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ORIGINAL ARTICLE

Development and characterization of a complete set of *Triticum aestivum–Roegneria ciliaris* disomic addition lines

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Abstract

Key message **A complete set wheat-*R. ciliaris* disomic addition lines (DALs) were characterized and the homoeologous groups and genome affinities of *R. ciliaris* chromosomes were determined.**

Abstract Wild relatives are rich gene resources for cultivated wheat. The development of alien addition chromosome lines not only greatly broadens the genetic diversity, but also provides genetic stocks for comparative genomics studies. *Roegneria ciliaris* (genome $S^cS^cY^cY^c$), a tetraploid wild relative of wheat, is tolerant or resistant to many abiotic and biotic stresses. To develop a complete set of wheat-*R. ciliaris* disomic addition lines (DALs), we undertook a euplasmic backcrossing program to overcome allocytoplasmic effects and preferential chromosome transmission. To improve the efficiency of identifying chromosomes from S^c and Y^c, we established techniques including sequential genomic in situ hybridization/fluorescence in situ hybridization (FISH) and molecular marker analysis. Fourteen DALs of wheat, each containing one pair of *R. ciliaris* chromosomes pairs, were characterized by FISH using four repetitive sequences [p*Ta794*, p*Ta71*, RcAfa and $(GAA)_{10}$] as probes. One hundred and sixty-two *R. ciliaris*-specific markers were developed. FISH and marker analysis enabled us to assign the homoeologous groups and genome affinities of *R. ciliaris* chromosomes. FHB resistance evaluation in successive five growth seasons showed that the amphiploid, DA2Y^c, DA5Y^c and DA6S^c had improved FHB resistance, indicating their potential value in wheat improvement. The 14 DALs are likely new gene resources and will be phenotyped for more agronomic performances traits.

Introduction

Wild relatives of common wheat are a vast resource of agronomic traits that can be transferred into wheat and have been used to broaden the genetic diversity of wheat (Dewey 1984; Sharma and Gill 1983) by wide hybridization and chromosome engineering. The production of alien disomic addition lines (DALs) in wheat is an initial step in mining and chromosome location of potentially useful genes prior to transfer to wheat chromosomes. Production of a complete set of wheat-alien addition lines allows determination of the homoeologous relationships between alien and corresponding wheat chromosomes, and dissection of the genetic effects of individual alien chromosomes in wheat background (Jiang et al. 1994). Using such genetic stocks, potentially useful genes for grain quality, and resistance to biotic and abiotic stresses have also been identified and characterized (Fasahat et al. 2012; Jauhar et al. 2009).

Roegneria ciliaris (Trin.) Nevski ($2n=4x=28$, genome $S^cS^cY^cY^c$), a perennial tetraploid species, within the

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Triticeae, is widely distributed in China, and has been cultivated as fine pasture fodder and lawn grass species. It has been also reported as a source of resistance to many wheat diseases, including Fusarium head blight, wheat streak mosaic virus, and barley yellow dwarf virus (Sharma et al. 1984; Weng and Liu 1989; Liu et al. 1990; Tomohiro 1997; Yang et al. 1999). Efforts have been made to produce hybrids between wheat and *R. ciliaris* since the 1980s (Jiang et al. 1992; Sharma and Gill 1983). Muramatsu et al. (1983) developed an alloplasmic amphiploid of *R. ciliaris* and wheat cultivar (cv.) Inayama komugi Jiang et al. (1992, 1993) developed DALs DA1S^c and DA1Y^c, but failed to identify DALs involving the other 12 chromosomes due to presumed alloplasmic effects. To overcome these effects, Wang et al. (2001) obtained a hybrid between *T. aestivum* cv. Chinese Spring (as female parent) and the Inayama komugi-*R. ciliaris* amphiploid. Among the derived euplasmic progenies, 10 wheat-*R.ciliaris* alien chromosome addition lines were developed, in which 5 were DALs, i.e., DA2S^c, DA3S^c, DA5Y^c, DA7S^c and DA7Y^c. Kong et al. (2008) showed that 18 wheat-*R. ciliaris* addition lines had been developed. However, due to lack of sufficient and accurate techniques for distinguishing the 14 *R. ciliaris* chromosome pairs, the identities of the alien chromosomes in the lines remained unclear.

The tetraploid *R. ciliaris* is presumed to contain two different genomes. However, the two genomes show high affinity and the differentiation has been very difficult (Liu et al. 2006; Lu and Bothmer 1989). Morris and Gill (1987) attempted to differentiate the S^c- and Y^c-genomes based on N- and C-banding patterns of *R. ciliaris* and the diploid S^c-genome species *Pseudoroegneria spicata*. Svitashev et al. (1998) reported the possibility of differentiating the two genomes by use of several RAPD primers that were specific for either the S^c- or Y^c-genomes. Wang et al. (2001) reported that FISH use of repetitive sequence pCbTaq4.14 as probe could identify the genomic affinities of the S^c- or Y^c-genomes. Wang et al. (2010) distinguished *R. ciliaris* chromosomes from those of the St, P, and Y genomes using GISH–FISH. More recently, Wang et al. (2017) reported that St_2-80 is a potential and useful FISH marker that can be used to distinguish St genome chromosomes from those of other Triticeae genomes. Although these methods might be feasible to assign homoeologous chromosomes to specific genomes, the lack of suitable genetic resources makes it difficult to verify the accuracy. Development of a complete set of wheat-*R. ciliaris* DALs would permit identification of all 14 chromosome pairs, and comparisons by FISH and amplification patterns with molecular markers would be helpful in assigning homoeologous chromosomes to the S^c or Y^c genomes.

The objectives of this study were to: (a) identify and characterize new wheat-*R. ciliaris* alien chromosome lines for the purpose of obtaining a complete set of wheat-*R. ciliaris* DALs; (b) establish a FISH-based karyotype of *R. ciliaris* chromosomes using a set of DALs; (c) develop molecular markers specific for each *R. ciliaris* chromosome; and (d) assign the genome affinity of each *R. ciliaris* chromosome. Fulfilment of these objectives will lay a solid foundation better utilization of useful genes from *R. ciliaris* in wheat breeding programs.

Materials and methods

Plant materials

A *Triticum aestivum* cv. Inayama komugi-*R. ciliaris* amphiploid ($2n = 10x = 70$, genome AABBDDS^{c}S^{c}Y^{c}Y^c), developed by Dr. Muramatsu et al. (1983), and seeds of it along with Inayama komugi (Ik) were obtained from the Wheat Genetic Resource Center, Kansas State University, Manhattan, Kansas, USA. *R. ciliaris* (Accession No. W614249) was introduced from the Western Regional Plant Introduction Station, Pullman, Washington, USA.

Single backcross populations derived from a cross between the amphiploid and wheat cv. Chinese Spring (CS) were used to develop disomic addition lines (DALs) (Table 1). Wheat varieties Sumai 3 and Mianyang 8545 were used as resistant and susceptible controls in Fusarium head blight (FHB) tests conducted at the Nanjing Agricultural University, Jiangsu Experiment Station. All materials are maintained at the Cytogenetics Institute, Nanjing Agricultural University (CINAU).

Molecular cytogenetic analysis

Chromosome preparations from root tip cells (RTC) were performed as described by Chen et al. (1995) with minor modifications.

GISH and FISH were conducted according to Zhang et al. (2004). For GISH, genomic DNA of *R. ciliaris* was labeled with fluorescein-12-dUTP by nick translation and used as a probe. Genomic DNA of CS was used for blocking with a probe: blocker ratio of 1:50. After GISH, the chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI).

Afa-family sequences were amplified by two-round PCR from *R. ciliaris* using Afa-specific primer pair (AS-A 5′-GATGATGTGGCTTTGAATGG-3′; AS-B 5′-GCATTT CAAATGAACTCTGA-3′). The first round used genomic DNA of *R. ciliaris* and products were used as a template for the second round PCR, in which the dUTP in the reaction solution was substituted by digoxigenin-11-dUTP (Roche). The products of this reaction were designated as RcAfa and used for FISH.

