Theor Appl Genet (1986) 73:66–71

Chromosomal alterations in the karyotype of triticale in comparison with the parental forms

2. Heterochromatin of the wheat chromosomes

N. L. Bolsheva, E. D. Badaeva, N. S. Badaev and A. V. Zelenin

Institute of Molecular Biology, USSR Academy of Sciences, Vavilov Street 32, Moscow 117334, USSR

Received December 20, 1985; Accepted June 30, 1986
Communicated by Yu. Gleba

Summary. Wheat chromosomes of the primary winter hexaploid and octoploid triticales and of the parental durum and common wheat varieties were studied using morphometric analysis. The size of some heterochromatic segments was shown to change in triticale. Telomeric and intercalary C-bands both increased and decreased in size whereas centromeric bands only increased. The size variability of C-bands in triticale B-genome chromosomes decreased in most of the cases and increased only for several specific C-bands. The C-bands of homologous B-genome chromosomes changed in the same direction in both triticale forms. The changes in size of the C-bands found in R-genome chromosomes detected earlier in these triticale forms (Badaeva et al. 1986) were shown to coincide in their pattern with the size changes of C-bands in homeological B-genome chromosomes. Our data are indicative of regular, directed chromosomal changes in the triticale karyotype.

Key words: Triticale – Ancestor forms – C-banding

Introduction

It has been reported that parental genomes change during hybridization between different species.

For instance, DNA content per triticale cell nucleus can be inferior to the overal DNA amount found in the parental rye and wheat forms (Kaltsikes 1971; Boyko et al. 1984; Badaev et al. 1984). The activity of nucleolar organizers in hybrid chromosomes also change in comparison to the parental forms and, as a rule, the nucleolar organizer of one of the parents is inactivated (Nawashin 1928; Cermeño et al. 1984). The total telomeric heterochromatin amount found in R-genome chromosomes has been reported to decrease in spring triticale relative to the standard rye varieties (Merker 1975; Gustafson and Bennett 1976; Zeller 1977; Seal and Bennett 1981; Pilch 1981a, b; Semyonov and Semyonova 1982). However, we have found that many C-bands of R-genome chromosomes in the primary winter triticale karyotype are larger than those found in the parental rye varieties (Badaeva et al. 1986). Seal (1982) has shown that many heterochromatic bands in triticale A- and B-genome chromosomes are smaller than those found in the studied common and durum wheat varieties. Unfortunately, among the forms investigated by Seal, only one durum wheat variety was a direct ancestor of one of the triticale varieties.

The present communication is concerned with a comparative analysis of differentially stained wheat chromosomes in the hexaploid and octoploid triticale forms as well as in the parental durum and common wheat varieties. We studied the same forms as Badaeva et al. (1986), which allowed us to compare the results of measuring the size of heterochromatic segments in B- and R-genome chromosomes in the triticale and parental varieties.

Materials and methods

The following forms were taken for the investigation: the primary hexaploid winter triticale (TPG-I/I-78) produced by crossing a durum wheat (line 482/76) with a diploid rye ('Kharkovskaja-60'); the primary octoploid winter triticale (AD 825) produced by crossing a winter common wheat ('Gostianum-237') with a diploid rye (VSKhI); their parental durum and common wheat varieties. The material was supplied by Dr. N. G. Maximov (All-Union Plant Breeding and Genetic Institute, VASKhNIL, Odessa).

The techniques of sample preparation, differential staining and morphometric analysis of chromosomes have been developed in the previous investigations (Bolsheva et al. 1984; Badaeva et al. 1986). Chromosome identification was done as described previously (Badaev et al. 1983) with some modification for 4A-4B chromosomes according to Lapitan et al. (1984). Fifty to sixty chromosomes from each homologous

group of the B-genome and a 4A chromosome were measured. The scheme of chromosomal segmentation for measurements is shown in Fig. 1. Other wheat chromosomes (A- and D-genomes) were investigated visually.

Results

1 Visual investigation

The durum wheat line 482/76. All of the analysed metaphase plates have 28 chromosomes (Fig. 2). Some of the plants display heteromorphism of 2B chromosome homologues: they either have or lack telomeric and subtelomeric segments in the long arm of this chromosome.

