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Molecular cytogenetic identification of wheat-*Elymus tsukushiense* introgression lines

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Summary

Elymus tsukushiense Honda (syn. *Roegneria kamoji* C. Koch) (2n = 6x = 42, $S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}$) is a hexaploid species, distantly related to bread wheat *Triticum aestivum* L. em Thell (2n = 6x = 42, AABBDD). Apart from the delineation of evolutionary relationships, this species is a potential source of resistance to scab, a devastating disease of wheat caused by *Fusarium graminearum* Schw. A standard C-banded karyotype was established identifying all 21 chromosome pairs of *E. tsukushiense*. By using C-banding and genomic *in situ* hybridization analyses, three wheat-*E. tsukushiense* chromosome addition lines, one ditelosomic addition line, and one disomic substitution line were identified in BC_2 progenies from wheat × *E. tsukushiense* hybrids. Twenty DNA markers specific for the seven homoeologous groups of the *Triticeae* were used to determine the homoeology of the added *E. tsukushiense* chromosomes. The *E. tsukushiense* chromosomes in the addition lines NAU702, NAU703, and NAU701 were identified as belonging to homoeologous groups 1, 3, and 5, and thus, were designated as 1Ets#1, 3Ets#1, and 5Ets#1, respectively. NAU751 was identified as a disomic substitution line with chromosome 3A of wheat replaced by chromosome 3Ets#1. Line NAU702 has a high level of resistance to scab and will be used in chromosomal engineering and development of improved wheat germplasm for scab resistance breeding.

Introduction

The perennial genus *Elymus*, comprised of about 150 species, is among the largest and most widely distributed genus within the *Triticeae* (Dewey, 1984). The nomenclature and evolutionary relationships of these species are still under discussion (Barkworth, 1992; Salomon et al., 1997). *Elymus tsukushiense* Honda (2n = 6x = 42, $S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}$, syn. *Roegneria kamoji* C. Koch) is a cross-pollinating hexaploid species native to the south-eastern and central parts of China, Korea, and Japan. *E. tsukushiense* is a distant relative of bread wheat, *Triticum aestivum* L. em. Thell (2n = 6x = 42, AABBDD), and an excellent source for resistance to wheat scab, caused by *Fusarium graminearum* Schw *(Gibberella zeae* Petch), a damaging disease in most parts of the world (Weng et al., 1989).

In order to transfer scab resistance to bread wheat, *E. tsukushiense* was reciprocally crossed with wheat and after two backcrosses a number of putative introgression lines were obtained (Weng & Liu., 1995; Wang et al., 1995; Wu et al., 1997).

C-banding and genomic *in situ* hybridization (GISH) analyses were used to identify alien chromatin in a wheat background (for review, see Friebe et al., 1996). Restriction fragment length polymorphism (RFLP) analysis was used to determine the genetic relationship of alien chromosomes with those of wheat (Sharp et al., 1989; Chen et al., 1994; Qi et al., 1997). In the present study, we established a standard karyotype of *E. tsukushiense* and used C-banding, GISH, and RFLP analyses for the cytogenetic characterization of wheat-*E. tsukushiense* introgression lines.

