

Comparison of C-banding patterns and *in situ* hybridization sites using highly repetitive and total genomic rye DNA probes of 'Imperial' rye chromosomes added to 'Chinese Spring' wheat*

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ABSTRACT

C-banding patterns of the seven 'Imperial' rye chromosomes and 11 derived rye telocentrics added to 'Chinese Spring' were analyzed and compared with *in situ* hybridization (ISH) patterns using biotin labeled highly repetitive rye DNA sequences pSc119, pSc74, and total rye genomic DNA as probes. C-banding and ISH analyses allow the identification of all individual rye chromosomes and most chromosome arms with these probes. A C-banded karyotype of the added rye chromosomes was constructed and data on chromosome lengths, arm ratios, and fraction lengths of diagnostic C-bands are given following the nomenclature system recently recommended for wheat. Similar telomeric and interstitial localized ISH patterns were observed with pSc119, pSc74, and total rye genomic DNA probes. In addition, pSc119 and total rye genomic DNA labels rye chromatin over their entire lengths, also permitting the detection of unbanded euchromatic rye chromatin transfers in wheat. All localized ISH sites correspond to dark C-banded regions; however, not all C-bands show up as hybridization sites.

1. INTRODUCTION

C-banding and *in situ* hybridization (ISH) are sensitive tools for chromosome and genome identification in plants. The C-banding pattern of cultivated rye, *Secale cereale* L., is well known (Gill and Kimber, 1974; Merker, 1973; Sarma and Natarajan, 1973; Verma and Rees, 1974; Vosa, 1974; Weimark, 1974) and C-banding analysis has been widely used to identify rye chromatin in wheat-rye amphiploids and chromosome addition, substitution, and translocation lines (for review see Appels, 1982; Gustafson, 1983; Lukaszewski and Gustafson, 1983).

Since the first report of Rayburn and Gill (1985a), using biotin labeling and ISH

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to map a highly repeated DNA sequence of rye on wheat chromosomes, ISH analysis with biotinylated repeated DNA probes has been used for chromosome and genome identification in the *Triticeae* (Friebe et al., 1992; Lapitan et al., 1986, 1987; Rayburn and Gill, 1985b, 1987; Xin et al., 1988) and for the physical mapping of defined DNA sequences (Clark et al., 1989; Gustafson et al., 1990; Heslop-Harrison et al., 1990; Le and Armstrong, 1991; Mukai et al., 1990, 1991). Furthermore, ISH analysis with biotinylated total genomic probes can also detect alien chromatin in wheat and its relatives (Anamthawat-Jónsson et al., 1990; Friebe et al., 1992; Le et al., 1989; McIntyre et al., 1990; Mukai et al., 1991; Mukai and Gill, 1991).

Driscoll and Sears (1971) produced a complete series of 'Imperial' rye chromosome addition lines to hexaploid wheat cv. 'Chinese Spring.' The C-banding patterns of the added 'Imperial' rye chromosomes were first described by Gill and Kimber (1974) and Darvey and Gustafson (1975) and a generalized C-banded karyotype of these chromosomes and their homoeology was established at the workshop on rye nomenclature and homoeology relationships (Sybenga, 1983). Unfortunately, up to now exact data on chromosome lengths, arm ratios, and locations of diagnostic C-bands of these chromosomes were lacking. Schlegel et al. (1986) suggested a nomenclature system for C-band designations in rye and, recently, Gill et al. (1991), following the suggestions of the 7th International Wheat Genetics Symposium, Cambridge, 1988, proposed a nomenclature system for description of chromosome bands and structural aberrations in wheat.

In the present study, we have re-analyzed the C-banding patterns of the 'Imperial' rye chromosomes and chromosome arms added to 'Chinese Spring' wheat and report data on chromosome lengths, arm ratios, and locations of diagnostic C-bands based on 30 measurements of each 'Imperial' rye chromosome. A standard C-banded karyotype of these chromosomes was constructed using C-band designations similar to those recommended for wheat. Furthermore, ISH patterns of the added 'Imperial' rye chromosomes and chromosome arms were analyzed using biotinylated highly repetitive rye DNA sequences pSc119, pSc74, and total rye genomic DNA and compared with C-band locations. The presented data are useful for characterizing manipulated wheat-rye derivatives.

