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Cytogenetic analysis of forms produced by crossing hexaploid triticale with common wheat

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Summary. Hexaploid triticales were crossed with common wheats, and the resultant froms were selected for either triticale (AD 213/5-80) or common wheat (lines 381/80, 391/80, 393/80). The cytogenetic analysis showed that all forms differ in their chromosome composition. Triticale AD 213/5-80 and wheat line 381/80 were stable forms with $2n=6x=42$. Lines 391/80 and 393/80 were cytologically unstable. In triticale AD 213/5-80, a 2R (2D) chromosome substitution was found. Each of the three wheat lines had a chromosome formed by the translocation of the short arm of IR into the long arm of the IB chromosome. In line 381/80, this chromosome seems to be inherited from the 'Kavkaz' wheat variety. In lines 391/80 and 393/80, this chromosome apparently formed de novo since the parent forms did not have it. The karyotype of line 381/80 was found to contain rye chromosomes 4R/7R, 5R and 7R/4R. About 15% of the cells in line 391/80 contained an isochromosome for the 5R short arm and also a chromosome which arose from the translocation of the long arms of the 5D and 5R chromosomes. About one-third of the cells in the common wheat line 393/80 contained the 5R chromosome. This chromosome was normal or rearranged. Practical applications of the C-banding technique in the breeding of triticale is discussed.

Key words: Triticale – C-banding – Substitutions – Translocations

Introduction

Distant hybridisation in combination with experimental polyploidy plays an important role in plant breeding and selection. However, artificial primary wheat-rye allopolyploids possess not only a number of advantages, such as resistance to stripe rust and powdery mildew, a large spike with multiflorous spikelets large grain, etc., but also certain shortcomings. These include cytological instability, lowered fertility of a spike, shrivelled grain and late ripening, which limited their direct application as new varieties. In order to improve the economical properties of hexaploid triticales, they are hybridised to octoploid forms or common wheat varieties (Kiss 1966).

On the other hand, the crossing of common wheat varieties with triticale forms has resulted in introducing, into the wheat genotypes, some important genes responsible for such economically important properties as frost resistance, lowered requirement for growth conditions, resistance to phytodeseases and other characteristics.

First generation hybrids produced by crossing hexaploid triticale with common wheat had 42 chromosomes of the genome composition *AABBDR*. In the metaphase I, chromosomes of D and R genomes, as a rule, formed univalents and were randomly distributed between the poles in the anaphase of the first and second divisions. This yielded gametes with the genome composition *ABD/R* having a different proportion of D/R chromosomes (Maximov and Maximova 1979).

Starting from the second generation, the formation process commenced in hybrid population which allowed one to select both hexaploid triticale and common wheat forms. In many plants of the second generation, the number of chromosomes deviated from the euploid level of parental species mainly towards a decrease in the number of chromosomes. The karyotypes of hybrids included a relatively high proportion of chromosome aberrations (Mettin and Liebelt 1982). The morphology of the forms depended on what rye chromosome or their parts had been included into the wheat genome by substitutions or translocations (Meister 1930; Sowa and Gustafson 1980).

The aim of this study was to conduct a cytogenetic analysis using the technique of C-banding on common wheat lines and hexaploid triticale produced by hybridisation of hexaploid triticale with common wheat. Our purpose was to elucidate the selection significance of genotypes having R/D chromosome substitutions and translocations.

Materials and methods

Four genotypes were selected from hybrid populations produced by crossing hexaploid triticale with common wheat varieties. Triticale AD 213/5-80 was isolated from the crossing of AD 206 × 'Kharkovscaya 63'. Hexaploid wheat line 381/80 was obtained by crossing AD 1 with wheat variety 'Kavkaz'. Lines 391/80 and 393/80 were selected from one and the same hybrid population – AD 206 × 'Polukarlic 71'. Seeds of the fifth generation were analysed.