Table 1 Materials used in this research and their chromosome constitutions

Materials	Chromosome number (2*n*)	Chromosome constitution	Reference
Chinese spring	42	AABBDD	
Inayama komugi	42	AABBDD	
R. ciliaris	28	$S^cS^cY^cY^c$	
Ik-*R.c* amphiploid	70	AABBDD$S^cS^cY^cY^c$	Muramatsu et al. (1983)
Disomic addition (DA) $1S^c$	44	AABBDD + $1S^c1S^c$	Jiang et al. (1992, 1993)
DA$1Y^c$	44	AABBDD + $1Y^c1Y^c$	Jiang et al. (1992, 1993)
DA$2S^c$	44	AABBDD + $2S^c2S^c$	Wang et al. (2001)
DA$3S^c$	44	AABBDD + $3S^c3S^c$	Wang et al. (2001)
DA$5Y^c$	44	AABBDD + $5Y^c5Y^c$	Wang et al. (2001)
DA$7S^c$	44	AABBDD + $7S^c7S^c$	Wang et al. (2001)
DA$7Y^c$	44	AABBDD + $7Y^c7Y^c$	Wang et al. (2001)
DA$6S^c$ (14-15K27)	44	AABBDD + $6S^c6S^c$	Kong et al. (2008)
DA$6Y^c$ (14-15K15)	44	AABBDD + $6Y^c6Y^c$	Kong et al. (2008)
DA$2Y^c$ (14-15K108)	44	AABBDD + $2Y^c2Y^c$	The present research
DA$3Y^c$ (14-15K132)	44	AABBDD + $3Y^c3Y^c$	The present research
DA$4S^c$ (14-15K271)	44	AABBDD + $4S^c4S^c$	The present research
DA$4Y^c$ (14-15K247)	44	AABBDD + $4Y^c4Y^c$	The present research
DA$5S^c$ (14-15K125)	44	AABBDD + $5S^c5S^c$	The present research

The three other FISH probes were repetitive DNA sequences p*Ta71* (45S rDNA), p*Ta794* (5S rDNA) and $(GAA)_{10}$. The $(GAA)_{10}$ was synthesized according to Cuadrado et al. (2008). The p*Ta71* clone is a 9 kb wheat rDNA repeat unit (Gerlach and Bedbrook 1979) containing 18S, 5.8S and 26S rRNA genes, and the intergenic spacer was used as the probe for 45S rDNA. Clone p*Ta794* is a *Bam*HI fragment of 5S rDNA, which has a 120 bp coding sequence (Gerlach and Dyer 1980). p*Ta71* and p*Ta794* were labeled with digoxigenin-11-dUTP by nick translation. Chromosomes were counter stained with DAPI.

FISH signals were visualized under a fluorescence microscope (Olympus BX60), and images were captured by Spot32 CCD camera and analyzed by Adobe Photoshop software.

Molecular marker analysis

A total of 1845 EST-SSR primer pairs evenly distributed in the seven homoeologous groups of wheat were used for molecular marker analysis. The primer pairs were designed from EST sequences that were physically mapped to wheat chromosomes (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi), and were synthesized by Invitrogen Life Technologies (Shanghai). The Ik-*R. ciliaris* amphiploid, common wheat, and wheat-*R. ciliaris* DALs were used for PCR to determine the specificity of markers for individual *R. ciliaris* chromosomes.

Total genomic DNA was extracted with SDS according to Sharp et al. (1989) and Devos et al. (1992). PCR was performed in 10 µL reaction volumes containing 25 ng of genomic DNA, 2 µmol/L of each primer pair, 2.5 mmol/L of dNTPs, 2.5 mmol/L of $MgCl_2$, 1×PCR buffer (10 mmol/L Tris–HCl, pH 8.5, 50 mmol/L KCl), and a 0.5 unit of *Taq* DNA polymerase. Amplification was performed for 3 min at 94 °C, followed by 32 cycles of 30 s at 94 °C, 45 s at 50–60 °C depending on the individual primers, 1 min 10 s at 72 °C, and a final step of 10 min at 72 °C. PCR products were separated in 8% polyacrylamide gels (acrylamide:bisacrylamide = 19:1 or 39:1) and visualized by silver staining.

Evaluation of FHB response

The Ik-*R. ciliaris* amphiploid, Ik, CS and a complete set of CS-*R. ciliaris* DALs were evaluated for FHB response in the greenhouse using the single-floret inoculation method over five successive growing seasons (2012–2017). Wheat varieties Sumai 3 and Mianyang 8545 were used as resistant and susceptible controls, respectively. Monosporic isolate Fg0609 of *F. graminearum* (kindly provided by Dr. Xu Zhang, Jiangsu Academy of Agricultural Sciences) was used in inoculations. At anthesis, 10 µL of a conidial suspension containing 1×10^5 macro conidia/mL was injected into the first floret of a central spikelet in one spike of each plant. FHB severity was measured as the percentage of diseased spikelets per spike at 21 days post-inoculation (Xiao et al. 2011).

Results

Screening and development of molecular markers specific for *R. ciliaris* chromosomes

A total of 1845 EST-SSR primer pairs previously mapped to seven homoeologous groups of wheat chromosomes were used for PCR using CS, Ik, Ik-*R.ciliaris* amphiploid and *R. ciliaris* as templates. Five hundred and fifty seven primers (30.2%) produced polymorphisms between wheat and *R. ciliaris*. Polymorphism rates for the seven homoeologous groups varied from 17.8 to 43.4% (Table 2).

Further PCR of the polymorphic markers performed on the wheat-*R. ciliaris* addition lines showed that 162 could be used as chromosome-specific markers for *R. ciliaris*. Individual *R. ciliaris* chromosomes were assigned to homoeologous groups based on known allocations of the markers to wheat chromosomes. Although the homoeologous relationships between *R. ciliaris* and wheat chromosomes were well conserved, there were exceptions, including $1Y^c$, $2S^c$, $7S^c$ and $7Y^c$ (Table S2). Bands of five markers specific for $1Y^c$ (1EST-111, 1EST-259, 1EST-1149, 1EST-258, 1EST-1134) were present in DA7S^c and DA7Y^c; bands of ten markers specific for $7S^c$ and $7Y^c$ (7EST-38, 7EST-203, 7EST-211, 7EST-216, 7EST-218, 7EST-220, 7EST-233, 7EST-234, 7EST-213, 7EST-138) were present in DA1Y^c; bands of three markers specific for $2S^c$ (2EST-971, 2EST-983, 2EST-997) were present in DA7S^c; and bands of one marker specific for $7S^c$ (7EST-133) were present in DA2S^c. Thus, chromosome rearrangements may have occurred between groups 1, 2 and 7.

Characterization of a complete set of wheat-*R. ciliaris* DALs

Jiang et al. (1992, 1993) developed two alloplasmic wheat-*R. ciliaris* addition lines carrying chromosomes 1S^c#1 and 1Y^c#1. Wang et al. (2001) developed euplasmic DALs involving only five other chromosomes (DA2S^c#1, DA3S^c#1, DA5Y^c#1, DA7S^c#1, DA7Y^c#1). To develop a complete set of wheat-*R. ciliaris* DALs, we repeated the cross between the CS and Ik-*R. ciliaris* amphiploid, and DALs carrying the remaining seven *R. ciliaris* chromosomes were successfully identified. The complete set of wheat-*R. ciliaris* DALs including those produced by Jiang et al. (1992, 1993) and Wang et al. (2001) is described below.

