The chromosome content and genotype of two wheat cell lines and of their somatic fusion product with oat

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Abstract Somatic hybridization seeks to genetically combine phylogenetically distant parents. An effective system has been established in bread wheat (Triticum aestivum L.) involving protoplasts from a non-totipotent cell line adapted to in vitro culture (T1) in combination with totipotent protoplasts harvested from embryogenic calli (T2). Here, we report the karyotype and genotype of T1 and T2. Line T1 carries nine A (A-genome of wheat), seven B (B-genome of wheat) and eight D (D-genome of wheat) genome chromosomes, while T2 cells have 12 A, 10 B and 12 D genome chromosomes. Rates of chromosome aberration in the B- and D-genomes were more than 25%, higher than in the A-genome. DNA deletion rates were 55.6% in T1 and 19.4% in T2, and DNA variation rates were 8.3% in T1 and 13.9% in T2. Rate of DNA elimination was B- > D- > A-genome in both T1 and T2. The same set of cytological and genetic assays was applied to a derivative of the somatic fusion between protoplasts of T1, T2 and oat (Avena sativa L.). The regenerant plants were near euploid with respect to their wheat complement. Six wheat chromosome arms—4AL, 3BS, 4BL, 3DS, 6DL and 7DL—carried small introgressed segments of oat chromatin. A genotypic analysis of the hybrid largely confirmed this cytologically-based diagnosis.

Keywords Chromosome content and genotype · Common wheat · Oat · Somatic hybrid

Abbreviations
T Triticum aestivum cv. Jinan 177
T1 Jinan 177 suspension cells
T2 Jinan 177 embryogenic calli
SSR Simple sequence repeats
GISH Genomic in situ hybridization
FISH Fluorescence in situ hybridization

Introduction
Numerous studies have been focused on analysis of somaclonal variation in vitro (Larkin et al. 1984; Lee and Phillips 1988; Pershina et al. 2003). The initial reports were concerned with determining changes in the number, structure, and morphology of the chromosomes, such as chromosome banding patterns (Larkin et al. 1984; Lee and Phillips 1988), and later studies examined variations in genomic DNA caused by mutations, the loss and rearrangement of genes, gene silencing and transposons or reverse transposons in subculture (Shaked et al. 2001; Polanco and Ruiz 2002; James and Reiner 2007). However, little was known about the content and genotype of cultured cells of allohexaploid wheat (Triticum aestivum L.) that had been grown for different periods of time.

Wheat (T. aestivum L., 2n = 42) is an important cereal. The genetic variability for some important traits, such as high quality, diseases and stress resistance, is limited in the cultivated wheat germplasm. Related or distant species of wheat are an important reservoir of useful genes (Liu et al. 2007). This paper presents a cytological and genotypic analysis of one somatic hybrid of wheat and oat, with the purpose to provide insights into the consequences of somatic hybridization in this species combination.
2005). There is thus an urgent need to broaden the wheat gene pool by introgressing genes for such traits from diverse cereal species. This kind of alien introgression line created can increase the genetic variation in wheat species. However, the sexual route with low crossability in achieving this limited the transfer of such genes (Wang et al. 2004; Liu et al. 2005). Somatic hybridization provides the possibility of overcoming sexual incompatibility of wheat with remote cereals (Xia et al. 2003). This technique has been used with some success as a means of introgressing genes from related grass and cereal species (Xia et al. 2003; Wang et al. 2005; Zhou and Xia 2005). However, when the donor species was only distantly related to wheat, fusions often failed to progress beyond undifferentiated calli or albino plants (Xiang et al. 1999; Li et al. 2001; Yue et al. 2001). In an attempt to overcome the difficulty of regenerating hybrids between distantly related species, a three-parent fusion system has been elaborated (Xiang et al. 2003a, 2004; Xu et al. 2003), based on two complementary wheat cell lines T1 and T2. The former is a non-totipotent cell line growing in long-term cell suspension, and the latter are totipotent cells taken from embryogenic calli (Xiang et al. 2003b). The third “parent” is an exotic donor species, the protoplasts of which are irradiated before fusion. Using this system, Xiang et al. (2003a) were able to regenerate a set of putative wheat/oat (Avena sativa L.) somatic hybrids and employed a combination of cytological and genetic assays to demonstrate the presence of a variable number of wheat/oat recombinant chromosomes. However, the contents and genotypes of the two wheat cell lines and their somatic fusion products with oat were unknown. This was critical for understanding the mechanism of the regeneration of hybrid plants.