Seeds were germinated at 26 °C. The seedlings with rootlets 0.5–2.0 cm long were first immersed in a 0.2% colchicine solution at 26 °C for 2–2.5 h, then into an ice water both for 1 h. The rootlets were fixed in 45% acetic acid at 2–4 °C for 4 h and subsequently hydrolysed in 0.2 N HCl at 60 °C 5 min. Squashed preparations were made from a cell suspension in 45% acetic acid. Coverslips were removed after freezing on dry ice. The preparations were kept in absolute ethanol. Prior to staining, the preparations were vacuum-dried at 10^{-1} mm Hg. This enabled a high quality of staining without storing problems. The differential staining of the chromosomes was carried out according to the technique based on the use of $Ba(OH)_2$ (Vosa and Marchi 1972; Shchapova 1974; Iordansky et al. 1978 a). Chromosomes were identified on the basis of the genetic nomenclature for wheat and rye chromosomes (Gill and Kimber 1974 a, b; Gerlach 1977; Seal 1982; Lukaszewski, Gustafson 1983; Badaev et al. 1983). At least 20 metaphase plates were analysed for each form.

Results

Triticale AD 213/5-80

The morphological features of the spikes and grains were typical of hexaploid triticale. It was a cytologically stable form with a chromosome number of $2n=6x=42$. The R genome was represented by six pairs of chromosomes, chromosome 2D substituting for chromosome 2R. In general, the C-banding pattern was typical of wheat and rye chromosomes. Heterochromatic C-bands were present in the centromeric regions of all chromosomes of the karyotype AD 213/5-80. Large C-bands were found in the telomeric regions of the long arms of 1R, 3R and 7R/4R chromosomes and in the telomeres of all rye chromosomes (Fig. 1). Faint and medium size telomeric C-bands were detected in chromosomes 7A, 1B, 3B, 4B and 6B of wheat. The C-banding pattern of chromosome 2D substituting for rye chromosome 2R allowed us to easily identify this chromosome both in the wheat varieties and in triticale.

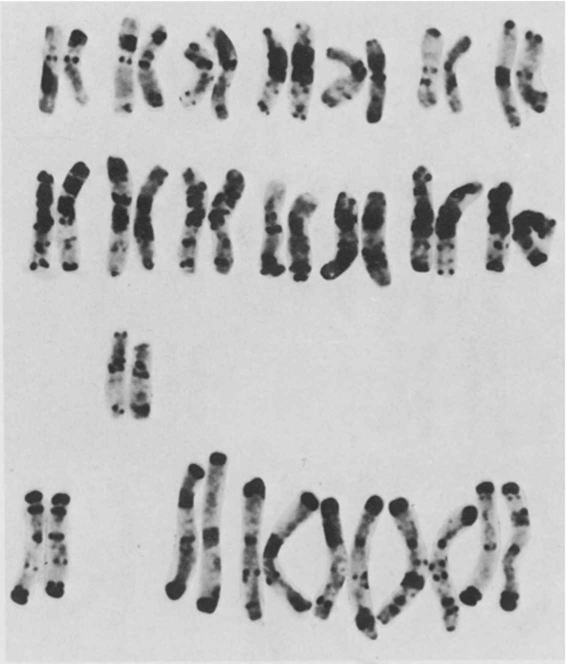
Fig. 1. The karyotype of triticale AD 213/5-80

Hairy necked wheat line 381/80

The stem below the spike had silking which indicated the presence of rye genetic material. It was a cytologically stable form with a chromosome number of $2n=6x=42$ (Fig. 2). The karyotype of this line had a chromosome formed as a result of the translocation of the short arm of 1R into the long arm of 1B. This chromosome seemed to be inherited from the 'Kavkaz' variety which had been used as a parental form in the crossing with hexaploid triticale AD 1. The karyotype of line 381/80 was found to have inherited rye chromosomes 4R/7R, 5R and 7R/4R from triticale AD 1. Thus, the genetic material of rye present in the genome of this line had different origins: the short arm of chromosome 1R was inherited from the parental 'Kavkaz' variety whereas the remaining rye chromosomes came from the maternal triticale form.

Rye chromosome 7R/4R in the karyotype of line 381/80 was a comparatively rare variant of chromosome polymorphism in which the telomere of the long arm had no C-bands and the intercalary C-bands were located approximately in the middle and in the subterminal region of the long arm. The telomeric C-band in chromosome 5R was duplicated. Since we had not

Fig. 2. The karyotype of common wheat line 381/80

Fig. 3. The karyotype of triticale AD 206

analysed the maternal triticale form, it was impossible to know whether these variants were inherited or whether they arose spontaneously as a result of chromosome rearrangements.

