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

1. Heterochromatic regions of R genome chromosomes

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Summary. R genome chromosomes were studied in two forms of primary triticales (hexaploid 'TPG-1/1-78' and octoploid 'AD 825') and in their parent rye forms (*Secale cereale* L. cv. 'Kharkovskaya 60' and 'VSKhI', respectively) using the methods of C-banding and morphometric analysis. The size of some heterochromatic segments was shown to alter in the karyotype of triticale. An increase in size was detected approximately in half of all telomeric C-bands; the size of the other C-bands either decreased or did not change. The frequencies of these alterations were 1 : 1. The variability in the size of telomeric C-bands in rye chromosomes diminished in both triticales studied. The two triticale forms inherited variants of R genome chromosome polymorphism predominantly with the medium size range of telomeric C-bands. The centromeric C-bands in both triticale forms either enlarged or did not alter. Possible mechanisms responsible for the observed pattern of alterations are discussed.

Key words: Rye – Triticale – C-banding – R genome – Alterations

Introduction

Triticale is a wheat-rye amphidiploid hybrid created to combine the most important characteristics of both parent species.

Many investigators have studied the cytogenetics of triticale (Darvey and Gustafson 1975; Merker 1975; Gustafson and Bennett 1976; Lukaszewski and Apolinarska 1981; Pilch 1981; Seal and Bennett 1981). These studies on various lines and cultivars of triticale indicated that the R genome chromosomes have been altered in the karyotype of triticale. D(R) chromosome substitutions were found in many triticales and the order of substitutions seemed to be non-random (Merker 1975; Gustafson and Bennett 1976). Apart from D(R) substitutions in triticale, the total amount of telomeric heterochromatin was found to be reduced in comparison with standard rye varieties (Merker 1975; Gustafson and Bennett 1976; Zeller 1977; Seal and Bennett 1981; Pilch 1981). Frequently this was due to a loss of one or more telomeric C-bands. It was supposed (Gustafson and Bennett 1976; Seal and Bennett 1981) that D(R) chromosome substitutions as well as telomeric C-band modifications resulted in the reduction of DNA content either per genome (in the case of substitutions) or per chromosome (in the case of C-band loss).

Substitutions of rye chromosomes for the chromosomes of the wheat D genome as well as the loss of telomeric C-bands from the R genome chromosomes are typical of spring triticales cultivated at lower latitudes (Lukaszewski and Apolinarska 1981; Seal and Bennett 1981); winter triticales from higher latitudes in general retain their complete set of rye chromosomes. Apparently, there are large differences between selection pressures in the breeding programmes of spring and winter triticale forms.

However, the lower amount of telomeric heterochromatin found in the R genome chromosomes of triticale when compared to randomly chosen rye varieties may reflect its lower amount in the parent rye forms of studied triticales rather than the loss of heterochromatin. To find out whether the heterochromatin of the R genome chromosomes is lost, one has to compare the R genome chromosomes of triticale directly with the parent rye variety.

The aim of this work was to compare the R genome chromosomes of triticales and their parent rye forms using the methods of visual and morphometric analysis.

Materials and methods

Two constant forms of primary hexaploid and octoploid winter triticale and their parent rye forms were used in this study. The primary hexaploid triticale 'TPG-1/1-78' was ob-

