Synthesis and cytological characterization of trigeneric hybrids of durum wheat with and without Ph1

Prem P. Jauhar, M. Doğramaci, and T.S. Peterson

Abstract: Wild grasses in the tribe Triticeae, some in the primary or secondary gene pool of wheat, are excellent reservoirs of genes for superior agronomic traits, including resistance to various diseases. Thus, the diploid wheatgrasses Thinopyrum bessarabicum (Savul. and Rayss) Á. Löve (2n = 2x = 14; JJ genome) and Lophopyrum elongatum (Host) Á. Löve (2n = 2x = 14; EE genome) are important sources of genes for disease resistance, e.g., Fusarium head blight resistance that may be transferred to wheat. By crossing fertile amphidiploids (2n = 4x = 28; JEE) developed from F1 hybrids of the 2 diploid species with appropriate genetic stocks of durum wheat, we synthesized trigeneric hybrids (2n = 4x = 28; ABJE) incorporating both the J and E genomes of the grass species with the durum genomes A and B. Trigeneric hybrids with and without the homoeologous-pairing suppressor gene, Ph1, were produced. In the absence of Ph1, the chances of genetic recombination between chromosomes of the 2 useful grass genomes (JE) and those of the durum genomes (AB) would be enhanced. Meiotic chromosome pairing was studied using both conventional staining and fluorescent genomic in situ hybridization (fl-GISH). As expected, the Ph1-intergeneric hybrids showed low chromosome pairing (23.86% of the complement), whereas the trigenerics with ph1b (49.49%) and those with their chromosome 5B replaced by 5D (49.09%) showed much higher pairing. The absence of Ph1 allowed pairing and, hence, genetic recombination between homoeologous chromosomes. Fl-GISH analysis afforded an excellent tool for studying the specificity of chromosome pairing: wheat with grass, wheat with wheat, or grass with grass. In the trigeneric hybrids that lacked chromosome 5B, and hence lacked the Ph1 gene, the wheat–grass pairing was elevated, i.e., 2.6 chiasmata per cell, a welcome feature from the breeding standpoint. Using Langdon 5D(5B) disomic substitution for making trigeneric hybrids should promote homoeologous pairing between durum and grass chromosomes and hence accelerate alien gene transfer into the durum genomes.

Key words: alien gene transfer, chiasma (xma) frequency, chromosome pairing, fluorescent genomic in situ hybridization (fl-GISH), homoeologous-pairing regulator, specificity of chromosome pairing, wheatgrass.

Résumé : Les graminées sauvages de la tribu des triticées, dont certaines appartiennent au pool génique primaire ou secondaire du blé, constituent d’excellents réservoirs de gènes d’intérêt agronomique dont la résistance à diverses maladies. Ainsi, les élitemes diploïdes, Thinopyrum bessarabicum (Savul. And Rayss) Á. Löve (2n = 2x = 14 ; génome JJ) et Lophopyrum elongatum (Host) Á. Löve (2n = 2x = 14 ; génome EE), sont d’importantes sources de gènes conférant la résistance aux maladies comme, par exemple, la résistance à la fusariose de l’épi, lesquels peuvent être transférés au blé. En croisant des amphidiploïdes fertiles (2n = 4x = 28 ; JEE), issus d’hybrides F1 entre les deux espèces diploïdes, avec des génotypes choisis de blé dur, les auteurs ont produit des hybrides trigénériques (2n = 4x = 28 ; ABJE) comprenant tant les génomes J et E des graminées sauvages que les génomes A et B du blé dur. Des hybrides trigénériques avec ou sans le gène de suppression des appariements homéologues, Ph1, ont été produits. En l’absence de Ph1, la probabilité de recombinaison génétique entre les chromosomes des génomes sauvages (JE) et ceux des génomes du blé dur (AB) serait accrue. L’appariement des chromosomes lors de la méiose a été examiné tant par des méthodes de coloration traditionnelles que par hybridation génomique in situ en fluorescence (fl-GISH). Tel qu’attendu, les hybrides trigénériques Ph1 ont montré peu d’appariement chromosomique (23,86 % du complément), tandis que les hybrides trigénériques ph1b (49,49 %) ou ceux chez lesquels le chromosome 5B avait été remplacé par le 5D (49,09 %) montraient bien davantage d’appariements. L’absence de Ph1 a rendu possible l’appariement et la recombinaison entre les chromosomes homéologues. L’approche Fl-GISH s’est avérée une excellente méthode pour étudier la spécificité des appariements chromosomiques : blé–élime, blé–blé ou élime–élime. Chez les hybrides trigénériques sans le chromosome Ph1, l’appariement et la recombinaison étaient accrues par rapport aux hybrides avec Ph1. En l’absence de Ph1, l’appariement et la recombinaison étaient accrues par rapport aux hybrides avec Ph1.


