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Cytological and molecular characterization of a chromosome interchange and addition lines in Cadet involving chromosome 5B of wheat and 6Ag of *Lophopyrum ponticum*

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Abstract. Efforts to transfer wheat curl mite (*Eriophyes tulipae* Keifer) resistance from *Lophopyrum ponticum* 10X (Podb.) Love to bread wheat (*Triticum aestivum* L.) have resulted in the production of a number of cytogenetic stocks, including an addition line of 6Ag, a "ditelo" addition line, and a wheat-*Lophopyrum* translocation line. Characterization of these lines with C-banding, in situ hybridization with a *Lophopyrum* species-specific repetitive DNA probe (pLeUCD2), and Southern blotting with pLeUCD2 and a 5S ribosomal DNA probe (pScT7) confirmed that the distal portion of the short arm of 6Ag was translocated onto the distal portion of 5BS (5BL.5BS-6AgS). It was also determined that the "ditelo" addition was an acrocentric chromosome of 6AgS.

Key words: Wheat – *Lophopyrum* – Translocation – Repetitive DNA sequence – C-banding – In situ hybridization

Introduction

Since *Lophopyrum* species possess several agronomically important features (i.e., cold, salt and drought resistance, pest and disease resistance, etc), they have long attracted the attention of wheat breeders in their attempts to improve wheat germ plasm (Larson and Atkinson 1973; Sears 1977; Cauderon 1978; Whelan et al. 1983; Knott 1989). Larson and Atkinson (1973) introduced wheat curl mite resistance, which is the vector of both wheat streak mosaic virus (WSMV) and the wheat spot mosaic agent, from *Lophopyrum* into wheat by substituting wheat chromosome 6D with *Lophopyrum ponticum* 10X (Podb.) Love chromosome 6Ag.

Several wheat-*Lophopyrum* recombinant stocks possessing wheat curl mite (*Eriophyes tulipae* Keifer) resistance have been produced. These include a 6DL.6AgS recombinant (Whelan and Hart 1988), a 6AgS.6AgS recombinant, and a noncompensating 5BL.6AgS recombinant (Whelan et al. 1986; Kim et al. 1992). The research described herein indicated that the wheat-*Lophopyrum* noncompensating translocation is not a Robertsonian translocation. Two types of *Lophopyrum* chromosome addition lines with 44 chromosomes are described and used for comparative purposes. These orignally appeared in the progenies of mono-6Ag additions (Whelan 1988). One type has 42 chromosomes plus a pair of small acrocentric chromosomes and another type has 42 plus 2 normal chromosomes. The acrocentric chromosome was defined as the whole arm of 6AgS and a very small proximal portion of 6AgL from the fact that (1) the plants containing this acrocentric chromosomes were resistant to curl mite colonization, (2) the acrocentric did not pair with either an L or S telocentric of the appropriate group 6 wheat chromosomes or any of the 'Cadet' chromosomes in crosses with 'Cadet'. The 44-chromosome plants showed resistance to curl mite colonization and the presence of 22^{II} in most of the meiocytes (E. D. P. Whelan, unpublished data). Therefore, these latter plants likely carry chromosome 6Ag in a disomic state. Hereafter, the 42 chromosome plus 2 acrocentric chromosome plants and 44-chromosome plants will be referred to as the 'Cadet'-acro 6Ag addition line and the 'Cadet'-6Ag addition line, respectively.

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Materials and methods

Lophopyrum-Wheat recombinant and Lophopyrum chromosome addition lines

The source of the addition and recombinant lines was the wild species *Lophopyrum ponticum* 10X (Podb.) Love. (Larson and Atkinson 1972). A wheat-*Lophopyrum* noncompensating recombinant, and two *Lophopyrum* chromosome addition lines were used in this study. The production of the wheat-*Lophopyrum* recombinant 5BL-6AgS (Whelan et al. 1986; Kim et al. 1992) and the *Lophopyrum* chromosome 6Ag addition lines, 'Cadet'-6Ag and 'Cadet'-acro 6Ag have been described elsewhere (Whelan 1988).

