologisches Zentralblatt* 99: 585–590.

SEARS ER, and OKAMOTO M. 1958. Intergenomic chromosome relationships in hexaploid wheat. *Proceedings of X International Congress of Genetics* 2: 258–259.

STEFANOWSKA G. 1988. Chromosome pairing in octoploid triticale (*Triticosecale* Wittmack.) depending on the initial wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) cultivars. *Genetica Polonica* 29: 257–264.

THOMAS JB, and KALTSIKES PJ. 1976. The genomic origin of the unpaired chromosomes in triticale. *Canadian Journal of Genetics and Cytology* 18: 687–700.