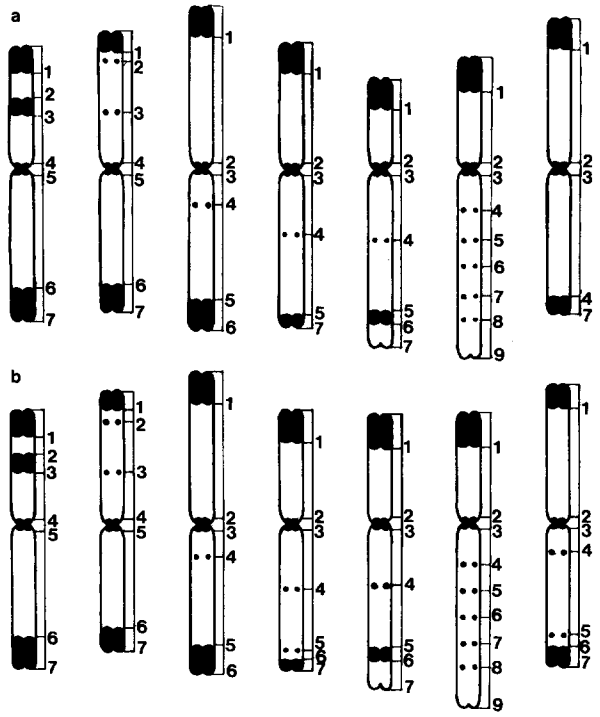

Fig. 1 a, b. R genome chromosomes divided into regions used in chromosome measurements. **a** rye 'Kharkovskaya 60' and triticale 'TPG-1/1-78'; **b** rye 'VSKhI' and triticale 'AD 825'

tained by crossing a tetraploid wheat (*Triticum durum* line '482/76') with diploid rye ('Kharkovskaya 60'), and the primary octoploid triticale 'AD 825' – by crossing a winter hexaploid wheat (*Triticum aestivum* cv. 'Gostianum 237') with a diploid rye variety 'VSKhI' ('Voronezhskaya SKhI'). All forms were a kind gift from Dr. N. G. Maximov (All-Union Plant Breeding and Genetic Institute, VASKhNIL, Odessa).

The preparation of chromosomes and the procedure of C-banding were carried out as described earlier (Bolsheva et al. 1984). Chromosomes were identified using the genetic nomenclature for wheat and rye chromosomes (Badaev et al. 1983; Lapitan et al. 1984). Chromosomes within a roughly identical range of spiralization with a clear C-banding pattern were taken for measurements. They were divided into regions according to the general C-banding pattern of rye chromosomes presented in Fig. 1. Measurements were carried out as described by Badaev et al. (1982). The Wilcoxon and Student criteria were used for statistical analysis of the results.

Results

1 Visual investigation

Secale cereale L. *cv. 'Kharkovskaya 60', 2n=2x=14.* This rye variety has a great chromosomal polymorphism manifested as the heteromorphism of homologues of 1R, 2R, 4R/7R, 5R and 7R/4R chromosomes and also as the differences between plants in the composition of heteromorphic variants (Fig. 2 a).

Triticale TPG-1/1-78. This is a hexaploid form containing all the chromosomes of the A, B and R genomes (Fig. 3). TPG-1/1-78 has inherited, from the parent rye variety, a 1R chromosome variant with a medium-sized telomeric C-band in the long arm and with an intercalary C-band localized approximately in the middle of

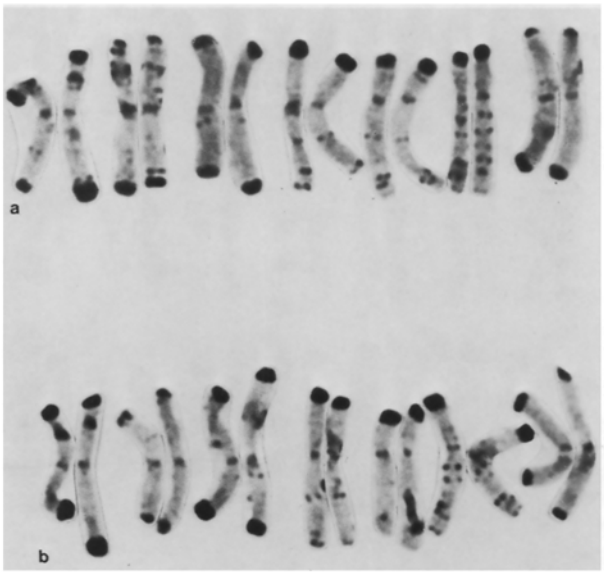

Fig. 2. a Karyotype of diploid rye 'Kharkovskaya 60'. Chromosomal polymorphism is manifested by the heteromorphism of homologues of 1R, 2R, 4R/7R chromosomes; **b** karyotype of diploid rye 'Voronezhskaya SKhI' (VSKhI)

