Theor Appl Genet (1996) 92: 591–598

Y.-C. Yang · N. A. Tuleen · G. E. Hart

Isolation and identification of *Triticum aestivum* L. em. Thell. cv Chinese Spring-*T. peregrinum* Hackel disomic chromosome addition lines

Received: 4 September 1995 / Accepted: 15 December 1995

Abstract Analyses of RFLPs, isozymes, morphological markers and chromosome pairing were used to isolate 12 *Triticum aestivum* cv Chinese Spring (genomes A, B, and D)-*T. peregrinum* (genomes S^v and U^v) disomic chromosome addition lines. The evidence obtained indicates that each of the 12 lines contains an intact pair of *T. peregrinum* chromosomes. One monosomic addition line, believed to contain an intact $6S^v$ chromosome, was also isolated. A CS-$7U^v$ chromosome addition line was not obtained. Syntenic relationships in common with the standard Triticeae arrangement were found for five of the seven S^v genome chromosomes. The exceptions were $4S^v$ and $7S^v$. A reciprocal translocation exists between $4S^l$ and $7S^l$ in *T. longissimum* and evidence was obtained that the same translocation exists in *T. peregrinum*. In contrast, evidence for syntenic relationships in common with the standard Triticeae arrangements were found for only one U^v chromosome of *T. peregrinum*; namely, chromosome $2U^v$. All other U^v genome chromosomes are involved in at least one translocation, and the same translocations were found in the U genome of *T. umbellulatum*. Evidence was also obtained indicating that the centromeric regions of 4U and $4U^v$ are homoeologous to the centromeric regions of Triticeae homoeologous group-6 chromosomes, that the centromeric regions of 6U and $6U^v$ are homoeologous to the centromeric regions of group-4 chromosomes, and that 4U and $4U^v$ are more closely related overall to Triticeae homoeologous group-6 chromosomes than they are to group-4 chromosomes.

Key words Wheat · *Triticum aestivum* · *Triticum peregrinum* · Chromosome addition lines · RFLPs

Communicated by J. W. Snape

Y.-C. Yang · N. A. Tuleen · G. E. Hart (✉)
Soil and Crop Sciences Department, Texas A&M University, College Station, TX 77843-2474, USA

Introduction

The gene pools of the wild relatives of wheat are a rich source of genetic variation for wheat improvement (Riley et al. 1968; Feldman 1977; Feldman and Sears 1981). The development of alien chromosome addition lines is a useful first step in analyzing and exploiting these genetic resources, and sets of wheat-alien chromosome addition lines have been developed with several wheat relatives, including *Triticum umbellulatum* (Zhuk.) Bowden, *Secale cereale* L., *Lophopyrum elongatum* (Host) Love, *T. longissimum* (Schweinf. & Muschli in Muschli) Bowden, and *Hordeum vulgare* L. (for a summary see Shepherd and Islam 1988).

Triticum peregrinum Hackel (syn. *Aegilops variabilis* Eig) ($2n=4x=28$, genomes U^v and S^v) has an abundance of morphological variation and possesses genes for drought, heat, and salt tolerance (Kimber and Feldman 1987). It is distributed in eastern and southern Mediterranean countries (Furuta 1981). Its two genomes were probably derived from *T. umbellulatum* (Kihara 1940; Kimber and Yen 1989) and either *T. longissimum* or *T. sharonense* L. (Zhang et al. 1992), respectively. Nine *Triticum aestivum* L. em. Thell.-*T. peregrinum* monosomic chromosome addition lines were developed and tentatively identified by Jewell and Driscoll (1983). Relationships between the *T. peregrinum* chromosomes contained in the addition lines and specific wheat chromosomes were not determined, however, and only three monosomic addition lines were identified as being derived from the U^v genome and one from the S^v genome.

The present paper reports the results of a study, using DNA markers, morphological markers, isozymes, and chromosome pairing analyses, that was designed to isolate the possible *T. aestivum* cv Chinese Spring-*T. peregrinum* disomic chromosome addition lines and to identify the *T. peregrinum* chromosomes contained in the lines.

Materials and methods

Plant materials

A cross was made between *T. aestivum* cv Chinese Spring (CS) and *T. peregrinum*. A 35-chromosome F_1 plant (genomes $ABDU^vS^v$) was identified and pollinated with CS. A BC_1 plant with 56 chromosomes (genomes $AABBDDU^vS^v$) was selected. Heads of this plant were emasculated and pollinated with CS, and a total of 72 seeds were produced. Chromosome counts were made on seedlings derived from these seeds, and plants with a minimum of 43 and a maximum of 46 chromosomes were saved. Chromosome pairing was analyzed and plants with 21 pairs (21″) and one univalent (21″+1′) were allowed to self-pollinate while plants with 21 pairs and two to four univalents were emasculated (one head) and pollinated with CS. Seeds resulting from these pollinations were germinated, and chromosome counts made from root-tip cells. Plants with 43 chromosomes were saved and selected plants with 21″+1′ were allowed to self-pollinate to produce plants with 22 pairs of chromosomes. To distinguish different putative alien chromosome addition lines and the alien chromosomes contained therein, a capital Roman letter was assigned to each BC1 derivative that had 21″ and up to four univalents, and an Arabic numeral was appended to the latter when 21″+1′ derivatives were isolated.

Aneuploid stocks used in the isozyme and RFLP analyses and the chromosome-pairing studies included the seven possible CS-*T. longissimum* (genome S^l) disomic chromosome addition lines (Feldman 1975, 1979a, b; Hart and Tuleen 1983b; Friebe et al. 1993); six CS-*T. umbellulatum* (genome U) disomic chromosome addition lines, namely, the 1U, 2U, 5U, 6U and 7U lines produced by Kimber (1967) and the 4U line, seedlings of which die shortly after reaching the three-leaf stage and which were derived from a CS-*T. umbellulatum* 21″+t1″ addition line carrying 4U and 4UL; the CS-*T. umbellulatum* 4US ditelosomic (Dt) addition line (N. A. Tuleen, unpublished data); and the CS-*T. umbellulatum* 4UL Dt addition line (Friebe et al. 1993). In addition, the 13 available CS-*T. longissimum* Dt addition lines (Friebe et al. 1993) (the $6S^lL$ Dt addition is not available) and the ten available CS-*T. umbellulatum* Dt addition lines (Friebe et al. 1995) (Dt addition lines are not available for either arm of both 3U and 6U) were used in chromosome-pairing studies.