2. MATERIALS AND METHODS

The material analyzed consisted of the wheat-rye amphiploid *Triticum aestivum* L. em Thell cv. 'Chinese Spring'/*Secale cereale* L. cv. 'Imperial' ($2n=8x=56$, AABBDDRR), seven disomic chromosome addition lines (1R-7R), and 11 derived ditelosomic chromosome addition lines (1RS, 1RL, 2RL, 3RS, 4RS, 4RL, 5RS, 5RL, 6RL, 7RS, and 7RL) of 'Imperial' rye to 'Chinese Spring.' Seed samples of the disomic addition lines were provided by E. R. Sears, University of Missouri, Columbia, MO, U.S.A., and seeds of the 'Chinese Spring'/'Imperial' amphiploid

and the ditelosomic chromosome addition lines were obtained from T. E. Miller, IPSR, Cambridge Laboratory, U.K.

Root tips were treated with 0.05% colchicine for 3 h in 1.5 ml Eppendorf tubes and fixed in ethanol/glacial acetic acid (3:1). C-banding analysis was according to the method described by Gill et al. (1991). Chromosome measurements were on 30 C-banded rye chromosomes of the chromosome addition lines 1R to 7R and 30 3B chromosomes of the 'Chinese Spring'/'Imperial' amphiploid. Positions of diagnostic C-bands were calculated as fractions of the total chromosome arm lengths from the centromere (fraction length).

For ISH, clones pSc119 and pSc74 and total genomic rye probes were used. Clones pSc119 and pSc74 derived from *Secale cereale*, containing highly repeated sequences from the 120-bp and 350-bp repeat families, respectively (Bedbrook et al., 1980; Jones and Flavell, 1982). All probes were labeled by the random primer method with biotin-16-dUTP. Details for slide preparation, ISH, and detection of hybridization sites using probes pSc119 and pSc74 were as described by Mukai et al. (1990) and Rayburn and Gill (1985a). For genomic ISH, total genomic rye DNA was used as a probe following the procedure of Mukai and Gill (1991). Ratios of 1:2, 1:4, and 1:8 of labeled rye DNA to unlabeled total genomic wheat blocking DNA were used in the hybridization mixture.

Photographs of C-banded chromosomes were taken with Kodak Imagelink HQ microfilm 1461 using a Leitz Dialux photomicroscope and for ISH a Nikon NCG 60 objective and either Kodak technical pan film 2415 or Fuji color negative film super HR II were used.

3. RESULTS

C-banding Analysis

All rye chromosomes present in the set of chromosome addition lines have differential staining of the centromeric regions. In addition, diagnostic telomeric and interstitial C-bands, allows the unambiguous identification of all individual rye chromosomes and most of the rye telocentric chromosomes. C-banding patterns of the rye telocentric chromosomes were always identical to those of the corresponding arms of the complete rye chromosomes from where they were derived.

The C-banding patterns of the rye chromosomes and chromosome arms present in the 'Chinese Spring'/'Imperial' addition lines are shown in Fig. 1 and a standard C-banded karyotype of these chromosomes is shown in Fig. 2. C-band designations are according to the nomenclature system for description of chromosome bands in wheat proposed by Gill et al. (1991). Using this system, five items are required in designating a particular band: the chromosome number according to the homoeologous groups 1 to 7 of *Triticeae*, the genome designation, the arm symbol (S=short arm, L=long arm), the region number, and the number within that region which is separated by a decimal point. Chromosome length data, arm

