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Molecular cytogenetic characterization of *Roegneria ciliaris* chromosome additions in common wheat

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Abstract The development of alien addition lines is important both for transferring useful genes from related species into common wheat and for studying the relationship between alien chromosomes and those of wheat. *Roegneria ciliaris* (2n=4x=28, $S^cS^cY^cY^c$) is reported to be a potential source of resistance to wheat scab, which may be useful in wheat improvement. The amphiploid common wheat-*R. ciliaris* and BC_1F_7 or BC_2F_6 derivatives were screened by C-banding, genomic in situ hybridization (GISH), fluorescent in situ hybridization (FISH) and restriction fragment length polymorphism (RFLP) for the presence of *R. ciliaris* chromatin introgressed into wheat. Six lines were identified as disomic chromosome additions (DA), one as a ditelosomic addition (Dt), two as double disomic additions (dDA) and one as a monosomic chromosome addition (MA). RFLP analysis using wheat homoeologous group-specific clones indicated that the *R. ciliaris* chromosomes involved in these lines belong to groups 1, 2, 3, 5 and 7. The genomic affinities of the added *R. ciliaris* chromosomes were determined by FISH analysis using the repetitive sequence pCbTaq4.14 as a probe. These data suggest that the *R. ciliaris* chromosomes in five lines belong to the S^c genome. Based on the molecular cytogenetic data, the lines are designated as DA2S^c#1, Dt2S^c#1L, DA3S^c#1, dDA1S^c#2+5Y^c#1, DA5Y^c#1, DA7S^c#1, DA7Y^c#1 and MA?Y^c#1. Based on the present and previous work, 8 of the 14 chromosomes of *R. ciliaris* have been transferred into wheat.

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Introduction

Wheat scab, caused by *Fusarium graminearum* Schw., is a devastating disease, especially in areas of high temperature and humidity during the flowering period. Several methods are being utilized to control the disease, among which the use of scab-resistant varieties is the most economic and effective. Among wheat cultivars, Sumai 3 represents the best source of scab resistance and has been successfully used to develop scab-resistant cultivars in breeding programs worldwide (Bai and Shaner 1994, 1996). As a result, most resistant germplasms have Sumai 3 in their pedigrees. To broaden the genetic base of scab resistance, new sources of resistance should be identified and introgressed into wheat.

Wild relatives of bread wheat (*Triticum aestivum* L. em. Thell., 2n=6x=42, AABBDD) provide a useful gene pool for wheat improvement, and many genes that confer resistance to diseases and pests have been introduced into wheat by wide hybridization (Friebe et al. 1996). In order to increase the diversity for scab-resistance genes, (Liu et al. 1990) screened 14 wheat relatives from 11 genera by single-floret inoculation; *Roegneria ciliaris* (Trin.) Nevski [syn. *Agropyron ciliare* (Trin) Franchet, syn. *Elymus ciliaris* (Trin) Tzvelev], *R. kamoji* (Trin.) Nevski (syn. *Elymus tsukushiense* Honda) and *Leymus racemosus* Lam (syn. *Elymus giganteus* L.) were found to have high levels of resistance to scab spread. Ban (1997) found that several indigenous Japanese *Agropyron* (*Elymus*) species also had high levels of resistance to wheat scab. One of the scab-resistant species, *Roegneria ciliaris* (2n=4x=28, $S^cS^cY^cY^c$) is a perennial tetraploid species distributed mainly throughout Asia and is well adapted to high-humidity environments. Sharma and Gill (1983) obtained a *R. ciliaris*-*T. aestivum* alloplasmic hybrid with *R. ciliaris* cytoplasm. Jiang et al. (1992, 1993)

attempted to produce a set of chromosome addition lines but, because of the alloplasmic condition, only chromosomes $1S^c$#1 and $1Y^c$#1, with fertility restoration (*Rf*) genes, were recovered. The $1S^c$#1 and $1Y^c$#1 chromosome addition lines were susceptible to scab.

Until now, the homoeology and the genome affinity of *R. ciliaris* chromosomes have not been established except for chromosomes $1S^c$#1 and $1Y^c$#1. Muramatsu et al. (1983) produced an alloplasmic wheat-*R. ciliaris* amphiploid. In order to avoid alloplasmic effects, they used *T. aestivum* cv. Chinese Spring (CS) as a female parent and crossed it with the amphiploid to obtain euplasmic progenies. This crossing scheme enabled the recovery of *R. ciliaris* chromosomes other than $1S^c$#1 and $1Y^c$#1. Wang et al. (1997) identified two addition lines among the euplasmic progenies by chromosome C-banding. In the present paper, we report the identification and molecular cytogenetic characterization of euplasmic wheat-*R. ciliaris* chromosome addition lines.