Lophopyrum species-specific molecular probe pLeUCD2 and 5S rDNA probe pScT7

The probe pLeUCD2 (Zhang and Dvorak 1990a) was obtained from Dr. J. Dvorak, Department of Agronomy and Range Science, University of California, Davis, Calif. The probe pScT7 (Scoles et al. 1988) was obtained from Dr. G. Scoles, Department of Crop Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

C-banding analyses

C-banding was applied to mitotic and meiotic chromosomes using procedures previously described by Jouve et al. (1980) and Friebe et al. (1990) with modification to the incubation time in 0.2 *N* HCl.

In situ hybridization

The technique of chromosome preparation for in situ hybridization has been described elsewhere (Le et al. 1989). Probe labelling and in situ hybridization were carried out according to the manufacturer's specifications (DIG Nucleic Acid Labelling and Detection, Boehringer Mannheim).

The chromosome preparations were treated with RNase (100 µg/ml in 2× SSC) at 37 °C for 30 min and washed sequentially in 2× SSC, 70% EtOH, 95% EtOH for 5 min each and then air dried. The chromosomal DNA was denatured in 70% formamide at 80 °C for 3.5 min and washed in chilled (−20 °C) 70% and 95% EtOH for 5 min each. After the slides were air dried completely, 15 µl of heat-denatured probe mixture (5 ng probe DNA/slide, 50% formamide, 10% dextran sulfate, 2× SSC, 10 µg of denatured and fragmented salmon sperm DNA/slide) was applied to each slide. The chromosomal DNA and probe DNA were denatured once again on the slide at 80 °C for 3 min. The slides were then incubated at 40 °C in a moisture chamber for 16 h. After this hybridization treatment the slides were dipped into 2× SSC at 40 °C for 5 min in order to float off the coverslips. The unhybridized and loosely hybridized probe DNAs were washed off by dipping the slides into the following solutions sequentially; 50% formamide in 2× SSC for 5 min at 40 °C, 2× SSC 3 times for 5 min each at 40 °C, 0.5× SSC 3 times for 5 min each at 40 °C, and 0.5× SSC 3 times for 5 min each at room temperature.

The Digoxigenin signals were detected at room temperature by the following procedure. The slides were dipped into Buffer I (0.1 *M* TRIS-Cl (pH 7.5), 0.15*M* NaCl) for 1 min. Then the slides were transferred to Buffer II (0.5% Blocking agent in Buffer I) for 30 min and back to Buffer I for 1 min. Then 100 µl of 1:5000 diluted ⟨Dig⟩ Ap-conjugated antibody in Buffer I was applied to each slide and the slide placed into a dark moisture chamber for 2 h. The unbound antibodies were washed off with Buffer I 3 times for 10 min each, and then the slides were equilibrated with Buffer III (0.1 *M* TRIS-Cl (pH 9.5), 0.1 *M* NaCl, 0.05 *M* $MgCl_2$) for 2 min. The Digoxigenin signals were generated with 300 µl of color-generating solution (35 µg NBT, 300 µg BCIP in 1.5 ml of Buffer III) per slide in a moisture chamber for 3–24 h in the dark. The appearance of signals was monitored with a phase contrast microscope, and the slides were washed in 1× TE for a few minutes after the maximum signals were developed. The slides were dried completely in a dessicator and mounted with Permount.

Plant genomic DNA extraction and Southern hybridization

The plant genomic DNA extraction protocol followed was as described by Dellaporta et al. (1983) with slight modifications (Kim et al. 1992). The genomic DNAs were digested with the restriction enzymes *Hae*III, *Taq*I, and *Bam*HI, electrophoresed in 1% agarose gels in 1× TAE buffer overnight at 35 V, and transferred to a nylon membrane (Hybond plus, Amersham) by the alkaline transfer method.

The insert DNAs from pLeUCD2 and pScT7 were labelled with [^{32}P] using the oligolabelling reaction, and the unincorporated nucleotides were separated using the Sephadex G50 spin column method (Maniatis et al. 1982). After 6 h of prehybridization with a solution composed of 5× SSPE, 5× Denhardt, 0.5% SDS, and 500 µg of denatured and fragmented salmon sperm DNA at 65 °C, the membranes were transferred to the hybridization solution, which was the same as the prehybridization solution but contained labelled and denatured probes. The membranes were incubated at 65 °C overnight with constant shaking and then washed twice in 2× SSPE, 0.1% SDS for 10 min each at room temperature, once in 1× SSPE, 0.1% SDS at 65 °C for 15 min, and twice in 0.1× SSPE, 0.1% SDS at 65 °C for 10 min each time. The membranes were then exposed to Kodak X-Omat 485 X-ray film at −70 °C for 1–5 days depending on the remaining radioactivity in the membranes.