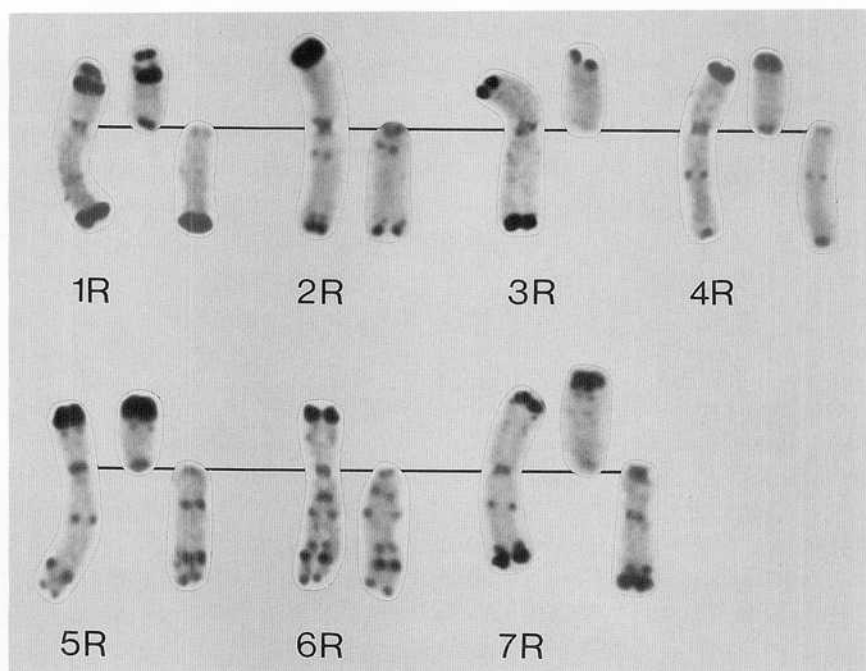


Fig. 1. C-banding patterns of the 'Imperial' rye chromosomes and rye chromosome arms added to 'Chinese Spring' wheat.

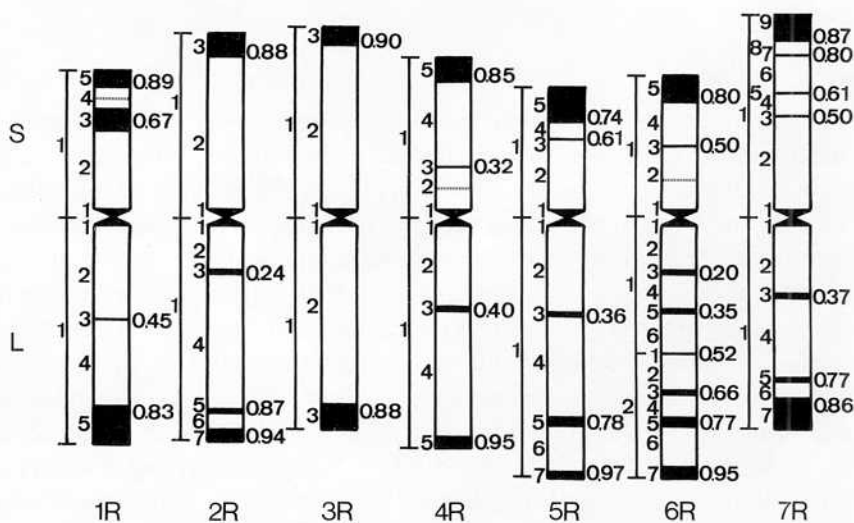


Fig. 2. Standard C-banded karyotype of the rye chromosomes present in the 'Chinese Spring'/'Imperial' chromosome addition lines (chromosome length data and fraction length data of diagnostic C-bands are based on measurements of 30 C-banded rye chromosomes).

