

X. Cai · S. Jones

## Direct evidence for high level of autosyndetic pairing in hybrids of *Thinopyrum intermedium* and *Th. ponticum* with *Triticum aestivum*

Received: 19 September 1996 / Accepted: 18 April 1997

**Abstract** Genomic in situ hybridization (GISH) was used to distinguish autosyndetic from allosyndetic pairing in the hybrids of *Thinopyrum intermedium* and *Th. ponticum* with *Triticum aestivum* cv ‘Chinese Spring’ (CS). All hybrids showed high autosyndetic pairing frequencies among wheat chromosomes and among *Thinopyrum* chromosomes. The high autosyndetic pairing frequencies among wheat chromosomes in both hybrids suggested that *Th. intermedium* and *Th. ponticum* carry promoters for homoeologous chromosome pairing. The higher frequencies of autosyndetic pairing among *Thinopyrum* chromosomes than among wheat chromosomes in both hybrids indicated that the relationships among the three genomes of *Th. intermedium* and among the five genomes of *Th. ponticum* are closer than those among the three genomes of *T. aestivum*.

**Key words** Autosyndetic and allosyndetic pairing · In situ hybridization · Wheat · Wheatgrass

ing those for disease and insect resistance, and salt, drought, and cold tolerance, have been characterized and, in some cases, transferred to common wheat (*Triticum aestivum* L. em. Thell.) from these wild species (McGuire and Dvorak 1981; Shepherd and Islam 1988; Jiang et al. 1994; Jones et al. 1995). The hybrids between wheat and these two *Thinopyrum* species have been extensively studied cytogenetically (Peto 1936; Stebbins and Pun 1953; Dewey 1962; Dvorak 1981a,b; Muramatsu 1990; Jauhar 1995). However, direct evidence for autosyndetic and allosyndetic pairing in the hybrids has not been obtained because of the limitation of conventional chromosome techniques (Dvorak 1979; Sharma and Gill 1983; Jauhar 1995).

In the study presented here, autosyndetic and allosyndetic pairing in the hybrids of *Th. intermedium* and *Th. ponticum* with *T. aestivum* were analyzed by genomic in situ hybridization. This provided direct evidence for autosyndetic and allosyndetic pairing in the hybrids.

### Introduction

*Thinopyrum intermedium* (Host) Barknorth & D.R. Dewey [syn. *Agropyron glaucum* Roem. et Schult., *A. intermedium* (Host) Beauv., *Elytrigia intermedia* (Host) Nevski,  $2n = 6x = 42$ ] and *Th. ponticum* (Podp.) Barknorth & D.R. Dewey [syn. *Lophopyrum ponticum* (Podp.) A. Love, *Agropyron elongatum* (Host) Beauv.,  $2n = 10x = 70$ ] are both important genetic resources for wheat improvement. Many desirable genes, includ-

### Materials and methods

The *Th. intermedium* accession, PI 264770 ( $2n = 6x = 42$ ), and *Th. ponticum* accession, PI 206624 ( $2n = 10x = 70$ ), were provided by the USDA Regional Plant Introduction Station, Washington State University, Pullman, Wash. Both were used as male parents to cross with *T. aestivum* cv. ‘Chinese Spring’ (CS). Viable hybrid seeds were obtained by conventional crossing techniques without special treatment. Chromosome numbers of the hybrids were checked by a procedure described previously (Cai and Liu 1989).

Confirmed hybrids were planted in a greenhouse and maintained with standard cultural practices.

To analyze chromosome pairing in the hybrids of *Th. intermedium* and *Th. ponticum* with *T. aestivum*, we collected anthers at metaphase I (MI) of meiosis from hybrid plants and fixed these in 3:1 ethanol-acetic acid for 2–3 days at room temperature. Slides were prepared according to Cai et al. (1996). Chromosome pairing in the hybrids of *Th. intermedium* and *Th. ponticum* with *T. aestivum* was evaluated by calculating the mean arm pairing frequency ( $c$ ) (Driscoll et al. 1979).

Communicated by B. S. Gill

X. Cai · S. S. Jones (✉)  
Department of Crop and Soil Sciences,  
Washington State University, Pullman, WA 99164-6420, USA  
Fax: +1 (509) 335-8674; e-mail: jones@wsu.edu

Genomic in situ hybridization (GISH) was performed on meiotic chromosomes at MI in pollen mother cells (PMCs) of the hybrid plants. The same GISH technique as described earlier (Cai et al. 1996) was employed for GISH analysis in the present study. Total genomic DNA of *Th. intermedium* and *Th. ponticum* was used as probes for GISH analysis of meiotic chromosomes in the hybrids of *Th. intermedium* and *Th. ponticum* with CS, respectively. Both probes were labeled with Biotin-16-dUTP by nick translation (Enzo Diagnostic Nick Translation Kit). Total genomic DNA of CS sheared by boiling in 0.4 M NaOH for 40–50 min was used as blocking DNA of GISH.

