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T. Naranjo

Chromosome structure of *Triticum longissimum* relative to wheat

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Abstract Homoeologous pairing at meiotic metaphase I was analyzed in *T. longissimum* × *T. aestivum* hybrids in order to reconfirm the homoeologous relationships of *T. longissimum* chromosomes to wheat. Hybrids between *T. longissimum* and 'Chinese Spring' carrying the *Ph1* gene or the *ph1b* mutation, which showed low and high pairing levels, respectively, were used. Chromosome arms associated at metaphase I were identified by C-banding. The homoeology of chromosomes $1S^l$, $2S^l$, $3S^l$, $5S^l$ and $6S^l$ to wheat group 1, 2, 3, 5, and 6 chromosomes, respectively, was confirmed. Chromsome arms $4S^lS$ and $7S^lS$ showed normal homoeologous relationships to wheat. The $4S^lL$ arm carries a translocated segment from $7S^lL$ relative to wheat. The $7S^lL$ arm seldom paired, likely because this arm lost a relatively long segment and received a very short segment in the interchange with $4S^lL$. Available data suggest that translocation $4S^lL/7S^lL$ arose in the evolution of *T. longissimum*, which implies that this species was not the donor of the B genome of wheat.

Key words C-banding · Homoeologous pairing · Homoeologous relationships · Wheat · *Triticum longissimum*

Introduction

The genomes of hexaploid wheat, *Triticum aestivum*, and of the other species within the tribe Triticeae are assumed to have evolved from a common ancestral genome. The chromosome structure of the genomes of wheat and related species may be comparatively studied from an analysis at metaphase I of homoeologous pairing in interspecific hybrids. Pairing between homoeologous chromosome arms is expected to occur when such chromosome arms maintain the same structure. The occurrence of pairing between arms from different homoeologous groups would indicate the existence of structural differences between genomes that arose as a result of translocations.

Two translocations which occurred in the evolution of wheat have been detected using this approach. Naranjo et al. (1987, 1988a,b) analyzed homoeologus pairing at meiotic metaphase I in different wheat-rye hybrids using a C-banding technique that allowed the identification of most of the wheat chromosomes and their arms. Pairing between 4AL, 7AS and 7DS, between 5AL, 4BL and 4DL and between 7BS, 5BL and 5DL indicated that the arms 4AL, 5AL and 7BS of hexaploid wheat are involved in two interchanges, a translocation between 5AL/4AL and a translocation between 4AL/7BS. The construction of genetic maps based on molecular markers confirmed these translocations (Anderson et al. 1992; Liu et al. 1992). Likewise, evolutionary translocations present in the R genome of rye relative to wheat have been inferred from both an analysis of homoeologous pairing in wheat-rye hybrids (Naranjo and Fernández-Rueda 1991; Naranjo 1992) and genetic maps (Devos et al. 1993).

Among the three genomes of hexaploid wheat, the A and D genomes were derived from *T. monococcum* and *T. tauschii*, respectively, while the source of the B genome remains undetermined (Kerby and Kuspira 1987). The species of the *Sitopsis* section of genus *Triticum*, *T. bicorne*, *T. longissimum*, *T. searsii*, *T. sharonense* and *T. speltoides*, have been implicated as putative B genome donors. Chromosome 7B of *T. turgidum* and *T. aestivum* is involved in the intergenome translocation 4AL/7BS that occurs at the early tetraploid stage (Naranjo 1990). Therefore, chromosome arm 7BS structurally differs from its counterparts of the *Sitopsis* section species. Whether or not there is any other structural difference between the B genome of wheat and the S genome of the *Sitopsis* section species

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T. Naranjo
Departamento de Genética, Facultad de Biologia, Universidad Complutense de Madrid, 28040 Madrid, Spain

should be considered in order to establish the donor of the B genome.

The homoeologous relationships of *T. longissimum* chromosomes to wheat have been established from studies on isozyme, storage protein and morphological markers, as well as from sporophytic and gametophytic compensation tests (Hart and Tuleen 1983; Jewell and Driscoll 1983; Netzle and Zeller 1984; Kota and Dvořák 1985; Levi et al. 1985; Millet et al. 1988; Hueros et al. 1991; Friebe et al. 1993). In these analyses, addition and substitution lines of *T. longissimum* in *T. aestivum* were mainly employed. Normal homoeologous relationships were identified for chromsomes $1S^l$, $2S^l$, $3S^l$, $5S^l$, and $6S^l$. Chromosomes $4S^l$ and $7S^l$ are homoeologous to both groups 4 and 7 chromosomes of wheat, which has suggested the presence of a reciprocal translocation in *T. longissimum* relative to the situation in wheat (Friebe et al. 1993).