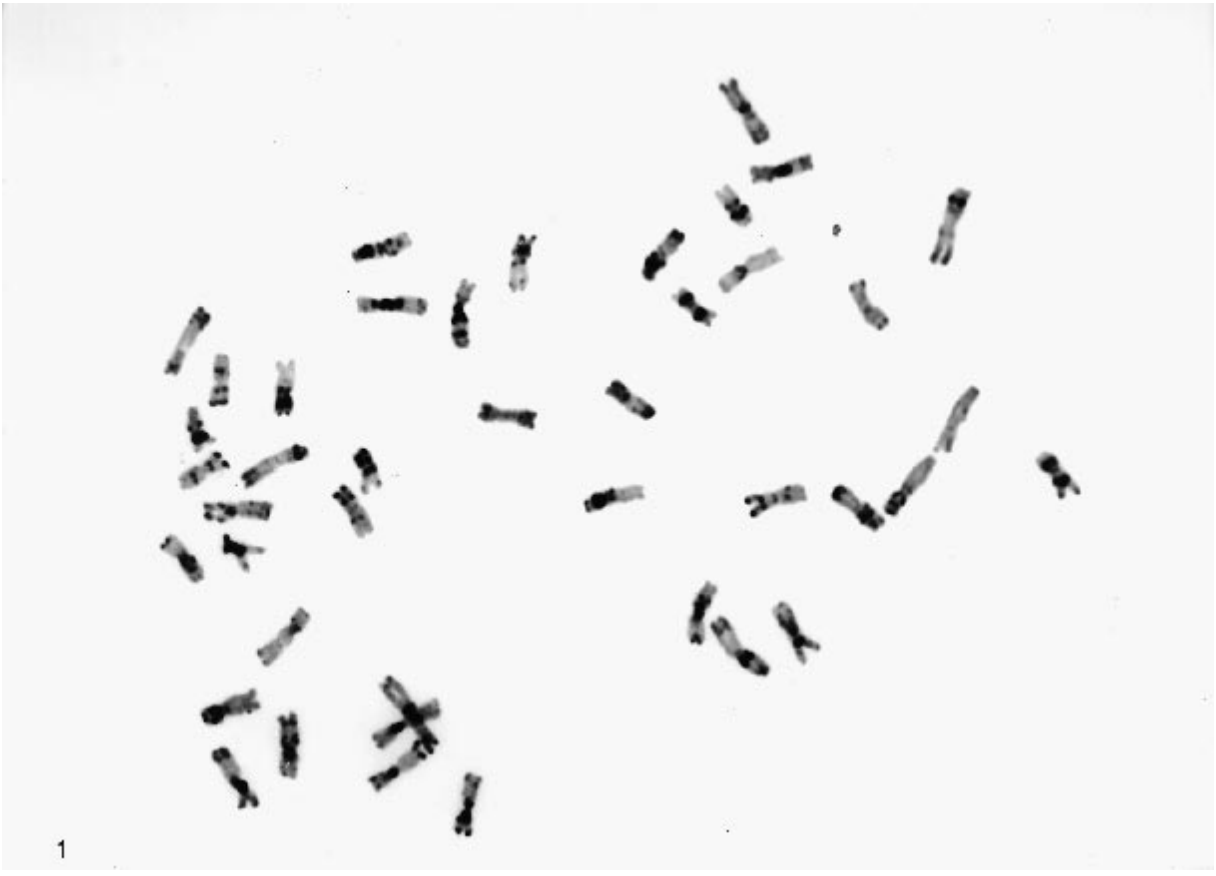

Figure 1. C-banded mitotic metaphase cell of *E. tsukushiense*.

Materials and methods

Elymus tsukushiense and *Elymus ciliaris* accessions were collected on the campus of Nanjing Agricultural University, China, and tested for resistance to scab as described by Weng et al. (1989). The production and preliminary identification of *Triticum aestivum* L. em. Thell cv. Chinese Spring (CS) × *E. tsukushiense* introgressive lines were reported previously (Weng et al., 1995; Wang et al., 1995; Wu et al., 1997). Eighteen derivatives were chosen on the basis of chromosome number, plant morphology, meiotic chromosome pairing, and C-banding analysis.

The standard C-banding protocol (Gill B.S. et al., 1991) was used for chromosome identification. For chromosome pairing analysis, anthers at meiotic metaphase I (MI) were fixed in ethanol/glacial acetic acid (3:1) for several days, and squashed in 1% acetocarmine. Pairing configurations were observed with a Zeiss microscope and documented using Kodak Imagelink HQ film 1461. The *E. tsukushiense* chromosomes were designated with letters from A to U, because their homoeology with wheat is unknown. The designation of the genetically identified *E. tsukushi-*

Table 1. DNA-markers used for identifying homoeology of *E. tsukushiense* chromosomes added to wheat