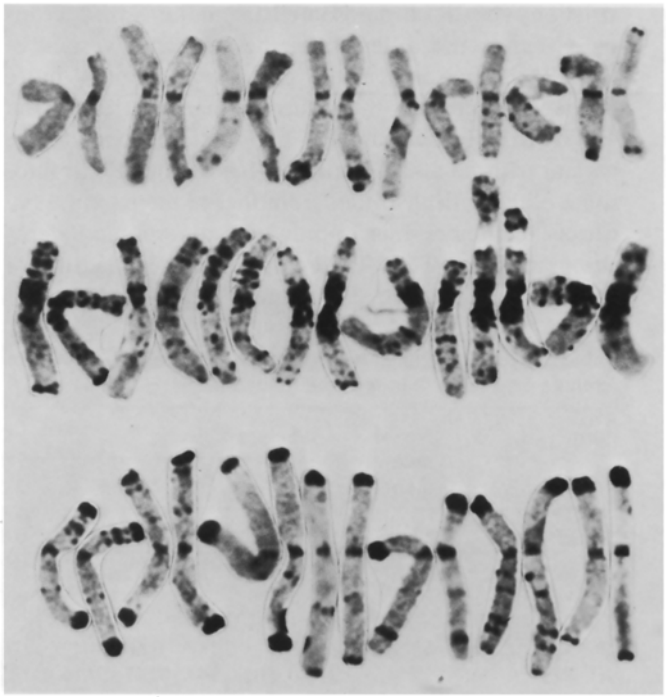

Fig. 3. Karyotype of primary hexaploid triticale 'TPG-1/1-78'

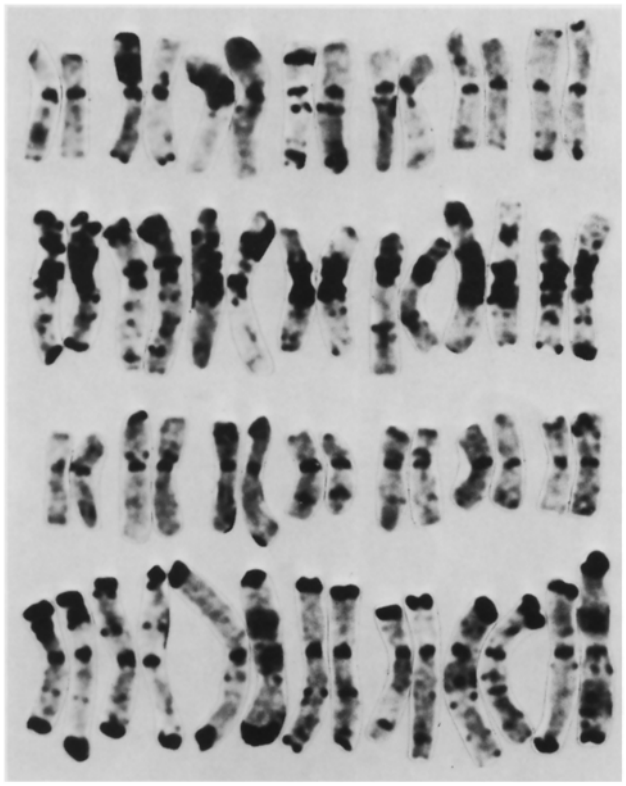

Fig. 4. Karyotype of primary octoploid triticale 'AD 825'

the long arm. The 1R chromosome of the triticale has an intense C-band in the middle of the secondary constriction. The 2R chromosome has medium-sized terminal C-bands and differs from the respective variant of the 2R chromosome in the parent rye form by more intensive intercalary C-bands on the short arm. The 4R/7R chromosome of TPG-1/1-78 corresponds to the variant without a subterminal C-band. The 5R chromosome of the triticale differs from that of the parental rye variety by the presence of only one subterminal heterochromatic band. The 7R/4R chromosome of the triticale corresponds to the variant with medium-sized telomeric C-bands.