Cytological staining techniques

Chromosome numbers were determined from somatic cells of root-tips utilizing the Feulgen technique. To observe chromosome pairing at meiotic metaphase-I, the aceto-carmine smear technique was used. Immature spikes were collected and placed in Carnoy's solution for 24 hours and then stored in 70% ethanol in the refrigerator until examined.

Morphological analyses

Plants with 22″ of chromosomes were first separated into groups based on morphological characteristics. The characters used included head types (compact, lax, short, long, etc.), glume color, presence or absence of awns and tenacious glumes, brittle or non-brittle rachis, and purple vs green coleoptiles. A number of these characteristics are known to be associated with specific homoeologous chromosome groups in the Triticeae (for a review see Miller and Reader 1987).

Isozyme analyses

Two enzyme structural gene loci, *Aco-U^v1* and *Adk-S^v1*, were analyzed. The wheat genes that encode aconitase (ACO) and adenylate kinase (ADK) are located in the long arms of the homoeologous group-6 (Chenicek and Hart 1987) and homoeologous group-7 chromosomes (Benito et al. 1990), respectively. Zymogram analysis of ACO and ADK isozymes was performed as described by Morden et al. (1986).

RFLP analyses

RFLP procedures were performed as described by Devey and Hart (1993). Five restriction enzymes, namely, *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I, were used. Four wheat cDNA probes and 26 gDNA probes (designated pTaTAM, abbreviated TAM) isolated by Devey and Hart (1993) were employed. In addition, two *Triticum tauschii* clones (pTtksu, abbreviated ksu) obtained from Dr. B. S. Gill (Gill et al. 1991), four barley gDNA probes (ABG) and a barley dehydrin clone (Hv6) obtained from Dr. A. Kleinhofs (Kleinhofs et al. 1993), and one DNA clone from a wheat genomic DNA library (WG) and one clone from a barley cDNA library (BCD) obtained from Dr. M. Sorrells (Anderson et al. 1992) were used.

Chromosome-pairing analyses

Based mainly on the results of RFLP analyses, selected crosses were made between the CS-*T. peregrinum* disomic chromosome addition lines and the available CS-*T. longissimum* and CS-*T. umbellulatum* Dt chromosome addition lines. Disomic additions 6U and $6S^l$ were also included in the crosses since neither of the two possible 6U Dt addition lines are available and only the short-arm Dt addition line is available for $6S^l$. Several of the *T. peregrinum* chromosome addition lines were also crossed with *T. peregrinum*. Chromosome pairing was analyzed at meiotic metaphase-I in F_1 plants from all of these crosses.

Results

CS-*T. peregrinum* chromosome addition lines

A total of 28 disomic chromosome addition lines (22″), three monosomic (21″+1′) and three monotelodisomic addition lines (21″+t1″) were derived. These lines were tentatively classified into homoeologous groups based on morphological characteristics and RFLP and isozyme analyses.

RFLP analyses

A total of 38 RFLP clones were used in the identification of CS-*T. peregrinum* disomic chromosomes addition lines (Table 1). All of the DNA clones used detect loci in only one of the seven homoeologous groups of wheat, except TAM46 which detects loci in chromosome groups 2 and 7. Thirty of the clones detected RFLPs in both the S^v and the U^v genomes and the other eight detected RFLPs only in one or the other of the two genomes.

The manner in which RFLPs were used to distinguish between and to identify the alien chromosome contained in CS-*T. peregrinum* addition lines will be illustrated with clone TAM12. The RFLP patterns observed with this clone are shown in Fig. 1. Two DNA fragments, 1.8 kb and 2.0 kb in size, were detected in *T. peregrinum* but not in CS. The 1.8-kb fragment was also detected in two addition lines, K-2 and R-1, and the 2.0-kb fragment in six lines, B-1, F-1, M-1, N-2, T-1 and U-1. Given that TAM12 is known

Table 1 Chromosomes in putative CS-*T. peregrinum* addition lines and in CS-*T. umbellulatum* and CS-*T. longissimum* addition lines to which DNA probes hybridized

Probes	CS-*T. peregrinum* addition lines	CS-*T. umbellulatum* and CS-*T. longissimum* addition lines	Chromosomal locations in hexaploid wheat or barley of DNA fragments to which probes hybridize
TAM2, -7	C-1 and D-1	$1S^{l}$ and 6U	1AL, 1BL, and1DL
TAM35	C-1 and A-1	$1S^{l}$ and 1U	1AL, 1BL, and1DL
TAM52	C-1 and A-1	$1S^{l}$ and 1U	1AS, 1BS, and 1DS
ABG452	C-1 and A-1	$1S^{l}$ and 1U	1H
TAM18, -23, -34	G-1 and L-1	$2S^{l}$ and 2U	2A, 2B, and 2D
BCD307	G-1 and L-1	$2S^{l}$ and 2U	2H
WG996	J-2 and L-1	$2S^{l}$ and 2U	2H
TAM11, -33	K-2 and F-1	$3S^{l}$	3AL, 3BL, and 3DL
TAM12	K-2 and F-1	$3S^{l}$	3AS, 3BS, and 3DS
TAM56	K-2	$3S^{l}$	3AS, 3BS, and 3DS
TAM59	K-1	$4S^{l}$	4B
Hv6	K-1 and D-1	$4S^{l}$ and 6U	4HS
ABG366	K-1 and L-2	$4S^{l}$ and 5U	4HL
TAM1, -16, -29	J-1 and L-2	$5S^{l}$ and 5U	5AL, 5BL, and 5DL
TAM38	J-1	$5S^{l}$	5A, 5B, and5 D
TAM41	J-1 and L-2	$5S^{l}$ and 5U	5AS and5 DS
TAM43	L-2	5U	5A and 5D
TAM53	J-1	$5S^{l}$	5AS, 5BS, and 5DS
TAM54	J-1 and L-2	$5S^{l}$ and 5U	5AS, 5BS, and 5DS
ABG3	J-1 and L-2	$5S^{l}$ and 5U	4HS
TAM6, -10	N-1 and H-2	$6S^{l}$ and 4U	6AS and 6BS
TAM9, -25, -74	N-1 and H-2	$6S^{l}$ and 4U	6AL, 6BL, and 6DL
ksuG8	D-1	6U	6BS
ksuG48	N-1 and D-1	$6S^{l}$ and 6U	6AS and 6BS
TAM4[a]	O-1	$7S^{l}$	7A and 7B
TAM13[a]	O-1	$7S^{l}$	7AS, 7BS, and 7DS
TAM45	O-1 and K-1	$7S^{l}$ and $4S^{l}$	7A, 7B, and 7D
TAM46	O-1 and K-1	$7S^{l}$ and $4S^{l}$	2A and 7B
TAM64	O-1	$7S^{l}$	7A, 7B, and 7D