Materials and methods

Plant materials

Wheat var. Chinese Spring (CS) was crossed with a *Triticum aestivum* cv. Inayama komugi (Ik)-*R. ciliaris* amphiploid (Kansas State University accession number TA3427). Based on chromosome number, plant morphology and meiotic chromosome pairing, 16 BC_1F_7 and BC_2F_6 derivatives were chosen for C-banding, genomic in situ hybridization (GISH), fluorescent in situ hybridization (FISH) and restriction fragment length polymorphism (RFLP) analyses. Two accessions of *Roegneria ciliaris* (TA12245 and TA12254) and the wheat cultivars Ik and CS were used as controls. TA3427, TA12245, TA12254 CS and Ik are maintained at the Wheat Genetics Resources Center, Kansas State University, Manhattan, Kansas, USA.

Chromosome identification was according to Gill and Kimber (1974) and Gill BS et al. (1991). Clones pCbTaq4.14 and pPlTaq2.5 were isolated originally from *Critesion bogdanii* (Wilensky) A. Löve (2n=2x=14, HH) and *Pseudoroegneria libanotica* (Hackle) D. R. Dewey (2n=2x=14, SS). The insert sizes of the two clones were 0.8 kb and 0.4 kb, respectively, The fragments were ligated to *Acc*I-digested pUC18 and cloned in *Escherichia coli* RR1 (Gill et al. 1988). Genomic DNA of *R. ciliaris* and the two repetitive-sequence clones were used as probes in GISH and FISH experiments following the protocol of Jiang and Gill (1994).

RFLP analysis was used to determine the homoeologous relationships of the added *R. ciliaris* chromosomes. DNA was isolated from leaf tissue and digested with restriction enzymes (*Eco*RI, *Hin*dIII, *Dra*I, *Eco*RV and *Alu*I). DNA isolation, electrophoresis, Southern blotting, hybridization and washing were performed as described by Faris et al. (2000).

Low-copy RFLP clones located on the seven homoeologous groups of wheat were selected from barley (*Hordeum vulgare* L.) cDNA (BCD), oat (*Avena sativa* L.) cDNA (CDO) and wheat genomic DNA (WG, KSU, and PSR) libraries. The clones have been described by Heun et al. (1991) (BCD, CDO and WG); Gill KS et al. (1991) (KSU) and Devos and Gale (1993) (PSR). Clones were kindly supplied by M.E. Sorrells (Cornell University, Ithaca, N.Y.) (BCD, CDO and WG) and M.D. Gale (John Innes Centre, Norwich, UK) (PSR).

The nomenclature of *R. ciliaris* chromosomes was according to Raupp et al. (1995).

Results

Characterization of the alloplasmic amphiploid of wheat-*R. ciliaris*

GISH analysis revealed the presence of 14 pairs of *R. ciliaris* chromosomes in the Ik-*R. ciliaris* amphiploid. Although many of the *R. ciliaris* chromosomes have distinct C-band patterns that allow their identification, some of them have C-band patterns similar to those of wheat making their identification difficult. C-banding and GISH analysis were used in combination for unambiguous chromosome identification. Some of the *R. ciliaris* chromosomes present in the amphiploid showed C-banding polymorphism comparable with those of the *R. ciliaris* accession analyzed by Jiang et al. (1993).

Forty-two clones that detect loci distributed on the seven wheat homoeologous groups were selected for RFLP analysis, and 34 were polymorphic between wheat and *R. ciliaris* (Table 1). Only a few polymorphisms between Ik and CS were observed. No polymorphism was found in the accession of *R. ciliaris* used in the amphiploid and the accessions TA12245 and TA12254. Clones that detect polymorphisms between wheat and *R. ciliaris* were further used to characterize the *R. ciliaris* introgression lines.

Clones pCbTaq4.14 and pPlTaq2.5 were used as probes in FISH analysis to determine the genome affinities of the added *R. ciliaris* chromosomes. Jiang et al. (1994) observed that clone pCbTaq4.14 was specific only to some H-genome species but hybridized to six pairs of S-genome chromosomes in *Pseudoroegneria spicata* (Pursh) A. Löve. In our study, FISH analysis using pCbTaq4.14 as a probe detected no hybridization sites on any of the wheat chromosome of cvs. CS and Ik. However, the probe detected FISH sites on seven chromosome pairs in *R. ciliaris (*TA12245 and TA12254) and in the wheat-*R. ciliaris* amphiploid (Fig. 1). Because this probe can hybridize to six pairs of *P. spicata* chromosomes, the chromosomes detected in the amphiploid most likely belong to the S^c genome.