DALs DA1S^c and DA1Y^c

Alloplasmic DALs DA1S^c and DA1Y^c developed by Jiang et al. (1992, 1993) were re-characterized by GISH/FISH and marker analysis. A total of 27 *R. ciliaris*-specific markers were developed based on wheat ESTs previously mapped on homoeologous group 1. PCR of a complete set of wheat-*R. ciliaris* DALs showed that these markers amplified specific amplicons only in DA1S^c and DA1Y^c; however, the amplification patterns for the two DALs were different, confirming that they both belong to homoeologous group1 but are from different genomes (Tables 3, S1). Twenty two ESTs were on $1S^c$, such as 1EST-255 (Table S1, Fig. 3a) and 3 were on $1Y^c$. 1EST-258 and 1EST-1134 had co-dominant markers that could be used to distinguish $1S^c$ and $1Y^c$ (Tables 3, 4). GISH/FISH using four probes, p*Ta794*, p*Ta71*, $(GAA)_{10}$ and RcAfa showed that $1S^c$ is metacentric (Fig. 1a) and $1Y^c$ is submetacentric (Fig. 1b); however, neither chromosome had FISH signals for probes p*Ta794* and $(GAA)_{10}$, although both had intensive RcAfa signals. $1S^c$ and $1Y^c$ had similar signal distributions at the telomeric regions (Figs. 1a, b, 4b). However, $1S^c$ was differentiated from $1Y^c$ because $1S^c$ had p*Ta71* signals on the short arm, confirming it as a satellited (SAT) chromosome (Fig. 4a). The spike morphologies of the two DALs were different. DA1S^c had similar morphology to Ik and DA1Y^c was awned and had a rod-like spike (Fig. 2).

Table 2 Analysis of molecular marker polymorphisms between wheat and *R. ciliaris*

Type of marker	Origin of the primer sequence	No. of primers	No. of polymorphic primers	Polymorphism rate (%)
EST-PCR	Wheat group 1	220	71	32.27
EST-PCR	Wheat group 2	275	49	17.80
EST-PCR	Wheat group 3	226	98	43.36
EST-PCR	Wheat group 4	267	97	36.33
EST-PCR	Wheat group 5	243	83	34.16
EST-PCR	Wheat group 6	389	102	26.22
EST-PCR	Wheat group 7	225	57	25.33
Total		1845	557	30.19

Table 3 Molecular markers specific for individual *R. ciliaris* chromosome

R. ciliaris chromosome	No. of specific markers	Name of specific markers		
		Type I	Type II	Type III
1S[c]	24	1EST-258-750 bp, 1EST-1134-370 bp	1EST-40-700 bp, 1EST-66-400 bp, 1EST-81-500 bp, 1EST-94-450 bp, 1EST-128-200 bp, 1EST-132-400 bp, 1EST-227-1200 bp, 1EST-236-800 bp, 1EST-243-1000 bp, 1EST-249-800 bp, 1EST-251-600 bp, 1EST-255- 1000 bp, 1EST-1112-500 bp, 1EST-1115-300 bp, 1EST-1119-1000 bp, 1EST-1123-1200 bp, 1EST-1132-300 bp, 1EST-1137-550 bp, 1EST-1140-750 bp, 1EST-1142-500 bp, 1EST-1146-750 bp, 1EST-1152-500 bp	
1Y[c]	5	1EST-258-650 bp, 1EST-1134-350 bp	1EST-111-750 bp, 1EST-259-400 bp, 1EST-1149-800 bp	
2S[c]	11	2EST-125-750 bp, 2EST-305-700 bp,2EST-705-750 bp, 2EST-971-310 bp, 2EST-992-600 bp, 2EST-997-450 bp, 2EST-1002-300 bp, 2EST-1008-450 bp	2EST-210-300 bp, 2EST-243-500 bp, 2EST-983-300 bp	
2Y[c]	15	2EST-125-300 bp, 2EST-305-750 bp, 2EST-705-1000 bp, 2EST-971-300 bp, 2EST-992-1300 bp, 2EST-997-1900 bp, 2EST-1002-400 bp, 2EST-1008-600 bp	2EST-58-1100 bp, 2EST-67-100 bp, 2EST-84-160 bp, 2EST-263-1800 bp, 2EST-235-200 bp, 2EST-948-550 bp, 2EST-1000-850 bp	
3S[c]	12	3EST-21-650 bp, 3EST-104-2000 bp, 3EST-118-400 bp, 3EST-124-1200 bp, 3EST-147-2000 bp, 3EST-154-600 bp, 3EST-165-1000 bp, 3EST-196-700 bp, 3EST-201-500 bp, 3EST-210-450 bp	3EST-169-450 bp, 3EST-186-500 bp	
3Y[c]	16	3EST-21-800 bp, 3EST-104-1000 bp, 3EST-118-300 bp, 3EST-124-2000 bp, 3EST-147-900 bp, 3EST-154-1700 bp, 3EST-165-750 bp, 3EST-196-600 bp, 3EST-201-300 bp, 3EST-210-550 bp	3EST-87-550 bp, 3EST-89-530 bp, 3EST-100-750 bp, 3EST-106-250 bp, 3EST-152-500 bp, 3EST-440-900 bp	
4S[c]	25	4EST-19-650 bp, 4EST-31-300 bp, 4EST-33-650 bp, 4EST-46-300 bp, 4EST-55-300 bp, 4EST-78-700 bp, 4EST-90-350 bp, 4EST-91-1500 bp, 4EST-142-450 bp, 4EST-171-300 bp, 4EST-174-410 bp, 4EST-454-750 bp	4EST-9-1000 bp, 4EST-28-500 bp, 4EST-63-200 bp, 4EST-100-400 bp, 4EST-104-500 bp,4EST-146-750 bp, 4EST-168-150 bp, 4EST-435-850 bp, 4EST-459-1500 bp, 4EST-470-600 bp, 4EST-475-450 bp, 4EST-476-1500 bp	4EST-145-400 bp
4Y[c]	20	4EST-19-350 bp, 4EST-31-500 bp, 4EST-33-750 bp, 4EST-46-650 bp, 4EST-55-750 bp, 4EST-78-750 bp, 4EST-90-600 bp, 4EST-91-1000 bp, 4EST-142-300 bp, 4EST-171-400 bp, 4EST-174-400 bp, 4EST-454-1000 bp	4EST-6-450 bp, 4EST-39-400 bp, 4EST-117-1000 bp, 4EST-124-400 bp, 4EST-173-750 bp, 4EST-450-700 bp, 4EST-474-220 bp	4EST-145-400 bp
5S[c]	9	5EST-10-450 bp, 5EST-18-250 bp, 5EST-62-1000 bp	5EST-66-800 bp, 5EST-67-600 bp, 5EST-74-450 bp, 5EST-79-700 bp, 5EST-81-600 bp, 5EST-83-1200 bp	
5Y[c]	15	5EST-10-150 bp, 5EST-18-150 bp, 5EST-62-750 bp	5EST-2-500 bp, 5EST-20-750 bp, 5EST-54-400 bp, 5EST-72-350 bp, 5EST-87-1000 bp, 5EST-85-650 bp, 5EST-1061-400 bp, 5EST-1072-700 bp, 5EST-1073-400 bp, 5EST-1074-450 bp, 5EST-1075-1500 bp, 5EST-1086-350 bp	
6S[c]	26	6EST-6-800 bp, 6EST-33-370 bp, 6EST-35-750 bp, 6EST-45-650 bp, 6EST-50-2000 bp, 6EST-53-430 bp, 6EST-54-300 bp, 6EST-75-1000 bp, 6EST-234-700 bp, 6EST-322-650 bp, 6EST-325-1000 bp, 6EST-335-350 bp, 6EST-349-450 bp, 6EST-379-300 bp, 6EST-392-2000 bp, CINAU15-700 bp	6EST-309-600 bp, 6EST-318-500 bp, 6EST-332-1500 bp, 6EST-341-1500 bp, 6EST-344-2000 bp, 6EST-358-370 bp, 6EST-371-350 bp, 6EST-389-800 bp, 6EST-402-1800 bp	6EST-306-500 bp

Table 3 (continued)

R. ciliaris chromosome	No. of specific markers	Name of specific markers		
		Type I	Type II	Type III
$6Y^c$	26	6EST-6-350 bp, 6EST-33-580 bp, 6EST-35-600 bp, 6EST-45-750 bp, 6EST-50-2000 bp, 6EST-53-450 bp, 6EST-54-400 bp, 6EST-75-1050 bp, 6EST-234-450 bp, 6EST-322-1500 bp, 6EST-325-1200 bp, 6EST-335-450 bp, 6EST-349-400 bp, 6EST-379-280 bp, 6EST-392-1000 bp,CINAU15-720 bp	6EST-317-750 bp, 6EST-327-1000 bp, 6EST-339-350 bp, 6EST-352-400 bp, 6EST-357-450 bp, 6EST-367-400 bp, 6EST-373-200 bp, 6EST-385-500 bp, 6EST-387-1700 bp	6EST-306-500 bp
$7S^c$	3	7EST-213-300 bp	7EST-133-500 bp	7EST-138-500 bp
$7Y^c$	10	7EST-213-600 bp	7EST-38-2000 bp, 7EST-203-500 bp, 7EST-211-800 bp, 7EST-216-300 bp, 7EST-218-500 bp, 7EST-220-350 bp, 7EST-233-800 bp, 7EST-234-550 bp	7EST-138-500 bp