Table 1. Chromosome lengths, standard deviations (σ), rye chromosome/3B length ratios, arm ratios (L/S), fraction length of diagnostic C-bands and *in situ* hybridization patterns using pSc119, pSc74, and total genomic rye DNA as probes of the 'Imperial' rye chromosomes added to 'Chinese Spring' wheat

Chromosome	Chromosome length (σ)	Rye chromosome/3B length ratio	Arm ratio (L/S)	Diagnostic C-bands	Fraction length	pSc119	pSc74	Total genomic rye DNA
1R	9.9 μm (0.9 μm)	0.96	1.5	1RS1.3	0.67	+	-	+
				1RS1.5	0.89	+	+	+
				1RL1.3	0.45	+	-	-
				1RL1.5	0.83	+	+	+
2R	10.8 μm (1.0 μm)	1.04	1.2	2RS1.3	0.88	+	+	+
				2RL1.3	0.24	-	-	-
				2RL1.5	0.87	-	+	-
3R	10.7 μm (0.9 μm)	1.03	1.1	3RS1.3	0.90	+	+	+
				3RL1.3	0.88	+	+	+
4R	10.4 μm (1.0 μm)	1.01	1.4	4RS1.3	0.32	-	-	-
				4RS1.5	0.85	+	+	+
				4RL1.3	0.40	+	-	-
5R	10.4 μm (1.1 μm)	1.01	2.0	5RS1.3	0.61	-	-	-
				5RS1.5	0.74	+	+	+
				5RL1.3	0.36	+	-	-
				5RL1.5	0.78	-	+	+
6R	10.7 μm (1.0 μm)	1.03	1.8	5RL1.7	0.97	+	-	-
				6RS1.3	0.50	+	-	-
				6RS1.5	0.80	+	+	+
				6RL1.3	0.20	-	-	-
				6RL1.5	0.35	+	-	-
				6RL2.1	0.52	+	-	-
				6RL2.3	0.66	-	+	+
7R	11.0 μm (1.0 μm)	1.06	1.0	6RL2.5	0.77	-	+	+
				6RL2.7	0.95	+	+	+
				7RS1.3	0.50	+	-	+
				7RS1.5	0.61	+	-	+
				7RS1.7	0.80	+	-	-
				7RS1.9	0.87	+	+	+
7R				7RL1.3	0.37	+	-	+
				7RL1.5	0.77	+	+	+
				7RL1.7	0.86	+	+	+

ratios (L/S), and fraction lengths of diagnostic C-bands from the centromere of individual rye chromosomes are given in Table 1.

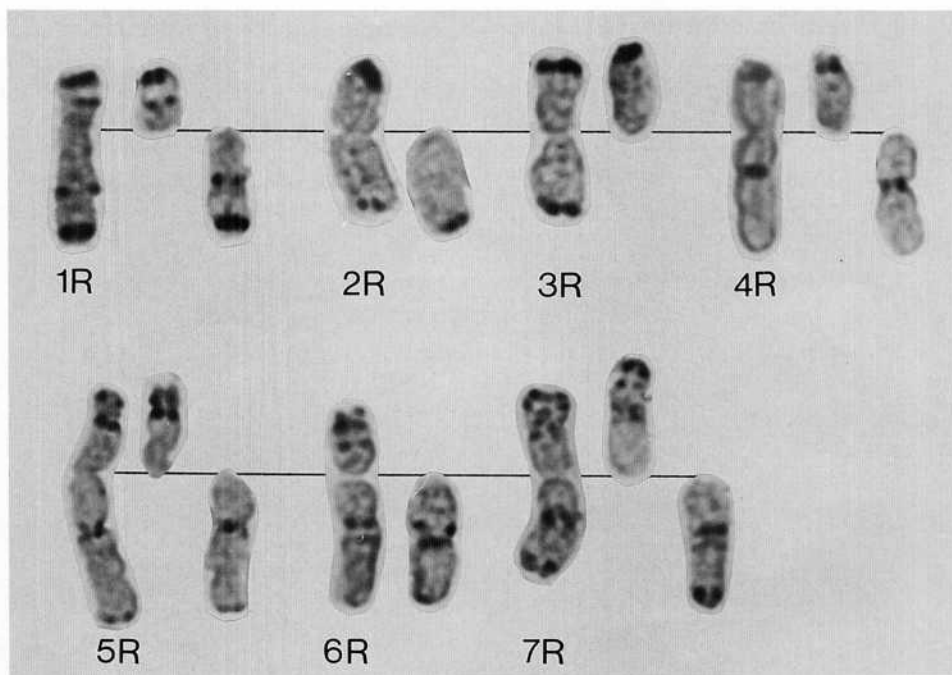


Fig. 3. *In situ* hybridization patterns of the 'Imperial' rye chromosomes and chromosome arms added to 'Chinese Spring' wheat using pSc119.