Hairy necked wheat line 381/80 constituted all the chromosomes of genomes A and B (in addition to the short arm of chromosome 1B) as well as chromosomes 1D, 2D, 3D, and 6D. The four chromosomes of genome D present in this line genetically determined the manifestation of morphological properties typical of a common wheat spike (the size and shape of a spikelet scale, its straight arm, the structure of the spikelet, etc.).

The karyotype of line 381/80 differed from the karyotype of AD 213/5-80 by having polymorphic variants of 3A, 5A, 7A and 3B chromosomes, namely, the presence of faint intercalary and terminal C-bands and the degree of their expression. Of the four forms studied only in this line did chromosome 7A have no terminal C-bands. Chromosome 6B of line 381/80 had a long secondary constriction (NOR) whereas none were found either in chromosome 6B of AD 213/5-80 or in the short arm of chromosome 1R translocated to chromosome 1B.

Wheat lines 391/80 and 393/80

Lines 391/80 and 393/80 were typical common wheats by the morphological properties of their spikes and grains and in the general habit of the plant. The marker trait of hairy neck indicated that they contained the genetic material of rye. Both lines were cytologically unstable. In the first one about half the cells were aneuploid with 2n = 41 whereas the second line showed both hypo-aneuploid cells with 2n = 41 and hyperaneuploid cells with 2n = 43. In line 393/80, about one-third of the cells were euploid with 2n = 42. Both lines had a chromosome whose presence was a result of an 1B/1R translocation. This chromosome was similar to the respective chromosome of line 381/80. In this case, however, chromosome 1B/1R was formed de novo by translocation since the parent forms AD 206 (Fig. 3) and 'Polukarlic 71' (Fig. 4) did not have it. Chromo-

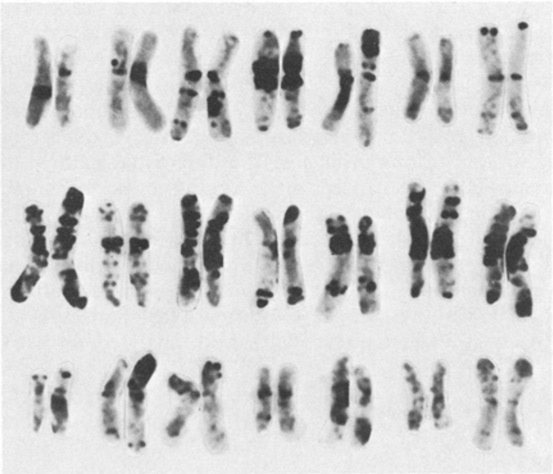

Fig. 4. The karyotype of *Triticum aestivum* var. 'Polukarlic 71'

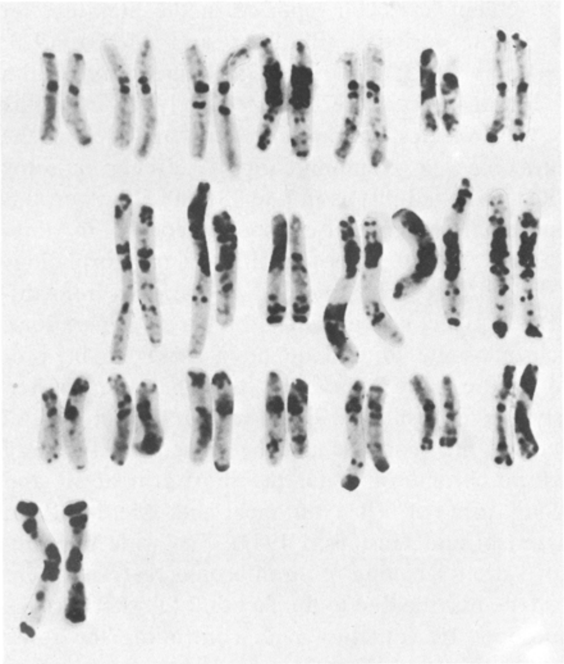

Fig. 5. The karyotype of common wheat line 391/80 with a normal chromosome set

Fig. 7. The karyotype of common wheat line 393/80 with a telocentric chromosome for the short arm of chromosome 5R