Type I: co-dominant markers; Type II: markers only specific for S or Y; Type III: markers common for S and Y

DALs $DA2S^c$ and $DA2Y^c$

Eighteen *R. ciliaris*-specific markers were developed based on homoeologous group 2 ESTs of wheat. Specific amplicons from these markers were produced only in $DA2S^c$ (Wang et al. 2001) and line 2014-2015K108 with $2n = 44$. However, their amplification patterns were different indicating that the added chromosomes in 2014-2015K108 belonged to homoeologous group 2, and could be designated as $2Y^c$ (Tables 3, S1). Three of the 18 markers were $2S^c$-specific, 7 were $2Y^c$-specific, and the remaining 8 were co-dominant, such as 2EST-705 (Table S1, Fig. 3b). GISH/FISH showed that $2Y^c$ is metacentric (Fig. 1d). Chromosomes $2S^c$ and $2Y^c$ showed different FISH patterns with two repetitive DNA probes. $2S^c$ had RcAfa and $(GAA)_{10}$ signals at the centromeric regions of the long arm (Figs. 1c, 4b). $2Y^c$ had RcAfa signals at the telomeric regions of both arms, and $(GAA)_{10}$ signals were present only at the distal region of the long arm (Figs. 1d, 4b). Plants carrying $DA2S^c$ and $DA2Y^c$ were slender and the spikes had tenacious glumes (Fig. 2) that are typical for alien group-2 chromosome addition lines.

DALs $DA3S^c$and $DA3Y^c$

Eighteen *R. ciliaris*-specific markers were developed based on homoeologous group 3 ESTs of wheat. Specific amplicons were amplified in $DA3S^c$ (Wang et al. 2001) and line 2014-2015K132 ($2n = 44$) and showed a different amplification pattern from $DA3S^c$. This indicated that the added chromosome also belonged to homoeologous group 3, but belonged to the Y^c genome. Two markers were $3S^c$ specific, such as 3EST-186 (Table S1, Fig. 3c), 6 were $3Y^c$-specific, and 10 were co-dominant, such as 3EST-147 (Table S1, Fig. 3d). GISH/FISH showed that $3Y^c$ is submetacentric (Fig. 1f). Chromosomes $3S^c$ and $3Y^c$ had different FISH patterns for two repetitive DNA probes; $3S^c$ had no $(GAA)_{10}$ signal, and the RcAfa signals were detected at the telomeric region of the long arm (Figs. 1e, 4b). In contrast, $3Y^c$ had no RcAfa signal, but had a pair of dispersed $(GAA)_{10}$ signals at the centromeric region of the long arm (Figs. 1f, 4b). The spike of $DA3S^c$ has long awns, and $DA3Y^c$ has a similar spike structure to CS (Fig. 2).

DALs $DA4S^c$ and $DA4Y^c$

Thirty two *R. ciliaris*-specific markers were developed based on homoeologous group 4 ESTs of wheat. Specific amplicons were amplified in lines 2014-2015K271 and 2014-2015K247, both of which had $2n = 44$. The two lines showed different amplification patterns for these markers, indicating that the added chromosomes in both lines belonged to homoeologous group 4, but were from different genomes (Tables 3, S1). Twelve markers were specific for

Table 4 Evaluation of Ik-*R.c* amphiploid and DALs for reaction to FHB

Materials	Percentage of diseased spikelets(%)					FHB response
Year	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017	
Sumai 3	**1.58**	**6.00**	**2.5**	**1.97**	**8.67**	**R**
Mianyang 8545	36.33	64.3	47.35	62.44	27.81	S
Ik-*R.c* amphiploid	**1.44**	**1.89**	**2.03**	**7.18**	**ND**	**R**
Chinese Spring	15.19	33.2	39.7	48.31	10.49	S
Inayama Komugi	50.3	91.21	89.9	94.56	91.84	S
Disomic addition (DA)1S^c (15-16K03)	ND	ND	4.07	1.46	8.57	R
DA1Y^c(15-16K09)	4.68	7.19	4.36	5.96	22.2	MR
DA2S^c(15-16K16)	31.85	67.39	36.95	65.69	56.73	S
DA2Y^c(15-16K26)	**11.27**	**5.56**	**6.03**	**9.77**	**5.86**	**R**
DA3S^c(15-16K30)	1.45	ND	1.89	1.43	39.78	MR
DA3Y^c(15-16K631)	ND	ND	78.2	94.1	94.64	S
DA4S^c(15-16K58)	7.53	8.29	3	2.95	56.03	MR
DA4Y^c(15-16K59)	50.5	ND	ND	72.22	52.11	S
DA5S^c(15-16K69)	44.98	48.62	42.25	74.56	3.2	S
DA5Y^c(15-16K72)	**3.19**	**5.5**	**5.13**	**5.27**	**4.7**	**R**
DA6S^c(15-16K80)	**6.45**	**2.35**	**2.49**	**7.56**	**15.8**	**R**
DA6Y^c(15-16K81)	56.72	58.5	68.18	80.31	47.63	S
DA7S^c(15-16K85)	31.85	14.3	42.14	84.97	3.81	S
DA7Y^c(15-16K86)	ND	27.58	58.86	ND	79.84	S

Lines that have shown high and stable resistance to FHB during 2012–2017 are represented in bold

R resistant, *MR* moderate resistant, *S* susceptible, *ND* no data

the alien chromosome in 2014-2015K271, such as 4EST-100 (Table S1, Fig. 3e), 7 were specific for the alien chromosome in 2014-2015K247, and 12 were co-dominant amplifying polymorphic bands in both lines, such as 4EST-19 (Table S1, Fig. 3f). Marker 4EST-145 was amplified similar specific bands in 4S^c and 4Y^c (Table 3). GISH/FISH showed that the alien chromosomes in 2014-2015K271 and 2014-2015K247 were submetacentric and metacentric, respectively. They showed different FISH patterns with two repetitive DNA probes. The alien chromosome pair in 2014-2015K271 had rich RcAfa signals distributed on both arms and its $(GAA)_{10}$ signals were located at the centromeric regions (Figs. 1g, 4b). However, the alien chromosome pair in 2014-2015K247 had RcAfa signals at the telomeric region of the short arm and $(GAA)_{10}$ signals mainly located at centromeric regions of the short arm (Figs. 1h, 4b). Based on FISH patterns from the $(GAA)_{10}$ and RcAfa probes, the signals were richer in the previously assigned S^c genome chromosomes (1S^c, 2S^c, 3S^c) than the homoeologous partners (1Y^c, 2Y^c, 3Y^c). We thus designated the alien chromosomes in lines 2014-2015K271 and 2014-2015K247 as DA4S^c and DA4Y^c, respectively. Both DA4S^c and DA4Y^c were tip-awned and had square spikes. The upper spikelets of DA4Y^c had low fertility (Fig. 2).