Results and discussion

In situ hybridization using the pLeUCD2 probe

As shown in Fig. 1A the distal half of the short arm of a pair of chromosomes were in situ labelled with the pLeUCD2 sequence. The sequence represents a *Lophopyrum* species-specific repetitive DNA sequence that was distributed evenly over all of the chromosomes of *Lophopyrum ponticum* (Zhang and Dvorak 1990a, b). Five cells which had a pair of translocated chromosomes were analyzed, and all of them showed that the distal half of the short arm had been replaced by a segment of a *Lophopyrum* chromosome. The break-point in the 5B wheat chromosome appeared to be in the middle of the 5BS arm. In situ labelling of the 'Cadet'-acro 6Ag addition and 'Cadet'-6Ag addition lines revealed a pair of acrocentric chromosomes and a pair of complete chromosomes that were labelled with the pLeUCD2 sequence (Fig. 1B, C), respectively. Therefore, these in situ results confirm the chromosome pairing (Table 1, Kim et al. 1992) and curl mite colonization resistance results of Larson and Atkinson (1973) and Whelan and Hart (1988). The *Lophopyrum*

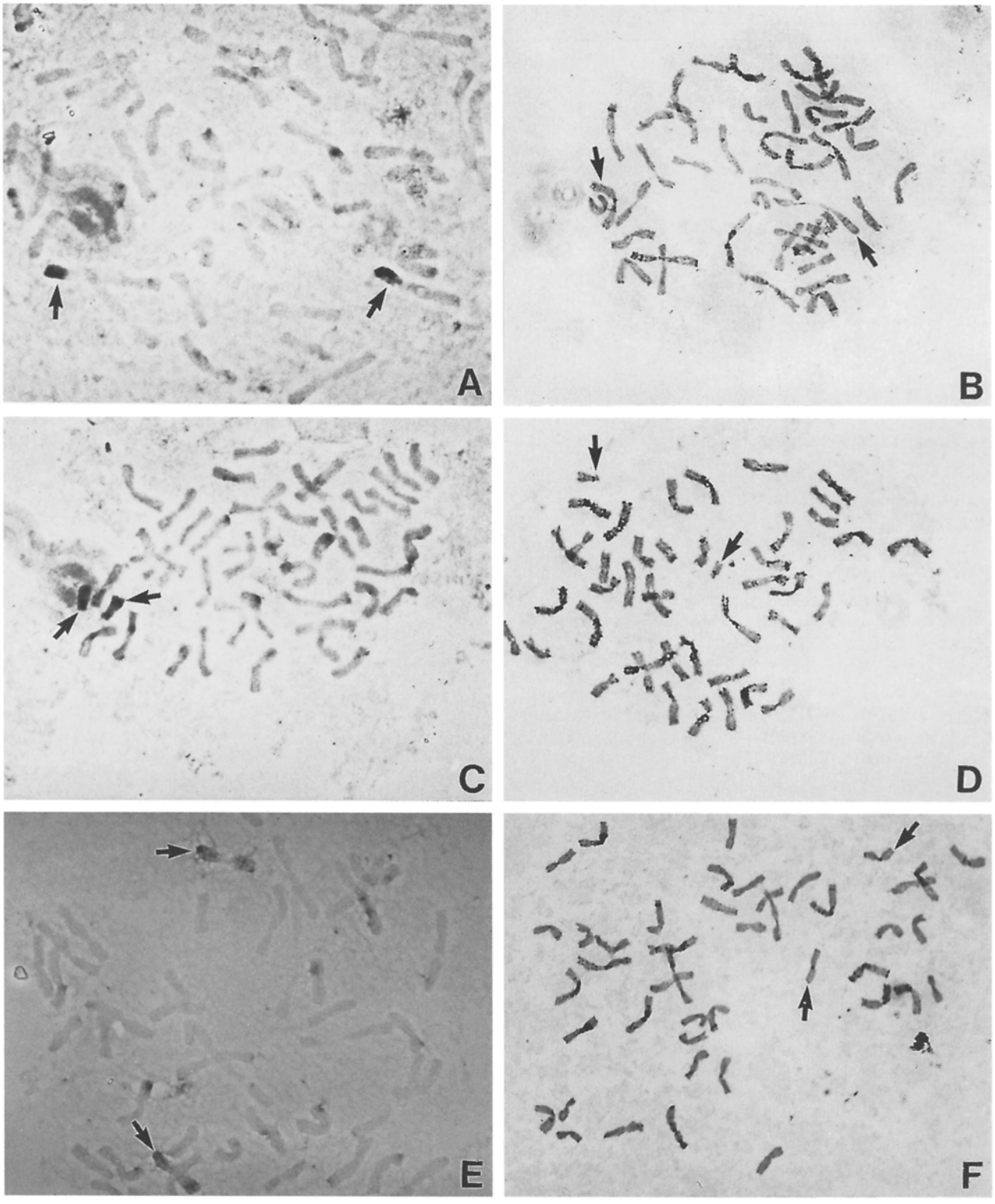

Fig. 1A–F. In situ-hybridized (**A**, **C**, **E**) and C-banded (**B**, **D**, **F**) somatic cells of 'Cadet' 5BS-6AgS (**A**, **B**), the 'Cadet'-6Ag acrocentric addition (**C**, **D**), and the 'Cadet'-6Ag addition (**E**, **F**). *Arrows* point to the recombinant chromosomes and *Lophopyrum* chromosome 6Ag

Table 1. The frequency of chromosome pairing configurations of F_1 hybrids from crosses between the translocation (5B-6Ag) and double ditelosomics of group 5 chromosomes of 'Chinese Spring'

Double-ditelosomic parent	Chromosome configurations and frequency (%)				
	Number of PMCs	$20^{II}+(t+t)1^{III}$	$20^{II}+t1^{II}L+t1S$	$20^{II}+t1^{II}S+t1L$	$20^{II}+1^{I}+t1L+t1S$