[a] Group-7 probes TAM4 and TAM13 detected two RFLPs between CS and *T. peregrinum*, one of which was not observed in any of the addition lines tested

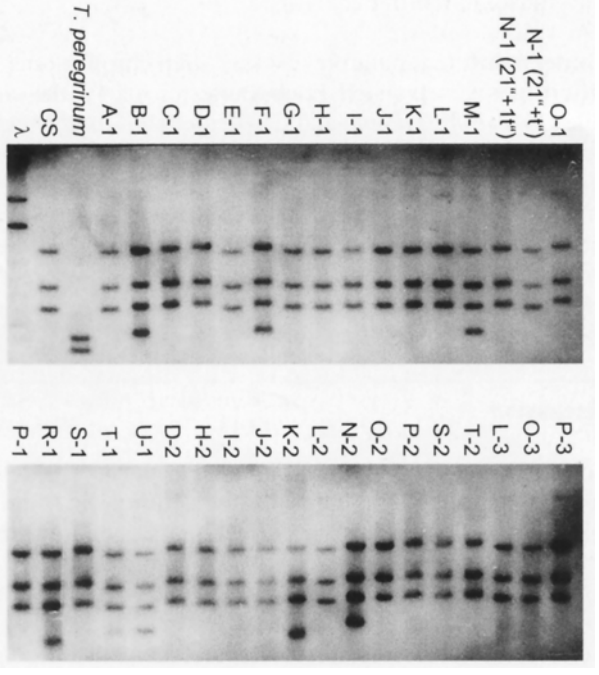

Fig. 1 Photograph of a Southern blot on which TAM12 has been hybridized to *Eco*RV-digested genomic DNAs from Chinese Spring, *T. peregrinum*, and CS-*T. peregrinum* addition lines, showing RFLPs in the B-1, F-1, M-1, R-1, T-1, U-1, K-2, and N-2 lines

to hybridize to DNA fragments located in CS chromosomes 3A, 3B, and 3D, these finding provide evidence that $3S^{v}$, or a segment thereof, is present in one of these two groups of addition lines and that $3U^{v}$, or a segment thereof, is present in the other group of lines.

Isozyme analyses

Different CS and *T. peregrinum* ACO-1 zymogram phenotypes were observed (Fig. 2). The ACO-1c band of S-1, T-1, D-2, H-2, and P-2 and of the CS-4U disomic addition line and the CS-4US Dt addition line had a higher relative staining intensity than the ACO-1c band of CS, indicating that an *Aco-1* gene and at least a part of a homoeologous group-6 chromosome are present in each of these lines. All of the other putative addition lines tested and all of the CS-*T. longissimum* disomic chromosome addition lines had the same phenotype as CS.

Different CS and *T. peregrinum* ADK-1 zymogram phenotypes were also observed (Fig. 3). All of the putative CS-*T. peregrinum* addition lines tested, except O-1, I-2 and O-2, had the same phenotype as CS. These lines had a ADK-1b band of higher relative staining intensity than CS and also exhibited ADK-1d, a band not produced by CS. An ADK-1b band of higher relative staining intensity and

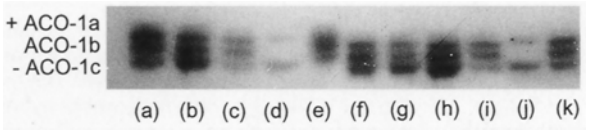

Fig. 2 Photograph of a starch gel showing ACO-1 zymogram phenotypes produced by Chinese Spring, *T. peregrinum*, and chromosome addition lines. (*a*) CS-*T. umbellulatum* 4UL addition line, (*b*) CS-*T. umbellulatum* 4US addition line, (*c*) CS, (*d*) *T. peregrinum*, (*e*) CS-*T.* umbellulatum 6U addition line, (*f*) H-2, (*g*) D-2, (*h*), P-2, (*i*) CS, (*j*) *T. peregrinum*, (*k*) O-1

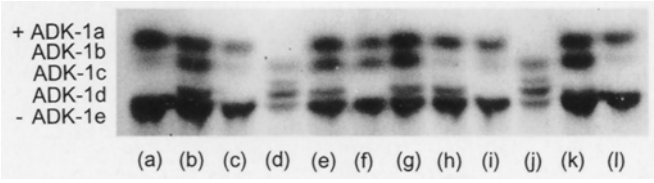

Fig. 3 Photograph of a starch gel showing ADK-1 zymogram phenotypes produced by Chinese Spring, *T. peregrinum*, and chromosome addition lines. (*a*) I-1, (*b*) I-2, (*c*) CS, (*d*) *T. peregrinum*, (*e*) O-1 (22″), (*f*) O-1 (21″+t″; non-purple coleoptile), (*g*) O-2 (22″), (*h*) O-2 (21″+t″; purple coleoptile), (*i*) CS, (*j*) *T. peregrinum*, (*k*) CS-*T. longissimum* $7S^l$ addition line, (*l*) H-2

ADK-1d were also produced, respectively, by short-arm and long-arm Dt addition lines derived from O-1. These results indicate that an *Adk-1* gene and at least part of a homoeologous group-7 chromosome are present in each of these lines.