Clone pPlTaq2.5 was used for FISH analysis on wheat, *R. ciliaris* and the wheat-*R. ciliaris* amphiploid. Twelve pairs of *R. ciliaris* chromosomes had pPlTaq2.5 FISH sites. Thus, this clone does not differentiate between S^c- and Y^c-genome chromosomes.

Characterization of wheat-*R. ciliaris* introgression lines

Chromosome C-banding and GISH analysis were used to identify the added *R. ciliaris* chromosomes; RFLP and FISH were used to determine the homoeologous group and genome affinity of the added chromosomes. On the basis of the plant morphology of the backcross derivatives and molecular cytogenetics analyses, the following lines were identified.

Disomic addition line (DA) $DA2S^c$#1 and ditelosomic addition line (Dt) $Dt2S^c$#1L

Table 1 RFLP analysis of *Roegneria ciliaris* chromosomes introduced into wheat using 42 genomic and cDNA wheat clones and 2 repetitive sequences as probes (+ polymorphism. – no polymorphism)

Clones	Line no. (2n=) Homeologous groups	TA-3427 (70)	99–1503 (44)	99–1506 (44)	99–1507 (44)	99–1511 (42+2t)	99–1512 (44)	99–1514 (46)	99–1515 (46)	99–1518 (44)	99–1520 (44)	99–1521 (44)
PSR161	1 L	–	–	–	–	–	–	–	–	–	–	–
BCD371	1 L	+	–	–	–	–	–	+	+	–	–	–
PSR109	2 S	+	–	+	–	–	–	–	–	–	–	–
PSR855	2 S	–	–	–	–	–	–	–	–	–	–	–
PSR388	2 L	+	–	+	–	+	–	–	–	–	–	–
KSUG53	3 S	+	–	–	–	–	–	–	–	–	–	–
PSR123	3 S	+	–	–	–	–	–	–	–	+	–	–
PSR598	3 S	+	–	–	–	–	–	–	–	+	–	–
CDO480	3 S	+	–	–	–	–	–	–	–	+	–	–
PSR116	3 L	+	–	–	–	–	–	–	–	+	–	–
PSR156	3 L	+	–	–	–	–	–	–	–	+	–	–
PSR903	3 L	+	–	–	–	–	–	–	–	+	–	–
PSR578	3 L	–	–	–	–	–	–	–	–	–	–	–
CDO105	3 L	–	–	–	–	–	–	–	–	–	–	–
PSR110	4 S	+	–	–	–	–	–	–	–	–	–	–
PSR139	4 S	+	–	–	–	–	–	–	–	–	–	–
CDO795	4 S	+	–	–	–	–	–	–	–	–	–	–
PSR164	4 L	+	–	–	–	–	–	–	–	–	–	–
BCD110	4 L	+	–	–	–	–	–	–	–	–	–	–
PSR157	4 L	+	–	–	–	–	–	–	–	–	–	–
CDO1387	4 L	+	–	–	–	–	–	–	–	–	–	–
PSR170	5 S	+	–	–	–	–	–	–	–	–	–	–
BCD1130	5 S	–	–	–	–	–	–	–	–	–	–	–
BCD1871	5 S	+	–	–	–	–	+	+	+	–	–	–
PSR115	5 L	+	–	–	–	–	+	+	+	–	–	–
PSR128	5 L	+	–	–	–	–	+	+	+	–	–	–
CDO1312	5 L	–	–	–	–	–	–	–	–	–	–	–
PSR964	6 S	+	–	–	–	–	–	–	–	–	–	–
PSR627	6 S	+	–	–	–	–	–	–	–	–	–	–
PSR371	6 L	–	–	–	–	–	–	–	–	–	–	–
PSR915	6 L	+	–	–	–	–	–	–	–	–	–	–
PSR142	6 L	+	–	–	–	–	–	–	–	–	–	–
BCD385	7 S	+	+	–	+	–	–	–	–	–	+	–
BCD349	7 S	+	+	–	+	–	–	–	–	–	+	–
PSR152	7 S	+	–	–	–	–	–	–	–	–	–	–
PSR150	7 S	–	–	–	–	–	–	–	–	–	–	–
CDO595	7 S	+	–	–	–	–	–	–	–	–	–	–
WG669	7 S	+	+	–	–	–	–	–	–	–	+	–
KSUD2	7 L	+	+	–	–	–	–	–	–	–	+	–
PSR311	7 L	+	+	–	–	–	–	–	–	–	+	–
PSR690	7 L	+	+	–	–	–	–	–	–	–	+	–
CDO686	7 L	+	–	–	–	–	–	–	–	–	–	–
pCbTaq4.14		+	–	+	+	–	–	+	+	+	–	–
pPlTaq2.5		+	+	+	+	–	+	+	+	–	+	+