Corresponding Editor: G.J. Scoles.

P.P. Jauhar,1 M. Doğramaci,2 and T.S. Peterson. United States Department of Agriculture– Agricultural Research Service, Northern Crop Science Laboratory, Fargo, ND 58105, USA.

1Corresponding author (e-mail: prem.jauhar@ndsu.nodak.edu).
2Present address: AGROTEK Seed Co., Altiayak Mh. 2401 Sok. No.2/1,Varsak-Antalya, Turkey.
5B (ainsi dépourvu de Phl), l’appariement blé-élyme était accru pour atteindre 2,6 chiasmas par cellule, une caractéristique bienvenue du point de vue du sélectionneur. L’emploi de la substitution disomique Langdon 5D (5B) lors de la synthèse d’hybrides trigénériques devrait favoriser l’appariement des homéologues et accélérer ainsi le transfert de gènes étrangers vers le blé dur.

Mots clés : transfert de gènes étrangers, fréquence des chiasmas, appariement chromosomique, hybridation génomique in situ en fluorescence (fl-GISH), régulateur d’appariement homéologue, spécificité de l’appariement chromosomique, élymes.

[Intaduit par la Rédaction]

Introduction

Durum wheat or macaroni wheat, *Triticum turgidum* L. (2n = 4x = 28; AABB genomes), is a natural hybrid with 2 related genomes but with genetically enforced diploid-like chromosome pairing effected by the homoeologous-pairing suppressor gene, Phl, in the long arm of chromosome 5B (Sears 1976; Feldman 1993; Gill et al. 1993; Jauhar and Joppa 1996; Jauhar et al. 1999: Martinez et al. 2001). It is an important cereal used for preparing pasta and semolina for human consumption worldwide. Several useful traits are present in wild species of the tribe Triticeae that may be transferred to wheat. Wide hybridization has contributed to genetic improvement of polyploid wheats (Friebe et al. 1996; Jauhar and Chibbar 1999; Jauhar 2003). The wheatgrass genera *Thinopyrum* and *Lophopyrum* provide important genetic resources for wheat improvement. Thus, the diploid wheatgrasses *Thinopyrum bessarabicum* (Savul. and Rayss) A. Löve (2n = 2x = 14; JJ genome) and *Lophopyrum elongatum* (Host) A. Löve (2n = 2x = 14; EE genome) are important sources of genes for salt tolerance and Fusarium head blight (FHB) resistance (Gorham et al. 1986; Dvořák and Ross 1986; Omielan and Epstein 1991; Jauhar and Peterson 2000a, 2000b; Fedak et al. 2003). These grasses are potential donors of FHB resistance to polyplloid wheats.

Diploid F1 hybrids (2n = 2x = 14; JE) between these wheatgrass species are sterile, whereas the derived amphidiploids (2n = 4x = 28; JJEE) have essentially diploid-like chromosome pairing and are highly fertile (Jauhar 1988). Trigeneric hybrids (2n = 4x = 28; ABJE) involving durum wheat and the 2 diploid wheatgrasses show limited pairing because of the presence of Phl (Jauhar 1992a), thereby restricting alien gene transfer into durum wheat. In trigeneric with 2 useful grass genomes, J and E, and without Phl, the chances of homoeologous pairing and hence genetic recombination between chromosomes of JE genomes and those of the AB genomes would be enhanced. We therefore synthesized several trigeneric hybrids using durum genotypes with and without Phl and studied chromosome pairing using conventional staining techniques. Fluorescent genomic in situ hybridization (fl-GISH) provides an excellent tool for discriminating between chromosomes of different genomes (Kosina and Heslop-Harrison 1996) and for studying the specificity of chromosome pairing, thereby elucidating genomic relationships (Heslop-Harrison and Schwarzacher 1996; Jauhar et al. 1999). The main objective of this study was to assess pairing between grass chromosomes (JE complement) and durum chromosomes (AB complement) in both the presence and absence of Phl. Chromosome pairing within the grass complement (JE) and within the durum complement (AB) was also analyzed.