In this work, our goal was to investigate the chromosome elimination and variation of T1 and T2, as well as the relationship between the genetic complementation of T1, T2, oat and somatic hybrid plant regeneration.

Materials and methods

Plant materials

Wheat protoplasts derived from two types of cell lines T1 and T2 of T. aestivum L. cv. Jinan 177, and oat protoplasts came from embryogenic calli A. sativa L. cv. Mapur. The T1 were harvested from a non-regenerable long-term (>10 years) cell suspension, and the T2 from 2-year-old embryogenic calli with regeneration frequency of 75% (number of calli which can differentiate to plants/number of calli analysed, %) (Xiang et al. 2003a). The oat cell line was 1.5-year-old embryogenic calli (Xiang et al. 2003a). The T1 and T2 lines were subcultured on MB liquid and solid media (Xia and Chen 1996) with 2.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). Protoplasts were isolated from T1 suspension after 3 days of subculture, T2 and oat embryogenic calli were isolated 7 days after subculture according to the method of Xia and Chen (1996). After washing in 0.6 M mannitol and 5 mM CaCl2, oat protoplasts were transferred onto 3.5 cm Petri dishes in a thin layer and irradiated with UV light at an intensity of 300 μW/cm² for 1 min before they were fused with T1 and T2 (Xiang et al. 2003a). T1 and T2 protoplasts were combined at the ratio of 1:1 and mixed in equal volumes with UV-treated protoplasts of oat. The fusion was carried out according to the improved PEG method (Xia and Chen 1996). When the fusion clones grew to small cell lines of 1.5–2.0 mm in diameter, they were transferred to the MB medium with 1 mg/L 2,4-D for proliferation. After subculture for 1–2 months, proliferating calli from the cell lines were moved to the MB medium containing 0.5 mg/L IAA and 0.5 mg/L zeatin for regeneration. Regenerated plantlets were transferred to seedling-strengthening MB medium containing 1–2 mg/L multi-effect triazal (MET) and 0.5 mg/L NAA for strengthening and rooting. All the regenerated plants containing four hybrid clones resembled wheat, but only plants of clone no. 94-1 can grow into soil, and then tassel normally (Xiang et al. 2003a). In this experiment, a lager number of roots from hybrid plants of clone no. 94-1 in different growth stages were analysed. Accessions of Triticum urartu Thum, Aegilops speltoides Tausch and Aegilops tauschii (Cross.) Schmal were kindly provided by the Quality and Resource Institute of the Agriculture Science Academy of China.

Karyotype analysis and in situ hybridization

Chromosome spreads were obtained from T1 and T2 cells and from the root tips of the regenerants and germinating seedlings, as described by Xiang et al. (2003b). Karyotypes of cv. Jinan 177 were derived from a sample of ten mitotic metaphase cell spreads. Karyotype classification followed the method of Sears (1969) and was based on the data for Chinese Spring wheat from Gill (1987). The parameters of the karyotypes were based on ten metaphase cell spreads. The arm ratios and the relative distances of the chromosomes from the hybrid cells selected were analysed. For in situ hybridization (ISH) purposes, genomic DNA of cv. Jinan 177, oat, T. urartu, Ae. tauschii, Ae. speltoides and the hybrid regenerants was isolated following Doyle and Doyle (1990). The pSc119.2 and pAs1 plasmids contain, respectively, B and D genome-specific repetitive sequences (Mukai et al. 1993). These were labelled for use as fluorescence ISH (FISH) probes with digoxigenin-11-dUTP, using a nick translation kit, according to the manufacturer’s
instructions (Boehringer Mannheim, Germany), as described elsewhere (Wang et al. 2005). Total oat genomic DNA was labelled in the same way to generate a genomic ISH (GISH) probe. The GISH and FISH methodology followed Wang et al. (2005), the former using a ratio of 1:50 labelled oat genomic DNA to unlabelled wheat genomic DNA, and the latter 1:50 labelled pSc119.2 to unlabelled genomic (T. urartu + Ae. tauschii) DNA, or 1:50 pAs1 to unlabelled genomic (T. urartu + Ae. speltoides) DNA. In sequential GISH and FISH experiments (pAs1/pSc119.2 and GISH/ pAs1/pSc119.2), the initial signal was rinsed before rehybridization with the subsequent probe.