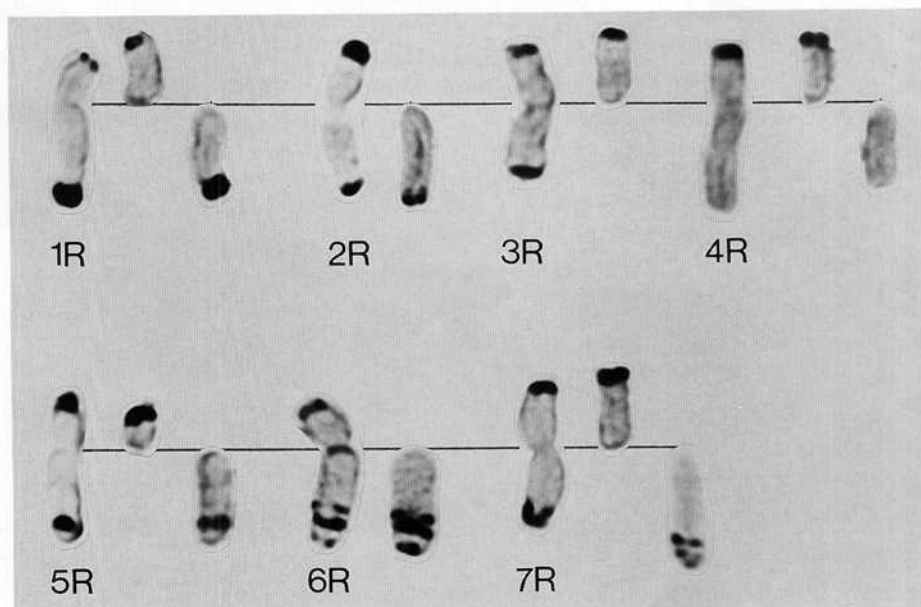


Fig. 4. *In situ* hybridization patterns of the 'Imperial' rye chromosomes and chromosome arms added to 'Chinese Spring' wheat using pSc74.