Materials and methods

Plant materials

F1 hybrids between the diploid wheatgrasses *Thinopyrum bessarabicum* (2n = 2x = 14; JJ genome) and *Lophopyrum elongatum* (2n = 2x = 14; EE genome) were sterile, and chromosome doubling of the F1 hybrids yielded highly fertile amphidiploids (2n = 4x = 28; JJEE) (Jauhar 1988). To combine both J and E genomes with durum genomes A and B, durum wheat was hybridized with the JJEE amphidiploids, and trigeneric hybrids (2n = 4x = 28; ABJE) with Phl were obtained. A heterozygous Langdon Ph mutant (Ph1 ph1b) and Langdon 5D(5B) disomic substitution line were used as female parents for making trigeneric hybrids without Phl.

Synthesis of trigeneric hybrids

Hybrids between durum wheat lines (‘Langdon’, Langdon (Ph1ph1b) and Langdon 5D(5B) disomic substitution) and JJEE amphidiploids were made using durum wheat as the female parent. Spikes of the durum lines were emasculated before anthesis and covered with glassine bags until the stigmas became receptive (3 to 5 days after emasculation). The emasculated spikes were then pollinated with fresh pollen of the amphidiploid using the individual anther technique; 1 anther usually pollinated 2 florets. A hormone mixture of 2,4-D (5 mg/L), gibberellic acid (75 mg/L), and Tween 20 (0.01%) was sprayed into each floret (~5 µL/floret) 24 h after pollination.

Embryos were rescued 14 to 20 days after pollination and cultured on Murashige and Skoog’s media supplemented with 2% sucrose, 8% purified agar, and 0.5 mg/L α-naphthaleonic acid. Cultured embryos were placed in a lighted growth room (at 21 °C and on a16 h light, 8 h dark regime) until root and shoot development was obtained (~ 2–4 wk). The plantlets were then transferred to Jiffy pots and placed into the growth room for 7 d to harden. The established plantlets were moved to a greenhouse, transplanted into 15-cm pots, and grown to maturity.

Chromosome studies

Spikes of the hybrid plants at the appropriate stages were fixed in Carnoy’s fluid (6 parts of ethanol : 3 parts of chloroform : 1 part of glacial acetic acid). For conventional staining, an anther from a floret was squashed in carbol fuchsin to obtain well-spread meiotic stages. When the squashed an-
ther had the appropriate metaphase I stage, the remaining 2 anthers from the same floret were saved in the fixative for fl-GISH analysis. Chromosome pairing was analyzed at meiotic metaphase I. Fl-GISH helped to distinguish between the durum and grass chromosomes.

To study the specificity of chromosome pairing (between the grass and durum chromosomes, within the durum complement AB, and within the grass complement JE), fl-GISH was used. Selected anthers at the appropriate stage were squashed in 45% acetic acid. Slides with well-spread meiotic metaphase I stages were frozen and stored at –80 °C. Sheared *Triticum urartu* and *Aegilops speltoides* DNA (~300–600 bp) labeled with biotin-14-dATP (using nick translation) was used as a probe, and autoclaved JJEE amphidiploid DNA was used as the blocker; 200 ng probe DNA (100 ng *T. urartu* and 100 ng *Ae. speltoides*) and 4000 ng blocking DNA were used per slide. A hybridization mixture, prepared for 4 slides, contained 50 µL deionized formamide, 20 µL 50% dextran sulfate, 10 µL 20X SSC (0.15 mol/L NaCl plus 0.015 mol/L sodium citrate), and 20 µL Tris–EDTA buffer containing the probe and blocking DNA. The slides were then hybridized for 24 h. After hybridization, detection of the biotinylated probe was accomplished with the use of fluorescein isothiocyanate-conjugated avidin. The slides were then counterstained using propidium iodide.

Slides were viewed under an epifluorescent microscope with the appropriate filter sets and a 100-watt adjustable UV light source. Images of appropriate chromosome preparations were captured digitally using a SPOT II digital camera and the manufacturer’s software (Diagnostic Instruments, Inc., Sterling Heights, Mich.). The images were then scored for chromosome pairing. Plates of suitable photographs were prepared using Paint Shop Pro version 5 (JASC Software Inc.) to capture individual images and make imposed overlays of the probe image onto the counterstain image. The combined images were then imported into Microsoft PowerPoint to prepare a composite plate.