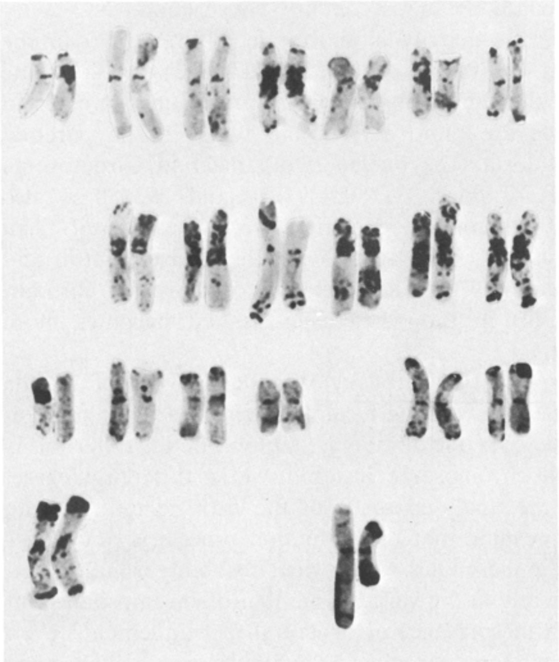

Fig. 6. The karyotype of common wheat line 391/80 with both 5D chromosomes being substituted by rearranged 5R

some 1B/1R, of differing origins, had a nearly identical morphology.

Cytologically unstable wheat line 391/80 had five types of cells. About 40% of the cells were aneuploid with $2n=41$ and, in each case, had identical chromosome composition, namely, that of wheat monosomal for 5D. Euploid cells were of four types. The first type (about one half of the cells) had the normal wheat chromosome set (Fig. 5). In the second type, both chromosomes 5D were substituted for with an isochromosome for the short arm of 5R and by a chromosome which resulted from the translocation of the long arms of chromosomes 5D and 5R (Fig. 6). The third and fourth types were intermediate between the first and second types: one of the 5D homologues was substituted for either by an isochromosome for the short arm of chromosome 5R or by a translocated 5D/5R chromosome. Chromosome 5D was easily identified by analysing the various karyotypes found in line 391/80; this chromosome was represented by one homologue in aneuploid cells and by one or two homologues in different variants of euploid cells in which it also could be substituted for by chromosome 5R. It is noteworthy that aneuploid cells never contained either an isochromosome for the short arm of 5R or a translocated 5D/5R chromosome.

Line 393/80 was even more unstable than line 391/80; over 70% of the analysed cells were aneuploid. The aneuploid cells were mostly hypo-aneuploid though some of them were hyperaneuploid. Hyperaneuploid cells contained a telocentric chromosome for the short arm of 5R added to the chromosome set of wheat. Hypo-aneuploid cells had three variants of the karyotype: 1) monosomic for 5D; 2) both chromosomes

5D were substituted for by a normal 5R; 3) both chromosomes 5D were substituted by a telos for the short arm of 5R.

Euploid cells were of four types: (1) with the normal chromosome set of wheat; (2) with both 5D homologues substituted for by normal chromosomes 5R; (3) with one normal 5R and one normal 5D (this variant was intermediate between the first and second variants); (4) with the pair of 5D and a telocentric chromosome for the short arm of 5R (Fig. 7).

Aneuploid cells, in which one homologue of chromosome 5D is absent from the normal chromosome set of wheat, prevailed among all variants.

Wheat lines 391/80 and 393/80 had the same C-banding pattern as wheat chromosomes which differed from that of wheat line 381/80 and triticale AD 213/5-80. Chromosomes 3B, 4B, 5B and 7A contained segments of telomeric heterochromatin.

Discussion

Triticale AD 206 is known to have a 2R (2D) chromosome substitution (Stepochkin 1979; Lukaszewski and Apolinarska 1981; Mettin and Liebelt 1982). AD 206 is a parent form of triticale AD 213/5-80 and of wheat lines 391/80 and 393/80. The studied triticale also has one 2R (2D) substitution. It is noteworthy that the offspring triticale form inherited the entire rye chromosome complement of the parent variety. Apparently, triticale with an entire set of rye chromosomes has an enhanced ability for adaptation to environmental conditions and has improved agronomical characteristics (Pilch 1981a, b; Lukaszewski and Apolinarska 1981; Seal and Bennett 1981). Therefore, winter triticale with the highest possible number of rye chromosomes will have advantages for breeding new triticale forms. At the same time, the detection of a R (D) chromosome substitution for the second homeologous group is not unexpected since many triticale forms (in particular, 'Armadillo', one of the first widespread varieties) also have a 2R(2D) chromosome substitution (Gustafson and Zillinsky 1973; Merker 1975; Gustafson and Bennett 1976).

