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Identification of a complete set of isogenic wheat/rye D-genome substitution lines by means of Giemsa C-banding *

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Summary. A complete set of isogenic wheat/rye D-genome substitutions were produced by crossing an inbred line of spring rye *Secale cereale* L. cv. "Prolific" to a tetraploid wheat, the A- and B-genomes of which had previously been extracted from hexaploid wheat, *Triticum aestivum* L. em Thell. cv. "Thatcher". After chromosome doubling, the derived hexaploid triticale (× *Triticosecale* Wittmack) was backcrossed to 6*x* "Thatcher" and selection for wheat/rye substitution lines was carried out in BCF_3 to BCF_6 families by using Giemsa C-banding. Five fertile disomic wheat/rye D-genome substitution lines were obtained and their chromosomal constitution was determined to be 1D/1R, 2D/2R, 7D/4R, 6D/6R, 7D/7R. The two remaining 3R and 5R substitutions are at the moment in a monosomic condition. Another 1D/7R substitution was detected but this plant was very weak and sterile, indicating that only substitutions between homoeologous chromosomes result in fertile, vigorous plants. Furthermore, many rye telocentrics as well as rye-rye and rye-wheat translocations were selected. Since all lines selected in this program share the same genetic background of "Thatcher" wheat, genetic heterogeneity is excluded. The material is very useful, therefore, for analyzing the effects of different rye chromosomes or chromosome segments in an otherwise homozygous background.

Key words: Wheat/rye substitution lines – C-banding

Introduction

Due to the close evolutionary relationship between rye (*Secale cereale* L.) and wheat (*Triticum* species), rye chromatin offers a great potential to increase the genetic variability and germplasm resources of cultivated hexaploid wheat, *Triticum aestivum* L. em Thell. Furthermore, many efforts have been made to combine the best characters of both genera in one species, which have lead to the production of triticale (× *Triticosecale* Wittmack). The first fertile amphiploid (AABBDDRR) between wheat and rye was obtained by Rimpau (1981). Since that time, triticale developed from a more or less experimental plant to a present-day commercial cereal crop (Bushuk and Larter 1982; Gupta and Priyadarsham 1982; Gustafson 1983; Kaltsikes et al. 1984; Skovmand et al. 1984).

Much or our knowledge of the genetic behavior of triticale has been obtained by analyzing the effects of specific rye chromosomes, chromosome arms or chromosome segments in wheat/rye addition, substitution and translocation lines. Often, however, the interpretation of the results was difficult due to genetic heterogeneity existing in either the wheat or rye component. To overcome these difficulties, a project was initiated at the University of Manitoba to establish a complete set of wheat/rye (D/R-genome) substitution lines in an isogenic wheat background. This paper describes the selection and identification of these lines.

Materials and methods

Employing a method developed by Kerber (1964), Kaltsikes et al. (1968, 1970) extracted the A- and B-genomes from a hexaploid wheat cv "Thatcher", genomically AABBDD. The ex-