Group	CLone	Group	Clone
1S	PSR596	4L	PSR104
1L	PSR544	5S	PSR929
2S	PSR126	5S	PSR628
2S	BCD433	5L	BCD1088
2L	BCD240	6S	PSR113
3S	KSUA6	6S	CDO1158
3S	BCD589	6L	CDO497
3L	PSR578	7S	BCD387
3L	PSR926	7L	PSR311
4S	PSR584	7L	WG466

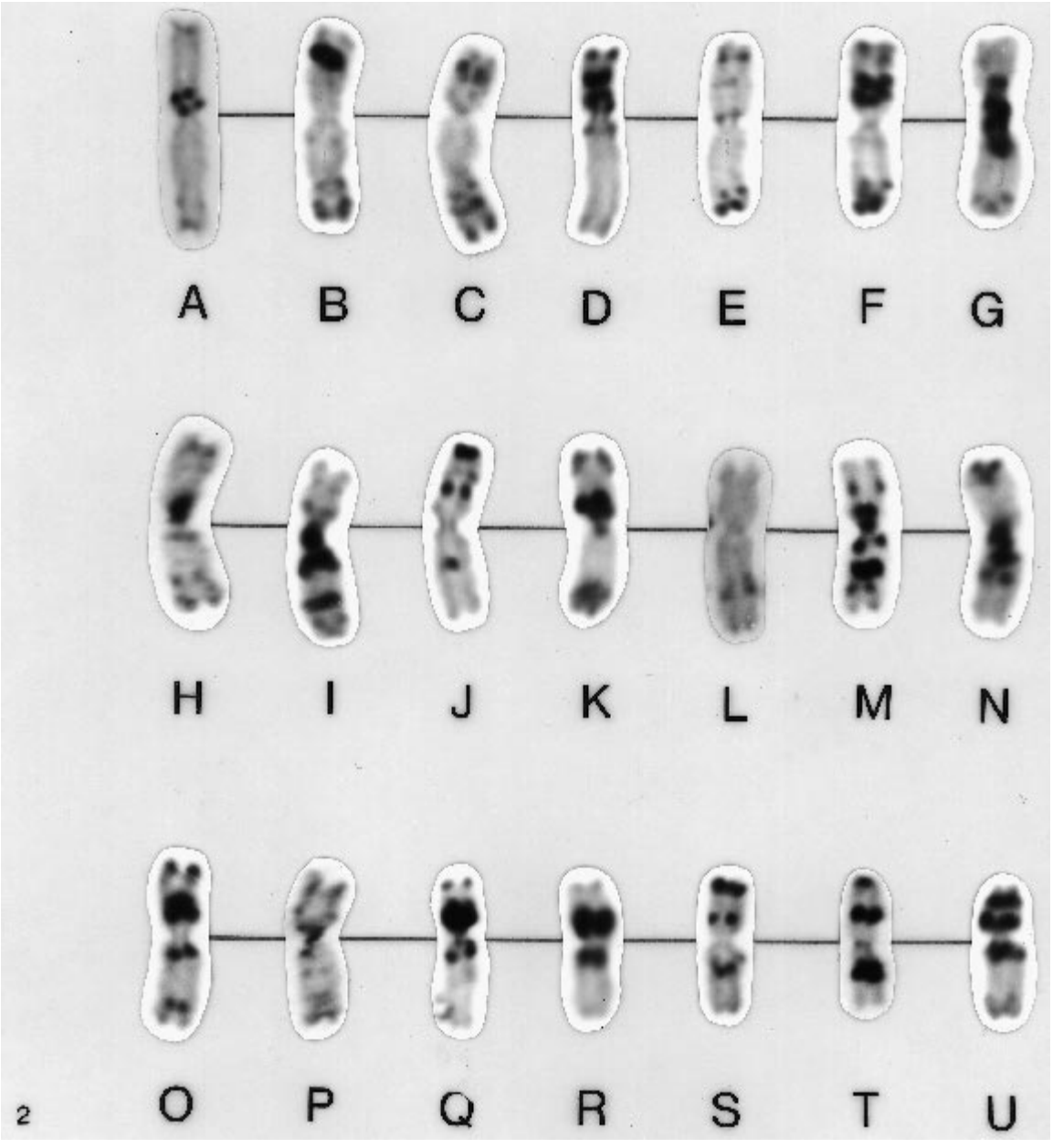

Figure 2. C-banded karyotype of *E. tsukushiense*.