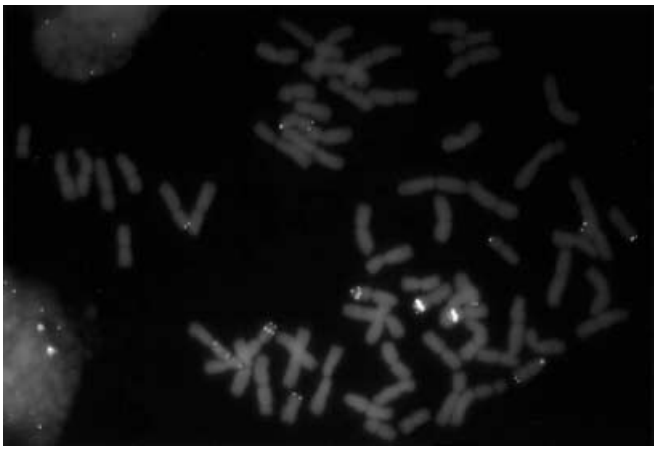

Fig. 1 FISH patterns of the amphiploid *T. aestivum-R. ciliaris* using pCbTaq4.14 as a probe

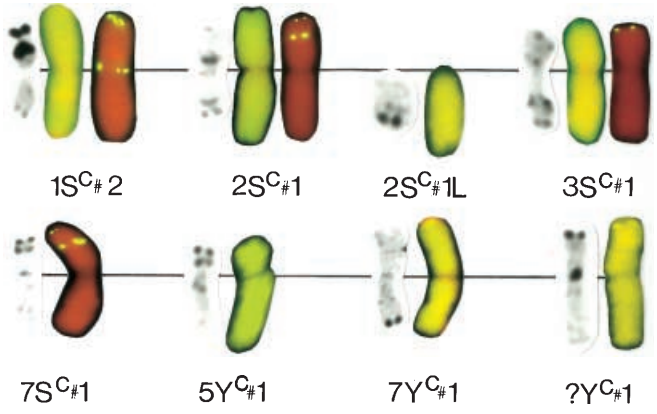

Fig. 2 C-banding, GISH and FISH patterns of *R. ciliaris* chromosomes added to wheat. *1S^{c}#2* Lines 99–1514, 99–1515, *2S^{c}#1* line 99–1506, *2S^{c}#1L* line 99–1511, *3S^{c}#1* line 99–1518, *7S^{c}#1* line 99–1507, *5Y^{c}#1* line 99–1512, *7Y^{c}#1* lines 99–1503, 99–1520, *?Y^{c}#1* line 99–1521

GISH showed that one pair of metacentric and one pair of telocentric chromosomes were present in lines 99–1506 (2n=44) and 99–1511 (2n=42+2t), respectively. The *R. ciliaris* chromosomes in 99–1506 have a small telomeric band and a large proximal band in the short arm, and a proximal and a telomeric band in the long arm (Figs. 2, 3). RFLP analysis using group 2-specific clones identified the added *R. ciliaris* chromosomes in line 99–1506 as belonging to homoeologous group 2 (Table 1). On the basis of Fig. 2 and Table 1, we identified the added telosome in line 99–1511 as the long arm of the *R. ciliaris* chromosome present in line 99–1506. Spikes of line 99–1506 have tenacious glumes, which is a group-2 short arm marker, whereas those of the line 99–1511 are free-threshing (Fig. 4). FISH analysis with clone pCbTaq4.14 revealed the presence of hybridization sites on the added *R. ciliaris* chromosome in line 99–1506, suggesting that this chromosome belongs to the S^{c} genome. Thus, lines 99–1505 and 99–1511 were designated as DA2S^{c}#1 and Dt2S^{c}#1L, respectively.

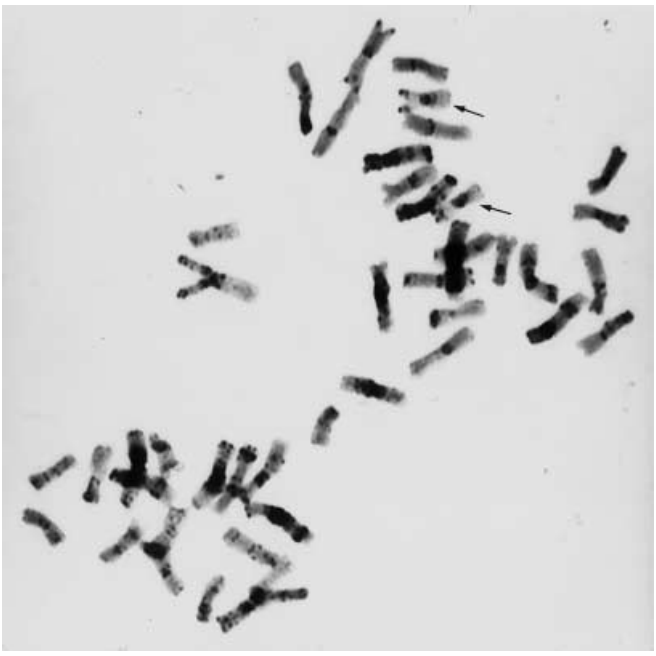

Fig. 3 C-banded mitotic metaphase of disomic chromosome addition line DA2S^{c}#1 (99–1506). The two 2S^{c}#1 chromosomes are indicated by the *arrows*