**Results**

Trigeneric hybrids (*2n = 4x = 28; ABJE*) with *Ph1* were obtained by crossing durum ‘Langdon’ with fertile *Th. bessarabicum/L. elongatum* amphidiploids (*2n = 4x = 28; JJEE*). Crosses of these amphidiploids with the heterozygote *Ph1Ph1b* mutant yielded trigeneric hybrids with either *Ph1* or *ph1b*, and these were easily distinguishable. The latter were selected for study of chromosome pairing. Crosses of the JJEE amphidiploids with Langdon 5D(5B) disomic substitution line yielded trigenerics without chromosome 5B and, hence, without *Ph1*. All trigeneric hybrids, with or without *Ph1*, had 28 somatic chromosomes.

The trigeneric hybrids were perennial and vigorous. As observed earlier by Jauhar (1992a), spike morphology and spikelet density in the trigeneric hybrids were intermediate between the male and female parents, although awns in the hybrids were greatly suppressed.

### Table 1. Chromosome pairing and chiasma frequency in trigeneric hybrids (*2n = 4x = 28; ABJE*) with *Ph1*.

<table>
<thead>
<tr>
<th>Trigeneric hybrid</th>
<th>No. of PMCs scored</th>
<th>Mean Range</th>
<th>Mean Range</th>
<th>Mean Range</th>
<th>Mean Range</th>
<th>Mean Range</th>
<th>Mean Range</th>
<th>Mean Range</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chr. no. (2n)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>H-15</td>
<td>28</td>
<td>0.02 (0–1)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.08 (0–2)</td>
<td>0.16 (0–2)</td>
<td>0.02 (0–1)</td>
<td>0.004 (0–1)</td>
</tr>
<tr>
<td>H-15</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.017 (0–2)</td>
</tr>
<tr>
<td>H-14</td>
<td>28</td>
<td>0.02 (0–1)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.08 (0–2)</td>
<td>0.16 (0–2)</td>
<td>0.02 (0–1)</td>
<td>0.004 (0–1)</td>
</tr>
<tr>
<td>H-13</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.004 (0–1)</td>
</tr>
<tr>
<td>H-7</td>
<td>28</td>
<td>0.02 (0–1)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.08 (0–2)</td>
<td>0.16 (0–2)</td>
<td>0.02 (0–1)</td>
<td>0.004 (0–1)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.02 (0–1)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.08 (0–2)</td>
<td>0.16 (0–2)</td>
<td>0.02 (0–1)</td>
<td>0.004 (0–1)</td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td>0.02 (0–1)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.14 (0–2)</td>
<td>0.08 (0–2)</td>
<td>0.16 (0–2)</td>
<td>0.02 (0–1)</td>
<td>0.004 (0–1)</td>
</tr>
</tbody>
</table>

Notes: Chr, chromosome; PMCs, pollen mother cells.

**Chromosome pairing in the presence of *Ph1***

Chromosome pairing at meiotic metaphase I in 5 trigeneric hybrids with *Ph1* varied among the hybrids,
Table 2. Chromosome pairing and chiasma frequency in trigeneric hybrids \((2n = 4x = 28; \text{ ABJE})\) with \(phib\) allele.