## Results

### CS × *Th. intermedium* and chromosome pairing in the hybrid

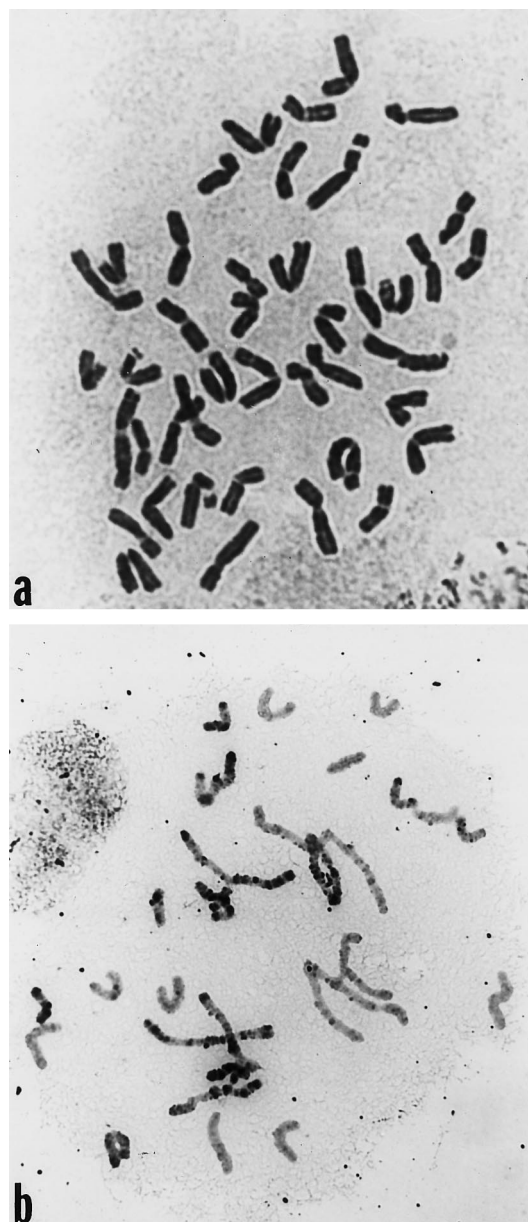
The *Th. intermedium* accession PI 264770 showed high cross-compatibility with CS. Seed set of the cross was 33.3%, and all hybrid plants had the expected chromosome number of  $2n = 42$  (Fig. 1a).

Chromosome pairing in the hybrid of *Th. intermedium* with CS was analyzed at MI of meiosis in PMCs by GISH. Since the probe used for GISH was the total genomic DNA of *Th. intermedium*, only *Th. intermedium* chromosomes in the hybrid showed hybridization signals (Fig. 1b). This hybrid showed high frequencies of autosyndetic pairing among wheat chromosomes (W/W) and among *Th. intermedium* chromosomes (T/T) (Table 1). The mean arm pairing frequencies of W/W and T/T were 0.33 and 0.44, respectively. Both T/T and W/W chromosome pairing had the same configurations, i.e., I + rodII + ringII + III + chain IV (Table 1). The allosyndetic pairing between CS and *Th. intermedium* chromosomes (W/T) was very low with a mean arm pairing frequency of 0.012. The W/T chromosome pairing configurations observed in the hybrid included rod bivalents, three kinds of trivalents (W/T/T, W/W/T and T/W/T), and one chain quadrivalent (W/W/T/T) (Table 1).

### CS × *Th. ponticum* and chromosome pairing in the hybrid

The *Th. ponticum* accession PI 206624 showed higher cross-compatibility with CS than did *Th. intermedium*. Seed set of this cross was 53.8%, and all hybrid plants had the expected chromosome number of  $2n = 56$  (Fig. 2a).