Microsatellite (SSR) analysis

DNA extracted from T1 cells, T2 calli and leaf of somatic hybrids and parental lines (Doyle and Doyle 1990) was used as template for the analysis of allelic constitution at 101 SSR loci, following Röder et al. (1998). The marker set covered the centromeric and subtelocentric regions of all the chromosomes present in T1 and T2, and the locations selected based on the previous GISH, FISH and karyotype analysis of hybrid. The relative distance (%; the distance from the centromere to SSR locus checked/the length of the arm involved × 100%) was measured according to the genetical map of Röder et al. (1998).

Results

The chromosome content of T1 and T2 and cv. Jinan 177

Of the 138 T1 cells analysed, 119 (86.2%) contained 22–25 chromosomes (Fig. 1a), while 104/124 (83.9%) of the T2 cells had 34–38 chromosomes (Fig. 1b). Some T1 and T2 cells included telocentric chromosomes, acrocentric chromosomes and dicentric chromosomes (see Suppl. Table 1, Fig. 1a, b). One or two chromosome fragments were present in ~87% of both the T1 and T2 cells. The karyotype of cv. Jinan 177 conformed to that of standard bread wheat (Gill 1987; Suppl. Table 2, Fig. 1c).

The genome content of T1 and T2 cells

FISH preparations based on hybridization with pSc119.2 and pAs1 succeeded in defining the B and D genome content of T1 and T2. From a sample of 136 T1 cells, 82 (~60%) contained seven B genome, and 83 (~61%) contained eight D genome chromosomes (Table 1, Fig. 2a, b); similarly, of the 95 T2 cells analysed, ~60% had ten B and ~54% 12 D genome chromosomes (Table 1, Fig. 2c, d). The range in the number of B and D genome chromosomes in T1 was, respectively, 6–8 and 7–9, and in T2, 9–11 and 10–12. Chromosomes 2B, 3B, 7B, 3D and 6D were present in most T1 and T2 cells, but 4B, 5B and 4D were rare. Chromosome abnormalities were present in, respectively, 27 and 39% of the T1 B and D genome chromosomes and 31 and 36% of T2 chromosomes (Table 2). Therefore, many eliminations and rearrangements including duplication of B- and D-genome chromosomes in T1 and T2 presented in the calli subculture. Chromosome arm ratios and relative lengths were used to infer the A genome content of T1 and T2 cells. Comparing the statistics of karyotype data of T1, T2 with cv. Jinan 177, it was revealed that A genome chromosomes of T1 and T2 were distributed mainly in 4A and 5A, whereas 3A was the lowest. About 61% of T1 cells contained nine A genome chromosomes, while ~57% of the T2 cells contained 12 (Table 1, Fig. 2a, b). The range in A genome chromosome number in T1 was 7–11, and in T2 11–15. Non-standard A genome chromosomes were present in 22% of T1 and 16% of T2 cells (Tables 1, 2).

Distinguishing between DNA deletion and modification in T1 and T2

Simple sequence repeats genotyping was employed to distinguish between DNA deletion and modification in T1 and T2 as a result of cell culture. Of the 58 loci assayed, 36 exposed genetic polymorphism between T1 and T2 and T
Of the 36 informative markers, 13 showed same patterns among T, T1 and T2, 15 between T1 and cv. Jinan 177 and 25 between T2 and cv. Jinan 177 (Table 3). The rates of DNA deletion were 55.6% (20/36) in T1, such as in Xgwm160 and Xgwm428 (Fig. 3), and