ense chromosomes in the introgression lines followed the nomenclature system of Raupp et al. (1995). The first number indicates the homoeologous group, followed by the species designation (because the genomic affinity to S^{ts}, H^{ts}, and Y^{ts} is not known). The #1 sign indicates that these are the first group 1, 3, and 5 *E. tsukushiense* chromosomes identified.

For genomic *in situ* hybridization analysis, total genomic DNA of *E. tsukushiense* was labeled with biotin-11-dUTP, and genomic DNA of CS was used as blocking DNA. The hybridization signal was detected with anti-biotin-FITC, and stained with propidium iodide (Jiang et al., 1994a, b). The GISH patterns were recorded using a Sensys CCD camera. The original signals were synthesized and tinted using IPLab and Adobe Photoshop software.

For RFLP analysis, 20 clones of the seven *Triticeae* homoeologous groups were selected (Table 1). The

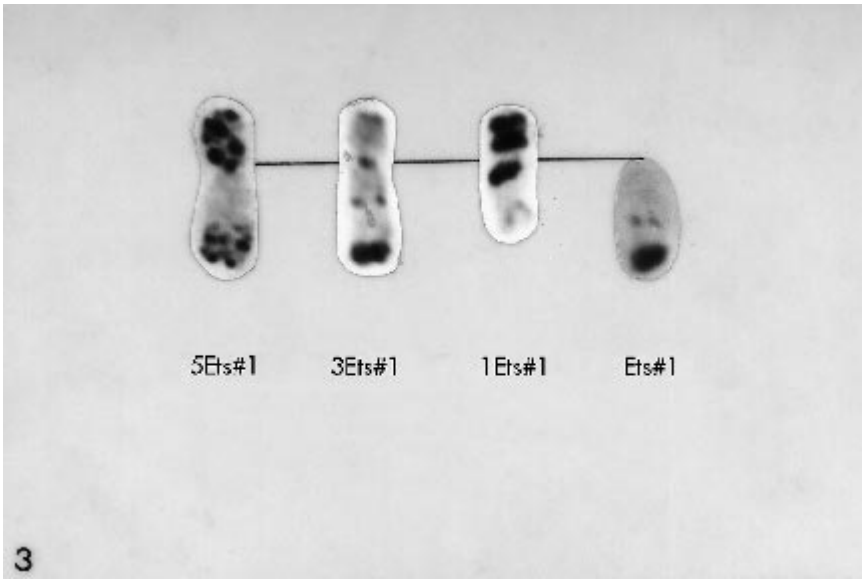

Figure 3. C-banding patterns of *E. tsukushiense* chromosome and telosomes added to wheat.

Table 2. Mean chromosome pairing per pollen mother cell (PMC) at metaphase I in wheat-*E. tsukushiense* addition and substitution lines

Line	Constitution	No. of	Chromosome pairing						tt"
		PMCs	I	Rod II	Ring II	Total II	III	IV	
NAU702 (TA7684)	DA1Ets#1 (2n = 44)	17	0.00	1.83	20.17	22.00			
NAU703 (TA7685)	DA3Ets#1 (2n = 44)	40	0.10	2.13	19.55	21.68	0.18		
NAU701 (TA7686)	MA5Ets#1 (2n = 43)	81	0.74	1.96	18.67	20.63	0.28	0.04	
NAU704 (TA7687)	Dt?Ets#1 (2n = 44tt)	13	0.00	1.85	19.15	21.00			1.00
NAU751 (TA6638)	DS3Ets#1(3A) (2n = 42)	6	0.00	1.33	19.67	21.00			
	CS*	81	0.17	1.46	19.44	20.90			

*data taken from Qi & Liu (1992).