<table>
<thead>
<tr>
<th>Trigeneric hybrid</th>
<th>Chr. no. ((2n))</th>
<th>No. of PMCs scored</th>
<th>IV Mean</th>
<th>IV Range</th>
<th>III Mean</th>
<th>III Range</th>
<th>Ring II Mean</th>
<th>Ring II Range</th>
<th>Rod II Mean</th>
<th>Rod II Range</th>
<th>I Mean</th>
<th>I Range</th>
<th>Per cell</th>
<th>Per II</th>
<th>% chr. paired</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-9</td>
<td>28</td>
<td>50</td>
<td>0.04</td>
<td>(0–1)</td>
<td>0.38</td>
<td>(0–2)</td>
<td>0.26</td>
<td>(0–2)</td>
<td>5.48</td>
<td>(3–9)</td>
<td>15.22</td>
<td>(9–18)</td>
<td>6.88</td>
<td>1.05</td>
<td>45.64</td>
</tr>
<tr>
<td>H-23</td>
<td>28</td>
<td>50</td>
<td>0.30</td>
<td>(0–2)</td>
<td>0.90</td>
<td>(0–3)</td>
<td>0.48</td>
<td>(0–4)</td>
<td>5.08</td>
<td>(1–10)</td>
<td>12.66</td>
<td>(6–19)</td>
<td>8.94</td>
<td>1.09</td>
<td>54.76</td>
</tr>
<tr>
<td>H-26</td>
<td>28</td>
<td>50</td>
<td>0.06</td>
<td>(0–1)</td>
<td>0.44</td>
<td>(0–2)</td>
<td>0.16</td>
<td>(0–1)</td>
<td>5.74</td>
<td>(3–9)</td>
<td>14.64</td>
<td>(10–20)</td>
<td>7.20</td>
<td>1.03</td>
<td>47.71</td>
</tr>
<tr>
<td>H-27</td>
<td>28</td>
<td>50</td>
<td>0.00</td>
<td>0</td>
<td>0.42</td>
<td>(0–2)</td>
<td>0.32</td>
<td>(0–3)</td>
<td>4.92</td>
<td>(2–8)</td>
<td>16.16</td>
<td>(9–22)</td>
<td>6.42</td>
<td>1.06</td>
<td>42.29</td>
</tr>
<tr>
<td>H-28</td>
<td>28</td>
<td>50</td>
<td>0.08</td>
<td>(0–1)</td>
<td>0.62</td>
<td>(0–3)</td>
<td>0.26</td>
<td>(0–2)</td>
<td>6.64</td>
<td>(3–10)</td>
<td>12.02</td>
<td>(8–18)</td>
<td>8.64</td>
<td>1.04</td>
<td>57.07</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.48</td>
<td></td>
<td>2.76</td>
<td></td>
<td>1.48</td>
<td></td>
<td>27.86</td>
<td></td>
<td>70.70</td>
<td></td>
<td>38.08</td>
<td>5.27</td>
<td>247.47</td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td></td>
<td>0.10</td>
<td>(0–2)</td>
<td>0.55</td>
<td>(0–3)</td>
<td>0.30</td>
<td>(0–4)</td>
<td>2.97</td>
<td>(1–10)</td>
<td>14.14</td>
<td>(6–26)</td>
<td>7.65</td>
<td>1.05</td>
<td>49.49</td>
</tr>
</tbody>
</table>

Table 3. Chromosome pairing and chiasma frequency in trigeneric hybrids \((2n = 4x = 28; \text{ ABJE})\) without chromosome 5B.

<table>
<thead>
<tr>
<th>Trigeneric hybrid</th>
<th>Chr. no. ((2n))</th>
<th>No. of PMCs scored</th>
<th>IV Mean</th>
<th>IV Range</th>
<th>III Mean</th>
<th>III Range</th>
<th>Ring II Mean</th>
<th>Ring II Range</th>
<th>Rod II Mean</th>
<th>Rod II Range</th>
<th>I Mean</th>
<th>I Range</th>
<th>Per cell</th>
<th>Per II</th>
<th>% chr. paired</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH00–20–1</td>
<td>28</td>
<td>50</td>
<td>0.06</td>
<td>(0–1)</td>
<td>0.40</td>
<td>(0–2)</td>
<td>0.86</td>
<td>(0–3)</td>
<td>5.82</td>
<td>(3–11)</td>
<td>13.20</td>
<td>(8–18)</td>
<td>8.52</td>
<td>1.13</td>
<td>52.86</td>
</tr>
<tr>
<td>GH00–20–12</td>
<td>28</td>
<td>50</td>
<td>0.06</td>
<td>(0–1)</td>
<td>0.38</td>
<td>(0–3)</td>
<td>0.56</td>
<td>(0–3)</td>
<td>6.12</td>
<td>(4–10)</td>
<td>13.26</td>
<td>(8–18)</td>
<td>8.18</td>
<td>1.08</td>
<td>52.64</td>
</tr>
<tr>
<td>GH00–18–1</td>
<td>28</td>
<td>50</td>
<td>0.06</td>
<td>(0–1)</td>
<td>0.40</td>
<td>(0–2)</td>
<td>0.86</td>
<td>(0–3)</td>
<td>5.82</td>
<td>(3–11)</td>
<td>13.20</td>
<td>(6–16)</td>
<td>8.52</td>
<td>1.13</td>
<td>52.86</td>
</tr>
<tr>
<td>GH00–18–2</td>
<td>28</td>
<td>50</td>
<td>0.10</td>
<td>(0–1)</td>
<td>0.66</td>
<td>(0–3)</td>
<td>0.38</td>
<td>(0–3)</td>
<td>5.52</td>
<td>(1–8)</td>
<td>13.82</td>
<td>(9–21)</td>
<td>7.90</td>
<td>1.06</td>
<td>50.64</td>
</tr>
<tr>
<td>GH00–20–13</td>
<td>28</td>
<td>50</td>
<td>0.04</td>
<td>(0–1)</td>
<td>0.32</td>
<td>(0–2)</td>
<td>0.06</td>
<td>(0–1)</td>
<td>4.48</td>
<td>(1–8)</td>
<td>17.80</td>
<td>(11–24)</td>
<td>5.36</td>
<td>1.01</td>
<td>36.43</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.32</td>
<td></td>
<td>2.16</td>
<td></td>
<td>2.72</td>
<td></td>
<td>27.76</td>
<td></td>
<td>71.28</td>
<td></td>
<td>38.48</td>
<td>5.41</td>
<td>245.43</td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td></td>
<td>0.06</td>
<td>(0–1)</td>
<td>0.43</td>
<td>(0–3)</td>
<td>0.54</td>
<td>(0–3)</td>
<td>5.55</td>
<td>(1–11)</td>
<td>14.26</td>
<td>(6–24)</td>
<td>7.70</td>
<td>1.08</td>
<td>49.09</td>
</tr>
</tbody>
</table>