Table 1 Frequency of pSc119.2 and pAs1 hybridization signals in T1 and T2 cell lines

| DNA probe | Material | Main chromosome no. | Cell no. observed | Signal number and frequency (%) |
|-----------|----------|----------------------|-------------------|----------------------------------|
|           |          |                      |                   | 6 (%)  | 7 (%)  | 8 (%)  | 9 (%)  | 10 (%) | 11 (%) | 12 (%) |
| pAs1      | T1       | 23                   | 45                | 7 (15.5) | 29 (64.4) | 9 (20.1) |
|           |          | 24                   | 58                | 11 (19.0) | 36 (62.0) | 11 (19.0) |
|           |          | 25                   | 33                | 6 (18.2)  | 19 (57.6) | 7 (21.2)  |
|           | T2       | 33                   | 38                | 8 (21.1)  | 10 (26.3) | 20 (52.6) |
|           |          | 34                   | 25                | 14 (31.8) | 25 (56.8) | 5 (11.4)  |
|           |          | 35                   | 32                | 6 (18.2)  | 9 (28.1)  | 17 (53.1) |
| pSc119.2  | T1       | 23                   | 44                | 14 (31.8) | 25 (56.8) | 5 (11.4)  |
|           |          | 24                   | 48                | 14 (29.2) | 30 (62.5) | 4 (8.3)   |
|           |          | 25                   | 31                | 7 (22.6)  | 19 (61.3) | 5 (16.1)  |
|           | T2       | 33                   | 19                | 5 (26.4)  | 11 (57.9) | 3 (15.7)  |
|           |          | 34                   | 23                | 5 (21.7)  | 14 (60.9) | 4 (17.4)  |
|           |          | 35                   | 15                | 4 (26.7)  | 9 (60.0)  | 2 (13.3)  |

Fig. 2 FISH profile of the mitotic metaphase chromosomes. a and b B and D genome chromosomes of T1 identified by their pSc119.2 and pAs1 patterns. c and d B and D genome chromosomes of T2 identified by their pSc119.2 and pAs1 patterns. Scale bars 10 μm
19.4% (7/36) in T2, such as in Xgwm335 (Fig. 3) and Xgwm273, and 16.7% (6/36) in both T1 and T2 (Table 3). This indicated that the rate of DNA deletion of T1 was higher than that of T2. The ratio of DNA modification was 8.3% (3/36) in T1 with examples such as Xgwm389 (Fig. 3) and Xgwm160 (Fig. 3). In T2 the ratio of DNA modification was 13.9% (5/36), with examples of Xgwm389, Xgwm335 and Xgwm428 (Table 3, Fig. 3). B-genomic DNA was eliminated at a faster rate than D-genomic DNA, which was deleted faster than A-genomic DNA in T1 and T2. Anyway, the rate of DNA deletion was greater in T1 than in T2, while the rate of DNA modification was greater in T2 than in T1 (Table 3). Therefore, there were many instances of DNA variation at different positions in T1 and T2, and within the calli of subcultures of wheat.

### Table 2  Chromosome constitution of the T1 and T2 lines

| A-genome chromosome | T1 No. of chromosome (%) | T2 No. of chromosome (%) | B-genome chromosome | T1 No. of chromosome (%) | T2 No. of chromosome (%) | D-genome chromosome | T1 No. of chromosome (%) | T2 No. of chromosome (%) |
|---------------------|--------------------------|--------------------------|---------------------|--------------------------|--------------------------|---------------------|--------------------------|--------------------------|
| 1A                  | 8 (8.89)                 | 5 (9.09)                 | 1B                  | 10 (8.93)                | 7 (8.33)                 | 1D                  | 14 (11.86)               | 12 (6.78)               |
| 2A                  | 9 (10.00)                | 7 (12.73)                | 2B                  | 16 (14.29)               | 8 (9.52)                 | 2D                  | 9 (7.63)                 | 26 (14.69)              |
| 3A                  | 4 (4.44)                 | 2 (3.64)                 | 3B                  | 16 (14.29)               | 16 (19.05)               | 3D                  | 14 (11.86)               | 21 (11.86)              |
| 4A                  | 21 (23.33)               | 9 (16.36)                | 4B                  | 4 (3.57)                 | 4 (3.76)                 | 4D                  | 5 (4.24)                 | 6 (3.39)                |
| 5A                  | 13 (14.44)               | 12 (21.82)               | 5B                  | 8 (7.14)                 | 3 (3.57)                 | 5D                  | 7 (5.94)                 | 15 (8.47)               |
| 6A                  | 7 (7.78)                 | 5 (9.09)                 | 6B                  | 12 (10.71)               | 8 (9.52)                 | 6D                  | 19 (16.10)               | 18 (10.17)              |
| 7A                  | 8 (8.89)                 | 6 (10.91)                | 7B                  | 16 (14.29)               | 12 (14.29)               | 7D                  | 4 (3.39)                 | 16 (9.05)               |
| XA                  | 20 (22.22)               | 9 (16.36)                | XB                  | 30 (26.79)               | 26 (30.95)               | XD                  | 46 (38.98)               | 63 (35.59)              |
| Total               | 90 (100)                 | 55 (100)                 | Total               | 112 (100)                | 84 (100)                 | Total               | 118 (100)                | 177 (100)               |