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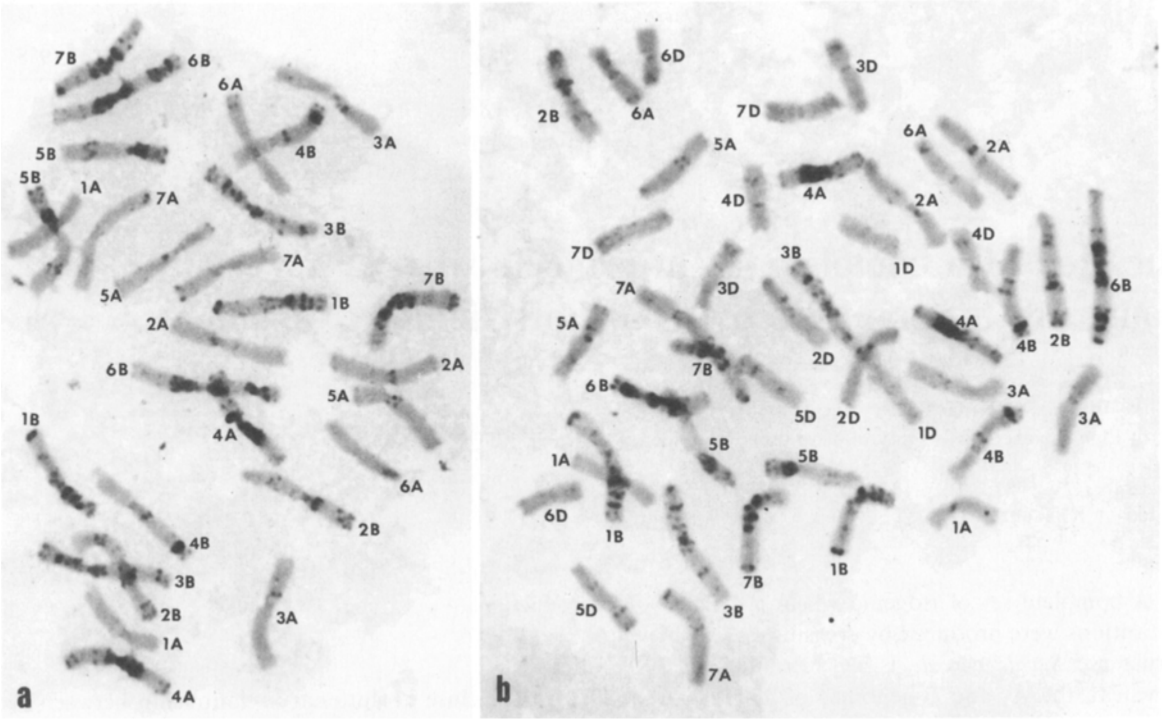

Fig. 1 a and b. C-banded mitotic metaphase chromosomes of complete cells of **a** tetraploid and **b** hexaploid Thatcher wheat

tracted tetraploid 4*x* "Thatcher" was used in the present study to produce an isogenic background ("Thatcher"), which in turn provided a source of chromosome substitutions involving the R-genome of rye and the D-genome of wheat. The following scheme was used in the derivation of the isogenic source: Tetraploid 4*x* "Thatcher" wheat was crossed with an inbred line of spring rye (cv "Prolific") and the hybrid was colchicine-doubled to produce a hexaploid ($2n=6x=42$) triticale, designated as 6A-639; 6A-639 was then crossed with hexaploid ($2n=6x=42$) wheat (cv "Thatcher"). Plants in F_3 and subsequent generations were selected that were homozygous for A- and B-genome chromosomes from "Thatcher" but segregated for chromosmes of rye (R) and D- ("Thatcher") genomes. The C-banding technique as described by Giraldez et al. (1979) was used to identify wheat/rye D-genome substitutions as they occurred. A C-banded karyotype of the 4*x* and 6*x* wheat cv "Thatcher", and of the triticale 6A-639 was established and used as a standard. At least two complete, well-spread mitotic metaphases of 10 plants per line were used for C-banding analysis.

Results and discussion

C-banding pattern of the tetra- and hexaploid wheat cultivar "Thatcher" and the rye chromosome complement of the hexaploid triticale 6A-639

Figure 1 illustrates the C-banding patterns of the tetra- (4*x*) and hexaploid (6*x*) "Thatcher", the source of the A- and B-genomes of triticale 6A-639 of the present study. The same specific C-bands were present in AB-genome components of both the original hexaploid "Thatcher" and the tetraploid component extracted from it by Kaltsikes et al. (1968). A more detailed karyotype of "Thatcher" wheat is shown in Fig. 2, in which the A- and B-genome chromosomes were taken from 4*x* "Thatcher" and those of the D-genome from 6*x* "Thatcher". The C-banding pattern of "Thatcher" wheat is similar to that described by Lukaszewski and Gustafson (1983) and Endo (1986) for *Triticum aestivum* cv. "Chinese Spring", which has been established by using aneuploids and telocentric lines. The close relationship between C-banding patterns of the A- and B-genome chromosomes of tetraploid (4*x*) and hexaploid (6*x*) "Thatcher", and the close similarities of D-genome chromosomes of 6*x* "Thatcher" and those reported for "Chinese Spring", were reassuring in the identification of specific wheat/rye chromosome substitutions in the present study.