Disomic addition line DA3S^{c}#1

Line 99–1518 was identified as a disomic addition line (2n=44) by GISH analysis. The *R. ciliaris* chromosomes are submetacentric and have proximal and telomeric C-bands in both arms (Fig. 2). The added *R. ciliaris* chromosomes in this line were found to be homoeologous to group-3 chromosomes by RFLP analysis (Table 1). FISH analysis using pCbTaq4.14 as a probe detected signals on the alien chromosome. Therefore, this line was designated as DA3S^{c}#1. Spikes of this line are awned. Because neither the amphiploid nor CS has long awns this trait is likely the result of outcrossing.

Disomic addition line DA5Y^{c}#1 and double disomic addition line (dDA) dDA1S^{c}#2+5Y^{c}#1

GISH analysis revealed the presence of a pair of acrocentric *R. ciliaris* chromosomes in line 99–1512 (2n=44). Two pairs of *R. ciliaris* chromosomes were identified in lines 99–1514 and 99–1515 by GISH; one chromosome pair is acrocentric and the other is metacentric.

The acrocentric alien chromosomes in lines 99–1512, 99–1514 and 99–1515 have the same C-banding pattern with a telomeric and an intercalary C-band in the short arm and a proximal band in the long arm. Another pair of chromosomes present in 99–1514 and 99–1515 have a C-banding pattern similar to that of the 1S^{c}#1 chromosome recovered by Jiang et al. (1993). According to these results, line 99–1512 was identified as a disomic

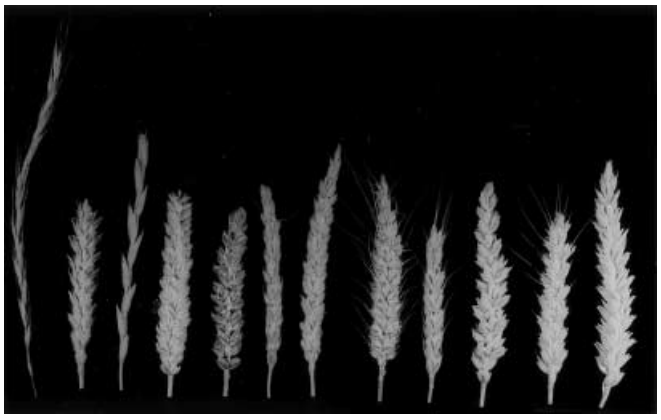

Fig. 4 Spike morphology of wheat-*R. ciliaris* addition lines. From *left* to *right*: *R. ciliaris*, Inayama komugi, Ik-*R. ciliaris* amphiploid, CS, dDA1S^c#2+5Y^c#1 (99–1514, 99–1515), DA2S^c#1 (99–1506), Dt2S^c#1L) (99–1511), DA3S^c#1 (99–1518), DA7S^c#1 (99–1507), DA5Y^c#1 (99–1512), DA7Y^c#1 (99–1503, 99–1520), MA?Y^c#1 (99–1521)

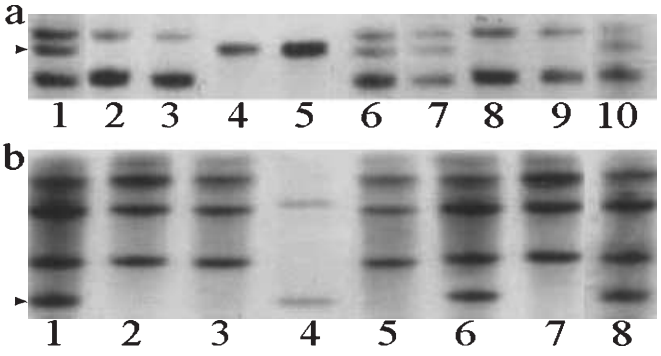

Fig. 5a, b RFLP analysis of wheat-*R. ciliaris* addition lines. **a** *Dra*I-digested DNA probed with group 5S-specific clone BCD1871: *1* Ik-*R. ciliaris* amphiploid, *2* CS, *3* Ik, *4*, *5 R. ciliaris*, *6*, *7* dDA1S^c#2+5Y^c#1 (99–1514, 99–1515), *10* DA5Y^c#1 (99–1512). **b** *Eco*RI-digested DNA probed with group 1L-specific clone BCD371: *1* Ik- *R. ciliaris* amphiploid, *2* CS, *3* Ik, *4 R. ciliaris*, *6*, *8*: dDA1S^c#2+5Y^c#1 (99–1514 and 99–1515). *Arrowheads* Polymorphic bands

addition line. Lines 99–1514 and 99–1515 are identical and were identified as double disomic addition lines (Fig. 2). The spikes of all these lines are lax (Fig. 4), which is typical for group-5 chromosome addition lines.