Secale cereale L. *cv. 'VSKhI', 2n=2x=14.* It is less polymorphic in the C-banding pattern than the rye 'Kharkovskaya 60', and the heteromorphism of homologues is detected only for 2R and 7R/4R chromosomes (Fig. 2 b).

Triticale AD 825. This is an octoploid form with all the chromosomes of the A, B, D and R genomes (Fig. 4). All the R genome chromosomes have more intensive intercalary C-bands than the parent rye variety. The triticale has inherited the 2R chromosome variant with medium-sized telomeric C-bands. The distribution of intercalary C-bands in the 6R long arm differs in the triticale and its parent rye form by the intensity of staining. The 7R/4R chromosome of 'AD 825' corresponds to the variant with a medium-sized telomeric C-band.

2 Morphometric investigation

The length of a heterochromatic region does not depend on the total length of a chromosome (Selesneva et al. 1977). Therefore, the heterochromatic segments of the related forms are compared in terms of their absolute lengths. The absolute length is used to characterize the size of a heterochromatic band. It is calculated as a mean of all measurements of this band in the analyzed chromosome. The variability of the band is characterized in terms of standard deviations calculated according to the formula

$$\sigma = \sqrt{\frac{I}{n-I} \sum (X_i - \bar{X})^2} .$$

The reliability of differences is determined for the related segments. The results of morphometric analysis for 1R, 2R, 3R, 4R/7R, 5R, 6R and 7R/4R chromosomes are presented in Tables 1–7, respectively. The

Table 1. Mean lengths of heterochromatic regions in IR chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.				
		1	2	3	5	7
'Khark-60'	63	0.92±0.16	0.82±0.64	0.65±0.16	0.40±0.11	1.04±0.28
'TPG-1/1-78'	69	0.96±0.16	0.60±0.25	0.60±0.12	0.50±0.09	1.10±0.16
P		0.995	0.999	0.120	0.999	0.995
'VSKhI'	62	0.86±0.16	0.60±0.42	0.62±0.14	0.39±0.18	1.07±0.23
'AD 825'	69	0.93±0.14	0.47±0.16	0.57±0.11	0.49±0.11	1.01±0.21
P		0.999	0.999	0.320	0.999	0.260

Table 2. The mean lengths of heterochromatic regions in the 2R chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.		
		1	5	7
'Khark-60'	55	0.68±0.19	0.43±0.09	0.82±0.37
'TPG-1/1-78'	63	0.74±0.15	0.46±0.12	1.08±0.24
P		0.913	0.964	0.766
'VSKhI'	60	0.56±0.21	0.47±0.12	0.69±0.25
'AD 825'	60	0.63±0.11	0.47±0.13	0.78±0.20
P		0.999	0.190	0.984

Table 3. The mean lengths of heterochromatic regions in the r̂ chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.		
		1	3	6
'Khark-60'	66	1.02±0.19	0.45±0.12	0.99±0.29
'TPG-1/1-78'	61	0.97±0.20	0.49±0.10	1.10±0.23
P		0.998	0.992	0.999
'VSKhI'	72	1.04±0.19	0.40±0.08	1.03±0.20
'AD 825'	57	0.93±0.17	0.44±0.09	0.89±0.21
P		0.993	0.874	0.999

Table 4. The mean lengths of heterochromatic regions in the 4R/7R chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.		
		1	3	7
'Khark-60'	67	1.04±0.25	0.45±0.10	0.45±0.11
TPG-1/1-78'	62	0.97±0.19	0.49±0.14	0.50±0.12
P		0.740	0.850	0.993
'VSKhI'	72	0.99±0.18	0.40±0.09	0.39±0.11
'AD 825'	61	0.93±0.17	0.48±0.13	0.45±0.10
P		0.930	0.999	0.999

total lengths of telomeric and centromeric C-bands for the haploid set of R genome chromosomes are:

	Telomeric	Centromeric
Rye 'Khark.-60'	11.45 μm	3.11 μm
Trit. 'TPG-1/1-78'	12.30 μm	3.36 μm
Rye 'VSKhI'	11.14 μm	2.96 μm
Trit. 'AD 825'	11.07 μm	3.33 μm .