Morphological analysis

Analysis of the morphological characters expressed by putative monosomic and disomic addition lines, and of the pairing behavior of the added chromosome contained in the lines (see below), was further used to identify groups of lines that were likely to contain the same added chromosome and to select one line from each group for detailed analysis. The 14 lines selected by this process, and the DNA clones that hybridized to the added chromosome contained in each line, are shown in Table 1.

The spikes of plants with chromosome A-1 are black, a characteristic known to be controlled by a gene located in chromosome 1U of *T. umbellulatum* [references for the Triticeae gene locations described in this paragraph are contained in Miller and Reader (1987)]. The spikes of plants with chromosomes G-1 and L-1 have short awns and tenacious glumes, characters known to be controlled by genes located in chromosomes of Triticeae homoeologous group 2. The spikes of plants with chromosome K-2 have a brittle rachis, a character controlled by a gene in the short arm of $3S^l$ in *T. longissimum*. The spikes of plants with chromosomes H-1 and L-2 have lax bases and compact tops, characters known to be controlled by genes in Triticeae homoeologous group-5 chromosomes. The coleoptiles of plants with chromosome O-1 are purple, a character known to be controlled by a gene in Triticeae chromosome 7.

Table 2 Meiotic pairing in progeny of crosses between CS-*T. peregrinum* disomic chromosome addition lines[a] and CS-*T. longissimum* and CS-*T. umbellulatum* ditelosomic addition lines[b]

Cross		Number of cells counted	% of cells with t1″
C-1 × CS-*T. longissimum*	21″ + $1S^lS$″	298	9.7
C-1 × CS-*T. longissimum*	21″ + $1S^lL$″	155	59.4
A-1 × CS-*T. umbellulatum*	21″ + 1US″	81	30.9
A-1 × CS-*T. umbellulatum*	21″ + 1UL″	104	34.6
G-1 × CS-*T. longissimum*	21″ + $2S^lS$″	283	43.8
G-1 × CS-*T. longissimum*	21″ + $2S^lL$″	138	55.1
L-2 × CS-*T. umbellulatum*	21″ + 2US″	185	11.4
L-2 × CS-*T. umbellulatum*	21″ + 2UL″	126	73.0
K-2 × CS-*T. longissimum*	21″ + $3S^lS$″	162	14.2
K-2 × CS-*T. longissimum*	21″ + $3S^lL$″	129	55.0
K-1 × CS-*T. longissimum*	21″ + $4S^lS$″	218	26.1
K-1 × CS-*T. longissimum*	21″ + $4S^lL$″	203	48.8
H-2 × CS-*T. umbellulatum*	21″ + 4US″	98	88.8
H-2 × CS-*T. umbellulatum*	21″ + 4UL″	81	79.0
J-1 × CS-*T. longissimum*	21″ + $5S^lS$″	53	0.0
J-1 × CS-*T. longissimum*	21″ + $5S^lL$″	132	49.2
L-2 × CS-*T. umbellulatum*	21″ + 5US″	82	31.7
L-2 × CS-*T. umbellulatum*	21″ + 5UL″	60	70.8
N-1 × CS-*T. longissimum*	21″ + $6S^lS$″	117	0.0
N-1 × CS-*T. longissimum*	21″ + $6S^l$″	96	12.5
D-1 × CS-*T. umbellulatum*	21″ + 6U″	51	74.5
O-2 × CS-*T. longissimum*	21″ + $7S^lS$″	249	30.5
O-2 × CS-*T. longissimum*	21″ + $7S^lL$″	214	30.8

[a] N-1 was crossed as a monosomic addition line because a disomic N-1 addition line has not been produced (see text)
[b] Disomic $6S^l$ and 6U addition lines were used in place of ditelosomic 6US, 6UL and $6S^lL$ addition lines because these ditelosomic addition lines are not available

Chromosome-pairing analyses

Thirteen different putative disomic alien chromosome addition lines were isolated. Each was testcrossed to the available CS-*T. longissimum* and CS-*T. umbellulatum* Dt addition lines and the frequency of formation of a t1″ in the progeny of each cross was determined. These results are shown in Table 2. It may be noted that U^v-genome chromosomes generally paired at a higher frequency with telosomes from the U genome than did S^v chromosomes with telosomes from the S^l genome.

Discussion

Robertsonian translocations often occur during the development of wheat-alien chromosome addition lines due to misdivision of the centromeres of monosomic chromosomes followed by the fusion of telosomes derived from different chromosomes (Dvorak 1980; Hart and Tuleen 1983a; Tuleen and Hart 1988). Consequently, reliable methods are needed to insure that the alien chromosomes present in addition lines are intact rather than translocated. In this study, analyses of isozymes, RFLPs, morphological characters, and chromosome pairing were used for this purpose.

Homoeologous group 1

Each of the five wheat homoeologous group-1 probes used in this study detected two different RFLPs between CS and *T. peregrinum* (Table 1). Presumably, one should be from the S^v genome and the other one from the U^v genome. C-1 was the only addition line in which a RFLP was detected with all five probes. RFLPs detected by these five probes were also observed in the CS-*T. longissimum* $1S^l$ disomic addition line, but only three of them are identical to the RFLPs observed in C-1 (data not shown). These results indicate that RFLPs exist between the *T. peregrinum* chromosome present in C-1 and $1S^l$ of *T. longissimum*. The *T. peregrinum* chromosome contained in C-1 paired with $1S^l$ and with $1S^lL$ and $1S^lS$ of *T. longissimum* (Table 2). These results indicate that C-1 contains an intact pair of *T. peregrinum* $1S^v$ chromosomes.