Since the D-genome chromosomes of wheat are of special interest, their C-banding patterns are described in some detail. All chromosomes of the D-genome show pronounced C-bands of the centromeres similar to those of the A- and B-genomes (Fig. 2). The following is a brief description of the most distinguishing features of D-genome chromosomes as observed in the present study:

1D shows a proximally and distally located C-band in the long arm and a faint subterminal and terminal band in the short arm. 2D together with 7D is the largest chromosome pair in the D-genome and exhibits small telomeric and more pronounced pericentromeric bands in both arms. In addition, a small intercalary band is located in the long arm and in the distal region of the

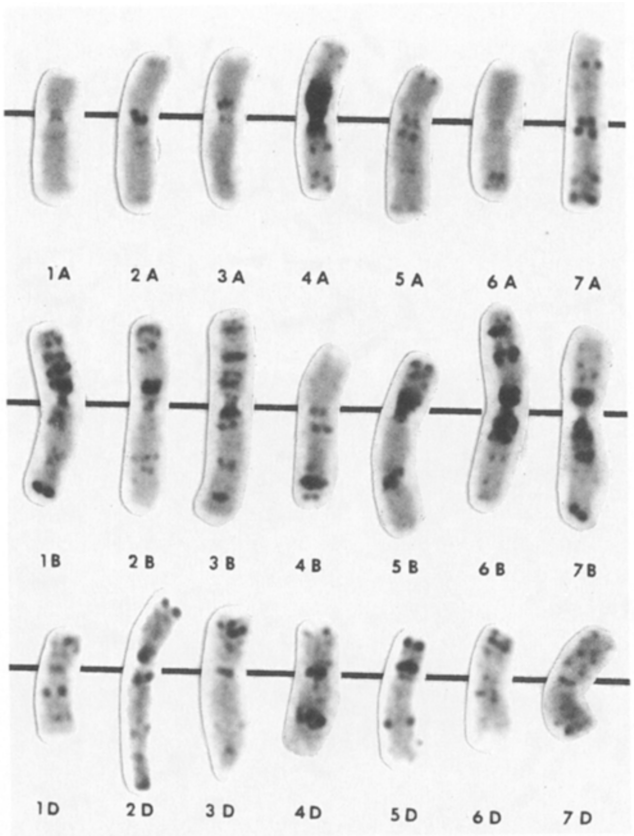

Fig. 2. C-banded karyotype of "Thatcher" wheat (A- and B-genome chromosomes were derived from 4*x* "Thatcher" and D-genome chromosomes from 6*x* "Thatcher")

short arm. 3D shows a subterminal C-band in the short arm and two faint bands in the distal region of the long arm. 4D is characterized by a pronounced C-band in the distal region of the long arm, faint bands on both sides of the centromere, and a small telomeric band in the short arm. 5D can be easily identified by the presence of a proximal and terminal band in the short arm and a distally located C-band in the long arm. The distal region of the short arm is known to carry an actively transcribed rDNA gene cluster, but fails to show a secondary constriction in the presence of nucleolar chromosomes from the B-genome. Chromosome 6D shows subterminal and terminal C-bands in the short arm and a distinct band adjacent to the centromere in the long arm. 7D is nearly metacentric and presents faint subterminal and terminal C-bands in one arm and proximal, intercalary and terminal bands in the other arm.