Although there is polymorphism for C-band size and C-band location, *T. longissimum* chromsomes show a distinctive C-banding pattern and can be distinguished from wheat chromsomes (Friebe et al. 1993). The aim of the work presented here was to study the chromosome structure of *T. longissimum* relative to wheat using homoeologous pairing at metaphase I in wheat × *T. longissimum* hybrids.

Material and methods

Plants of *Triticum aestivum* (genome constitution AABBDD, $2n = 6x = 42$) of the normal and *ph1b* mutant (Sears 1977) lines of cv 'Chinese Spring' were crossed to *Triticum longissimum* (genome constitution S^lS^l, $2n = 14$) accession TL01, a low-pairing genotype (Ceoloni et al. 1986), which was kindly supplied by M. Feldman, Department of Plant Genetics, The Weizmann Institute of Science, Rehovot, Israel. Three normal hybrids and three *ph1b* hybrids were used for this study. The hybrids were grown in a controlled environment chamber at 16°–18 °C after vernalization for 8 weeks at 6°–8 °C.

Anthers at metaphase I of the hybrids were fixed in acetic acid-alcohol(1:3) and stored at 0°–4 °C for a minimum of 2 months. The fixed material was squashed and stained according to the C-banding technique of Giráldez et al. (1979). The chromosomes of wheat, and their arms, were identified according to Naranjo et al. (1987). For the identification of *T. longissimum* chromosomes, seeds of *T. longissimum* were germinated on moist filter paper in petri dishes. Growing roots 1–2 cm long were excised and immersed in tap water at 0 °C for 24–36 h to accumulate metaphase cells and shorten the chromosomes. The tips were fixed in acetic acid-alcohol (1:3), stored at 0°–4°C for 2 months and stained with the C-banding procedure. Each homologous pair of the chromosome complement showed a distinctive C-banding pattern that could be recognized in the cells at metaphase I of the hybrids. The assignment of *T. longissimum* chromosomes to the seven homoeologous groups and the arm designation, S or L, were carried out by virtue of pairing with wheat chromosomes in the hybrids. On average, 100 pollen mother cells (PMCs) per plant were scored in each of the two type of hybrids.

Results and discussion

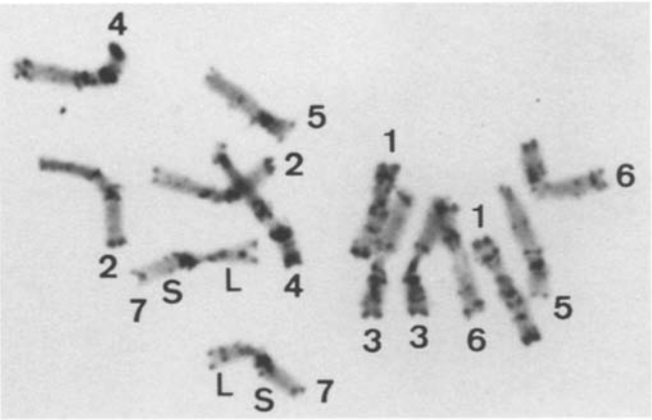

Fig. 1 C-banded mitotic metaphase of *T. longissimum*. *Numbers* indicate the homoeologous group and are located close to the telomere of the S arm. Designation of the *S* and *L* arms of chromosome $7S^l$ was not size dependent (see text)

The mitotic metaphase of Fig. 1 shows the C-banding pattern of *T. longissimum* chromosomes. The assignment of chromosomes and arm designations, which were based on pairing with wheat chromosomes, are in agreement with those of Friebe et al. (1993) with the exception of the arms of chromosome $7S^l$. Friebe et al. (1993) designated the S and L arms of chromosome $7S^l$ on the basis of size. This designation is, however, contradictory with the arm homoeology of chromosome $7S^l$ to wheat deduced from homoeologous pairing and from the location of genes for puple coleoptile, a group 7 chromosome S arm marker (Friebe et al. 1993). For this reason, the designation of $7S^lS$ and $7S^lL$ was interchanged in the present investigation. In fact, homoeology rather than length was the criterion used for designating the S and L arms of almost metacentric chromosomes of wheat (Gill et al. 1991) and chromosome $6S^l$ of *T. longissimum* (Friebe et al. 1993)

All *T. longissimum* chromosomes, and their arms, could be identified at metaphase I in the PMCs of the hybrids (Fig. 2a,b). Chromosomes 2A and 2D and their arms could not be distinguished from one another. The fact that 2AS, 2BS and 2DS are homoeologous, as are 2AL, 2BL and 2DL (Naranjo 1994), and the fact that $2S^lS$ paired with 2BS and $2S^lL$ paired with 2BL suggested that associations of $2S^lS$ and $2S^lL$ with chromosomes 2A and 2D involved the short and the long arms of these chromosomes, respectively.