Other markers detected by the five group-1 probes were in either A-1 or D-1, two long-arm markers being in D-1 and two other long-arm markers and the only short-arm marker in A-1 (Table 1). The markers detected in A-1 were also observed in the CS-1U disomic chromosome addition line. The results of RFLP analyses indicated that a RFLP also exists between the *T. peregrinum* chromosome present in A-1 and the 1U of *T. umbellulatum* (data not shown). The markers detected in D-1 were also observed in the CS-6U disomic chromosome addition line. These results suggest that a translocation exists in the *T. peregrinum* chromosomes present in A-1 and D-1 relative to the homoeologous group-1 chromosomes of hexaploid wheat. The fact that the markers detected in D-1 were also observed in the CS-*T. umbellulatum* 6U addition line indicates that this translocation is also present in *T. umbellulatum* and did not arise during the development of these addition lines. Further discussion of D-1 will be presented below.

The spikes of A-1 are black, a characteristic known to be controlled by a gene located in 1U of *T. umbellulatum*. The *T. peregrinum* chromosome contained in A-1 pairs only with 1U and with 1UL and 1US of *T. umbellulatum* (Table 2). These results indicate that A-1 contains an intact pair of *T. peregrinum* $1U^v$ chromosomes.

Homoeologous group 2

Each of the five wheat homoeologous group-2 probes that were used detected two RFLPs between CS and *T. peregrinum* (Table 1). One of the RFLPs was in L-1 and in the CS-*T. umbellulatum* 2U addition line. The spikes of L-1 have short awns and tenacious glumes, characters that are known to be controlled by genes located in chromosomes of Triticeae homoeologous group 2. The *T. peregrinum* chromosome contained in L-1 pairs only with 2U and with 2UL and 2US of *T. umbellulatum* (Table 2). These results indicate that L-1 contains an intact pair of *T. peregrinum* $2U^v$ chromosomes.

Four of the five group-2 probes detected a RFLP in G-1 and the fifth clone, WG996, detected a RFLP in J-2 (Table 1). All of these RFLPs were also present in the CS-*T. longissimum* $2S^l$ addition line. These results indicate that G-1 and J-2 contain at least part of *T. peregrinum* $2S^v$. Since J-2 also contains part of $6S^v$ (see below), a translocation between $2S^v$ and $6S^v$ may have arisen during the development of these addition lines.

The reason that the marker detected by WG996 is present in J-2 but not in G-1 has not been determined. There may be a translocation between the *T. peregrinum* chromosomes contained in G-1 and J-2. However, no markers were detected in G-1 by homoeologous group-6 probes. The DNA fragments that hybridized to WG996 were mapped in the centromeric region of barley chromosome 2 (Kleinhofs et al. 1993), indicating that the translocated *T. peregrinum* segment, if any, that differentiates lines G-1 and J-2 is not small. However, the *T. peregrinum* chromosome contained in G-1 pairs well with $2S^l$ and with $2S^lL$ and $2S^lS$ of *T. longissimum* but not with other *T. longissimum* chromosomes, indicating that the translocated segment, if any, must be small. In summary, a translocation is unlikely and it is more likely that the fragment to which clone WG996 hybridized in J-2 is located in $2S^v$ and not in a translocated $6S^v$ segment.

The spikes of G-1 have short awns and tenacious glumes. These results and the aforementioned findings indicate that G-1 contains an intact pair of $2S^v$ chromosomes of *T. peregrinum*.

Homoeologous group 3

Each of the four wheat homoeologous group-3 probes that were used detected two RFLPs between CS and *T. peregrinum* (Table 1). One of the two RFLPs detected with each of the probes was observed in K-2 and the same RFLPs were present in the CS-*T. longissimum* $3S^l$ addition line. The spikes of K-2 have a brittle rachis, a character that is known to be controlled by a gene located in *T. longissimum* $3S^lS$. The *T. peregrinum* chromosome contained in K-2 pairs only with $3S^lL$ and $3S^lS$ of *T. longissimum*. This indicates that K-2 contains an intact pair of *T. peregrinum* $3S^v$ chromosomes.

The second RFLP detected by three of the group-3 probes was observed in F-1. TAM56, however, did not detect a RFLP in any of the CS-*T. peregrinum* addition lines but did detect one in the 7U addition line derived from *T. umbellulatum*. This indicates that a translocation may exist between 3U and 7U of *T. umbellulatum* and $3U^v$ and $7U^v$ of *T. peregrinum*. Unfortunately, since neither a $7U^v$ addition line nor a 3U addition line has been isolated, this hypothesis can not be tested. The results indicate that F-1 contains an intact pair of the *T. peregrinum* chromosome designated $3U^v$.

Homoeologous group 4

Only three homoeologous group-4 probes, TAM59, Hv6 and ABG366, were used. The barley DNA fragments that

hybridize to Hv6 and ABG366 are located in the short arm and long arm, respectively, of barley chromosome 4H (Kleinhofs et al. 1993). Hv6 and ABG366 each detected two RFLPs between CS and *T. peregrinum* while TAM59 detected only one RFLP.

The RFLP detected by TAM59 and one of the two RFLPs detected by Hv6 and ABG366 were in K-1 and in the CS-*T. longissimum* $4S^l$ addition line. Two wheat homoeologous group-7 probes, TAM45 and TAM46, also detected RFLPs in the K-1 and $4S^l$ lines. Since a translocation involving $4S^lL$ and $7S^lL$ exists in *T. longissimum* (Friebe et al. 1993), the same translocation appears to exist in *T. peregrinum*. Furthermore, the *T. peregrinum* chromosome contained in K-1 paired only with $4S^lL$ and $4S^lS$ of *T. longissimum*. The aforementioned results indicate that K-1 contains an intact pair of the *T. peregrinum* chromosome designated $4S^v$.