Note: Chr, chromosome; PMCs, pollen mother cells.
Fig. 1. Conventional meiotic chromosome preparations of trigeneric hybrids (2n = 4x = 28; ABIE) with Ph1 (A and B), with chromosome 5D substituted for 5B (C and D), and with ph1b (E–G). V, pentavalent; IV, quadrivalent; III, trivalent; II, bivalent; I, univalent. (A) 1 rod II + 26 I. Note restricted pairing in the presence of Ph1. (B) 2 III (arrowheads) + 22 I. The formation of 2 trivalents in the presence of Ph1 is rare, however. (C) Meiotic metaphase I with 6 rod II + 16 I in the absence of chromosome 5B; note that almost 43% of the chromosome complement is paired up. (D) Another cell with 1 IV (arrow) + 1 III (arrowhead) + 6 rod II + 9 I; almost 68% of the complement is paired in the absence of 5B. (E) Meiotic metaphase I with 7 rod II (1 II separated) + 14 I in the presence of ph1b; 50% of the complement is paired. (F) A cell with 1 III (arrowhead) + 1 ring II + 1 rod II + 21 I in the presence of ph1b. (G) A cell with 1 III (arrowhead) + 5 rod II + 15 I in the presence of ph1b.
19.64% to 30.71% of the total chromosome complement being paired (Table 1). On an average, 23.86% of the complement showed pairing. Most of the pairing was limited to bivalent formation (Fig. 1A), and most bivalents were rod configuration, as shown by a chiasma frequency of 1.04 per bivalent. Trivalents and quadrvivalents were also observed, albeit with low frequency (Table 1). However, a cell with 2 trivalents, a rare event, and 22 univalents was observed (Fig. 1B). Chromosome pairing in these trigeneric hybrids was obviously restricted because of the presence of Ph1.

### Chromosome pairing in the presence of *ph1b* allele

Data on pairing in 5 trigeneric hybrids with *ph1b* allele (Table 2) revealed a substantial variation among the hybrids: the proportion of complement showing pairing varied from 42.29% to 57.07% with an overall mean of 49.49%. Bivalent formation (mostly rod bivalents) accounted for most of the pairing (Figs. 1E–1G). Figure 1E, for example, shows 7 rod bivalents plus 14 univalents. Trivalent frequency (0.55 III per cell) in the *ph1b*-trigeneric hybrids (Table 2) was higher than in the *Ph1*-trigenerics described earlier. Multivalent formation above the trivalent level was very low.

### Chromosome pairing in the absence of chromosome 5B

Five of the trigeneric hybrids synthesized by crossing Langdon 5D(5B) disomic substitution with the JJEE amphidiploids were analyzed. Trigeneric hybrids without chromosome 5B (and hence without *Ph1*) showed substantial pairing, ranging from 36.43% to 52.86% (Table 3); on an average, almost 52% of the complement showed pairing. Ring bivalent, trivalent, and quadrvivalent formations also occurred and were higher than in the *Ph1*- and *ph1b*-trigeneric hybrids already described. Thus, Fig. 1D shows a meiotic metaphase I cell with 1 IV + 1 III + 6 II + 9 I (almost 68% of the complement is paired up.) Quadrvivalent formation could have involved chromosomes of all 4 genomes, A, B, J, and E.

### Specificity of chromosome pairing

Total pairing in the *Ph1*-trigeneric hybrids was low (Table 1) because of restriction imposed by the *Ph1* gene on homoeologous chromosome pairing. However, in the presence of the *ph1b* allele (Table 2) or in the absence of chromosome 5B and hence without *Ph1* (Table 3), pairing is substantially increased. FI-GISH analysis allows the study of the specificity of chromosome pairing, whether wheat with wheat, grass with grass, or wheat with grass (Table 4).