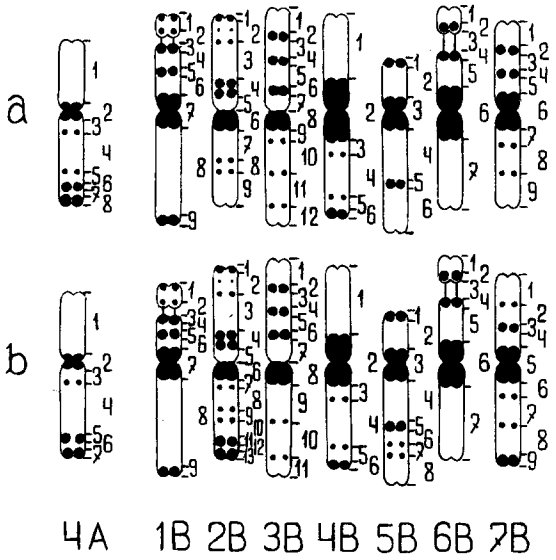

Fig. 1a, b. Scheme of chromosomal division into regions for measurements. **a** Chromosomes of durum wheat line 482/76 and triticale TPG-I/I-78; **b** Chromosomes of common wheat 'Gostianum 237' and triticale AD 825

The triticale TPG-I/I-78. This is a hexaploid from with a complete set of A-, B- and R-genome chromosomes. From the parental durum wheat variety, TPG-I/I-78 has inherited a 2B chromosome variant lacking terminal and subterminal bands in the long arm.

The common wheat 'Gostianum-237'. All analysed metaphase plates have 42 chromosomes (Fig. 3). The homologues of the 2B, 3B, 3D, 4A, and 5B chromosomes are rather polymorphic. For instance, the 2B chromosome either has or lacks a telomeric band in its long arm. A subterminal band is sometimes absent from the long arms of the 3A and 3B chromosomes. The 3D chromosome occasionally has a paracentric inversion in its short arm. A telomeric band is absent from the long arm of some 4A chromosomes. The long arm of the 5B chromosome comprises four median bands, the most proximinal of these is much longer than the other three in the majority of cases, but it happens that all four bands are identical in size.

The triticale AD 825. This is an octoploid form with a complete set of A-, B-, D-, and R-genome chromosomes. From the parental wheat variety, it has inherited a 2B chromosome variant with a telomeric band in the long arm, 3A and 3B chromosome variants without subterminal bands in the long arm, a normal 3D chromosome, a 4A chromosome variant with a terminal band in the long arm, and a 5B chromosome variant with one large and three small median bands in the long arm.