The second RFLP detected by probes Hv6 and ABG366 was observed in D-1 and L-2, respectively. The available evidence indicates, however, that D-1 and L-2 contain *T. peregrinum* chromosomes $6U^v$ and $5U^v$, respectively. The RFLPs detected by ABG366 were also observed in the CS-*T. umbellulatum* 5U addition line. These results indicate that a translocation may exist in *T. peregrinum* involving $4U^v$ and $5U^v$ and in *T. umbellulatum* involving 4U and 5U (see also King et al. 1994). See below for further discussion of D-1 and L-2.

The CS-*T. umbellulatum* chromosome 4U disomic addition line was developed by Kimber (1967) and was studied by Sharp et al. (1989). The latter authors were able to locate only one of the two group-4 DNA markers that they studied in the added chromosome present in this line. Five DNA probes, namely, TAM6, TAM9, TAM10, TAM25 and TAM74, detected RFLPs in the H-2 line, but all are group-6 probes (Table 1) and four of the probes detected the same RFLP in the CS-4U addition line (the fifth probe, TAM74, was not tested on the CS-4U line). TAM6 and TAM10 hybridize to DNA fragments located in the 6S arms of CS and TAM9, TAM25, and TAM74 hybridize to fragments located in the 6L arms (Devey and Hart 1993). Furthermore, *Xtam6-6B* and *Xtam10-6B* are located in the proximal part of the *T. turgidum* 6BS linkage map, *Xtam74-6B* in the distal part of the 6AL linkage map, and *Xtam25-6A* and *Xtam25-6B* in the distal parts of the 6AL and 6BL linkage maps, respectively (Chen et al. 1994). This indicates that the centromere of the alien chromosome present in H-2, and possibly most or all of one arm of the chromosome, belong to Triticeae homoeologous group 6. As noted above, a gene encoding ACO-1, another Triticeae group-6 marker, is located in the *T. peregrinum* chromosome present in H-2 and in the *T. umbellulatum* chromosome present in the 4U addition line.

The *T. peregrinum* chromosome present in H-2 paired well with 4UL and 4US (Table 2). Therefore, it appears that H-2 contains an intact pair of the *T. peregrinum* chromosome designated $4U^v$. The *T. peregrinum* chromosome present in H-2 and the *T. umbellulatum* chromosome present in the 4U addition line, however, appear to be more closely related to wheat homoeologous group-6 chromosomes than they are to homoeologous group-4 chromosomes. It should be noted also that all of the RFLPs detected by the probes that hybridize to fragments located in the long arm of wheat group-6 chromosomes are located in the short arm of 4U and vice versa (data not shown).

Homoeologous group 5

Seven probes detected RFLPs in L-2 (Table 1) and in the CS-*T. umbellulatum* 5U addition line. The spikes of L-2 have a lax base and a compact top, characters that are known to be controlled by genes located in Triticeae homoeologous group-5 chromosomes. The *T. peregrinum* chromosome contained in this line pairs with telosomes 5UL and 5US of *T. umbellulatum*. This indicates that L-2 contains an intact pair of *T. peregrinum* $5U^v$ chromosomes.

Seven wheat group-5 probes (including three long-arm probes and three short-arm probes) and one barley group-4 probe detected RFLPs in J-1 (Table 1) and in the CS-*T. longissimum* $5S^l$ addition line. The spikes of J-1 have a lax base and a compact top. The *T. peregrinum* chromosome contained in J-1 paired with $5S^lL$ of *T. longissimum*. No pairing was observed between the added chromosome in J-1 and the CS-$5S^lS$ addition line. In hybrids between J-1 and *T. peregrinum*, however, 61% of meiotic metaphase-I cells contained one or more ring bivalents, while in hybrids between CS and *T. peregrinum* only 10% of meiotic metaphase I cells contained one or more ring bivalents. Also, in hybrids between *T. peregrinum* and R-1 (a 21″+1″ addition line known to contain a $3S^vS.1S^vL$ centric fusion translocation) 12% of meiotic metaphase-I cells contained a ring bivalent (data not shown). These finding provide strong evidence that J-1 contains an intact *T. peregrinum* $5S^v$.

Homoeologous group 6

Six of the seven wheat homoeologous group 6 probes detected two RFLPs between CS and *T. peregrinum*. The seventh probe, ksuG8, detected only one RFLP (Table 1). As noted above, TAM6, TAM9, TAM10, TAM25, and TAM74 detected RFLPs in the $4U^v$ addition line. Other RFLPs detected by these five probes and the one RFLP detected by probe ksuG48 were observed in N-1. With two exceptions, the same RFLPs were observed in the CS-*T. longissimum* $6S^l$ addition line. (TAM74 was not tested on the $6S^l$ addition line and ksuG48 did not reveal a RFLP in the line.) This suggests that the *T. peregrinum* chromosome present in N-1 is $6S^v$.

The *T. peregrinum* chromosome in N-1 is present either as a monosome (21″+1′) or a monosome plus a telosome for one of its arms (21″+t1″). When plants with 21″+t1″ were self-pollinated, no plants with 22″ were observed in the 130 progeny examined. This behavior is identical to that of $6S^l$ in the CS-*T. longissimum* addition line series (N.A. Tuleen, unpublished results). The $6S^v$ chromosome present in N-1 pairs with $6S^l$ of *T. longissimum* but $6S^vS$ was unable to pair with $6S^lS$. The results of RFLP analy-

ses indicate, however, that the telosome in N-1 is $6S^vS$. Also, in hybrids between N-1 and *T. peregrinum*, 81% of the cells observed at meiotic metaphase-I contained one or more ring bivalents. These results indicate that N-1 contains *T. peregrinum* chromosome $6S^v$.