In the *Ph1*-trigenerics, intergeneric pairing, i.e., wheat with grass chromosomes, was also restricted. In the 30 meiotic metaphase cells analyzed, a total of 32 wheat chromosomes paired with 29 grass chromosomes with a total of 29 chiasmata, which is essentially 1 intergeneric chiasma per cell (Table 4). Thus, Fig. 2A shows a total of 5 rod bivalents: 2 wheat with wheat, 2 grass with grass, and 1 wheat with grass. In the presence of the *ph1b* allele, homoeologous pairing was substantially elevated (Table 2). Figure 2B, for example, shows a trivalent and rod bivalent involving pairing between wheat and grass chromosomes.

In the absence of chromosome 5B, which carries the *Ph1* gene, trigeneric hybrids showed substantially elevated chromosome pairing (Figs. 2C and 2D). Figure 2C shows 2 trivalents and 1 rod bivalent formed by wheat and grass chromosomes, whereas Fig. 2D shows a pentavalent formed by 3 wheat and 2 grass chromosomes, and 2 trivalents each involving 1 wheat and 2 grass chromosomes.

### Discussion

Although the corresponding (homoeologous) chromosomes of the A and B genomes of durum wheat are capable of synopsis, the principal homoeologous-pairing suppressor gene, *Ph1*, in the long arm of chromosome 5B restricts pairing only to homologous partners (Sears 1976; Jauhar and Joppa 1996) as demonstrated in *Ph1*- and *ph1c*-haploids, and in haploids deficient for 5B (Jauhar et al. 1999). Such a regulated chromosome pairing ensures diploid-like meiosis and disomic inheritance in durum wheat, thereby helping to maintain the meiotic integrity of its 2 genomes. Moreover, *Ph1* inhibits or restricts pairing between alien chromosomes and durum chromosomes in intergeneric hybrids, impeding alien gene transfer into durum wheat (Knot 1987; Jauhar 1991, 1992a; Jauhar and Almouslem 1998). Therefore, for successful gene transfer, methods of promoting wheat–alien pairing must be used. Such approaches include the production of hybrids without *Ph1* by using a wheat parent without chromosome 5B or with a *ph* mutation. Using a grass genotype that suppresses the activity of *Ph1* would also promote homoeologous pairing.

The trigeneric hybrids (2n = 4x = 28; ABJE) have 7 chromosomes each of the A, B, J, and E genomes. The corresponding chromosomes of the A and B genomes are homoeologous and show very little pairing in the presence of *Ph1* (Jauhar et al. 1999). The 7 chromosomes of the E genome show close genetic correspondence to 7 homoeologous groups of wheat (Dvořák 1980) and are also probably homoeologous to chromosomes of the J genome (Jauhar 1990). In the 5 trigeneric hybrids with *Ph1*, 19.64% to 30.71% (overall mean 23.86%) of the chromosome complement paired, showing genotypic variation in pairing. Earlier,
Fig. 2. Fluorescent genomic in situ hybridization (fl-GISH) analysis of chromosome pairing at meiotic metaphase I of trigeneric hybrids (2n = 4x = 28; ABJE) with \(Ph1\) (A), with \(ph1b\) (B), and without chromosome 5B (i.e., without \(Ph1\)) (C and D). The A and B genomes were probed with biotinylated *Triticum urartu* and *Aegilops speltoides* genomic DNA while the J and E genomes were blocked with autoclaved genomic DNA of the JJEE amphidiploid. The chromosome preparations were counterstained with propidium iodide (PI) and the probe detected with fluorescein isothiocyanate (FITC). The wheat chromosomes fluoresce green and the grass chromosomes fluoresce red. V, pentavalent; IV, quadrivalent; III, trivalent; II, bivalent; I, univalent. (A) Cell with 5 rod II (5 arrows) + 18 I. Note, 2 wheat II (grey arrows), 2 grass II (blue arrows), 1 wheat × grass bivalent (grey arrowhead), and 9 I of durum (green), and 9 I of the grass parent (red). (B) Meiotic metaphase I cell showing 2 chain III (2 arrows) + 1 ring II + 3 rod II + 14 I. The cell also shows both intra-complement (within wheat and within grass) and inter-complement (between wheat and grass) pairing. Note 1 grass III (blue arrow) + 1 III (involving 1 wheat and 2 grass chromosomes) (grey arrowhead) + 1 grass ring II + 1 grass rod II + 2 wheat × grass rod II + 10 wheat I (green) + 4 grass I (red). Note elevated pairing in the presence of the \(ph1b\) allele, with homoeologous pairing between wheat and grass chromosomes. (C) Cell without 5B showing 1 IV (blue arrow) + 2 III (grey arrow and grey open arrowhead) + 4 rod II + 10 I. Probing with AB genomic DNA shows that the IV is constituted of grass chromosomes (blue arrow), 2 rod II within wheat, 1 rod II within grass, 1 III (2 wheat and 1 grass chromosomes) (grey arrow) + 1 III (1 wheat and 2 grass chromosomes) (grey open arrowhead) + 1 rod II (1 wheat and 1 grass chromosome) (grey arrowhead) + 6 wheat I (green), and 4 grass I (red). (D) Another cell without chromosome 5B showing 1 V (grey arrow) + 2 III + 4 rod II + 9 I. Note complex homoeologous pairing showing a pentavalent composed of 3 wheat and 2 grass chromosomes (grey arrow), 2III (each constituted by 1 wheat and 2 grass chromosomes), 3 grass rod II + 1 wheat rod II + 7 wheat I (green) + 2 grass I (red).
Jauhar (1992a) also observed 25.8% pairing in Phl-trigenerics. This pairing would be possible because of partial inactivation of Phl by the genotype of the grass parent, a phenomenon that is not uncommon in wide hybrids (Dvorfák 1987; Chen et al. 1989; Jauhar 1992b; Jauhar and Almouslem 1998). Thus, the use of appropriate genotypes of wheatgrass could promote homoeologous pairing involving the grass and durum chromosomes and facilitate alien gene transfer into durum wheat.