With a few exceptions, the overall C-banding pattern of "Thatcher" wheat is very similar to that described by Lukaszewski and Gustafson (1983) and Endo (1986) for "Chinese Spring". Some of these differences may be the result of differences in the C-banding technique applied. For example, in the karyotype published by Endo (1986), chromosome 1A lacks C-bands, whereas Lukaszewski and Gustafson (1983) detected a faint proximal band in the long arm, which could also be seen in 1A of "Thatcher". However, there is variation in C-banding patterns between different cultivars of wheat (Iordansky et al. 1978; Endo and Gill 1983, 1984) that clearly reflects the existence of polymorphism for C-heterochromatin, a very common phenomenon in many animal and plant species.

With two exceptions, no major differences in C-banding patterns could be observed between the tetraploid (4*x*) and hexaploid (6*x*) lines of "Thatcher" wheat. Chromosome 3B of 4*x* "Thatcher" shows a distinct distally located C-band in the long arm, whereas this band was less pronounced (sometimes undetectable) in 3B of 6*x* "Thatcher". The other difference concerns the terminal C-band of the short arm of chromosome 6B, which is more pronounced in 6*x* than in 4*x* "Thatcher" (Fig. 1 a and b). The overall similarity between banding patterns of hexaploid "Thatcher" and the tetraploid (AABB) component extracted from it indicates that no major structural changes occurred during the extraction process and subsequent propagation of these lines. The A- and B-genome of 4*x* and 6*x* "Thatcher" can be considered isogenic; however, it is recognized that some residual heterozygosity due to meiotic recombination in the initial stage of extraction process may still persist.

The C-banding pattern of mitotic metaphase chromosomes of the hexaploid triticale 6A-639 is shown in Fig. 3 (a). No differences in C-banding patterns, of the A- and B-genome chromosomes were observed between those present in the triticale and those of the wheat cultivar "Thatcher". The rye "Prolific" chromosome complement of the triticale 6A-639 showed a very typical C-binding pattern, making possible unambiguous identification of all 7 chromosome pairs as well as 9 of the total of 14 chromosome arms. The remaining 5 arms (2Rp, 3Rq, 4Rp, 5Rp, 6Rp) were similar in that they showed only one large, terminally located C-band, although arms differed in length.

With two exceptions, no variation in C-banding patterns of the rye chromosome complement was detectable between different plants of the triticale 6A-639. One difference involved a modified rye chromosome 4R that lacked the terminal C-band in the short arm, 4Rp22 (according to the rye band nomenclature proposed by Schlegel et al. 1986), and the other was a modified 7R, which showed an amplification of the subterminal C-band in the long arm (7Rq22). The latter led to the formation of one large terminal block of C-heterochromatin (Fig. 4).

Modified rye chromosomes that show either amplification or deletion in the amount of C-heterochromatin have been reported earlier, and provide useful material for analyzing the effects of C-heterochromatin upon various plant characteristics (Gustafson 1983).