RFLP probes were: BCD (barley cDNA), CDO (oat cDNA), and WG (wheat genomic DNA, all supplied by Dr M.E. Sorrells, Cornell University, Ithaca, New York); PSR (wheat cDNA or genomic DNA, supplied by Dr M.D. Gale, John Innes Centre, Norwich, U.K.); and KSU, (*Aegilops tauschii* genomic DNA) (Gill K.S. et al., 1991). DNA extraction, restriction enzyme digestion, Southern blotting, probe labeling and hybridization are described in Qi et al. (1997).

Results

C-banded karyotype of E. tsukushiense

E. tsukushiense has 21 pairs of metacentric or submetacentric chromosomes that are similar in size and arm ratio to those of wheat. However, the C-banding patterns of the *E. tsukushiense* chromosomes are distinct in size, position and/or intensity from those of common wheat allowing the identification of all 21 chromosome pairs of the complement (Figures 1 and 2). A large amount of C-banding polymorphism was present among different plants of the accession analyzed.

Analyses of T. aestivum-E. tsukushiense introgression lines

Eighteen lines were analyzed by C-banding, GISH, and molecular markers. Based on these results, five lines were identified that contained complete *E. tsukushiense* chromosomes or chromosome arms. Three lines (NAU702, NAU701, and NAU751) were

identified by using molecular markers, while the fourth line (NAU703) was identified by a combination of C-banding and GISH labelling. No molecular markers were detected for an *E. tsukushiense* telocentric addition line. The introgression lines are described below.

Disomic addition line 1Ets#1: Line NAU702 had 2n = 44 chromosomes. The morphology and C-banding pattern of the added chromosome corresponds to chromosome U of *E. tsukushiense* parent accession (Figures 2, and 3). Two group-1 probes hybridized to fragments on chromosome U in NAU702 (Figure 4). Thus, this chromosome was designated as 1Ets#1. Chromosome 1Ets#1 is the smallest *E. tsukushiense* chromosome. The short arm has prominent proximal and distal C-bands, and a prominent proximal C-band is present in the long arm (Figure 3). The alien origin of this chromosome was further confirmed by GISH analysis. The line NAU702 is cytogenetically stable and forms 22 bivalents at metaphase I (Table 2).

Disomic addition line 3Ets#1: Line NAU703 is a disomic addition line with 2n = 44 chromosomes. The added *E. tsukushiense* chromosome is submetacentric, with a prominent C-band at the telomere and a smaller C-band in the middle of the long arm (Figure 3). The C-banding pattern of the added chromosome is different from all the chromosomes of the *E. tsukushiense* parent accession. GISH analysis revealed a pair of labeled chromosomes in this line. Line NAU703 formed $0.10^{I}+21.68^{II}+0.18^{III}$ at metaphase I. The added *E. tsukushiense* chromosome in NAU703 is identical to the *E. tsukushiense* chromosome in the disomic substitution line 96–1059–2 (NAU751).

Monosomic addition line 5Ets#1: Line NAU701 was identified as monosomic addition line with 2n = 43 chromosomes. The added chromosome present in this line corresponds to chromosome C of *E. tsukushiense* (Figure 1). Chromosome C is acrocentric with C-bands present in the distal regions of the short and long arms. GISH analysis confirmed the C-banding result, and detected only one labeled chromosome in line NAU701. The monosomic addition line NAU701 formed $0.74^{I} + 20.63^{II} + 0.28^{III} + 0.004^{IV}$ at meiotic metaphase I (Table 2). The homoeology of chromosome C with that of wheat was determined by mapping three group-5 clones (Table 3). Therefore, the *E. tsukushiense* chromosome was designated as 5Ets#1.