The frequency of autosyndetic pairing among wheat chromosomes (W/W) and among *Th. ponticum* chromosomes (T/T) was extremely high in this hybrid (Fig. 2b and Table 1). The mean arm pairing frequencies of W/W and T/T were 0.66 and 0.91, respectively. In contrast, the allosyndetic pairing frequency between wheat and *Th. ponticum* chromosomes (W/T) was extremely low with a mean arm pairing frequency of 0.007 (Table 1). The W/W chromosome pairing in this hybrid



**Figs. 1a, b** Chromosomes of F<sub>1</sub> hybrid between CS and *Th. intermedium*. **a** Somatic chromosomes,  $2n = 42$ . **b** GISH patterns of meiotic chromosomes at MI in PMCs; *Th. intermedium* chromosomes (dark, 11 I + 1 ring II + 4 rod II) show hybridization signals and CS chromosomes (light, 12 I + 1 rod II + 1 III + 1 IV) do not

showed the same configurations as the hybrid between *Th. intermedium* and CS, although the frequencies differed (Table 1). Among the T/T chromosome pairing configurations, bivalents and trivalents showed much higher frequencies than quadrivalents and quinquivalents in the hybrid. Additionally, a high frequency of T/T ring bivalents was observed in the hybrid. Hexavalents and heptavalents formed by *Th. ponticum* chromosomes were observed at low frequencies in the hybrid (Table 1). The W/T chromosome pairing

**Table 1** Mean wheat/wheat (W/W), *Thinopyrum/Thinopyrum* (T/T), and wheat *Thinopyrum* (W/T) chromosome pairing in hybrids of CS with *Th. intermedium* and *Th. ponticum* (ranges in parentheses)

Hybrids	W/W		T/T		W/T																			
	I	II	III	IV	c <sup>a</sup>	I	II	III	IV	V	VI	VII	c	II	III	IV	VIII	c						
Chromosome no.	Ring	Rod	Ring	Rod	Chain	Ring	Rod	Ring	Rod	Chain	Chain	Chain	Chain	Rod	Rod	Rod	Rod	Rod	Rod					
CS × <i>Th. ponticum</i>	7.28 (2-13)	1.89 (0-5)	3.49 (0-6)	0.89 (0-5)	0.05 (0-1)	0.66	4.00 (0-8)	7.54 (1-12)	2.89 (0-10)	1.49 (0-3)	0.08 (0-1)	0.44 (0-2)	0.41 (0-3)	0.18 (0-1)	0.03 (0-1)	0.91	0.18 (0-1)	0.03 (0-1) <sup>b</sup>	0.03 (0-1) <sup>c</sup>	—	0.03 (0-1) <sup>e</sup>	0.03 (0-1) <sup>f</sup>	0.007	
CS × <i>Th. intermedium</i>	12.87 (6-8)	0.47 (0-2)	3.13 (0-6)	0.21 (0-2)	0.01 (0-1)	0.33	9.74 (6-25)	0.43 (0-2)	4.09 (2-7)	0.53 (0-3)	—	0.03 (0-1)	—	—	—	0.44	0.33 (0-4)	0.04 (0-1)	0.05 (0-1)	0.03 (0-1) <sup>d</sup>	0.01 (0-1)	—	—	0.012