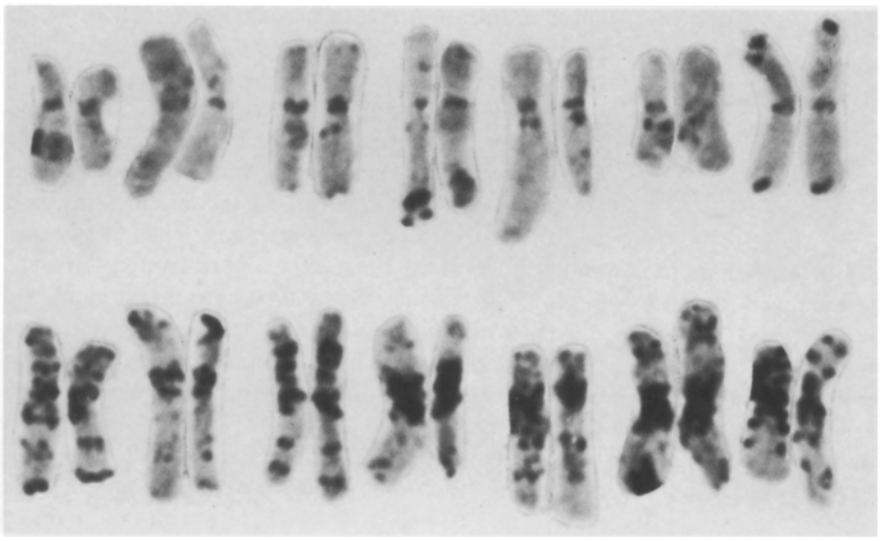

Fig. 2. Karyotype of the durum wheat line 482/76

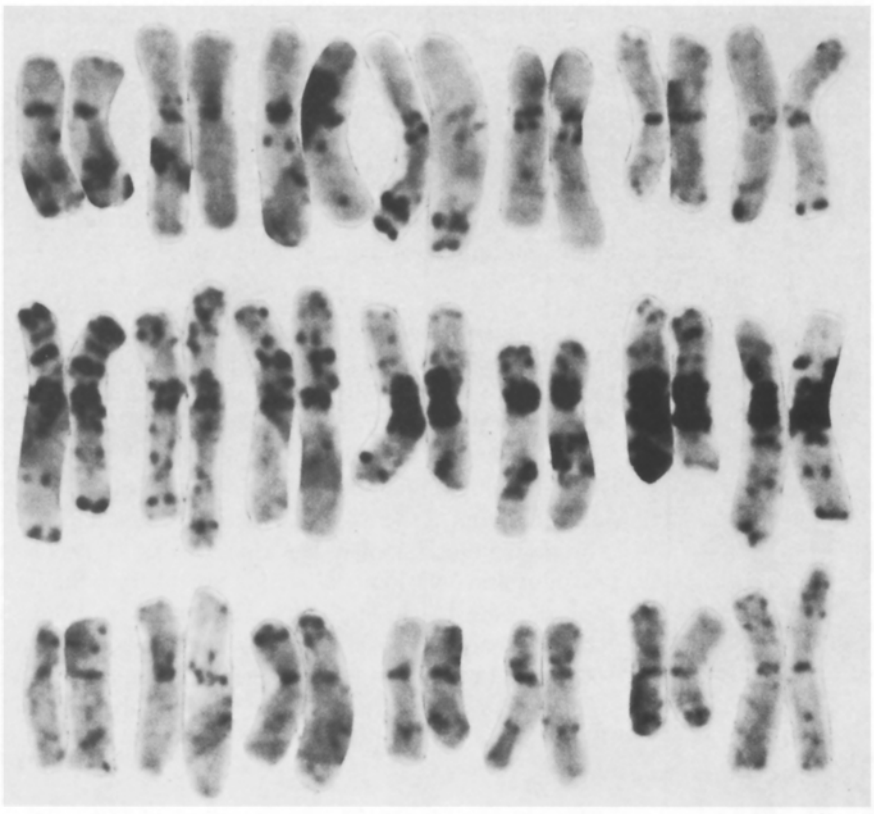

Fig. 3. Karyotype of the common wheat 'Gostianum 237'

The TPG-I/I-78 and AD 825 karyotypes have been presented in a previous publication (Badaeva et al. 1986).

Visual comparison has shown that most C-bands in the chromosomes of both triticale studied are larger than in corresponding parental forms.

2 Morphometric analysis

The results of morphometric analysis on heterochromatin bands of the B-genome chromosomes and the 4A chromosome for the two studied triticale forms and for the parental durum and common wheat forms are presented in Tables 1, 2 and 3.

The overall length of heterochromatic C-bands per haploid set of the B-genome plus the 4A chromosome is as follows:

– in the durum wheat line 482/76, 23.10 μm (telomeric bands, 2.80 μm; intercalary bands, 6.62 μm; centromeric bands, 13.68 μm)
– in triticale TPG-I/I-78, 24.01 μm (telomeric bands, 2.93 μm; intercalary bands, 6.73 μm; centromeric bands, 14.35 μm)
– in the common wheat 'Gostianum-237', 21.48 μm (telomeric bands, 3.30 μm; intercalary bands, 5.86 μm; centromeric bands, 12.32 μm)
– in triticale AD 825, 22.92 μm (telomeric bands, 3.39 μm; intercalary bands, 6.16 μm; centromeric bands, 13.37 μm).

Discussion

As can be seen from the results, the size of some C-bands in the B-genome chromosomes and in the 4A chromosome of triticale has changed relative to the parental wheat forms. For instance, the size of eight bands changed significantly ($P \geq 0.95$) in TPG-I/I-78: one telomeric band increased and one decreased, two intercalary bands increased and one decreased, four centromeric bands increased; seventeen bands changed significantly in AD 825: two telomeric bands increased and three decreased, three intercalary bands increased and two decreased, seven centromeric bands increased.