Disomic substitution line 3Ets#1(3A): Line NAU751 had 2n = 42 chromosomes and was identified as disomic substitution line. The *E. tsukushiense* pair is identical to the *E. tsukushiense* chromosomes present in the disomic addition line NAU703. The four group-3 probes not only detected specific loci on the chromosome 3Ets#1, but also revealed the absence of the specific bands for wheat chromosome 3A (Figure 5).

Ditelosomic addition line with unknown homoeology: A pair of telocentric *E. tsukushiense* chromosomes is added in line NAU704 (2n = 44tt). The telocentric chromosome has a prominent telomeric and a smaller interstitial C-band (Figure 3). GISH analysis confirmed the C-banding data, and detected a pair of labeled telocentric chromosome in this line (Figure 6). Meiotic analysis showed that the telosomic chromosome generally paired as a rod bivalent (Table 2). No specific loci for the *E. tsukushiense* chromosome arm were detected by any of the 20 probes used in this study.

Lines with no detectable E. tsukushiense chromatin: Line 96–1055–4 had 2n = 44 chromosomes. C-banding analysis did not detect any *E. tsukushiense* chromosome in this line. GISH analysis also failed to detect *E. tsukushiense* chromosome in this line, suggesting that this line is probably tetrasomic for a wheat chromosome.

DNA probes from group 2, 4, 6, and 7 did not detect *E. tsukushiense* chromatin in any of the remaining 12 lines.

Discussion

E. tsukushiense is a self-pollinating species (Salomon, personal communication), and a large amount of C-banding polymorphism was observed among different plants of the accession analyzed. A similar amount of variation in C-band size and C-band position as well as structural rearrangements were observed previously in the cross-pollinating hexaploid species *Agropyron intermedium* (Host) P. B. (syn. *Thinopyrum intermedium* (Host) Barkworth and Dewey) (2n = 6x = 42, genomically $E_1E_1E_2E_2XX$) (Friebe et al., 1992). However, the C-banding patterns of the *E. tsukushiense* chromosomes are distinct from those of wheat and permitted the identification of all 21 chromosome pairs.

Three *E. tsukushiense* chromosomes were recovered as chromosome addition and substitution lines