Wheat lines produced by the hybridisation of the common wheat variety 'Polukarlic 71' with a similar triticale form, AD 206, have inherited only a part of the chromosome 1R translocated to the long arm of 1B. In certain cells, a normal or rearranged chromosome 5R was found. The latter is a translocated 5D/5R chromosome, a telocentric chromosome or an isochromosome for the short arm of 5R. All the lines have the hairy neck character associated with the 5R chromosome. Chromosome 5R is present in all three wheat lines produced by hybridisation of hexaploid triticales with common wheats. This is inconsistent with the data of Merker (1975) on the order in which rye chromosomes are substituted for by wheat chromosomes in secondary triticale populations. Line 381/80 also contains chromosome 4R/7R which, according to previous data (Merker 1975; Sowa and Gustafson 1980; Zillinsky 1980), comes second after 2R in the frequency of substitution in secondary triticale populations.

All three of these studied lines have chromosome 1B/1R from different origins: from the 'Kavkaz' variety in line 381/80 whereas in other studied lines it arises spontaneously as a result of translocation. Such a rearrangement has been reported in the literature for many wheat varieties (Blüthner and Mettin 1973; Mettin et al. 1973; Zeller 1973; Bennett and Smith 1975; Iordansky et al. 1978b; Merker 1982), including the Soviet varieties 'Aurora' and 'Kavkaz'. Most of the varieties have a common origin, i.e. chromosome 1B/1R is inherited just as in line 381/80. However, this chromosome, which arises spontaneously in lines 391/80 and 393/80, does not differ in its morphology from the chromosome inherited by varieties from different genotypic backgrounds for many generations. The chromosome formed de novo seems to be produced in the same manner as the one risen earlier, namely, as a result of the irregular separation of bivalents in the anaphase and the subsequent fusion of telocentric chromosomes for the short arm of 1R and the long arm of 1B (Blüthner and Mettin 1973; Lukaszewski and Gustafson 1983). The wide distribution of varieties having a chromosome rearrangement of this type may be due to the fact that the short arm of chromosome 1R contains genes controlling the resistance to certain phytodiseases.

The functioning of nucleolar organizers in the studied forms is of great interest. The nucleolar organizer of chromosome 1R is inactive in triticale since the length of the secondary constriction (NOR) reflecting its activity is shorter in triticale than in the parent rye variety (Badaev et al. 1982). At the same time, the nucleolar organizers of chromosome 1B in triticale are more active than those in the parental wheat form. The nucleolar organizer in chromosome 1B/1R of lines 381/80, 391/80 and 393/80 is also inactive (secondary constriction is not expressed) – an observation which agrees with the data of Mettin and Blüthner (1973). The nucleolus organizer of chromosome 6B in the wheat line studied becomes more active.

As was shown by cytogenetic analysis of wheats obtained by crossing hexaploid triticales with common wheats, only one of them is stable while the other forms exhibit chromosome instability to a different degree. Since we analysed seeds of the fifth generation, one may presume that the formation process is not terminated in these lines. Cytological instability is manifested not merely in the variable number of chromosomes but also in the presence of structural rearrangements, even when the form contains the euploid number of chromosomes. Such structural rearrangements include the fusion of homeologous chromosome parts, and therefore, the formation of a new chromosome type. Cytological instability is also caused by the unbalanced composition of chromosomes belonging to the fifth homeologous group.

Cytological instability in terms of chromosome number in a particular variety or form can be revealed using conventional methods of research. However, structural rearrangements of chromosomes and their unbalanced composition can be detected at the euploid level only by the technique of differential staining of chromosomes (C-banding). C-banding can be a source of additional information in estimating the cytological stability of new varieties and forms as well as in determining the genome and chromosome structure of their karyotypes which is significant for evaluating their application in selection.

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