In both triticale forms, the centromeric bands can only increase in size whereas the telomeric and intercalary bands are capable of both increasing and decreasing. However, the corresponding chromosomal C-bands can change in the two forms studied in a similar manner. The telomeric band found in the long arm of the 4A chromosome has decreased in AD 825 as well as in TPG-I/I-78 in which, however, this change is

Table 1. Sizes of the C-bands in the IB, 2B, 3B chromosomes of the triticale and parental wheat forms. X = mean length in μm; σ = standard deviation; P = confidence coefficient

	C-bands	No.	3	5	7	9	
Chromosome 1B	Line 482/76	X	0.50	0.56	0.67	0.71	
		σ	0.12	0.13	0.19	0.13	
	TPG-I/I	X	0.53	0.55	0.76	0.81	
		σ	0.11	0.11	0.20	0.16	
	P		0.84	0.07	0.96	0.97	
	'Gost. 237'	X	0.48	0.59	0.66	0.75	
		σ	0.16	0.12	0.11	0.17	
	AD 825	X	0.51	0.64	0.97	0.74	
		σ	0.13	0.12	0.27	0.11	
	P		0.81	0.87	0.99	0.61	
	C-bands	No.	1	4	6	11	13
Chromosome 2B	Line 482/76	X	0.54	0.91	0.55		
		σ	0.12	0.26	0.15		
	TPG-I/I	X	0.59	0.85	0.57		
		σ	0.12	0.17	0.13		
	P		0.87	0.80	0.77		
	'Gost. 237'	X	0.43	0.77	0.53	0.44	0.13
		σ	0.09	0.22	0.14	0.14	0.17
	AD 825	X	0.49	0.80	0.66	0.53	0.32
		σ	0.09	0.19	0.18	0.10	0.14
	P		0.99	0.82	0.99	0.99	0.99
	C-bands	No.	2	4	6	8	
Chromosome 3B	Line 482/76	X	0.73	0.57	0.60	0.86	
		σ	0.15	0.11	0.52	0.21	
	TPG-I/I	X	0.78	0.60	0.56	1.03	
		σ	0.16	0.10	0.26	0.26	
	P		0.96	0.40	0.72	0.99	
	'Gost. 237'	X	0.55	0.58	0.50	0.95	
		σ	0.11	0.14	0.12	0.24	
	AD 825	X	0.68	0.70	0.48	1.10	
		σ	0.10	0.18	0.11	0.26	
	P		0.99	0.99	0.77	0.97	

Table 2. Sizes of the C-bands in the 4A and 4B chromosomes of the triticale and parental wheat forms. X = mean length in μm; σ = standard deviation; P = confidence coefficient

	C-bands	No.	2	6	8
Chromosome 4A	Line 482/76	X	0.49	0.88	0.55
		σ	0.09	0.15	0.08
	TPG-I/I	X	0.62	0.84	0.53
		σ	0.14	0.37	0.13
	P		0.99	0.84	0.84
	'Gost. 237'	X	0.49	0.74	0.48
		σ	0.12	0.17	0.20
	AD 825	X	0.54	0.66	0.42
		σ	0.09	0.14	0.10
	P		0.99	0.99	0.97
	C-bands	No.	2	6	
Chromosome 4B	Line 482/76	X	3.11	0.46	
		σ	0.62	0.08	
	TPG-I/I	X	3.34	0.47	
		σ	0.90	0.10	
	P		0.90	0.23	
	'Gost. 237'	X	2.84	0.47	
		σ	0.66	0.23	
	AD 825	X	3.09	0.51	
		σ	0.58	0.08	
	P		0.96	0.27	

not significant ($P > 0.81$). The telomeric band in the short arm of the 2B chromosome has increased in AD 825 while its absolute increase in TPG-I/I-78 is not significant ($P > 0.87$). In the remaining cases, changes in the telomeric band size were found for one of the forms whereas no reliable changes were detected for the other form, or the bands did not change in both forms. The size of the telomeric bands did not change in opposite directions in the triticale forms studied. The intercalary bands at the nucleolar organizer of the 6B chromosome increased in both triticale forms as well as those in the distal region of the short arm of the 3B chromosome. The intercalary band found in the distal region of the long arm of the 4A chromosome decreased in both triticale forms but this change was not significant ($P > 0.84$) in TPG-I/I-78. In the remaining cases, the intercalary bands either changed in one of the forms or did not change in both triticale forms; the size of these bands did not change in opposite directions. The centromeric bands increased in the 1B, 3B and 4A chromosomes in both triticale forms. These bands also increased in the 4B chromosome, but the change was not significant ($P > 0.90$) in TPG-I/I-78. No changes in the centromeric bands were detected in the remaining cases.