Table 5. The mean lengths of heterochromatic regions in the 5R chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.		
		1	3	6
'Khark-60'	56	1.03±0.22	0.47±0.15	0.44±0.08
'TPG-1/1-78'	60	1.13±0.21	0.47±0.10	0.47±0.10
P		0.989	0.320	0.020
'VSKhI'	62	1.12±0.15	0.47±0.08	0.50±0.09
'AD 825'	56	0.97±0.19	0.49±0.11	0.50±0.07
P		0.999	0.500	0.160

Table 6. The mean lengths of heterochromatic regions in the 6R chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.	
		1	3
'Khark-60'	57	1.12±0.16	0.47±0.15
'TPG-1/1-78'	60	1.12±0.18	0.47±0.11
P		0.840	0.030
'VSKhI'	45	1.13±0.22	0.42±0.08
'AD 825'	51	1.25±0.24	0.47±0.10
P		0.992	0.973

Table 7. The mean lengths of heterochromatic regions in the 7R/4R chromosome (μm) and the reliabilities of differences between C-bands of the related forms

Form	No. of measurements	Segment no.		
		1	3	7
'Khark-60'	50	1.00±0.22	0.44±0.11	0.69±0.20
'TPG-1/1-78'	51	1.29±0.27	0.48±0.12	0.62±0.15
P		0.999	0.944	0.820
'VSKhI'	56	0.88±0.22	0.42±0.08	0.77±0.19
'AD 825'	57	1.03±0.19	0.47±0.10	0.67±0.18
P		0.999	0.976	0.999

Discussion

Crop cereals represent an interesting model for the investigation of the evolutionary process at the chromosomal level. This stems from the fact that man has significantly accelerated the evolution of crop plants by applying such pressures as mutagenesis, and distant and related hybridization. In contrast to natural species, as a rule parental forms are known in the breeding

material. It allows one to compare the original material with the resultant varieties. Grain crops are chosen as a model for evolutionary studies since they have large chromosomes with a rich C-banding pattern specific for chromosomes of each species.

As has been shown earlier (Savchenko et al. 1982), the amount of heterochromatin (particularly centromeric) is higher in a maize variety obtained by the method of experimental mutagenesis without hybridization than in the parent form. At the same time, the polymorphism of heterochromatic regions is expressed to a greater degree. Consequently, mutagenesis can alter the amount of heterochromatin and the C-banding pattern. The present study has been undertaken in order to find out whether the chromosomes would change upon distant hybridization when allopolyploid forms are created.

The comparison of R genome chromosomes in primary triticales with those found in their parental forms has shown that the rye chromosomes of triticale, especially 1R, 2R and 3R, have more intense intercalary and centromeric C-bands. The morphometric investigation of differentially stained rye chromosomes demonstrates that wheat-rye hybridization modifies some heterochromatic regions of R genome chromosomes.