DALs DA5S^c and DA5Y^c

Twenty one *R. ciliaris*-specific markers were developed based on homoeologous group 5 ESTs of wheat. Specific amplicons could be amplified only in DA5Y^c (Wang et al. 2001) and line 2014-2015K125 with $2n = 44$. Line 2014-2015K125 showed a different amplification pattern than DA5Y^c, indicating that the added chromosomes also belonged to homoeologous group 5, but represented a different genome and, therefore, could be designated as 5S^c. Six markers were 5S^c specific, such as 5EST-79 (Table S1), 12 were 5Y^c specific, and 3 were co-dominant, such as 5EST-10 (Table S1, Fig. 3g). GISH/FISH showed that 5S^c is submetacentric (Fig. 1i). Chromosomes 5S^c and 5Y^c had different FISH patterns for two repetitive DNA probes; 5S^c had RcAfa signals at the distal regions of the short arms and $(GAA)_{10}$ signals were located at the centromeric regions of the long arm. The presence of p*Ta71* signals on the short arm of chromosome 5S^c revealed that it is another SAT chromosome (Figs. 1i, 4a). Chromosome 5Y^c has the largest arm ratio and can be differentiated from other *R. ciliaris* chromosomes by the presence of $(GAA)_{10}$ signals at the centromeric region and p*Ta794* signals at the distal region of the short arm (Figs. 1j, 4c). DA5S^c has tip-awns and a spindle-shaped

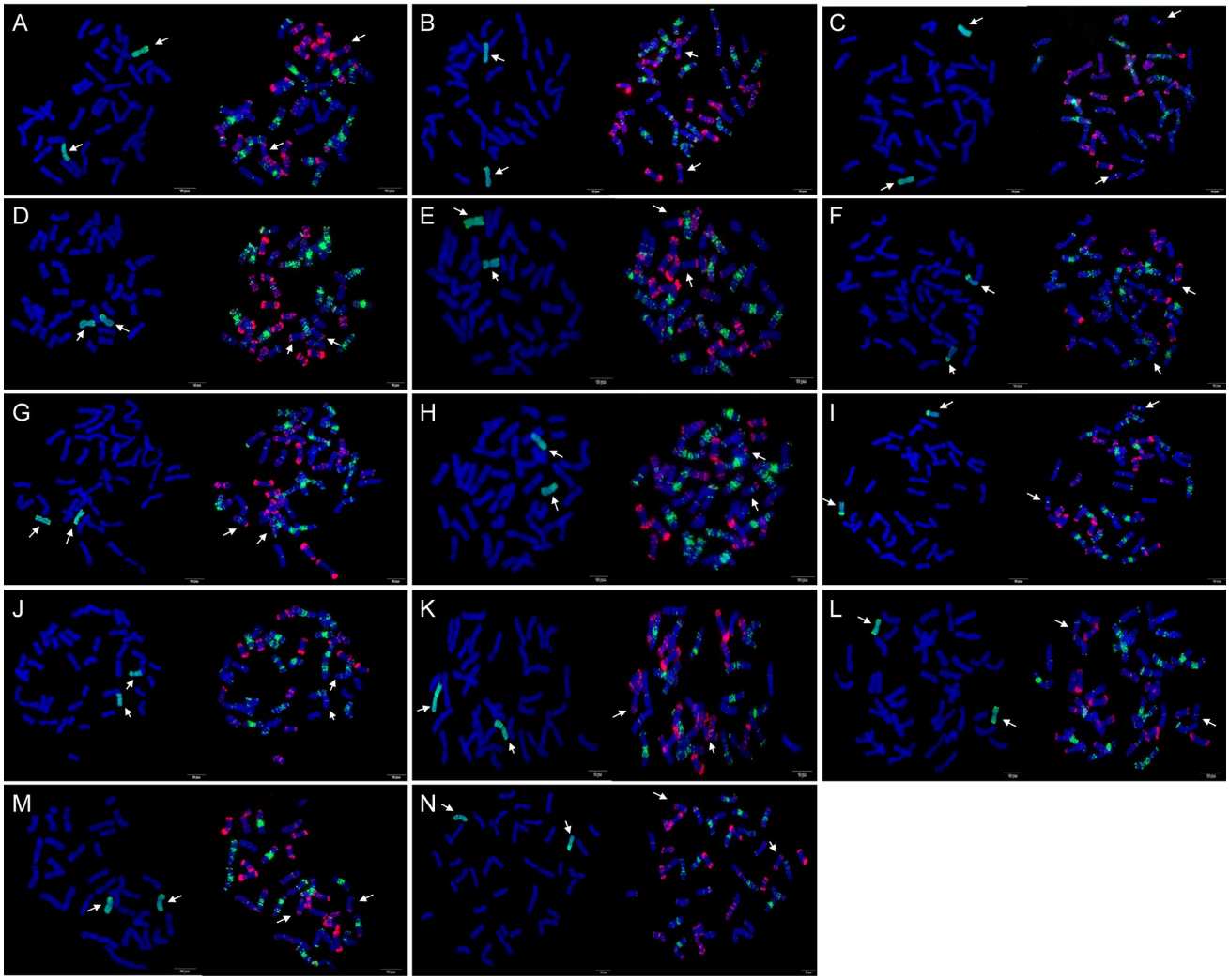

Fig. 1 Chromosome sequential GISH/FISH (right) on root tip cells of a set of wheat-*R. ciliaris* DALs. Arrows show one pair of *R. ciliaris* chromosomes using genomic DNA of *R. ciliaris* (green), RcAfa (red) and $(GAA)_{10}$ (green) as probes. **a**–**n** are GISH (left) and FISH (right) for addition lines DA1S^c, DA1Y^c, DA2S^c, DA2Y^c, DA3S^c, DA3Y^c, DA4S^c, DA4Y^c, DA5S^c, DA5Y^c, DA6S^c, DA6Y^c, DA7S^c and DA7Y^c, respectively. Scale bars, 10 µm

spike, and DA5Y^c has a long loose spike with low spikelet density (Fig. 2).

DALs DA6S^c and DA6Y^c

Thirty five *R. ciliaris*-specific markers were developed based on homoeologous group 6 wheat ESTs. Specific amplicons could be amplified only in lines 2014-2015K27 and 2014-2015K15 with $2n = 44$. The two lines showed different amplification patterns, indicating their added chromosomes belong to same homoeologous group 6, but to different genomes (Tables 3, S1). Nine markers were specific for the alien chromosomes pair in 2014-2015K27, such as 6EST-358 (Table S1), 9 were specific for the alien chromosome in 2014-2015K15, and 16 were co-dominant, such as CINAU15 (Table S1, Fig. 3h). Marker 6EST-306 amplified similar specific bands in 6S^c and 6Y^c (Table 3). GISH/FISH showed that the alien chromosomes in 2014-2015K27 and 2014-2015K15 are metacentric and submetacentric, respectively. They showed different FISH patterns with two repetitive DNA probes. The alien chromosome pair in 2014-2015K27 had similar RcAfa signal distribution to 4S^c, and no $(GAA)_{10}$ signal was detected (Figs. 1k, 4b). However, the alien chromosome pair in 2014-2015K15 had very few FISH signals, with a single $(GAA)_{10}$ signal at the middle region of the short arm (Figs. 1l, 4b). As in the case of 4S^c and 4Y^c lines 2014-2015K27 and 2014-2015K15 were designated as DA6S^c and DA6Y^c, based on the abundance of the FISH signals. DA6S^c has a unique short compact spike morphology compared with other DALs whereas DA6Y^c has a long speltoid spike (Fig. 2).