5A	97	59.8	37.1	2.1	1.0
5B	92	0.0	97.8	0.0	2.2
5D	111	68.5	28.8	0.9	1.8

From Kim et al. (1992)

chromosome segment in the 5BL.5BS-6AgS translocation line appeared to be smaller than the acrocentric segment in the 'Cadet'-6Ag acrocentric addition line.

C-banding analyses and Southern hybridization using the 5S rDNA probe

C-banding of the mitotic chromosomes of the translocation line showed that approximately one-third of the distal portion of the short arm of wheat chromosome 5B, which can be distinguished from others by its morphology and the presence of two prominent bands in the middle of the long arm (Gill et al. 1991), had been replaced by *Lophopyrum* chromatin. Reference to the standard C-banded karyotype of hexaploid wheat, *T. aestivum*, suggests that the breakpoint in 5BS would likely be between the Giemsa dark bands 5BS 2.1 and 2.3 (Gill et al. 1991). C-banded meiotic chromosome analyses also confirmed the above observation (Fig. 2).

Mukai et al. (1990) showed that the 5S rDNA locus is located in the Giemsa light 2.2 band, between Giemsa dark 5BS bands 2.1 and 2.3. Therefore, the 5S rDNA probe pScT 7 was also utilized to characterize the recombinant chromosome. In *Taq*I digests, a 500-bp restriction fragment located in chromosome 5BS (Dvorak et al. 1989) was missing in the wheat-*Lophopyrum* recombinant line (Fig. 3). Therefore, the breakpoint in 5BS appears to be between the 5S rDNA locus and chromosome band 5BS 2.1. There were no corresponding restriction fragments from *L. Ponticum* in any of the wheat-*Lophopyrum* recombinant and addition lines in both the *Taq*I and *Bam*HI digests (Fig. 2). The same results were obtained using pTA 71, an 18S + 26S rDNA clone, which indicates that the 6Ag chromosome carries neither 5S nor 18S + 26S rDNAs (results not shown).