Other RFLPs detected by ksuG48 and the RFLP detected by ksuG8 were observed in D-1 and in the CS-6U disomic chromosome addition line. RFLPs detected by the 1L probes TAM2 and TAM7 and by Hv6, a homoeologous group-4 probe, were present in D-1 and the CS-6U addition line. These results suggest that the *T. peregrinum* chromosome present in D-1 and chromosome 6U both contain DNA segments from three different Triticeae homoeologous groups, namely, groups 1, 4 and 6, and that a multiple translocation involving chromosomes from these three groups may have arisen during the evolution of *T. umbellulatum* prior to its hybridization with *T. longissimum*. *XksuG48-6A* is located in the distal portion of the *T. turgidum* 6AS linkage map, and both *XksuG8-6B* and *XksuG48-6B* are located in the distal portion of the *T. turgidum* 6BS linkage map (Chen et al. 1994). The barley DNA fragments that hybridize to probe Hv6 map close to the centromere on the barley chromosome 4H linkage map (Kleinhofs et al. 1993). The DNA fragments that hybridize to 1L probes TAM2 and TAM7 have not been mapped. These results indicate that the centromeres of 6U and of the *T. peregrinum* chromosome present in D-1 both belong to Triticeae homoeologous group 4.

The karyotype of the *T. peregrinum* chromosome contained in D-1 is the same as that of *T. umbellulatum* 6U (data not shown) and the chromosome pairs well with 6U (Table 2). The results obtained indicate that D-1 contains an intact $6U^v$ chromosome of *T. peregrinum*.

Homoeologous group 7

TAM4 detected one RFLP between CS and *T. peregrinum* while the other four homoeologous group-7 probes used detected two RFLPs (Table 1). One of the RFLPs detected by TAM13, TAM45, TAM46, and TAM64 and the RFLP detected by TAM4 were observed in O-1 and in the CS-*T. longissimum* $7S^l$ addition line.

The second RFLP detected by TAM45 and TAM46 was discussed above (homoeologous group 4) while the second RFLP detected by TAM13 and TAM64 was not observed in any of the addition lines tested.

The color of the coleoptile of O-1 is purple, a character known to be controlled by a Triticeae 7S gene. The *T. peregrinum* chromosome present in O-1 pairs well with $7S^lL$ and $7S^lS$ of *T. longissimum*. These results indicate that O-1 contains *T. peregrinum* chromosome $7S^v$, that the second RFLP detected by TAM13 and TAM64 was produced by a *T. peregrinum* chromosome that has not been isolated in an addition line, and that the fragments that hybridized to TAM45 and TAM46 are duplicated and located in chromosomes of homoeologous groups 4 and 7.

ADK-1c was not expressed in any of the addition lines tested (see Results). The gene that encodes this isozyme is probably located in $7U^v$. ADK-1b and ADK-1d are produced by the short and long arms of $7S^v$, respectively, in O-1 and by *T. peregrinum*. This indicates that *Adk-1* is duplicated in $7S^v$ both in *T. peregrinum* and in O-1, I-2, and O-2. A similar duplication was not observed in the CS-$7S^l$ disomic chromosome addition line, indicating that the duplication arose during the evolution of *T. peregrinum* after the hybridization of *T. longissimum* and *T. umbellulatum*.

DNA fragments that hybridized to TAM45 and TAM46 are duplicated and located in chromosomes $4S^l$ and $7S^l$ of *T. longissimum* and $4S^v$ and $7S^v$ of *T. peregrinum*. The basis for these duplications is unknown. Since a translocation involving $4S^lL$ and $7S^lL$ of *T. longissimum* exists, the same translocation appears to exist in *T. peregrinum*, and the duplication and translocation must have arisen during the evolution of *T. longissimum* prior to its hybridization with *T. umbellulatum*.

Table 3 Chinese Spring-*T. peregrinum* disomic chromosome addition lines produced[a]

T. peregrinum chromosome	CS-*T. peregrinum* addition line	*T. peregrinum* chromosome	CS-*T. peregrinum* addition line
$1S^v$	C-1	$1U^v$	A-1
$2S^v$	G-1	$2U^v$	L-1
$3S^v$	K-2	$3U^v$	F-1
$4S^v$	K-1	$4U^v$	H-2
$5S^v$	J-1	$5U^v$	L-2
$6S^v$	N-1	$6U^v$	D-1
$7S^v$	O-1	$7U^v$	None

[a] $6S^v$ was isolated in a monosomic addition line and the other 12 *T. peregrinum* chromosomes were isolated in disomic addition lines

Concluding remarks

Twelve of the fourteen possible CS-*T. peregrinum* disomic chromosome addition lines and one monosomic addition line were tentatively isolated and identified (Table 3). *T. peregrinum* chromosomes $1S^v$, $2S^v$, $3S^v$, $4S^v$, $5S^v$, $6S^v$, and $7S^v$ are most likely contained in addition lines C-1, G-1, K-2, K-1, J-1, N-1, and I-2, respectively, and chromosomes $1U^v$, $2U^v$, $3U^v$, $4U^v$, $5U^v$, and $6U^v$ are most likely contained in lines A-1, L-1, F-1, H-2, L-2, and D-1, respectively. The CS-$7U^v$ chromosome addition line was not isolated.