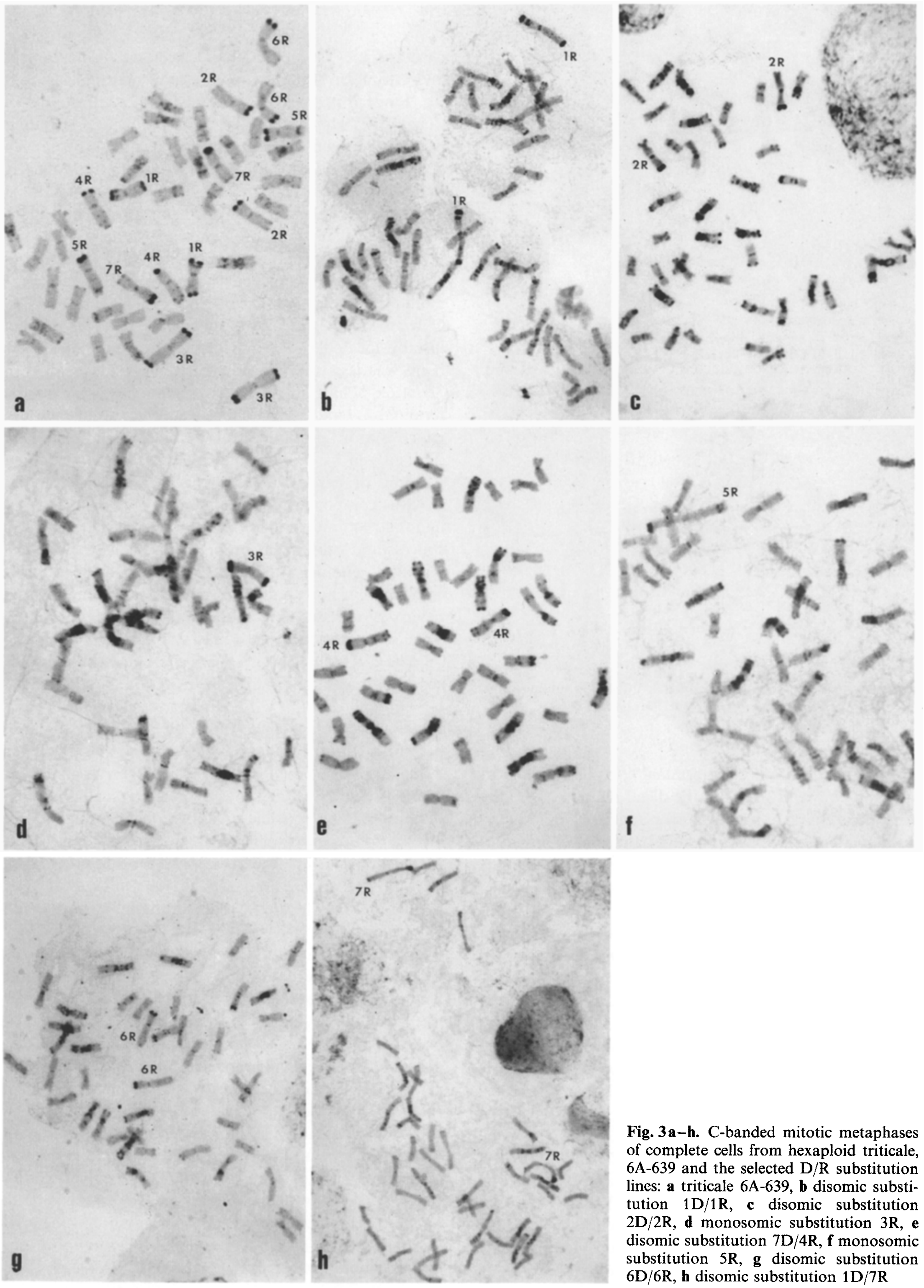

Fig. 3a–h. C-banded mitotic metaphases of complete cells from hexaploid triticale, 6A-639 and the selected D/R substitution lines: **a** triticale 6A-639, **b** disomic substitution 1D/1R, **c** disomic substitution 2D/2R, **d** monosomic substitution 3R, **e** disomic substitution 7D/4R, **f** monosomic substitution 5R, **g** disomic substitution 6D/6R, **h** disomic substitution 1D/7R

Selection of the D/R substitution lines

Selection was carried out in F_3 to F_6 progenies of 16 F_2 plants of the cross 6*x* triticale 6A-639 × 6*x* "Thatcher". From the self-pollinated 42-chromosome F_1 hybrid, AABBDR, it should be theoretically possible to select 128 (2^7) different homozygous combinations between the R- and D-genome chromosomes (Merker 1976). Those containing only one chromosome pair of rye in an otherwise isogenic wheat background are of special interest. Once this set has been established, however, any D/R combination could be obtained by intercrossing the appropriate lines.