The C-banding pattern on the acrocentric *Lophopyrum* chromosome was composed of a very dark centromeric band, a faint band in the interstitial location, and another faint band at a telomeric position of the long arm (Fig. 1D). The 6Ag chromosome in the chromosome addition line was characterized by the prominent centromeric C-band, which was identical to

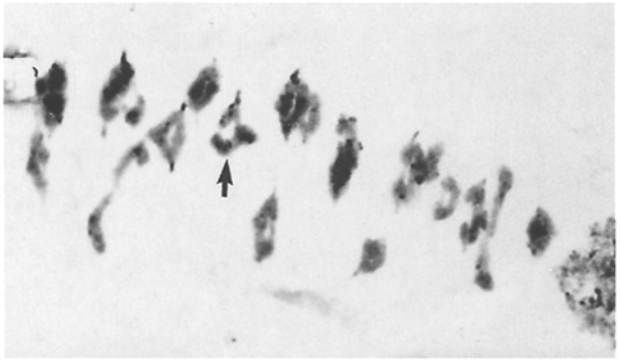

Fig. 2. A C-banded meiocyte at MI of 'Cadet' 5BS-6AgS. *Arrow* points to the recombinant chromosome 5BS-6AgS

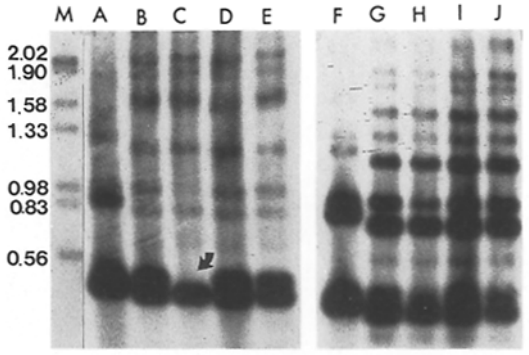

Fig. 3. Autoradiograms of genomic DNAs (10 μg each) digested with *Taq*I (*A–E*) and *Bam*HI (*F–J*) and probed with a 5S rDNA probe, pScT7. *M* Lambda marker III, *A, F L. ponticum*, *B, G* 'Cadet', *C, H* 'Cadet' 5BS-6AgS, *D, I* 'Cadet'-6Ag acrocentric addition, *E, J* 'Cadet'-6Ag addition. *Arrow* indicates the missing 500-bp fragment

that on the acrocentric chromosome (Fig. 1 F). In addition, there were faint bands in the interstitial and telomeric regions of the short arm, as on the acrocentric chromosome, and a prominent band at the telomeric region of the long arm. It was therefore apparent that the acrocentric and the 6Ag chromosome in the addition lines are homologous since they share identical C-banding patterns. Moreover, on the basis of banding patterns and a comparison of the size

of the *Lophopyrum* chromosome segment in the 'Cadet' 5BL.5BS-6AgS translocation and 6AgS the *Lophopyrum* chromosome in the 'Cadet' 5BL.5BS-6AgS interchange represents almost all of the short arm of 6AgS.

The schematic drawings of the C-banding pattern and the breakpoints of the chromosomes of 5B, 5BL.5BS-6AgS, 6Ag, and the 6Ag acrocentric are shown in Fig. 4. Two different breakpoints on 6Ag and one on wheat chromosome 5B have led to the recombinant and telocentric addition lines. A breakpoint in the long arm of 6Ag proximal to the centromere gave rise to an acrocentric fragment that consists of the entire short arm of 6Ag plus a short segment of the long arm of 6Ag. To form the translocated chromosome 5BL.5BS-6AgS, simultaneous breaks were required in the short arms of both 6Ag and 5B followed by the translocation of the 6AgS fragment to the short arm of 5B.

The reason for a lack of chromosome pairing between the translocated 5BL.5BS-6AgS and telocentric 5BS (Table 1) would be that the distal half of 5BS has been replaced with the alien chromosome 6AgS in the 5BL.5BS-6AgS recombinant. It has been hypothesized that chromosome pairing is initiated from the telomeric regions between homologues (Gillies 1984; Loidl 1986). If the telomeric regions are not homologous, pairing initiation would be inhibited.

Southern analyses using pLeUCD2

Southern blotting was performed on the DNAs of parents and recombinants using pLeUCD2 as a probe (Fig. 5). *L. ponticum*, which is the *Lophopyrum* parent of the recombinant, showed strong bands, while the wheat parent, *T. aestivum* var 'Cadet', showed a very faint restriction fragment (0.56 kb) that was very strong in *L. ponticum* and also relatively strong in the 5BL.5BS-6AgS recombinant and addition lines (Fig. 5). The recombinant and chromosome addition lines showed additional restriction fragments that corresponded in size to 0.7, 1.1, and 1.3 kb. The intensity of each restriction fragment slightly increased in the order of 'Cadet' 5BL.5BS-6AgS, 'Cadet'-6Ag acrocentric addition, and 'Cadet'-6Ag addition lines (the same amount of DNA was loaded in each lane). These results correspond to the in situ hybridization results with pLeUCD2; the latter showed a similar difference in amount of *Lophopyrum* chromatin in these lines.