Introgression of oat chromatin into wheat

About 71% of the cells of the hybrid regenerants contained 46–48 chromosomes (mean 47.2), with ~58% containing oat chromosome introgression segments distributed on six wheat chromosomes. Based on sequential ISH experiments, it was possible to identify 12–16 of the B genome and 11–13 of the D genome chromosomes (Table 4, Fig. 4a, b). By comparing their karyotype with that of cv. Jinnan 177, 17 A genome chromosomes and three chromosomal abnormalities were detected (Table 4). Almost the whole wheat genome was represented in the hybrid nuclei, along with duplicated, deleted or re-arranged chromosomes. The GISH/FISH analysis identified the presence of oat chromatin on chromosomes 3B and 4B (Fig. 4a, c), and 3D, 6D and 7D (Fig. 4b, c). A further introgression site was identified indirectly on chromosome 4A (Fig. 4c). An analysis of the arm ratios of the introgressed chromosomes, and the relative distances between the centromere and the introgression break point are given in Table 5.

Of the 58 SSR loci used to genotype the hybrid regenerants, 32 were amplified in all of the samples. Of the 32 informative markers, only 14 (43.8%) identified differences between hybrid plants and their parents. The alleles from both parents were present at nine of above 14 loci, only the oat allele was amplified at two loci, while at the remaining three loci, the alleles were biparental and novel (Table 6). The relative distance (%) between the centromeres and the SSR loci were measured. SSR bands were located on 4AL, 3BS, 4BL, 3DS, 6DL and 7DL chromosome arms, and the relative distances (%) of fragments ranged from 3.10 to 76.36% (Table 6), in agreement with the results of sequential GISH and FISH (Table 5). Oat alleles were also amplified by primers targeting SSR loci on chromosome arms 3AS, 3AL, 6AL and 7AL, none of which were identified by GISH as sites of introgression (Table 6). This observation is suggestive of the occurrence of many submicroscopic introgression events.

### Discussion

By using the pSc119.2 with high repetitive sequences of B-genome from rye (Secale cereale L.), and pAs1 with insertion of repetitive sequences of D-genome from Ae. tauschii for two-colour FISH, Mukai et al. (1993) established the ideogram of B- and D-genomes and a pair of 4A chromosomes of Chinese Spring wheat. Using N-, C-banding, GISH, FISH and SSR markers in combination with karyotypes data, heterogeneric chromatin in many wheat hybrids were localized into the wheat chromosomes (Jiang et al. 1993; Nagy et al. 2002; Malysheva et al. 2003; Silkova et al. 2006). Our previous result had also confirmed that FISH analysis with pSc119.2 and pAs1, in combination with karyotype data and GISH, could differentiate all...
of the A-, B- and D-genome chromosomes of the cultivar wheat Jinan 177 and localize small donor chromosomes in somatic hybrid of Jinan 177 with *Agropyron elongatum* (Wang et al. 2005). Here, we have extended this analysis to the three-cell fusion system (Xiang et al. 2003a), which has uncovered chromosome content and genotype of two wheat cell lines and of their somatic hybrid plant with oat.