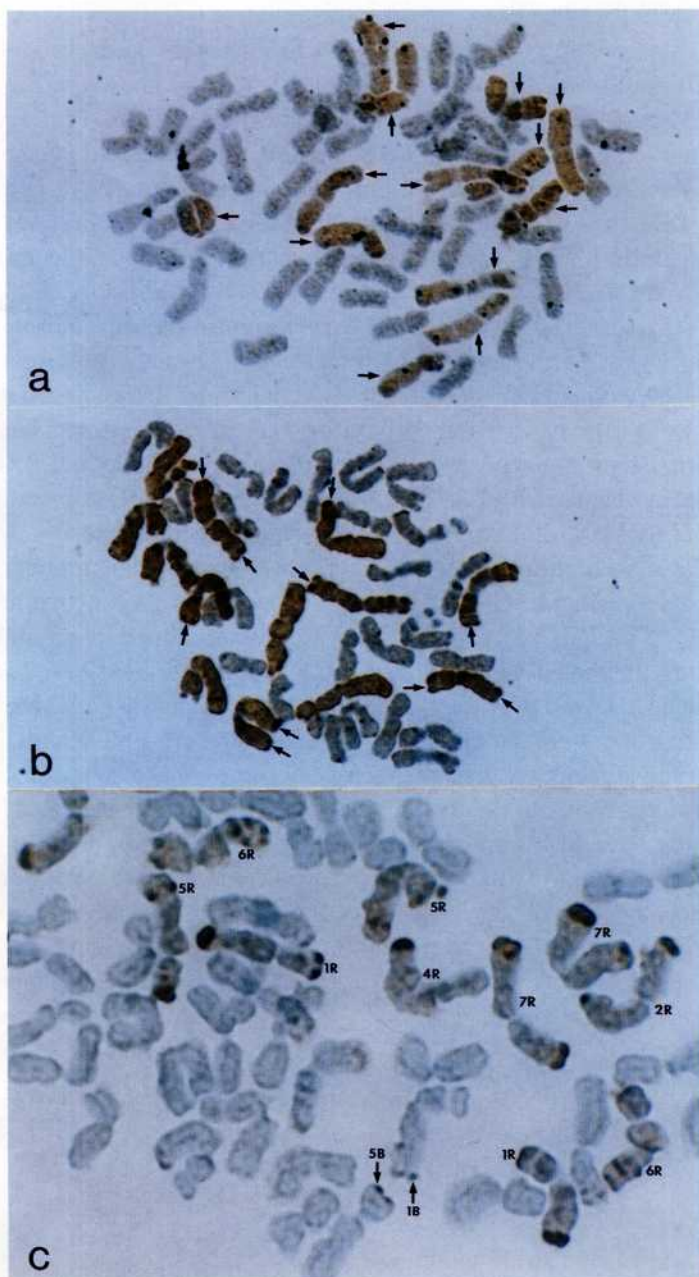


Fig. 5. *In situ* hybridization patterns of mitotic metaphase chromosomes of the amphiploid 'Chinese Spring'/'Imperial' using labeled total genomic rye DNA and unlabeled total genomic wheat blocking DNA at ratios: a) 1:2, b) 1:4, and c) 1:8.

In situ hybridization analysis

The ISH patterns of the 'Imperial' rye chromosomes and chromosome arms added to 'Chinese Spring' wheat using probe pSc119 are shown in Fig. 3. Fig. 4 shows the ISH patterns of these chromosomes using probe pSc74. Probe pSc119 produces dispersed labeling pattern over the entire length of all rye chromosomes and rye telocentrics and, in addition, chromosome specific telomeric and interstitial localized ISH sites. Localized and mainly telomeric ISH sites were obtained with pSc74. Both pSc119 and pSc74 give chromosome-specific ISH patterns allowing the identification of all rye chromosomes. In addition, pSc119 also has localized ISH patterns in all B-genome chromosomes and chromosomes 4A, 2D, 3D, and 5D of wheat permitting the identification of these chromosomes (data not shown here). A comparison of C-banded regions and ISH sites obtained with pSc119 and pSc74 is given in Table 1. Differences in ISH patterns between these probes were most pronounced in rye chromosome arms 5RL and 6RL, whereas pSc119 lights up bands 5RL1.3, 5RL1.7, 6RL1.5, and 6RL2.1 compared with bands 5RL1.5, 6RL2.3, 6RL2.5, and 6RL2.7 revealed by pSc74.

The ISH patterns of mitotic metaphase chromosomes of the amphiploid 'Chinese Spring'/'Imperial' using labeled total genomic rye DNA as a probe are shown in Figs. 5 and 6. The ISH patterns of the rye chromosomes depend on the ratio of labeled rye and unlabeled wheat blocking DNA. Using a ratio of 1:2 of labeled rye to unlabeled wheat DNA all rye chromosomes show dispersed labeling over their entire lengths, whereas all wheat chromosomes are unlabeled (Fig. 5a). A similar dispersed labeling pattern of rye chromosomes was also observed when the ratio of labeled rye DNA to unlabeled wheat DNA was increased to 1:4 (Fig. 5b). However, using this ratio some rye chromosomes in addition have some localized ISH sites at the telomeres (marked with arrows in Fig. 5b). No labeling of any wheat chromosome was observed when a 1:4 ratio of labeled rye and unlabeled wheat DNA was used in the hybridization mix. By increasing the amount of unlabeled blocking wheat DNA to a ratio of 1:8, rye chromosomes showed a