RFLP analysis using group-5-specific clones revealed that the *R. ciliaris* chromosomes common to lines 99–1512, 99–1514 and 99–1515 belong to group 5 (Fig. 5, Table 1). The other pair of *R. ciliaris* chromosomes present in lines 99–1514 and 99–1515 were group-1 chromosomes according to RFLP analysis using group-1-specific clones (Fig. 5, Table 1). Only one pair of *R. ciliaris* chromosomes in lines 99–1514 and 99–1515 was labeled, and no FISH sites were detected on any chromosome in line 99–1512. The common chromosome added in these three lines is 5Y^c#1, and the other pair of *R. ciliaris* chromosomes present in the double disomic addition lines is 1S^c#2.

Disomic addition line DA7S^c#1 and disomic addition line DA7Y^c#1

GISH and C-banding identified lines 99–1507, 99–1503 and 99–1520 as disomic additions (2n = 44). The added *R. ciliaris* chromosomes present in line 99–1507 have a unique C-banding pattern, with a subtelomeric and a telomeric band in the short arm The added *R. ciliaris* chromosomes in lines 99–1503 and 99–1520 are submetacentric and have interstitial C-bands in both arms and a large telomeric C-band in the long arm (Fig 2).

RFLP analysis using group-7 clones revealed that the *R. ciliaris* chromosomes present in lines 99–1507, 99–1503 and 99–1520 belong to homoeologous group 7. However, clones KSUD2, PSR311 and PSR690 mapped only on the *R. ciliaris* chromosomes present in lines 99–1503 and 99–1520. The FISH signal of clone pCbTaq4.14 was detected only in line 99–1507. The *R. ciliaris* chromosome pair in line 99–1507 is 7S^c#1, and the added chromosome pair in lines 99–1503 and 99–1520 is 7Y^c#1. Clone WG669 was genetically mapped on the short arm of wheat chromosome 4D but physically located on wheat chromosome 7D (Nelson et al. 1995; Boyko et al. 1999). In the present study, WG669 was located on the *R. ciliaris* chromosome 7Y^c#1. Therefore, WG669 is more likely a group-7-specific clone. The spikes of both lines DA 7S^c#1 and DA 7Y^c#1 are awned, which is also likely the result of outcrossing.

Monosomic addition (MA) line MA?Y^c#1

The somatic chromosome number of line 99–1521 was determined to be 2n=43. GISH and C-banding analysis identified this line as a monosomic addition line. The *R. ciliaris* chromosome added has a telomeric C-band in the short arm (Fig. 2). No polymorphism was found with any of the RFLP probes used in this study (Table 1). No pCbTaq4.14 FISH signal was detected on this chromosome, indicating it belongs to the Y^c-genome. Further work is necessary to determine the homeology of the added *R. ciliaris* chromosome in this line.

Discussion

Combined C-banding, GISH, FISH and RFLP analyses identified ten lines that represented eight *R. ciliaris* chromosome additions designated as DA7Y^c#1, DA2S^c#1, DA7S^c#1, DA2S^c#1L, DA5Y^c#1, DA1S^c#2+5Y^c#1, DA3S^c#1 and MA?Y^c#1.

Sharma and Gill (1983) initiated hybridization between *R. ciliaris* and wheat to investigate the polyploid nature and genome evolution in the genus *Elymus*, to understand the genetic relationships of the S^c and Y^c genomes of *Elymus* with those of wheat and to introgress disease-resistance genes from *Elymus* into wheat. However, because of the incompatibility between the cytoplasm of *R. ciliaris* and the nucleus of wheat, plants without