### Table 3

SSR profiles of cv. Jinan 177 and T1 and T2 cell lines

| Primer | Location | The amplification result of wheat and its calli | Deletion and modification of genomic DNA in T1 and T2 |
|--------|----------|-----------------------------------------------|-----------------------------------------------------|
|        |          | Jinan 177 | T1 | T2 |                                           |
| Xgwm359 | 1AS     | T         | T  | T  |                                           |
| Xgwm47  | 2AL     | T         | T  | T  |                                           |
| Xgwm674 | 3AL     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm369 | 3AS     | a + b     | a  | a + b | D in T1                                      |
| Xwmc258 | 4AL     | a + b     | 0  | 0   | D in T1 and T2                              |
| Xwmc262 | 4AL     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm160 | 4AL     | a + b     | a + N| a + b | D in T1, M in T1                            |
| Xgwm637 | 4AL     | T         | T  | T  |                                           |
| Xgwm494 | 6AL     | T         | T  | T  |                                           |
| Xgwm63  | 7AL     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm140 | 1BL     | T         | T  | T  |                                           |
| Xgwm273 | 1BS     | a + b+c   | a  | N   | D in T1 and T2, M in T2                     |
| Xgwm297 | 3BL     | T         | T  | T  |                                           |
| Xgwm247 | 3BL     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm547 | 3BS     | T         | T  | T  |                                           |
| Xpsp3030| 3BS    | a + b+c   | a + b| a + b | D in T1 and T2                              |
| Xgwm285 | 3BS     | T         | T  | T  |                                           |
| Xgwm231 | 3BS     | a + b+c + d| a + b+c| a + b | D in T1 and T2                              |
| Xgwm389 | 3BS     | a         | a + N| a + N | M in T1 and T2                              |
| Xgwm77  | 3BS     | T         | T  | T  |                                           |
| Xwmc69  | 3BS     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm107 | 4BL     | a + b+c + d| a + b+c + d+e| a + b+c | D in T1                                      |
| Xgwm165 | 4BL     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm368 | 4BS     | T         | T  | T  |                                           |
| Xgwm335 | 5BL     | a         | a  | N   | D in T2, M in T2                            |
| Xgwm219 | 6BL     | T         | T  | T  |                                           |
| Xgwm357 | 7BS     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm157 | 1 DL    | T         | T  | T  |                                           |
| Xgwm337 | 1 DS    | a + b+c   | a + b| a + b+c | D in T1                                      |
| Xgwm314 | 3DL     | T         | T  | T  |                                           |
| Xgwm456 | 3DS     | a + b     | a + b| a + b+N+N | M in T2                                      |
| Xgdm98  | 6DL     | a + b     | 0  | a + b | D in T1                                      |
| Xgwm428 | 7DL     | a + b     | 0  | a + b+N | D in T1, M in T2                            |
| Xgdm46  | 7DL     | a + b     | b  | a   | D in T1 and T2                              |
| Xpsp3079| 7DL     | a + b+c + d| a + b+c + N| a + b+c | D in T1 and T2, M in T1                     |
| Xgwm437 | 7DL     | a + b     | 0  | a + b | D in T1                                      |

| Rate of D (%) | T1 | T2 |
|---------------|----|----|
| Rate of M (%) | T1 | T2 |

of T1 | 55.6 | 19.4 |

M modification type

T non-informative; N novel allele

a, b, c the band amplified; 0 no amplification; D locus absent; M modification type

Chromosome loss and variation in the T1 and T2 cell lines

Variation in chromosome number and the formation of chromosomal aberrations are common in cells cultured in vitro over many generations (Larkin et al. 1984; Song et al. 2000). Here we have shown that the T1 cell line has lost...
about 18, while \( T_2 \) has lost only about eight chromosomes (Table 1, Fig. 2), as would be expected given that \( T_1 \) has been in culture for a much longer period than \( T_2 \). The evidence is that particular chromosomes tend to be more highly prone to loss, while others are lost rather rarely, as has been noted in other studies (Lee and Phillips 1988; Doğramaci-Altunetepe et al. 2001). The former group includes chromosomes 4A, 5A, 3B, 7B, 3D, 6D, and the latter chromosomes 3A, 6A, 4B, 5B and 4D (Table 2). As for the chromosomal abnormalities of \( T_1 \) and \( T_2 \), the frequencies in D-, B-groups were higher than that of the A-group, with the order of D- > B- > A-group in both \( T_1 \) and \( T_2 \) (Table 2).

### Hybrid chromosome number and regeneration ability

Previous studies have indicated that the totipotency of a hybrid cell line was dependent on the number of chromosomes present, so those having a chromosome number close to the bread wheat somatic number of 42 tended to be more fit than those which were either hypo- or hyper-ploid (Xia et al. 1996; Xiang et al. 2003a, 2004). The majority of the regenerants from the wheat/oat fusion had a chromosome number in the range 46–48, far less than the sum of the three fusion parents’ chromosomes (93–108). Thus, there must have been a massive and rapid phase of chromosome elimination following the fusion, particularly involving the oat chromosomes (aided by the pre-fusion irradiation treatment), but also involving chromosomes inherited from \( T_1 \) and \( T_2 \).