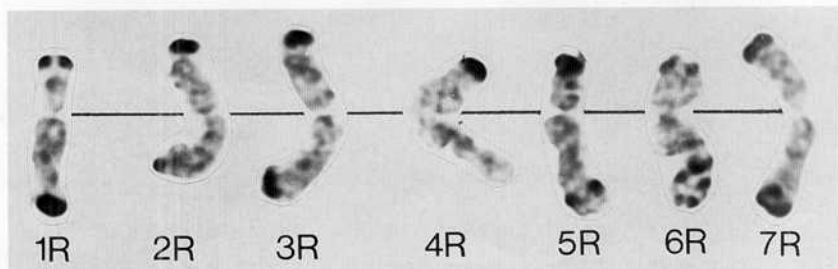


Fig. 6. *In situ* hybridization patterns of individual 'Imperial' rye chromosomes present in the amphiploid 'Chinese Spring'/'Imperial' using labeled total genomic rye DNA and unlabeled total genomic wheat blocking DNA at a ratio of 1:8.

dispersed labeling over their entire lengths and in addition localized telomeric and interstitial ISH sites, allowing the identification of individual rye chromosomes (Fig. 5c, Fig. 6). Also, some chromosomes of wheat showed localized ISH sites. In general, the localized ISH sites observed with total genomic rye DNA correspond to the ISH sites observed with pSc119 and pSc74 and a comparison with C-banded regions is given in Table 1.

4. DISCUSSION

The set of 'Imperial' rye chromosomes added to 'Chinese Spring' wheat has been considered as the standard rye chromosome set as suggested in the workshop report on rye chromosome nomenclature and homoeology relationships and the C-banding patterns of these chromosomes have been established (Sybenga, 1983). The C-banding patterns of the 'Imperial' rye chromosomes and rye telocentrics reported here is similar to that described earlier, allowing the identification of all seven chromosomes and most chromosome arms. Additional C-bands were observed in some chromosome arms (4RS1.3, 5RS1.3, 5RL1.7, 6RL2.1, 7RS1.3, 7RS1.5, 7RS1.7) which were not detected earlier, caused by the higher resolution of the C-banding technique used in the present study. However, according to Sybenga (1983) rye chromosome 1R should also have a C-band in region 1RS1.2 close to the subterminal C-band (1RS1.3) in the short arm and rye chromosome 3R should also have a C-band close to the centromere in region 3RS1.2. In the present study, these C-bands were not observed in the 'Imperial' rye chromosomes and corresponding telocentrics added to 'Chinese Spring'. This discrepancy cannot be explained by differences in the C-banding techniques used in both studies, since we detected these bands in other cultivars of rye (Friebe et al., 1989; Friebe et al., 1992; Friebe and Larter, 1988; Heun et al., 1990).

Chromosome length data of rye chromosomes present in the 'Chinese Spring'/'Imperial' addition lines are small compared to those of hexaploid wheat cv. 'Chinese Spring' (Gill et al., 1991). This discrepancy is caused by the different pretreatments used in both studies. In the present study, measurements were carried out on colchicine pretreated chromosomes, whereas those on 'Chinese Spring' wheat were based on ice-water pretreated chromosomes. Colchicine pretreatment usually results in smaller and more condensed chromosomes compared to ice-water pretreatment. To make the measurement data comparable we used the total length of chromosome 3B ($10.34 \mu\text{m}$, $\sigma=0.96 \mu\text{m}$) as a standard and calculated the rye chromosome/3B ratio for each of the seven rye chromosomes. The ISH analysis shows that pSc119 produces a dispersed labeling over the entire chromosome lengths and additional localized ISH sites in all chromosomes of rye and in 11 chromosome pairs of wheat, confirming earlier reports (Lapitan et al., 1986, 1987; McIntyre et al., 1990; Rayburn and Gill, 1985a). All rye chromosomes, but no wheat chromosomes, are labeled at telomeric and some interstitial

regions with probe pSc74. Both probes had chromosome-specific ISH sites which allow the identification of individual rye chromosomes.

The ISH patterns, using pSc119 and pSc74, of the 'Imperial' rye chromosomes reported here are similar to that described for 'Chaupon' rye (Lapitan et al., 1986, 1987). Some minor differences (i.e., presence of ISH site using pSc74 in 4RL1.3 in 'Chaupon' and absence of this site using the same probe in 'Imperial' rye chromosome 4R) are probably caused by polymorphic variation which is common within and between cultivars of rye.