<sup>a</sup> Mean arm pairing frequency

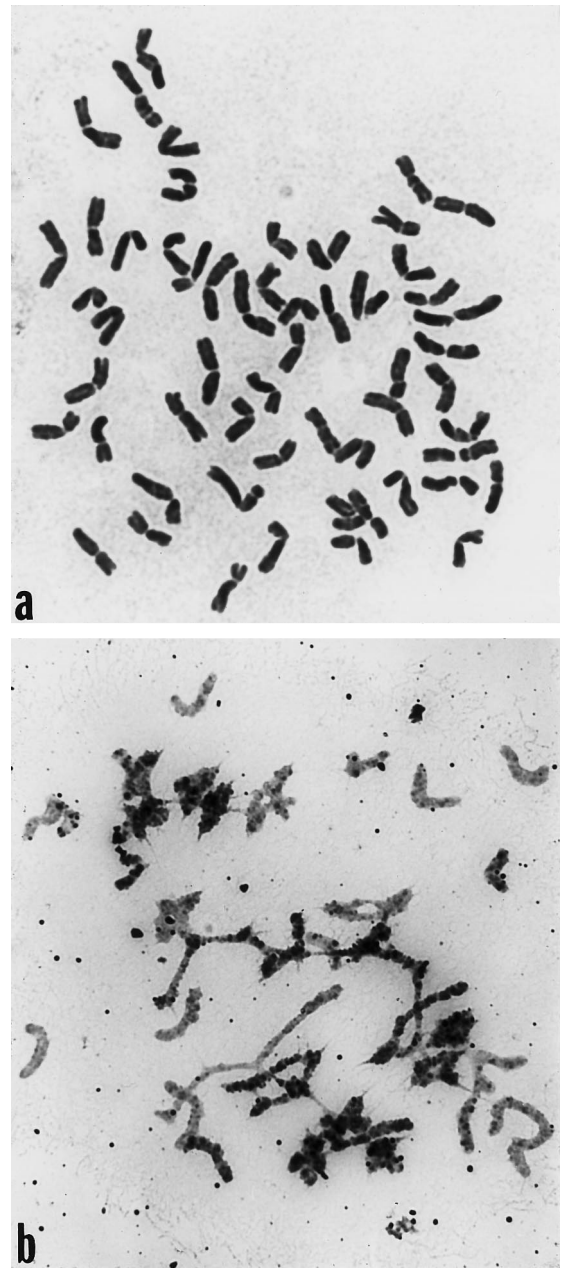
<sup>b</sup> W/T/T

<sup>c</sup> W/W/T

<sup>d</sup> T/W/T

<sup>e</sup> W/W/T/T

<sup>f</sup> W/W/W/T/T/T/T/T



**Figs. 2a, b** Chromosomes of F<sub>1</sub> hybrid between CS and *Th. ponticum*. **a** Somatic chromosomes, 2n = 56. **b** GISH patterns of meiotic chromosomes at MI in PMCs; *Th. ponticum* chromosomes (dark, 3 I + 9 ring II + 2 rod II + 1 III + 1 VII) show hybridization signals, CS chromosomes (light, 9I + 3 ring II + 3 rod II) do not

configurations in the hybrid included rod bivalents, two types of trivalents (W/T/T and W/W/T), one chain quadrivalent (W/W/T/T), and one chain octavalent (W/W/W/T/T/T/T/T) (Table 1).

## Discussion

GISH patterns of meiotic chromosomes at MI in the hybrid of *Th. intermedium* with CS indicated that

chromosome pairing in the hybrid mainly occurred among wheat chromosomes and among *Th. intermedium* chromosomes and that allosyndetic pairing between wheat and *Th. intermedium* chromosomes was very rare (Table 1 and Fig. 1b). The much higher frequencies of autosyndetic pairing than allosyndetic pairing in the hybrid of *Th. intermedium* with CS demonstrated that the relationships among *T. aestivum* genomes and among *Th. intermedium* genomes are much closer than the relationship between *T. aestivum* and *Th. intermedium* genomes.

The frequency of autosyndetic pairing among *Th. intermedium* chromosomes was higher than that among wheat chromosomes in the hybrid of *Th. intermedium* with CS (Table 1). This suggested that the three genomes of *Th. intermedium* are more closely related to each other than the three genomes of *T. aestivum*. The W/W and T/T autosyndetic pairing showed the same configurations, i.e., I + II (ring and rod) + III + IV (chain), in the hybrid of *Th. intermedium* with CS. In the hybrid, the frequency of trivalents formed by *Th. intermedium* chromosomes was two and half times higher than that of trivalents formed by CS chromosomes, and the frequency of T/T ring bivalents was lower than that of W/W ring bivalents (Table 1). *T. aestivum* has been considered to be a typical allohexaploid although the affinity between the A and D genomes of *T. aestivum* is higher than that between the A and B, or B and D genomes (Jauhar et al. 1991). Therefore, it can be concluded that the higher frequency of T/T autosyndetic pairing than that of W/W autosyndetic pairing resulted from the higher homoeology among the three genomes of *Th. intermedium* than the three genomes of *T. aestivum* rather than the homology between two specific genomes of *Th. intermedium*. *Th. intermedium* can thus be designated as an allohexaploid.

Both wheat and *Th. intermedium* chromosomes showed high autosyndetic pairing frequencies in the hybrid between *Th. intermedium* and CS carrying the homoeologous pairing suppressor gene, *Ph1* (Table 1). The mean arm pairing frequency of W/W in the hybrid, 0.33, is much higher than those of CS euploids carrying *Ph1* (Riley and Chapman 1958; Kimber and Riley 1963; McGuire and Dvorak 1982; Jauhar 1991). This indicated that W/W homoeologous chromosome pairing was promoted in the hybrid in the presence of *Ph1* and that *Th. intermedium* was the carrier of the promoter(s). The promoter(s) also probably affected autosyndetic pairing among *Th. intermedium* chromosomes themselves in the hybrid.

Chromosome pairing in the hybrid of *Th. ponticum* with CS occurred mainly among wheat chromosomes and among *Th. ponticum* chromosomes. The frequency of allosyndetic pairing between wheat and *Th. ponticum* chromosomes was very low in the hybrid (Table 1; Fig. 2b). These results demonstrated that the relationships among *T. aestivum* genomes and among decaploid *Th. ponticum* genomes are much closer than the

relationship between the genomes of these two species. *Th. ponticum* showed a higher autosyndetic pairing frequency ( $c = 0.91$ ) than wheat chromosomes ( $c = 0.66$ ) in the hybrid of *Th. ponticum* with CS. This indicated that the genomes of *Th. ponticum* are more closely related to each other than are the genomes of wheat.