Table 2 RFLP markers located on *R. ciliaris* chromosomes

Line no.	Chromosome constitution (Accession numbers)	Mapped markers
99–1506	DA2S^c#1 (NAU801=TA7705)	PSR109–2S, PSR388–2L
99–1511	Dt2S^c#1L (NAU802=TA7711)	PSR388–2L
99–1518	DA3S^c#1 (NAU803=TA7706)	PSR598–3S, PSR480–3S, PSR123–3S, PSR116–3L, PSR156–3L, PSR903–3L
99–1507	DA7S^c#1 (NAU804=TA7707)	BCD349–7S, BCD385–7S
99–1512	DA5Y^c#1 (NAU805=TA7708)	BCD1871–5S, PSR128–5L, PSR115–5L
99–1514 & 99–1515	dDA1S^c#2+5Y^c#1 (NAU821=TA7710)	BCD1871–5S, PSR128–5L, PSR115–5L, BCD371–1L
99–1503 & 1520	DA7Y^c#1 (NAU806=TA7709)	BCD349–7S, BCD385–7S, CDO669–7S,KSUD2–7L, PSR311–7L, PSR690–7L

1S^c#1 or 1Y^c#1 had reduced vigor or were sterile. Thus, only chromosomes 1S^c#1 or 1Y^c#1 with the *Rf* genes were recovered as chromosome addition lines. In the present study, we used the *R. ciliaris–T. aestivum* amphiploid as the male parent in a backcross scheme, which allows the recovery of not only 1S^c#1 and 1Y^c#1, but also other *R. ciliaris* chromosomes without *Rf* genes in an euplasmatic background.

Although an effort was made to produce a complete set of euplasmic wheat-*R. ciliaris* addition lines, only eight different introgression lines were obtained in the present study. Low transmission frequency of some of the alien chromosomes probably makes their recovery difficult.

Morris and Gill (1987) attempted to differentiate the S^c- and Y^c-genome chromosomes based on the N- and C-banding patterns of *R. ciliaris* and diploid S- genome species. Svitashev et al. (1998) reported several RAPD primers that were S- or Y-genome specific. The Y-genome donor of *Roegneria* species is unknown. Differentiation between the S^c- and Y^c-genome chromosomes by GISH is difficult because no competitor DNA is available.

A number of repetitive sequences are genome-specific and have been used to determine the genome affinity of certain chromosomes (Rayburn and Gill 1985; Gill et al. 1988; Tsujimoto and Gill 1991; Jiang et al. 1994; Dvorak and Dubcovsky 1996; Tsujimoto et al. 1997; Svitashev et al. 1998; Nagaki et al. 1998). In the present study, the genome specificity of clone pCbTaq4.14 was confirmed by FISH analysis on the DA1S^c#1 and DA1Y^c#1 lines produced by Jiang et al. (1993). FISH sites of clone pCbTAQ4.14 were detected only on chromosome 1S^c#2, confirming that this probe is S^c-genome-specific. In the present study, three S^c-genome chromosomes and two Y^c-genome chromosomes were identified by FISH analysis of the wheat-*R. ciliaris* addition lines. RFLP analysis using pCbTAQ4.14 as probe indicated that signals were only detected in lines containing S^c-genome chromosomes. RFLP analysis with pPlTAQ2.5 as a probe detected hybridization signals in lines DA7Y^c#1, DA7S^c#1, DA5Y^c#1 and dDA1S^c#2+5Y^c#1 but not in line DA3S^c#1.

Eighteen molecular markers were located on specific *R. ciliaris* chromosomes (Table 2). These markers can be used in further research as molecular markers to screen for useful gene(s) located on the corresponding chromosomes. Clones KSUG53–3L, PSR170–5S showed polymorphism between wheat and *R. ciliaris*, but DA3S^c#1, DA5Y^c#1, and dDA1S^c#1+5Y^c#1 produced no polymorphic bands. Therefore, these latter clones should be located on chromosomes 3Y^c#1 and 5S^c#1 of *R. ciliaris*, respectively. When we used clones PSR152–7S, CDO595–7S and CDO686–7L as probes, wheat and *R. ciliaris* showed polymorphism, but neither line DA7S^c#1 nor DA7Y^c#1 showed the polymorphic bands. It is likely that the homologous sequences of these clones are located on other *R. ciliaris* chromosomes that were not recovered in the present study. None of the clones used were located on the *R. ciliaris* chromosome in line MA?Y^c#1. C-banding pattern of this chromosome was different from that of 1Y^c#1. More markers belonging to group 2, 3, 4 and 6 should be used in further research for determining the homoeologous relationship of this chromosome.

In addition to characterizing the *R. ciliaris* chromosomes, our second objective was to transfer useful genes of *R. ciliaris* into wheat. *R. ciliaris* is a useful source for genes for resistance to wheat scab. The 1S^c#1 and 1Y^c#1 alloplasmic addition lines were tested for 2 years in the field for their resistance to scab. Both lines were found to be susceptible, indicating that these two chromosomes do not carry scab-resistance genes (Wang et al. unpublished). We are currently screening the materials identified in the present study for their scab resistance.