### Table 4  Chromosome content of a bread wheat/oat somatic hybrid derivative

| A-genome chromosome | No. of chromosome | B-genome chromosome | No. of chromosome | D-genome chromosome | No. of chromosome | Abnormal chromosome | No. of chromosome |
|---------------------|-------------------|---------------------|-------------------|---------------------|-------------------|---------------------|-------------------|
| 1A                  | 2                 | 1B                  | 2                 | 1D                  | 2                 | X                   | 2                 |
| 2A                  | 3                 | 2B                  | 2                 | 2D                  | 1                 |                     |                   |
| 3A                  | 1                 | 3B                  | 3                 | 3D                  | 1                 |                     |                   |
| 4A                  | 3                 | 4B                  | 1                 | 4D                  | 2                 |                     |                   |
| 5A                  | 4                 | 5B                  | 1                 | 5D                  | 2                 |                     |                   |
| 6A                  | 2                 | 6B                  | 1                 | 6D                  | 3                 |                     |                   |
| 7A                  | 2                 | 7B                  | 3                 | 7D                  | 1                 |                     |                   |
| XB                  | 1                 |                     |                   |                     |                   |                     |                   |
| YB                  | 1                 |                     |                   |                     |                   |                     |                   |
| Total               | 17                | Total               | 15                | Total               | 12                | Total               | 3                 |

\( XB \) and \( YB \) unidentified likely B genome chromosome, based on its FISH profile

\( X, Y \), unidentified chromosome

### Fig. 4  Sequential FISH and GISH preparations of metaphase chromosomes of a bread wheat/oat somatic hybrid derivative. FISH profiling to identify B (a) and D (b) genome chromosomes. c GISH profile to identify chromosome segments introgressed from oat. Scale bars 10 \( \mu \)m
Genetic complementation of \( T_1, T_2 \) and \( A. \) sativa with hybrid plant regeneration

When bread wheat is pollinated by either \( H. \) bulbosum or maize, the pollen parent’s chromosomes are eliminated during the first few mitotic divisions of the hybrid zygote, a process which has been exploited for the production of dihaploids (Inagaki and Tahir 1990; Chen et al. 1999). Even in compatible wide crosses, which occur in nature and are maintained by polyploidization, a spectrum of genetic and cytological events leads to various modifications (Kashkush et al. 2003; Birchler et al. 2005; James and Reiner 2007; Ma and Gustafson 2008). Thus, it is unsurprising that such events also affect the somatic hybridization process. The \( T_1 \) cell line has lost the capacity to regenerate, while \( T_2 \) cells remain totipotent (Xiang et al. 2003a, 2004; Xu et al. 2003). \( T_2 \) protoplasts cannot divide in vitro, while \( T_1 \) cells readily form non-regenerable calli. In combination, these two lines are able to contribute both the ability to grow in vitro and totipotency. Thus, it is plausible to suggest that the loss of totipotency of the \( T_1 \) cell line is due to the absence of a critical chromosome(s) and that this absence can be complemented in the fusion nucleus by the contribution of the \( T_2 \) line genome, where the critical chromosome(s) is still represented. A similar argument can be made for the ability to divide in in vitro culture. In addition, GISH/FISH pattern indicated the introgression of oat chromatin to the 4AL, 3BS, 4BL, 3DS, 6DL and 7DL of the hybrid wheat (Table 5, Fig. 4). It is interesting that 10 SSR loci near to the introgression position showed the same profile between the hybrid and oat (Table 6). Therefore, we suggested that hybrid plant regenerated through genetic complementation of \( T_1 \) and \( T_2 \) and oat. Somatic hybrid derivatives have also been recovered from other combinations with wheat, including oat, foxtail millet and maize (Xiang et al. 2003a, 2004; Xu et al. 2003), which may be explained by the genetic