Therefore, in spite of slight differences in the overall content of heterochromatin in B-genome chromosomes of the triticale and parent wheat varieties, we have found changes in the C-banding pattern of B-genome chromosomes in triticale forms. In the triticale forms studied, the corresponding bands changed in one and the same direction in a number of cases; no changes of the C-bands in the opposite directions were detected. One may assume, therefore, that the C-banding pattern of B-genome chromosomes in winter triticale changes in a regular manner.

The results of the morphometric investigation show that the size of 4A and 4B chromosomes changes similarly. Visual investigation revealed an increase in the size of most of the centromere bands of A and D chromosomes. It suggests that all homeologous chromosomes of wheat change in triticale in the same way.

Table 3. Sizes of the C-bands in the 5B, 6B, 7B chromosomes of the triticale and parental wheat forms

	C-bands	No.	1	4	5	7
Chromosome 5B	Line 482/76	X	0.54	0.97	0.66	0.66
		σ	0.12	0.26	0.16	0.14
	TPG-I/I	X	0.53	0.96	0.68	0.59
		σ	0.09	0.25	0.13	0.20
	P		0.37	0.59	0.10	0.95
	'Cost. 237'	X	0.51	0.79	0.58	0.47
		σ	0.12	0.20	0.12	0.13
	AD 825	X	0.44	0.80	0.63	0.47
		σ	0.08	0.21	0.10	0.13
	P		0.99	0.40	0.97	0.02
	C-bands	No.	2	4	6	
Chromosome 6B	Line 482/76	X	0.49	0.46	3.21	
		σ	0.11	0.15	0.74	
	TPG-I/I	X	0.60	0.56	3.27	
		σ	0.15	0.13	0.66	
	P		0.99	0.98	0.45	
	'Cost. 237'	X	0.51	0.41	2.59	
		σ	0.11	0.11	0.68	
	AD 825	X	0.53	0.51	2.66	
		σ	0.14	0.14	0.58	
	P		0.65	0.99	0.81	
	C-bands	No.	2	4	6	
Chromosome 7B	Line 482/76	X	0.56	0.62	2.26	
		σ	0.13	0.12	0.58	
	TPG-I/I	X	0.52	0.60	2.29	
		σ	0.13	0.12	0.47	
	P		0.62	0.78	0.43	
	C-bands	No.		3	5	9
	'Gost. 237'	X		0.58	2.13	0.54
		σ		0.10	0.58	0.15
	AD 825	X		0.47	2.13	0.47
		σ		0.09	0.37	0.12
	P			0.99	0.29	0.97

The changes in the R-genome chromosomes of the same triticale forms relative to the parental rye forms have been described previously (Badaeva et al. 1986). We compared now the changes in the telomeric and centromeric bands of B- and R-genome homeologous chromosomes taking account of a translocation between chromosomes 4 and 7 in *S. cereale* (Koller and Zeller 1976). These changes were in the same direction with a high significance ($P > 0.95$) in eight cases and with a probability close to the significant one ($0.82 < P < 0.95$) in three cases. A significant change in C-band size was found for only one of the homeologous in six cases. The changes were in opposite directions in one case (the telomeric band in the long arm of chromosomes belonging to the seventh homeologous group in AD 825).

The incomplete coincidence of the changes in C-bands of the B- and R-genome chromosomes in the two studied triticale forms may stem from genotypic differences between these forms, in particular, from the presence of D-genome chromosomes in AD 825, as well as from the fact that AD 825 has over 20 generations while TPG-I/I-78 only two.

Factors inducing changes in the size of C-bands have been discussed previously in the work of Badaeva et al. (1985). Changes in the content of heterochromatin in triticale chromosomes relative to the parent varieties of wheat and rye may be brought about by changes in the system of polymorphism, over-replication or under-replication of DNA in heterochromatic chromosomal regions, molecular drive, unequal crossing-over, etc.