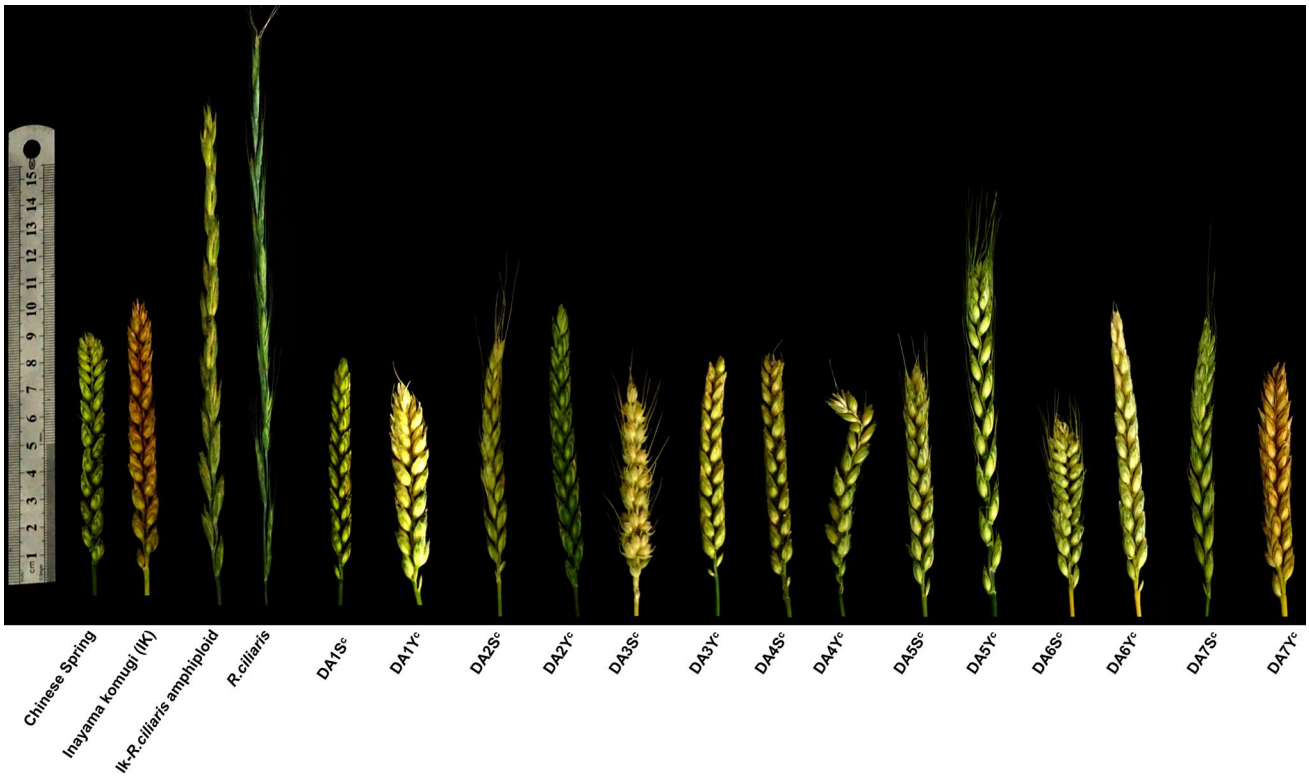

Fig. 2 Spike morphology of a complete set of wheat-*R. ciliaris* DALs. CS: Chinese spring; Ik: Inayama komugi; Amphiploid: Ik–*R.c* amphiploid; *R.c*: *R. ciliaris*; 5–18: DA1S^c, DA1Y^c, DA2S^c, DA2Y^c, DA3S^c, DA3Y^c, DA4S^c, DA4Y^c, DA5S^c, DA5Y^c, DA6S^c, DA6Y^c, DA7S^c and DA7Y^c

DALs DA7S^c and DA7Y^c

DA7S^c and DA7Y^c were developed by Wang et al. (2001). The two lines were further characterized by molecular marker and FISH analysis. A total of 11 *R. ciliaris*-specific markers were developed based on homoeologous group 7 ESTs of wheat. Specific amplicons were amplified in DA7S^c and DA7Y^c (Tables 3, S1). The two lines showed different amplification patterns, confirming the added chromosomes belonged to the same homoeologous group, but different genomes. 7EST-133 was the only marker allocated to 7S^c, 8 markers were allocated to 7Y^c, such as 7EST-234 (Table S1), and 7EST-213 was a co-dominant marker, which could distinguish 7S^c and 7Y^c in a single amplification (Table 3). Marker 7EST-138 amplified the same specific bands in 7S^c and 7Y^c (Table S1, Fig. 3i). GISH/FISH revealed that chromosome 7S^c had RcAfa signals and very weak $(GAA)_{10}$ signals at the telomeric regions of the long arm (Figs. 1m, 4b). Chromosome 7Y^c had more intensive RcAfa signals at the distal regions of both arms (Figs. 1n, 4b). DA7S^c had tip-awns and a slender spike, whereas DA7Y^c has a spindle-shaped spike (Fig. 2).

The standard FISH-based karyotype of *R. ciliaris* chromosomes

Four repetitive DNA sequences [p*Ta794*, p*Ta71*, RcAfa and $(GAA)_{10}$] were used as probes for FISH analysis. Based on the FISH patterns of the added alien chromosomes in 14 wheat-*R. ciliaris* DALs, we established a FISH-based karyotype of *R. ciliaris* chromosomes (Fig. 4d).

FISH using p*Ta71* as probe showed that there are two SAT chromosomes in *R. ciliaris*. The NOR of 1S^c was at the middle of the short arm and the NOR of 5S^c was in the telomeric region of its short arm; this chromosome pair also had less intensive signals on the long arms. The two chromosomes can be distinguished from other *R. ciliaris* chromosomes based on this feature (Fig. 4a). The presence of p*Ta794* FISH signals on the short arm is a unique character for identifying 5Y^c (Fig. 4c).

FISH using two repetitive DNA probes showed that RcAfa signaling is much more abundant than $(GAA)_{10}$ on *R. ciliaris* chromosomes. RcAfa signals were distributed on 11 *R. ciliaris* chromosomes, except 3Y^c, 5Y^c and 6Y^c. Most signals were located at the telemetric regions on both arms of 1S^c, 4S^c, 6S^c, 1Y^c, 2Y^c and 7Y^c, on the short arms