The frequencies of association at metaphase I between the arms of *T. longissimum* chromosomes and wheat chromosomes in the two types of hybrids analyzed are given in Table 1. The absence of the pairing suppressor gene *Ph1* accounts for the high pairing level reached in the *ph1b* hybrids.

From the results of pairing, normal homoeologous relationships for both arms of chromosomes $1S^l$, $2S^l$, $3S^l$ and $6S^l$ to wheat chromosomes were deduced. Rare associations between $2S^lL$ and $4S^lL$ and between $6S^lL$ and 4BS were observed in only 1 PMC. The short arm of chromosome $5S^l$ is homoeologous to 5AS, 5BS and

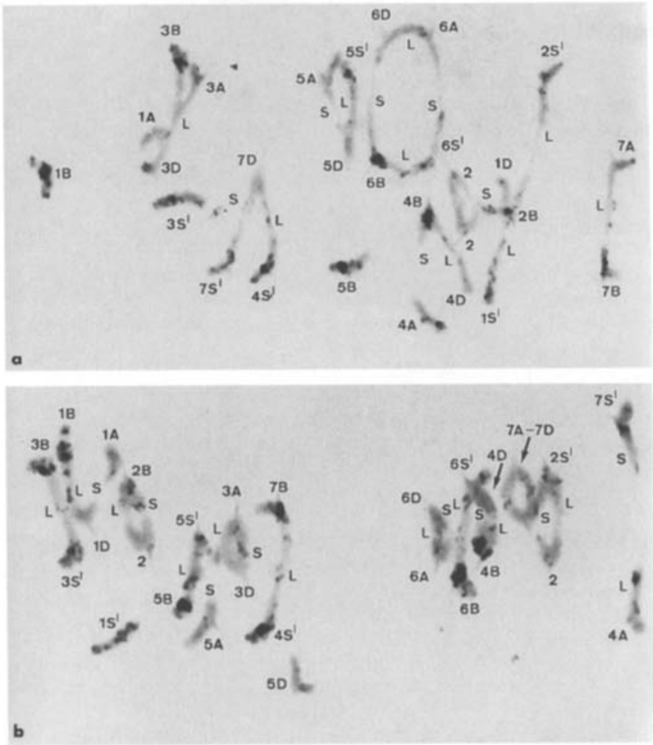

Fig. 2a,b Meiotic metaphase I cells of *ph1b* mutant wheat × *T. longissimum* hybrids. Wheat and *T. longissimum* chromosomes and the arms associated are identified. Chromosomes 2A and 2D that could not be distinguished from one another are indicated by the number 2. In **b**, the ring bivalent 7A-7D is indicated (*arrows*), but individual chromosomes 7A and 7D could not be identified

5DS. The $5S^lL$ arm often paired with 7BS, 5BL and 5DL and, at a much lower frequency, with 5AL. While the $5S^lL$-7BS association was distally located, the association between $5S^lL$ and 5AL involved an interstitial segment. The different location of these two arm associations results from the structure of the arms 5AL and 7BS, which are involved in the double translocation 5AL/4AL/7BS of wheat. The 5AL arm carries a translocated segment from 4AL. Thus, 5AL can only form chiasmata with group 5 chromosomes in the interstitial segment. The end segment of 7BS was translocated from 5AL and pairs with group 5 chromosomes. Chromosome $5S^l$ shows normal homoeologous relationships to chromosomes 5B and 5D, which are not involved in any translocation.

The results of pairing appears to confirm the homoeology of chromosomes $1S^l$, $2S^l$, $3S^l$, $5S^l$, and $6S^l$ to wheat previously established (Hart and Tuleen 1983; Jewell and Driscoll 1983; Netzle and Zeller 1984; Kota and Dvořák 1985; Levi et al. 1985; Millet et al. 1988; Hueros et al. 1991; Friebe et al. 1993).

The short arm of $4S^l$ is homoeologous to 4BS and 4DS. The frequency of association between $4S^lS$ and 4AS was very low (Table 1). This result is explained by the behavior of 4AS; this arm seldom pairs because of the structural modification of chromosome 4A by a pericientric inversion, which occurred in the primitive tetraploid wheat (Naranjo 1990). The long arm of chromosome $4S^l$ was associated with 7AL, 7BL or 7DL in the distal region. Interstitial bonds between $4S^lL$ and 4BL or 4DL were observed at a low frequency. The homoeologous relationships identified from this result indicate that $4S^lL$ carries a translocated segment from $7S^lL$.