Clearly, the pairing in the Phl-trigenerics is restricted. The Phl-regulated pairing is limited to closely related chromosomes and, thus, helps assess genomic relationships (Jauhar and Joppa 1996). In the presence of Phl the pairing between chromosomes of the A and B genomes of durum is as low as between durum chromosomes and grass (J and E genome) chromosomes. This trend of pairing remains the same in the trigenerics with ph1b or in those without chromosome 5B, i.e., the degree of pairing between chromosomes of the A and B genomes is comparable to that between durum chromosomes and grass chromosomes.

In the ph1b-trigenerics, pairing among chromosomes of the A and E genomes is higher than that among chromosomes of the A and B genomes. However, in the absence of Phl regulation, homoeologous chromosomes of different genomes can show substantial differences in the degree of pairing. Thus, in ph1b-haploids (2n = 3x = 21; ABD genomes) of bread wheat, A-D pairing is much higher than A-B or B-D pairing (Jauhar et al. 1991).

Our main interest is in the wheat—grass chromosome pairing, i.e., between AB chromosomes and JE chromosomes, because such pairing would be crucial to alien gene transfer into durum. Fl-GISH allows the study of the specificity of chromosome pairing between and within genomes or haploid complements (Mukai 1996; Cai et al. 1998; Jauhar et al. 1991). In the absence of Phl, we found, on an average, 1 bivalent per cell with 1 chiasma between durum and grass chromosomes. However, in the presence of the mutant ph1b allele or in the complete absence of chromosome 5B that carries the Phl gene, pairing was substantially elevated. In the ph1b-trigenerics, 49.5% of the total chromosome complement paired, with 7.6 chiasmata per cell. Fl-GISH on 42 pollen mother cells showed that 1.2 chiasmata per cell were formed between wheat and grass chromosomes. Therefore, one approach to bring about alien gene transfers in wheat would be to use ph mutants in the hybridization programs (Friebe et al. 1996; Jauhar 2001, 2003).

In the trigeneric hybrids in which chromosome 5B was replaced by 5D, total chromosome pairing was essentially similar to that in the ph1b-trigenerics. However, the wheat—grass pairing was greatly elevated, i.e., 2.6 chiasmata per cell as revealed by fl-GISH, a welcome feature from the breeding standpoint. Using Langdon 5D(5B) disomic substitution, which lacks chromosome 5B and hence the Phl gene, to make trigeneric hybrids would promote infidelity of pairing between durum and grass chromosomes and, hence, help capture desirable exchanges and accelerate alien gene transfer into the durum genomes. The presence of 2 important, agronomically useful grass genomes in the trigeneric hybrids would perhaps increase chances of transferring useful alien chromatin to durum wheat.

Acknowledgements

We thank Drs. Xiwen Cai, Michael McMullen, and Steven Xu for critically reading the manuscript and giving several suggestions. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or imply approval to the exclusion of other products that also may be suitable.

References