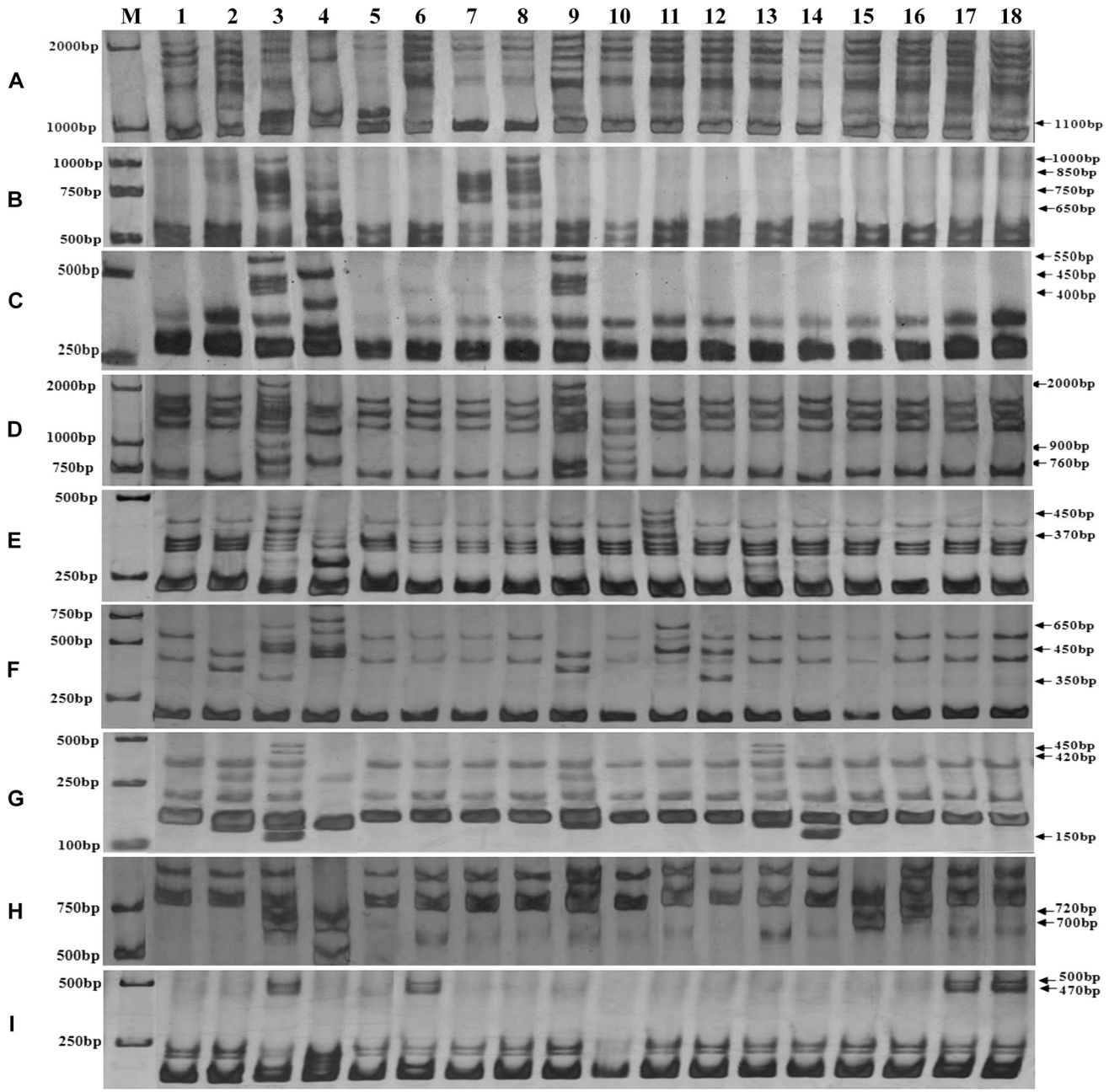

Fig. 3 Amplification of specific markers in a set of wheat-*R. ciliari* DALs and their parental lines. Electrophoresis of **a**: 1EST-255; **b**: 2EST-705; **c**: 3EST-186; **d**: 3EST-147; **e**: 4EST-100; **f**: 4EST-19; **g**: 5EST-10; **h**: CINAU15; **i**: 7EST-138. Arrows show specific bands of *R. ciliaris*. **m**: DNA ladder DL2000; **1**, Chinese Spring; 2, Ik: Inayama komugi; 3, Ik-*R.ciliaris* amphiploid; 4, *R. ciliaris*; 5, DA1S^c; 6, DA1Y^c; 7, DA2S^c; 8, DA2Y^c; 9, DA3S^c; 10, DA3Y^c; 11, DA4S^c; 12, DA4Y^c; 13, DA5S^c; 14, DA5Y^c; 15, DA6S^c; 16, DA6Y^c; 17, DA7S^c; 18, DA7Y^c

of 4S^c and 4Y^c, and on the long arms of 3S^c and 7S^c. The signals of 2S^c were located at the pericentric region of the long arm. In general, FISH signals from RcAfa were more intense in genome S^c than in Y^c. However, we found that 2Y^c had more RcAfa signals than 2S^c, and 7Y^c had more than 7S^c (Fig. 4b).

$(GAA)_{10}$ signals were distributed on 9 *R. ciliaris* chromosomes. The signals were mainly located at the centromeres or pericentromeric regions. Signals on 2Y^c and 7S^c were located at the telomeres of the long arms; those of 2S^c, 3Y^c, 5S^c and 5Y^c were at centromeric regions of the long arms; those of 4Y^c and 6Y^c were at centromeric regions of the

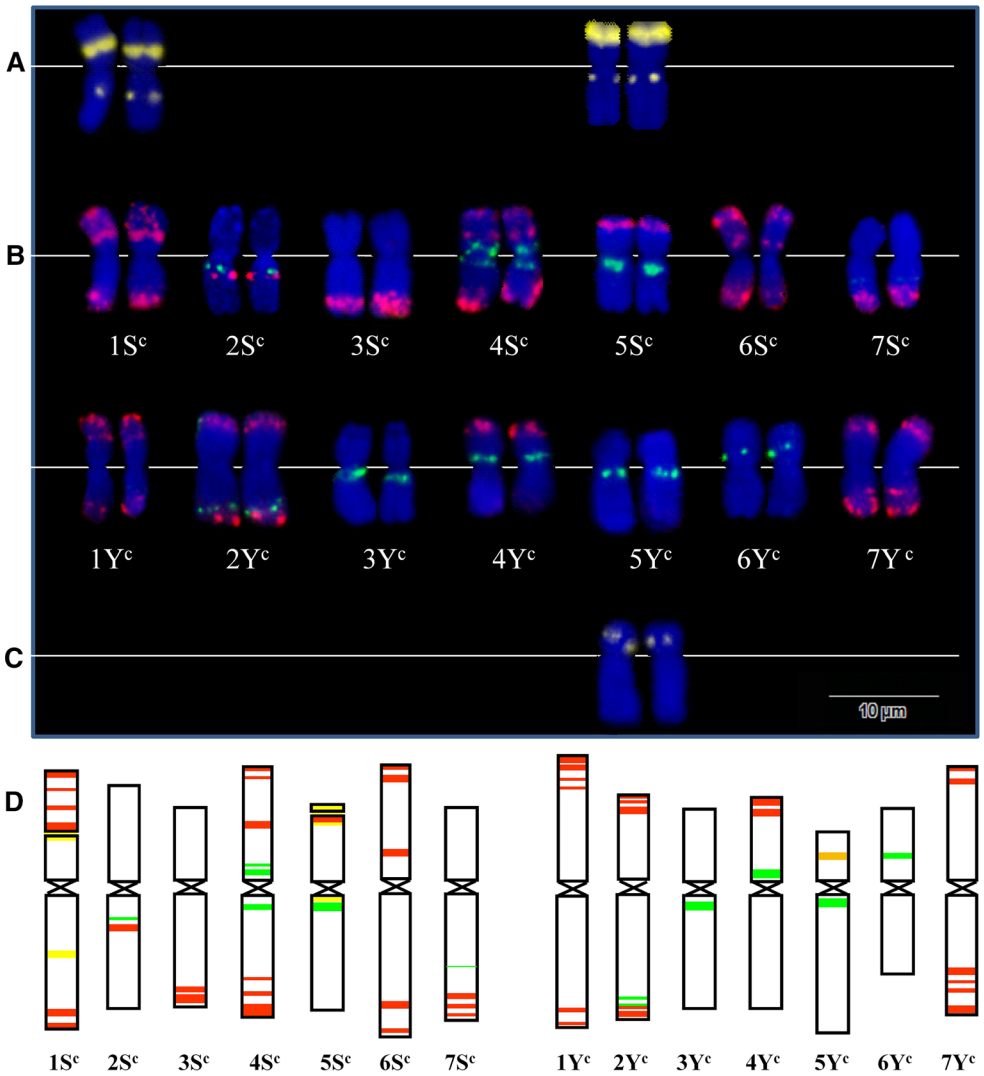

Fig. 4 FISH-based karyotype of *R. ciliaris* chromosomes. **a** FISH pattern of *R. ciliaris* chromosomes $1S^c$ and $5S^c$ using p*Ta71* (yellow) as probes. **b** Arranged *R. ciliaris* chromosomes after FISH using RcAfa (red) and $(GAA)_{10}$ (green) repetitive DNA as probes. **c** FISH pattern of *R. ciliaris* chromosome $5Y^c$ using p*Ta794* (yellow) as probe. Scale bar, 10 µm. **d** Idiogram of FISH-based karyotype of *R. ciliaris* chromosomes showing the distribution of RcAfa (red), $(GAA)_{10}$ (green), p*Ta71* (yellow) and p*Ta794* (orange) signals

short arms, and those of $4S^c$ were located at the centromeric regions.

To summarize, 12 *R. ciliaris* chromosome pairs can be unambiously distinguished from each other by sequential GISH/FISH using four probes. However, $1Y^c$ and $7Y^c$ showed similar FISH patterns and differentiation required marker analysis.

Roegneria ciliaris is a useful genetic resource for Fusarium head blight resistance

To evaluate the potential use of *R. ciliaris*, the Inayama komugi-*R. ciliaris* amphiploid and complete set of DALs were evaluated for reaction to FHB over 5 successive growth seasons (2012–2017). Compared with the background varieties (Ik and CS) and susceptible control (Mianyang 8545), the amphiploid and three DALs ($DA2Y^c$, $DA5Y^c$ and $DA6S^c$) showed stable FHB resistance (Table 4) confirming that *R. ciliaris* is a useful genetic resource for FHB resistance and that the above three chromosomes may confer resistance. The amphiploid showed a similar level of resistance to Sumai 3, the resistant control. However, the three DALs were less resistant than the amphiploid, indicating that the full resistance of *R. ciliaris* is controlled by the additive effects of genes located on three chromosomes. Crosses between these DALs and FHB susceptible varieties have been made to confirm the effects of these chromosomes in different wheat backgrounds.