Chromosome arm $7S^lS$ is homoeologous to the short arm of chromosomes 7A and 7D and to the segment of the 4AL arm that was translocated from 7BS (Table 1). The $7S^lL$ arm seldom paired, which indicates that this arm differs structurally from 7AL, 7BL and 7DL. Structure modification of the arms $7S^lL$ and $4S^lL$ may be the result of only one chromosome rearrangement, an unequal reciprocal translocation between $4S^lL$ and $7S^lL$ where the translocated segment from $7S^lL$ to $4S^lL$ was relatively long and the translocated segment from $4S^lL$ to $7S^lL$ was very short.

Table 1 Frequency (%) of association at meiotic metaphase I between chromosome arms of *T. longissimum* and *T. aestivum* in interspecific hybrids with the *Ph1* gene or with the *ph1b* mutation (*W* any of the three wheat genomes)

Arms being bound	Hybrid type[a]		Arms being bound	Hybrid type	
	ph1b	*Ph1*		*ph1b*	*Ph1*
$1S^lS$-1WS	21.3	2.7	$1S^lL$-1WL	86.0	17.0
$2S^lS$-2WS	60.3	2.0	$2S^lL$-2WL	67.7	9.0
$3S^lS$-3WS	53.0	2.7	$3S^lL$-3WL	70.0	6.3
$4S^lS$-(4BS, 4DS)	24.3	0.3	$4S^lL$-(4BL, 4DL)	2.0	0.0
$4S^lS$-4AS	0.3	0.0	$4S^lL$-7WL	48.0	4.7
			$4S^lL$-$2S^lL$	0.3	0.0
$5S^lS$-5WS	30.7	1.3	$5S^lL$-(7BS, 5BL, 5DL)	75.0	20.3
			$5S^lL$-5AL	2.0	0.0
$6S^lS$-6WS	44.0	2.0	$6S^lL$-6WL	74.7	3.3
$6S^lS$-4BS	0.3	0.0			
$7S^lS$-(4AL, 7AS, 7DS)	75.3	7.0	$7S^lL$-7WL	0.7	0.0

[a] Three hundred PMCs were scored for each hybrid type

Netzle and Zeller (1984) pointed out that the alien chromosome present in addition line D of *T. aestivum–T. longissimum* accession 7011 (Feldman 1975) can substitute for both the deficiency of chromosome 4B or chromosome arm 7BL. Hart and Tuleen (1983) found that the *T. longissimum* genes *Adh*-S^l1, *Lpx*-S^l2, and *Ep*-S^l1 are expressed in addition line D. In wheat, these markers are located on chromosomes of groups 4, 5 and 7, respectively. From these findings they suggested that the alien chromosome present in addition line D appears to be translocated relative to the situation in wheat. Friebe et al. (1993), who identified the *T. longissimum* chromosome present in addition line D as $4S^l$, reported that two different $4S^l$ addition lines, which were derived from *T. longissimum* accessions TL20 and No. 4, respectively, also expressed group 4 short arm and group 7 long arm isozymes. The presence in *T. longissimum* of a translocation between chromosomes $4S^l$ and $7S^l$ was concluded from the observation of seven bivalents at diakinesis-metaphase I in all F_1 hybrids between accession No. 4 and six different accessions of *T. longissimum*. Furthermore, the C-banding analysis realized by Friebe et al. (1993) did not detect any large structural difference in any of the 17 *T. longissimum* accessions that they used, accession TL01 that was analyzed in this work being included.

The presence of translocation $4S^lL/7S^lL$ in both accession 7011 and accession 6012 of *T. longissimum* is also supported by the finding that both accessions carry a reciprocal translocation relative to *T. searsii* where the translocated segments have unequal length (Feldman et al. 1979). All these data suggest that translocation $4S^lL/7S^lL$ should be an ancient rearrangement arisen during the evolution of *T. longissimum*.

Most studies concerning the origin of the B genome of wheat point to the conclusion that this genome is monophyletic and is derived from a diploid progenitor among the species of the *Sitopsis* section (Kerby and Kuspira 1987). A polyphyletic origin of the B genome also has been suggested (Sarkar and Stebbins 1956; Zohary and Feldman 1962). This hypothesis postulates that two or more amphiploids could arise from crosses between the donor of the A genome and different diploid species of the *Sitopsis* section. Hybridization between these amphiploids might produce new chromosome combination where the A genome would remain unchanged and the second genome, the B genome, would consist of chromosomes from two or more diploid species. The structural difference between the B genome of wheat and the S^l genome of *T. longissimum* precludes that this species was the donor of the B genome. But, if the origin of the B genome was polyphyletic, the possibility exists that any of chromosomes 1B, 2B, 3B, 5B or 6B could have been derived from *T. longissimum*.

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