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References

Bai GH, Shaner G (1994) Scab of wheat: prospect for control. Plant Dis 78:760–799

Bai GH, Shaner G (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis 80:975–979

Ban T (1997) Evaluation of resistance to *Fusarium* head blight in indigenous Japanese species of *Agropyron* (*Elymus*). Euphytica 97:39–44

Boyko EV, Gill KS, Mickelson-Young L, Nasuda S, Raupp WJ, Gill BS (1999) A high density genetic linkage map of *Aegilops tauschii*, the D-genome progenitor of bread wheat. Theor Appl Genet 99:16–26

Devos KM, Gale MD (1993) The genetic maps of wheat and their potential in plant breeding. Outlook Agric 22:93–99

Dvorak J, Dubcovsky J (1996) Genome analysis of polyploid species employing variation in repeated nucleotide sequences. In: Jauhar PP (ed) Methods of genome analysis in plants. CRC Press, Boca Raton, Fla., pp 133–145

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hotspot in wheat. Genetics 154:823–835

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to disease and pest: current status. Euphytica 91:59–87

Gill BS, Kimber G (1974) Giemsa C-banding and the evolution of wheat. Proc Natl Acad Sci USA 71:4086–4090

Gill BS, Morris KLD, Appels R (1988) Assignment of the genomic affinities of chromosomes from polyploid *Elymus* species added to wheat. Genome 30:70–82

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Gill KS, Lubbers EL, Raupp, WJ, Cox TS, Gill BS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34: 437–447

Jiang J, Gill BS (1994) Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheat. Chrom Res 2:59–64

Jiang J, Raupp WJ, Gill BS (1992) *Rf* genes restore fertility in wheat lines with cytoplasm of *Elymus trachycaulus* and *E. ciliaris*. Genome 35:614–620

Jiang J, Chen P, Friebe B, Raupp, WJ, Gill BS (1993) Alloplasmic wheat-*Elymus ciliaris* chromosome addition lines. Genome 36:327–333

Jiang J, Morris KLD, Gill BS (1994) Introgression of *Elymus trachycaulus* chromatin into common wheat. Chrom Res 2:3–13

Liu DJ, Weng YQ, Chen PD (1990) Transfer of scab resistance from *Roegneria* C. Koch (*Agropyron*) species into wheat. In: Kimber G (ed) Proc 2nd Int Symp Chrom Eng Plants. University of Missouri, Columbia, MO, USA, pp 166–176

Morris KLD, Gill BS (1987) Genome affinities of individual chromosomes based on C- and N-banding analyses of tetraploid *Elymus* species and their diploid progenitor species. Genome 29:247–252

Muramatsu M, Kaneta S, Ikeda R, Uetsuki T, Takahashi K (1983) Hybridization of Japanese indigenous *Agropyron (Roegneria)* species with hexaploid wheat and cytogenetics of some of the F_1, BC_1 and amphiploid plants. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Maruzen, Kyoto, pp 1041–1048

Nagaki K, Tsujimoto H, Sasakuma T (1998) H-genome specific repetitive sequence, pEt2, of *Elymus trachycaulus* in part of *Afa* family of Triticeae. Genome 41:134–136

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH (1995) Molecular mapping of wheat: major genes and rearrangements in homeologous groups 4, 5, and 7. Genetics 141:721–731

Raupp WJ, Friebe B, Gill BS (1995) Suggested guidelines for the nomenclature and abbreviation of the genetic stocks of wheat, *Triticum aestivum* L. em Thell., and its relatives. Wheat Inf Serv 81:50–55

Rayburn AL, Gill BS (1985) Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J Hered 78: 78–81

Sharma HC, Gill BS (1983) New hybrids between *Agropyron* and wheat. Theor Appl Genet 66:111–121

Svitashev S, Bryngelsson T, Li XM, Wang RC (1998) Genome-specific repetitive DNA and RAPD markers for genome identification in *Elymus* and *Hordelymus*. Genome 41:120–128

Tsujimoto H, Gill BS (1991) Repetitive sequences from polyploid *Elymus trachycaulus* and the diploid progenitor species: detection and genomic affinity of *Elymus* chromatin added to wheat. Genome 34:782–789

Tsujimoto H, Mukai Y, Akagawa K, Nagoki K, Fujigaki J, Yamamoto M, Sasakuma T (1997) Identification of individual barley chromosomes based on repetitive sequences: conservative distribution of Afa-family repetitive sequences on the chromosomes of barley and wheat. Genes Genet Syst 72:303–309

Wang XE, Chen PD, Liu DJ (1997) Development of euplasmic *T. aestivum-R.ciliaris* disomic addition line D and ditelosomic addition line tBL. Chinese Journal Genetics:127–131