Recently, pSc119 was subcloned into pSc119.1, pSc119.2, and pSc119.3, carrying 745-bp, 611-bp, and 167-bp inserts, respectively (McIntyre et al., 1990). Only the 611-bp insert showed internal repetition with a subrepeat length of 119 bp. ISH analysis showed that pSc119.1 hybridized only to rye chromosomes, which were labeled over the entire lengths, whereas pSc119.2 produced mainly localized telomeric and interstitial ISH sites on all rye chromosomes and in addition also localized ISH sites in 11 chromosome pairs of wheat.

In the present study the original clone pSc119 was used as a probe and, thus, both dispersed and localized ISH patterns were observed. The dispersed labeling of all rye chromosomes is probably caused by hybridization of the 745-bp sequence, whereas the localized ISH sites on rye and wheat chromosomes reflect hybridization of the 611-bp sequence.

The ISH pattern using total rye genomic DNA as a probe depends on the ratio of labeled rye DNA to unlabeled total genomic wheat blocking DNA in the hybridization mix. Using ratios of 1:2 and 1:4, all rye chromosomes are labeled over their entire lengths and can be easily distinguished from chromosomes of wheat which are unlabeled. By increasing the amount of unlabeled wheat blocking DNA to a ratio of 1:8 rye chromosomes show a dispersed labeling over their lengths and in addition chromosome-specific telomeric and interstitial ISH sites, permitting the identification of individual rye chromosomes.

Similar telomeric and interstitial ISH patterns were produced with pSc119 and pSc74. In general, more chromosome-specific ISH sites were obtained with the former probe. Almost identical localized ISH patterns were also observed in the rye chromosomes of the amphiploid 'Chinese Spring'/'Imperial' when labeled total genomic rye DNA and unlabeled wheat blocking DNA were used at a 1:8 ratio in the hybridization mix. All localized ISH sites observed either with pSc119, pSc74, or total genomic rye DNA correspond to dark C-banded regions, but not all C-banded regions show hybridization with these probes.

The C-banding technique is known to selectively stain all regions of constitutive heterochromatin which is composed of highly repeated DNA families (for review see Appels, 1982). Since DNA composition may vary between different C-bands, ISH analysis using highly repeated DNA sequences will light up only those regions which are carrying the same DNA repeats as the probes. The difference in ISH patterns observed with pSc119 and pSc74 in 5RL and 6RL reflects

differences in the composition of the repeat families at these sites.

Labeled total genomic rye DNA was previously used to detect rye chromatin in wheat-rye hybrids and in T1RS-1BL lines (Heslop-Harrison et al., 1990; Le and Armstrong, 1991; Le et al., 1989) and for identifying rye chromosomes in a *Hordeum chilense*—*Secale africanum* hybrid (Anamthawat-Jónsson et al., 1990). Recently, labeled total genomic rye DNA was used to identify rye chromatin in radiation induced terminal and intercalary wheat-rye translocation lines carrying resistance genes against the Hessian fly derived from rye chromosome arm 6RL (Friebe et al., 1991; Mukai et al., 1991). In these studies, an intercalary rye transfer was detected having a size of about 1 μm , showing the high resolution of genomic ISH analysis. Furthermore, labeled total genomic *Agropyron intermedium* DNA was used to detect *Ag. intermedium* chromatin in leaf rust resistant wheat-*Ag. intermedium* substitution and translocation lines (Friebe et al., 1992).

Both C-banding and ISH analyses permit the identification of individual rye chromosomes and are highly sensitive techniques for detecting rye chromatin in wheat. At present, more chromosome specific markers are obtained with the C-banding technique. However, C-banding stains only constitutive heterochromatin whereas ISH analysis, using pSc119 or total genomic rye DNA, also permits the detection of euchromatic rye chromatin. Both techniques should be used for characterizing manipulated wheat-rye introgression lines.

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