We believe that changes in the size of triticale C-bands cannot be attributed solely to the selection of parent polymorphism variants. The polymorphism of C-bands in the triticale B-genome mainly decreases when compared to the parental wheat forms but to a far lesser degree than in the case of R-genome chromosomes. This is due to the fact that the polymorphism of C-bands in the parental wheat varieties is lower than in the rye varieties. However, some bands become more polymorphous in triticale. For instance, the standard deviation σ is greater in the triticale forms than in the parent wheat and rye varieties for the centromeric band of the 1B chromosome in AD 825 and the 4A chromosome in TPG-I/I-78, for the intercalary bands in the long arm of the 4A and 5B chromosomes in TPG-I/I-78, and for the telomeric band in the short arm of the 6R chromosome in AD 825. The polymorphism of certain C-bands increases in triticale, apparently owing to changes in the size of heterochromatic segments.

The cytophotometric analysis of Feulgen-stained nuclei in these triticale forms and in the parental wheat and rye varieties has shown that the DNA content in triticale is significantly ($P > 0.99$) lower than the overall DNA amount of the parent forms (Badaev et al. 1984), while the total content of heterochromatin is higher in triticale. This inconsistency may be accounted for by the fact that the DNA content in triticale does not only change in heterochromatic regions.

It is difficult to presume what the nature of changes found in the size of C-bands in triticale is. Nevertheless, these changes must have a functional significance since they occur in a regular and directed manner.

References

Badaev NS, Badaeva ED, Bolsheva NL, Zelenin AV (1983) Identification of common wheat A and D genome chromosomes by use of substitutions and translocations between

homeologous chromosomes. Dokl Acad Nauk SSSR 273: 994–996

Badaev NS, Badaeva ED, Bolsheva NL, Boyko EV, Zelenin AV (1984) Regular chromosome changes in the triticale karyotype. In: 3rd Nat Conf Cytogenet, Bulgaria, pp 485–488

Badaeva ED, Badaev NS, Bolsheva NL, Zelenin AV (1986) Chromosome alterations in the karyotype of triticale in comparison with parent forms. 1. Heterochromatic regions of R-genome chromosomes. Theor Appl Genet 72:518–523

Bolsheva NL, Badaeva ED, Kurochkina AI, Badaev NS (1984) Comparative investigation of the different stained chromosomes of two related forms of rye. Genetika 20:2025–2030

Boyko EV, Badaev NS, Maximov NG, Zelenin AV (1984) Does DNA content change in the course of triticale breeding? Cereal Res Commun 12:99–100

Cermeno MC, Orellana J, Santos JL, Lacadena JR (1984) Nucleolar organizer activity in wheat, rye and derivatives analyzed by a silver-staining procedure. Chromosoma 89: 370–376

Gustafson JP, Bennett MD (1976) Preferential selection for wheat-rye substitutions in 42-chromosome triticale. Crop Sci 16:688–693

Kaltsikes PJ (1971) The mitotic cycle in the amphidiploid (triticale) and its parental species. Can J Genet Cytol 13: 656–662

Koller OL, Zeller FJ (1976) The homeologous relationships of rye chromosome 4R and 7R with wheat chromosomes. Genet Res 28:177–188

Lapitan NL, Sears RG, Gill BS (1984) Translocations and other karyotypic structural changes in wheat-rye hybrids regenerated from tissue culture. Theor Appl Genet 68: 547–555

Merker A (1975) Chromosome comparison of hexaploid triticale. Hereditas 80:41–52

Nawashin M (1928) "Amphiplastic" – eine neue karyologische Erscheinung. Z Indukt Abstamm Verarbungsl (Suppl) 2: 1148–1152

Pilch J (1981) Rye chromosome constitution and the amount of telomeric heterochromatin of the widely and narrowly adapted CIMMYT triticales. Z Pflanzenzücht 87:58–68

Pilch J (1981) Analysis of the rye chromosome constitution and the amount of telomeric heterochromatin in the widely and narrowly adapted hexaploid triticales. Theor Appl Genet 60:145–149

Seal AG, Bennett MD (1981) The rye genome in winter hexaploid triticales. Can J Genet Cytol 23:647–653

Seal AG (1982) C-banded wheat chromosomes in wheat and triticale. Theor Appl Genet 63:39–47

Semyonov VJ, Semyonova EV (1982) Polymorphism of rye chromosomes for heterochromatic bands in some varieties of rye and in lines of triticale. Genetika 18:1856–1867

Zeller FD (1977) Evidence for a IR/3R translocation in the Kharkov-Dakold wheat-rye addition set. Can J Genet Cytol 19:745–748