The hybrid between *Th. ponticum* and CS carrying *Ph1* showed a very high frequency of autosyndetic pairing among wheat chromosomes (W/W) and among *Th. ponticum* chromosomes (T/T) (Table 1). The mean arm pairing frequency of W/W autosyndetic pairing in the hybrid, 0.66, is much higher than those of CS euploids with *Ph1* (Riley and Chapman 1958; Kimber and Riley 1963; McGuire and Dvorak 1982; Jauhar 1991). This  $c$  value is also higher than that of CS euploid without *Ph1* (Jauhar 1991). This demonstrated that *Th. ponticum* carries promoter(s) expressing strong promotion for homoeologous chromosome pairing.

## References

- Cai XW, Liu DJ (1989) Identification of an 1B/1R wheat-rye chromosome translocation. *Theor Appl Genet* 77:81–83
- Cai XW, Jones SS, Murray TD (1996) Characterization of an *Agropyron elongatum* chromosome conferring resistance to *Cephalosporium* stripe in common wheat. *Genome* 39:56–62
- Dewey DR (1962) The genome structure of intermediate wheatgrass. *J Hered* 53:282–290
- Driscoll CJ, Bielig LM, Darvey NL (1979) An analysis of frequencies of chromosome configurations in wheat and wheat hybrids. *Genetics* 91:755–767
- Dvorak J (1979) Metaphase I pairing frequencies of individual *Agropyron elongatum* chromosome arms with *Triticum* chromosomes. *Can J Genet Cytol* 21:243–254
- Dvorak J (1981a) Genome relationships among *Elytrigia* (= *Agropyron*) *elongata*, *E. stipifolia*, “*E. elongata* 4 $\times$ ”, *E. caespitosa*, *E. intermedia*, and “*E. elongata* 10 $\times$ ”. *Can J Genet Cytol* 23:481–492
- Dvorak J (1981b) Chromosome differentiation in polyploid species of *Elytrigia*, with special reference to the evolution of diploid-like chromosome pairing in polyploid species. *Can J Genet Cytol* 23:287–303
- Jauhar PP (1995) Meiosis and fertility of F<sub>1</sub> hybrids between hexaploid bread wheat and decaploid tall wheatgrass (*Thinopyrum ponticum*). *Theor Appl Genet* 90:865–871
- Jauhar PP, Riera-Lizarazu O, Dewey WG, Gill BS, Crane CF, Bennett JH (1991) Chromosome pairing relationships among the A, B, and D genomes of bread wheat. *Theor Appl Genet* 82:441–449
- Jiang J, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. *Euphytica* 73:199–212
- Jones SS, Murray TD, Allan RE (1995) Use of alien genes for the development of disease resistance in wheat. *Annu Rev Phytopathol* 33:429–443
- Kimber G, Riley R (1963) The relationships of the diploid progenitors of hexaploid wheat. *Can J Genet Cytol* 5:83–88
- McGuire PE, Dvorak J (1981) High salt-tolerance potential in wheatgrasses. *Crop Sci* 21:701–705
- McGuire PE, Dvorak J (1982) Genetic regulation of heterogenetic chromosome pairing in polyploid species of the genus *Triticum* sensu lato. *Can J Genet Cytol* 24:57–82
- Muramatsu M (1990) Cytogenetics of decaploid *Agropyron elongatum* (*Elytrigia elongata*) (2n = 70). I. Frequency of decavalent formation. *Genome* 33:811–817

- Peto FH (1936) Hybridization of *Triticum* and *Agropyron* II. Cytology of the male parents and F<sub>1</sub> generation. Can J Res 14 Sec C:203–214
- Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715
- Sharma HC, Gill BS (1983) New hybrids between *Agropyron* and wheat. 2. Production, morphology and cytogenetic analysis of F<sub>1</sub> hybrids and backcross derivatives. Theor Appl Genet 66:111–121
- Shepherd KW, Islam AKMR (1988) Fourth compendium of wheat-alien chromosome lines. In: Miller TE, Koeloner RMD (eds), Proc 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp 1373–1398
- Stebbins GL, Pun FT (1953) Artificial and natural hybrids in the *Gramineae*, tribe *Hordeae*. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the *Triticinae*. Genetics 38:600–608