Since the principal amount of heterochromatin in the rye genome is located in telomeric C-bands, the summed total linear length of all the telomeric bands may roughly characterize the overall amount of heterochromatin found in the haploid rye genome. Morphometric investigation shows that the R genome chromosomes of triticale either contain more telomeric heterochromatin (in hexaploid triticale its amount increases by 7.4%) than the parent rye variety, or the amount of telomeric heterochromatin practically does not change (its content is 0.6% less in 'AD 825' than in the rye 'VSKhI'). The investigation of individual R genome chromosomes shows that 5 telomeric bands in 'TPG-1/1-78' are larger than those found in the rye 'Kharkovskaya 60', one segment is smaller, and the lengths of 6 bands are equal to respective segments of chromosomes in the parent rye form. In 'AD 825', the C-bands are also longer in 5 cases, shorter in 4 cases, and three C-bands do not differ from those of the rye 'VSKhI'. The centromeric heterochromatin bands are longer in the both triticales: in 'TPG-1/1-78' by 8.0%, in 'AD 825' by 11.1%.

One may ask what is the nature of the changes which occur in the heterochromatin amount of R genome chromosomes of triticale compared to the parental rye variety? The following explanations can be suggested.

1 Modification of the chromosome polymorphism system

Numerous studies show that rye is very polymorphic in large heterochromatic segments and in the position of some intercalary bands (Bennett et al. 1977; Lelley et al. 1978; Giraldež et al. 1979). Rye varieties used as parental forms in the breeding of triticale also show great polymorphism.

Since polymorphism for the size of each telomeric C-band is followed by an increase in the variation of its size plotted against its mean length, we decided to use the standard deviation as a measure of polymorphism of an individual C-band. Our data show that each C-band is characterized by an individual degree of polymorphism independent of its absolute size. In general, the length of telomeric C-bands varies to a far less degree in both triticales studied.

The parent rye forms of the studied triticales are extremely polymorphic in the heterochromatin band sizes while the triticale inherited chromosome variants with C-bands of a medium size. This seems to be due to the fact that different polymorphic variants of rye chromosomes or their combination in a zygote have varying chances to develop into a viable plant, i.e. the selection of chromosomal polymorphic variants can occur at the zygote level. The triticale which inherits variants of rye chromosomes with medium sizes of telomeric bands seems to have selective advantages.

Therefore, rye chromosomes with medium variants of polymorphism are selected for in triticales while variability in the sizes of heterochromatic segments significantly decreases. These factors can make the rye and its triticale differ in the size of some C-bands, even in the absence of real alterations. However, the selection of polymorphic variants cannot account for the whole pattern of the observed alterations. Changes of C-band lengths may be caused by the factors described below.

2 Underreplication of DNA in heterochromatin bands

Telomeric heterochromatic bands comprise families of highly repeated DNA sequences (Appels et al. 1978; Bedbrook et al. 1980; Bennett 1980; Jones and Flavell 1982a, b) and were shown to be late-replicating (Lima-de-Faria and Jaworska 1972). Since the cell cycle in triticale is shorter than that in rye (Bennett and Kaltsikes 1973), the DNA of telomeric heterochromatin bands in some R genome chromosomes may be underreplicated and their linear length subsequently decreased.

3 Molecular drive

Alterations in the sizes of centromeric and telomeric C-bands may also result from molecular drive. Molecular drive is a hypothetical way of evolution involving the movement of DNA sequences within chromosomes into homologous and non-homologous chromosomes (Dover et al. 1982; Lewin et al. 1982). It is possible that molecular drive causes an interaction of wheat and rye genomes in the set of triticale and increases the size of centromeric C-bands of rye chromosomes.

4 Meiotic chromosome pairing

Meiotic chromosome pairing may change the telomeric C-band sizes of rye chromosomes. The studies of meiosis in wheat-rye amphihaploids and triticales indicate that non-homologous rye chromosomes are associated mainly via telomeric heterochromatin regions (Sanchež-Monge 1980; Schlegel 1980; Schlegel et al. 1980). The inheritable material interchange when telomeric C-bands interact in meiosis.

5 Minor chromosome aberrations

Minor chromosome aberrations may cause alterations in the lengths of C-bands. It is possible that the appearance of such

aberrations involves mobile dispersed elements which often cause more or less profound genome rearrangements (Ilyin et al. 1978; Ströbel and Geissler 1982).

It is possible that the sizes of heterochromatin bands are modified by several factors.

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