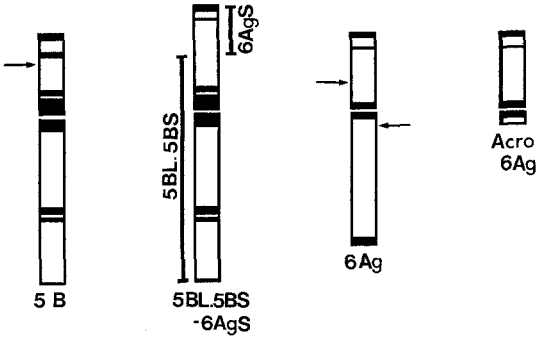

Fig. 4. Schematic drawing of C-banded chromosomes of 5B, 5BS-6AgS, 6Ag, and acrocentric 6Ag. *Arrows* indicate possible breakpoints that may give rise to 5BS-6AgS and acrocentric 6AgS

Conclusion

Because of the limited genetic resources for certain traits in the cultivated crop species, the introgression of alien chromatin carrying desirable genes would be very significant for crop improvement (Sears 1972; Knott and Dvorak 1976; Cauderon 1978; Whelan 1988). The wheat-*Lophopyrum* derivatives described in this paper carry resistance to colonization by the wheat curl mite, which is the vector for wheat streak mosaic virus (WSMV). Thus, the primary gene pool of wheat has been enhanced by the resistance derived from *Lophopyrum*. The conventional methodology of chromosome pairing analysis (Table 1) was able to detect the presence of alien chromatin. However, as demonstrated in this study, the application of techniques such as in situ hybridization and C-banding was able to greatly enhance the characterization of the translocation. The recombinant chromosome has been shown to be composed of the whole arm of 5BL and proximal half of 5BS plus approximately two-thirds of the distal

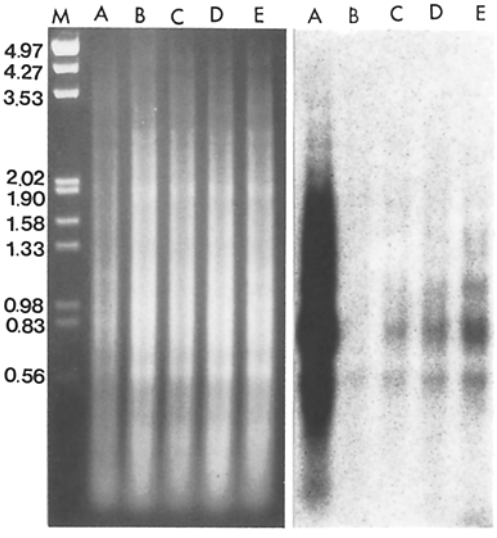

Fig. 5. Photograph of ethidium bromide-stained gel (*left*) and its autoradiogram (*right*). Genomic DNAs were digested with *Hae*III and probed with pLeUCD2. *M* Lambda marker III, *A* *L. ponticum* (6 µg), *B* 'Cadet' (10 µg), *C* 'Cadet' 5BS-6AgS (10 µg), *D* 'Cadet'-6Ag acrocentric addition (10 µg), *E* 'Cadet'-6Ag addition (10 µg)

portion of 6AgS. The breakpoint in 5BS should be between the 5S rDNA locus and chromosome band 5BS 2.1 (in the Giemsa light 2.2 band). The breakpoint in 6AgS is between the minute intercalary band and the centromere. Because the 'Cadet' 5BL.5BS-6AgS line carries the curl mite resistance, which is absent from any of the current wheat species (Kim et al. 1992), this disease resistance gene locus must be on the distal two-thirds of 6AgS.

The acrocentric chromosomes in the 'Cadet'-6Ag addition line contains the whole arm of 6AgS plus a very small proximal portion of 6AgL. The 'Cadet'-6Ag addition line contains the whole 6Ag chromosome. Both addition lines are also resistant to the curl mite colonization. It would also be of interest to analyze the previously described 6AS.6AgS (Whelan and Lukow 1990) and 6DL.6AgS (Whelan and Hart 1988) recombinant chromosomes to determine if these are true Robertsonian translocations.

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