Discussion

Transferring desirable genes from wild relatives into common wheat has been an important strategy in wheat breeding since the last century. Genes conferring genes of interest have been introduced into wheat through wide hybridization and chromosome engineering. Wheat–alien chromosome addition lines are important bridge materials for alien gene

transfer. In addition, complete sets of wheat–alien DALs are valuable materials for comparative genome analysis between wheat and wild relatives, and for investigating the origin and evolution of different genomes (Du et al. 2014; Friebe et al. 2000).

Roegneria ciliaris is a tetraploid species that possesses resistance or tolerance to various biotic and abiotic stresses (Sharma et al. 1984; Weng and Liu 1989; Chang et al. 2011). Although a complete set of wheat-*R. ciliaris* DALs are critical for exploring novel genes of interest and for allocating them to specific chromosomes, this has been hindered by the presence of alloplasmic effects (Jiang et al. 1992, 1993) and lack of efficient methods for determining the identities of alien chromosomes in the wheat background (Wang et al. 2001). This has been especially difficult in the case that *R. ciliaris*, which is an allotetraploid species.

Taking advantage of the huge numbers of EST sequences with known chromosome locations and high conservation between homoeologous chromosomes from different genomes of Triticeae species, we developed 162 EST-based PCR markers specific for *R. ciliaris* (Chen et al. 2003; Li et al. 2011). These markers not only helped us to determine the identities of the alien chromosomes added to wheat, but also enabled the determination of their homeologies with wheat chromosomes. Homoeologous chromosomes from the two genomes were differentiated from each other and from wheat chromosomes in a single-round PCR. For example, PCR using 3EST-147 produced three specific bands (2000, 900 and 760 bp), which were present only in the amphiploid, DA3S^c and DA3Y^c. DA3S^c and DA3Y^c shared the same 760 bp amplicon, whereas the 2000 and 900 bp amplicons were specific for 3S^c and 3Y^c, respectively (Fig. 3d). These markers provided a rapid and robust approach for identifying and tracing each *R. ciliaris* chromosome in the wheat background.

All the molecular markers were developed according to ESTs with known chromosome locations. Marker analysis generally revealed parallel homoeologous relationships between *R. ciliaris* and wheat chromosomes (Table S2). However, we observed exceptions for chromosomes 1Y^c, 2S^c, 7S^c and 7Y^c. The specific bands of five markers for 1Y^c were also observed in DA7S^c and DA7Y^c, and specific bands of ten markers for 7S^c and 7Y^c were observed in DA1Y^c. Similar phenomena occurred for chromosomes 2S^c and 7S^c. Since all the EST-based markers were derived from the coding sequences, we suggest that during the evolution of *R. ciliaris* there were multiple structural rearrangements involving chromosomes 1Y^c and 7S^c/7Y^c, and between 2S^c and 7S^c.

Up to now, the origin of the Y genome and its relationship with the S genome was an unsolved issue. Chromosome pairing analysis showed that the St and Y genomes had low affinity (Liu et al. 2006; Lu and Von Bothmer, 1989). However, Liu et al. (2006) preferred the explanation that St and Y may have the same origin based on ITS sequence analysis. Zhang et al. (2009) suggested that the Y genome originated from the St genome. This was inferred from a gene encoding plastid acety1-CoA carboxylase. Lei et al. (2016) also suggested that Y genome is closely related to the St genome; however, our data do not support the same origin for the two genomes. Molecular marker analysis showed that, among the 162 *R. ciliaris*-specific markers, only 3 (4EST-145, 6EST-306 and 7EST-138) produced the same size amplicons for the S and Y genomes, indicating the presence of diversification of the two genomes during evolution. There were fewer co-dominant markers than genome-specific markers in homoeologous groups 1, 5 and 7. However, the numbers of the marker pairs in groups 2, 3, 4, 6 were not very different.

Some workers suggested that St and Y have high affinity (Lu and Bothmer 1989, 1990, 1991; Lu et al. 1990) making it difficult to distinguish homoeologous chromosomes from the two genomes. Morris and Gill (1987) investigated the genomic affinities of individual chromosomes based on chromosome C- and N-banding of species including tetraploid *Elymus* and their diploid progenitor. Both the S and Y genomes had very few N-bands, but abundant C-bands. No significant difference was observed for the two genomes. In Triticeae species, tandem repeated sequences have been used as cytogenetic markers for chromosome identification (Badaeva et al. 2010; Mukai et al. 1992) and characterization of alien addition, substitution and translocation lines (Qi et al. 2010; Yuan and Tomita 2009). The conserved p*Ta794* and p*Ta71* FISH signals were frequently detected on homoeologous group 1 and group 5 chromosomes in Triticeae species (Mukai et al. 1992). In *R. ciliaris*, p*Ta71* signals were detected on 1S^c and 5S^c, and p*Ta794* loci were located on 5Y^c. The major loci of p*Ta71* were on the short arms of 1S^c and 5S^c, and minor loci were on the long arms. Liu et al. (2017) reported p*Ta794* FISH signals on several chromosome pairs, but this may be due to polymorphisms between different accessions.

Tsujimoto and Gill (1991) developed several genome-specific clones and proposed that pCbTaq4.14 is H genome specific and pPlTaq2.5 is S genome specific. Wang et al. (2001) used these clones for FISH to characterize wheat-*R. ciliaris* alien chromosome lines. They found that even though there were some differences in hybridization signal distribution on chromosomes from different genomes, it was still difficult to assign each chromosome to a specific genome. Recently, Wang et al. (2017) developed a new FISH marker for S^t genome St_2-80. FISH using this probe in *R. ciliaris* showed that 14 of the chromosomes showed S^t-type signal patterns. This probe may be useful to differentiate the two genomes; however, due to lack of the complete set of alien addition lines, they failed to determine the homoeologous group of each chromosome.

We established a FISH karyotype of *R. ciliaris* by FISH using $(GAA)_{10}$ and RcAfa as probes. Compared with the Y^c genome, the S^c genome chromosomes have more intensive RcAfa FISH signals. However, the chromosomes we designated as $2S^c$ and $7S^c$ have less RcAfa signals than their corresponding $2Y^c$ and $7Y^c$. Therefore, we propose that their genome affinities should be inverted, i.e., $2S^c$ should be $2Y^c$, $7S^c$ should be $7Y^c$, and vice versa. The distribution of RcAfa FISH signals on S^c genome chromosomes is similar to D genome chromosomes, hinting a closer relationship of the S and D genomes. This is consistent with the conclusion of Liu et al. (2007). If this is true, we can differentiate the homoeologous chromosomes from the two genomes by FISH using RcAfa as probe. However, this needs to be further validated by FISH by the development of S^c- or Y^c-genome specific probes.

The distribution of GAA in Triticeae species is considered to correspond with the distribution of N-bands, which are mainly composed of heterochromatin (Pedersen and Langridge 1997). C- and N-banding analysis in tetraploid species of *Elymus* and their diploid progenitors have demonstrated that the S^c chromosomes are characterized by the presence of rich terminal C-bands on one or both arms and absence of N-bands, whereas the Y chromosomes are characterized by the presence of centromeric C- and N-bands on most chromosomes (Morris and Gill 1987). Our results showed that $(GAA)_{10}$ signals were distributed on four S^c and five Y^c chromosomes. We failed to observe a correlation between C- or N-bands with the $(GAA)_{10}$ signal distribution.

With the help of a complete set of DALs, the genome affinity and homoeologous group of each alien chromosome was determined. This information is basic and also critical for further alien gene transfer. There have been reports of several valuable traits in *R. ciliaris* for wheat improvement. Five successive years of evaluation of FHB reaction indicated that introduction of genes on at least three chromosomes could improve FHB resistance in wheat. We also observed diverse morphological changes of the DALs when compared with the wheat recipient variety, indicating potential value for crop improvement. The DALs will be further phenotyped for resistances to biotic and abiotic stresses, grain quality and other agronomic traits. DALs conferring useful traits will used as initial materials for the development of alien translocations, which can be used in wheat breeding and for gene mining.

Author contribution statement XEW and HYW designed the project, LNK and XYS performed the experiments, JX designed the primer pairs, HJS designed the FISH probes, KLD performed part of the marker analysis, CXL, PS and RS evaluated FHB response, CXY and SZZ analyzed the morphological characters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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