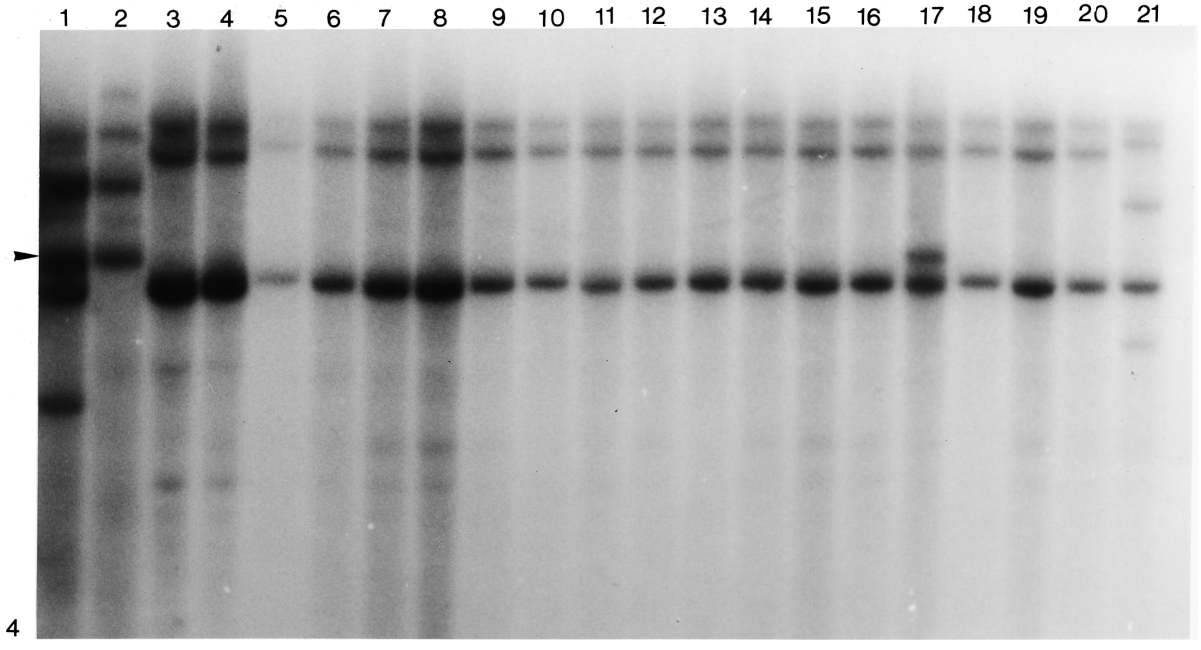

Figure 4. Hybridization of homoeologous group 1 probe PSR 596 to *Eco*RV-digested genomic DNA of the *E. tsukushiense* and C.S. × *E. tsukushiense* BC_2 derivatives, *E. ciliaris*, *E. tsukushiense* (lane 1, 2), Yangmei 5, C.S. (lane 3, 4), disomic addition NAU702 (lane 17). Arrow shows polymorphic band.

Table 3. Molecular markers mapped on the wheat-*E. tsukushiense* addition and substitution lines

Lines	Constitution	Mapped markers
NAU702	DA1Ets#1	PSR596-1S, PSR544-1L
NAU701	MA5Ets#1	PSR929-5S, PSR628-5S, BCD1088-5L
NAU751	DS3Ets#1(3A)	KSUA6-3S, BCD589-3S, PSR578-3L, PSR926-3L

and were identified genetically by RFLP analysis. Chromosome 1Ets#1 is overall similar in length, arm ratio, and C-banding pattern to chromosome $1Y^c$, which was transferred from the tetraploid species *E. ciliaris* (Trin.) Tzvelev (2n = 4x = 28, genomically $S^cS^cY^cY^c$) to wheat (Jiang et al., 1993), suggesting that this chromosome might belong to the Y^{ts} genome of *E. tsukushiense*. However, GISH and *in situ* hybridization analysis using different repetitive probes showed that the genome allocation of *Elymus* chromosomes is difficult, because of complex hybridization patterns observed in the polyploid species (Tsujimoto & Gill, 1991; Jiang et al., 1994c). Thus, further work is needed to verify the genomic origin of chromosome 1Ets#1.

Chromosome 5Ets#1 is similar in morphology and C-banding pattern to a corresponding chromosome designated as C of the *E. tsukushiense* parent accession. The arm ratio of this chromosome further supports its homoeology with group 5 chromosomes of the Triticeae (Gill B.S. et al., 1991). However, the C-banding pattern of chromosome 5Ets#1 is different from any of the S^c and Y^c genome chromosomes of *E. ciliaris* and from the S^t and H^t chromosomes of the tetraploid species *E. trachycaulus* (2n = 4x = 28, genomically $S^tS^tH^tH^t$ (Jiang et al., 1993, 1994c).

Chromosome 3Ets#1 and the *E. tsukushiense* telocentric chromosome of unknown homoeology are different in C-banding pattern from any of the chromosomes or chromosome arms present in the *E. tsukushiense* parent accession. The lack of correspondence is probably caused by the large amount of C-banding polymorphisms and/or structural chromosomal rearrangements that are present in this species.