D-genome substitution lines of all seven pairs of rye chromosomes could be selected (Fig. 3b–h). The 3R and 5R substitutions are, at present, only in the monosomic condition, but disomic substitutions for these chromosomes should be obtainable in one or more further generations. A C-banding analysis was also used to identify the substituted D-genome chromosome pair in each of the five disomic D/R substitution lines. Their identity was determined as follows: 1D/1R, 2D/2R, 7D/4R, 6D/6R, 7D/7R. With the exception of the 7D/4R substitution, vigor and fertility of all substitutions were good, with about 100 seeds being obtained per self-pollinated plant. In contrast, the 7D/4R substitution exhibited reduced seed-set. Furthermore, one plant was obtained that was disomic for a 1D/7R substitution; however, it was very weak and sterile.

Morphologically, the 4R and 5R substitutions could easily be identified by the appearance of red coleoptiles in the 7D/4R line and the hairy neck phenotype in the 5R substitution. These traits are determined by genes located on 4Rq and 5Rq, respectively (Schlegel et al. 1986; Zeller and Cermeño, 1988).

The homoeologous relationship of the chromosomes of wheat has been established by Sears (1966) using nullisomic-tetrasomic analysis. All 21 chromosome pairs of hexaploid wheat could be partitioned into 7 homoeologous groups, each consisting of one chromosome pair of the A-, B- and D-genome. The ability of a given chromosome pair to compensate nullisomy for another chromosome pair is a measure of genetic similarities between chromosomes and reflects the evolutionary relationship between species.

It is generally accepted that all genomes within the Triticinae were derived from a common ancestor. The chromosomes of rye are sufficiently related to those of wheat, both genetically and functionally, to allow substitution to occur. Furthermore, their relationship to the homoeologous groups of Triticinae is now well established from extensive studies of the mapping of genes governing morphological and biochemical traits within these species, as well as from studies of the similarities of the genomic arrangement of specific DNA sequences