Syntenic relationships in common with the standard Triticeae arrangement and with the S^l genome of *T. longissimum* were found for five of the seven chromosomes in the S^v genome of *T. peregrinum*. A $4S^l$–$7S^l$ reciprocal translocation is known to exist in *T. longissimum* and the results of the present study indicate that the same translocation exists in the S^v genome of *T. peregrinum*. In contrast, the evidence obtained indicates that only one U^v-genome chromosome, namely, $2U^v$, has the standard Triticeae chromosomal arrangement, all other U^v-genome chromosomes being involved in at least one translocation and with the

same translocations being present in the U genome of *T. umbellulatum*. The results also indicate that the centromeric regions of the chromosomes designated 4U and $4U^v$ are homoeologous to the centromeric regions of Triticeae homoeologous group-6 chromosomes, that the centromeric regions of the chromosomes designated 6U and $6U^v$ are homoeologous to the centromeric regions of Triticeae homoeologous group-4 chromosomes, and that 4U and $4U^v$ are more closely related overall to homoeologous group-6 chromosomes than they are to homoeologous group-4 chromosomes. Identification of all of the chromosomal rearrangements that separate the U^v and U genomes from Chinese Spring is likely to require the construction of a detailed genetic map of one of these genomes in a manner analogous to the map of *S. cereale* constructed by Devos et al. (1993). If the results of this or a similar type of study confirm the 4U, $4U^v$, 6U, and $6U^v$ homoeologies indicated by the present study, interchanging the designations of 4U and 6U and of $4U^v$ and $6U^v$ should be considered

References

Anderson JA, Ogihara Y, Sorrells ME, Tanksley SD (1992) Development of a chromosomal-arm map for wheat based on RFLP markers. Theor Appl Genet 83:1035–1043

Benito C, Gallego FJ, Frade JM, Zaragoza C, Figueiras AM (1990) Chromosomal location of adenylate kinase isozymes in Triticeae species. Theor Appl Genet 79:157–160

Chen Z, Devey M, Tuleen NA, Hart GE (1994) Use of recombinant substitution lines in the construction of RFLP-based genetic maps of chromosomes 6A and 6B of tetraploid wheat (*Triticum turgidum* L.). Theor Appl Genet 89:703–712

Chenicek KJ, Hart GE (1987) Identification and chromosomal locations of aconitase gene loci in Triticeae species. Theor Appl Genet 74:261–268

Devey M, Hart GE (1993) Chromosomal localization of intergenomic RFLP loci in hexaploid wheat. Genome 36:913–918

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Dvorak J (1980) Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can J Genet Cytol 22:237–259

Feldman M (1975) Alien addition lines of common wheat containing *Triticum longissimum* chromosomes. Proc 12th Int Bot Congr 2:506

Feldman M (1977) Historical aspects and significance of the discovery of wild wheats. Stadler Genet Symp 9:121–144

Feldman M (1979a) New evidence on the origin of the B genome of wheat. In: Proc 5th Int Wheat Genet Symp, New Delhi, India. Vol 1, pp 120–132

Feldman M (1979b) Genetic resources of wild wheat and their use in breeding. Monografia Genet Agraria 4:9–26

Feldman M, Sears ER (1981) The wild gene resources of wheat. Sci American 244:102–112

Note added in proof

A 20″ + t1″ plant with $4U^v$ and $4U^vS$ substituted for 6B has been produced and 20″ + t1″ plants that putatively contain $4U^v$ and $4U^vS$ substituted for 6A and for 6D have also been produced. The plants have normal morphology and a high rate of seed set. Also, it has been determined that male gametes that contain $4U^v$ substituted for either 6A or 6B or 6D will compete with normal male gametes during the gametophytic generation.

Friebe M, Tuleen N, Jiang J, Gill BS (1993) Standard karyotype of *Triticum longissimum* and its cytogenetic relationship with *T. aestivum*. Genome 36:731–742

Friebe B, Jiang J, Tuleen N, Gill BS (1995) Standard karyotype of *Triticum umbellulatum* and the characterization of derived chromosome addition and translocation lines in common wheat. Theor Appl Genet 90:150–156

Furuta Y (1981) Intraspecific variation in *Aegilops variabilis* and *Ae. kotschyi* revealed by chromosome pairing in F_1 hybrids. Jpn J Genet 56:495–504

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

Hart GE, Tuleen NA (1983a) Chromosomal locations of eleven *Elytrigia elongata* (=*Agropyron elongatum*) isozyme structural genes. Genet Res 41:181–202

Hart GE, Tuleen NA (1983b) Characterizing and selecting alien genetic material in derivatives of wheat-alien species hybrids by analyses of isozyme variation. In: Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp 377–385

Jewell DC, Driscoll CJ (1983) The addition of *Aegilops variabilis* chromosomes to *Triticum aestivum* and their identification. Can J Genet Cytol 25:76–84

Kihara H (1940) Verwandtschaft der *Aegilops*-Arten im Lichte der Genomanalyse. Ein Uberblick Zuechter 12:49–62

Kimber G (1967) The addition of the chromosomes of *Aegilops umbellulata* to *Triticum aestivum* (var. Chinese Spring). Genet Res 9:111–114

Kimber G, Feldman M (1987) Wild wheat, an introduction. Special report 353. College of Agriculture, University of Missouri-Columbia

Kimber G, Yen Y (1989) Hybrids involving autotetraploid *Triticum umbellulatum*. Genome 32:1–5

King IP, Purdie KA, Liu CJ, Reader SM, Pittaway TS, Orford SE, Miller TE (1994) Detection of interchromosomal translocations within the Triticeae by RFLP analysis. Genome 37:882–887

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Miller TE, Reader SM (1987) A guide to homoeology of chromosomes within the *Triticeae*. Theor Appl Genet 74:214–217, erratum 680

Morden CW, Doebley J, Schertz KF (1986) A manual of techniques for starch-gel electrophoresis of Sorghum isozymes. A miscellaneous publication of the Texas Agricultural Experiment Station, College Station

Riley R, Chapman V, Johnson R (1968) The incorporation of alien disease resistance in wheat by genetic interference with the regulation of meiotic synapsis. Genet Res 12:199–219

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78:342–348

Shepherd, KW, Islam, SKMR (1988) Fourth compendium of wheat-alien chromosome lines. In: Miller TE, Koebner RMD, (eds) Proc 7th Int Wheat Genet Symp, Cambridge, England, pp 1373–1395

Tuleen NA, Hart GE (1988) Isolation and characterization of wheat-*Elytrigia elongata* chromosome 3E and 5E addition and substitution lines. Genome 30:289–292

Zhang H-B, Dvorak J, Waines JG (1992) Diploid ancestry and evolution of *Triticum kotschyi* and *T. peregrinum* examined using variation in repeated nucleotide sequences. Genome 35:182–191