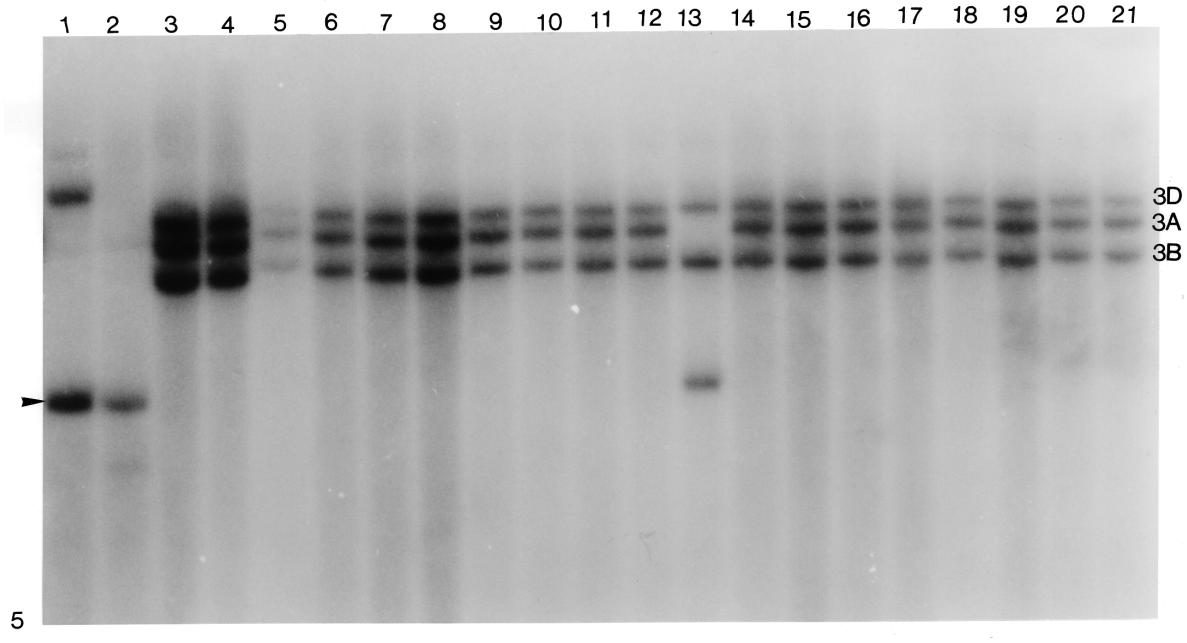

Figure 5. Hybridization of homoeologous group 3 probe PSR 929 to EcoRV-digested genomic DNA of the *E. tsukushiense* and C.S. × *E. tsukushiense* BC_2 derivatives, *E. ciliaris*, *E. tsukushiense* (lane 1, 2), Yangmei 5, C.S. (lane 3, 4), disomic substitution NAU751 (lane 13). Arrow shows polymorphic band. The specific band of wheat chromosome 3A was missing in NAU751.

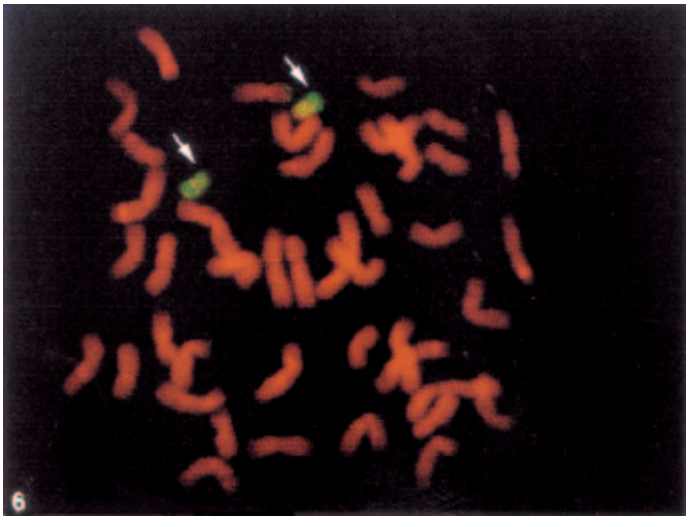

Figure 6. GISH pattern of the ditelosomic addition line NAU704 using genomic *E. tsukushiense* DNA as a probe.

The 20 probes detected no specific loci on the telosomic chromosome in NAU704. Further analysis is necessary to determine the homoeology of this chromosome arm.

Line NAU702 showed a high level of resistance to scab in field trials in Nanjing over the past several years. The present analysis identified the added *E. tsukushiense* chromosome of this line as belonging to homoeologous group 1 of wheat. Further work is underway to produce compensating translocations, which will be useful in breeding superior scab-resistant wheat cultivars.

Acknowledgements

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