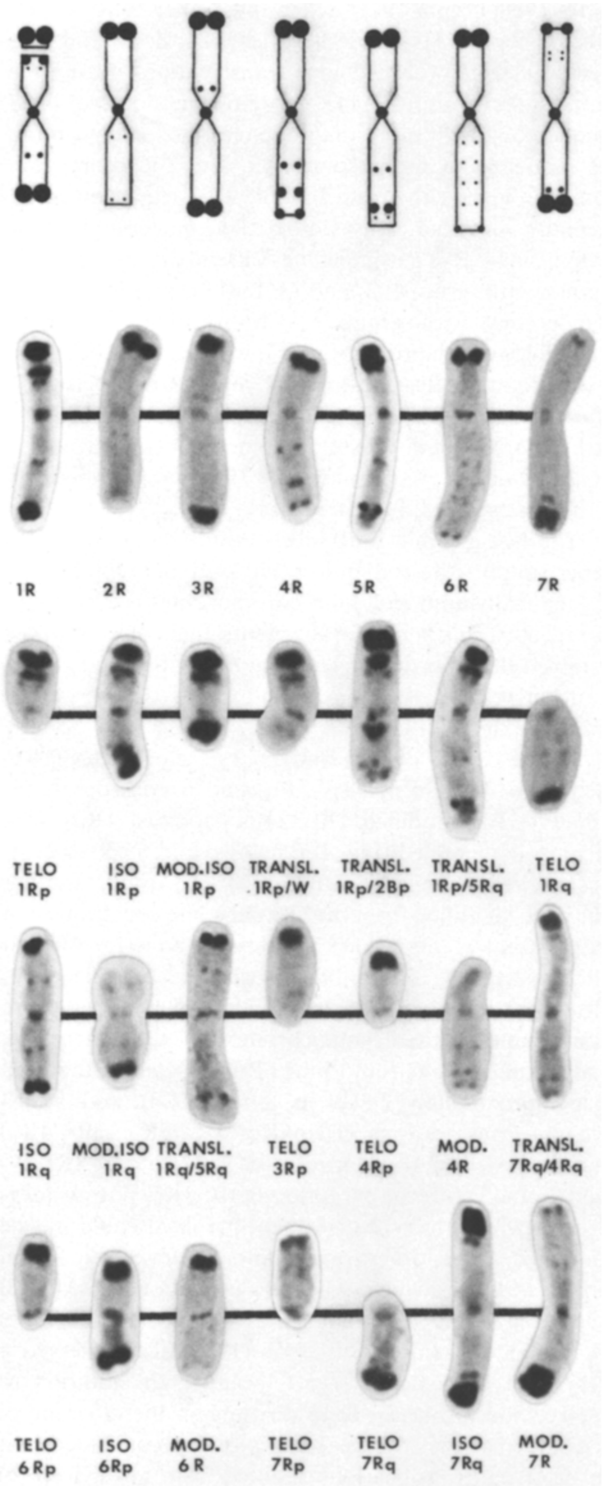

Fig. 4. C-banded idiogram and karyotype of the rye chromosome complement of the triticale, 6A-639, and chromosome modifications detected in the offsprings of the cross 6A-639 × 6*x* "Thatcher" wheat

(Sears 1968; Gupta 1971; Zeller and Koller 1981; Appels 1982; Zeller and Hsam 1983; Miller 1984; Zeller and Cermeño 1988). However, due to translocations during the course of evolution, some chromosomes of rye show homoeology with more than one chromosome group in the Triticinae. Chromosomes 1R and 5R of rye are homoeologous with group 1 and 5 of Triticinae; however, there are some indications of partial homoeology of 5R with group 4. Rye chromosomes 2R and 3R are homoeologous with groups 2 and 3 but also show partial homoeology with group 6. Chromosome 4R shows homoeology with groups 4 and 7, whereby the short arm of 4R compensates for group 4 and 4Rq for group 7. Chromosome 6R shows a large degree of homoeology with group 6, but also exhibits some affinity to groups 2, 3 and 7. Finally, rye chromosome 7R shows homoeology with groups 7 and 4 of Triticinae.

The five disomic D/R substitutions reported in this paper support the contention that only the replacement of genetically homoeologous chromosomes is effective in producing fertile and vigorous plants. Only one plant was obtained that was disomic for a non-homoeologous substitution (1D/7R) but this plant was extremely weak, grass-like and sterile.

In the course of this study, 7 rye telocentrics (1Rp, 1Rq, 3Rp, 4Rp, 6Rp, 7Rp, 7Rq) and 6 rye isochromosomes (1Rp, modified 1Rp, 1Rq, modified 1Rq, 6Rp, 7Rq) were identified (Fig. 4). The 4Rp and 6Rp telocentrics showed a similar C-banding pattern to one another but were identified from the chromosome constitution of their parent plants, which were monosomic for 4R and 6R, respectively. The modified isochromosomes 1Rp and 1Rq were found in the progenies of plants that were monosomic for these same chromosomes. Furthermore, one modified rye chromosome 6R was detected that had lost approximately 2/3 of its long arm. In addition, 3 rye-rye translocations (1Rp/5Rq, 1Rq/5Rq, 7Rq/4Rq) and 2 rye-wheat translocations (1Rp/2Bp and 1Rp/W) were found. With the exception of the 1Rp/W translocation, in which the wheat segment involved could not be identified, all the other translocations were centric fusion products. Only 2 wheat-wheat translocations were found and these involved chromosomes 4A and 7D.

Lukaszewski and Gustafson (1983) also observed a very high frequency of rye telocentrics in addition to rye-rye and rye-wheat translocations in the offspring of triticale × wheat crosses. These workers concluded that most of the translocations resulted from misdivision of univalents at meiosis with subsequent fusion of telocentric chromosomes.

In the present study, most of the telocentrics, isochromosomes and translocations are currently either in the monosomic condition or are together with other rye chromosomes or chromosome arms. Selection for homozygous lines will be continued. In addition to the selected wheat/rye substitutions, this material could prove useful in analyzing the effects of rye chromatin on various characters of wheat.

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