### Table 5

| Chromosome | Cell number | Relative distance (%) from centromere to the breaking point | Arm ratio |
|-----------|-------------|----------------------------------------------------------|----------|
| 4A        | L           | 27                                                       | 60.55 ± 11.12 | 1.45 ± 0.09 |
| 3B        | S           | 32                                                       | 21.63 ± 10.19 | 1.50 ± 0.13 |
| 4B        | L           | 33                                                       | 29.71 ± 11.25 | 1.02 ± 0.07 |
| 3D        | S           | 38                                                       | 40.57 ± 12.33 | 1.46 ± 0.12 |
| 6D        | L           | 31                                                       | 58.58 ± 11.02 | 1.41 ± 0.13 |
| 7D        | L           | 40                                                       | 27.29 ± 11.57 | 1.08 ± 0.08 |

| L chromosome long arm; S chromosome short arm |
|------------------------------------------------|
| *a* The distance from the centromere to the break point/the length of the arm involved × 100% |

### Table 6

| Primer      | Location | Band pattern | Relative distance (%) from the centromere | Primer      | Location | Band pattern | Relative distance (%) from the centromere |
|-------------|----------|--------------|------------------------------------------|-------------|----------|--------------|-------------------------------------------|
| Xgwm369    | 3AS      | P, N         | 76.36                                    | Xpsp3030    | 3BS      | O            | 21.21                                    |
| Xgwm674    | 3AL      | P            | 3.33                                     | Xgwm165     | 4BL      | P            | 19.50                                    |
| Xwmc258    | 4AL      | O            | 56.00                                    | Xgwm107     | 4BL      | P            | 3.54                                     |
| Xwmc262    | 4AL      | P            | 59.33                                    | Xgwm456     | 3BS      | P            | 38.1                                     |
| Xgwm494    | 6AL      | P            | 3.10                                     | Xgdm98      | 6DL      | P            | 67.05                                    |
| Xgwm63     | 7AL      | P            | 68.97                                    | Xpsp3079    | 7DL      | P            | 29.31                                    |
| Xwmc231    | 3BS      | P            | 11.95                                    | Xgwm428     | 7DL      | P, N         | 22.24                                    |

| P both parental alleles; O oat allele; N novel allele; T wheat allele |
|---------------------------------------------------------------|
| *a* The distance from the centromere to SSR locus checked/the length of the arm involved × 100% |

Fig. 5 SSR profiles of cv. Jinan 177 (lane \( T \)), oat (lane \( O \)) and a bread wheat/oat somatic hybrid derivative (lane \( H \)), based on primers directed to loci on wheat chromosome arms 3AS, 4AL, 3BS, 4BL, 6DL and 7DL. Oat alleles indicated by an arrow, wheat alleles by a thin arrowhead, novel alleles by a full arrowhead.
complementation between the T1 and T2 cell lines and the donor species. But no hybrid progenies were produced in these plants from the “Triple parents” (Xiang et al. 2003a, 2004; Xu et al. 2003). In our early reports, hybrid cells from high capacity for regeneration suspension of wheat cv. Jinan 177 with wheatgrass DNA were fertile and heredity stable (Wang et al. 2005). Thus, we suggest that besides the genetic complementation, some other factors also affect the fertility and genetic stability of these distant hybrid plants. This is worthy of further research (Fig. 5).

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References

Birchner JA, Riddle NC, Auger DL, Veitia RA (2005) Dosage balance in gene regulation: biological implications. Trends Genet 21:219–226

Chen CX, Sun JS, Zhu LH (1999) RFLP variations of common wheat doubled haploid progenies from wheat × maize crosses. Acta Bot Sin 41:55–59

Doğramaci-Altuntepe M, Peterson TS, Jauhar PP (2001) Anther complementation between the T1 and T2 cell lines and the donor species. But no hybrid progenies were produced in these plants from the “Triple parents” (Xiang et al. 2003a, 2004; Xu et al. 2003). In our early reports, hybrid cells from high capacity for regeneration suspension of wheat cv. Jinan 177 with wheatgrass DNA were fertile and heredity stable (Wang et al. 2005). Thus, we suggest that besides the genetic complementation, some other factors also affect the fertility and genetic stability of these distant hybrid plants. This is worthy of further research (Fig. 5).

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References

Birchner JA, Riddle NC, Auger DL, Veitia RA (2005) Dosage balance in gene regulation: biological implications. Trends Genet 21:219–226

Chen CX, Sun JS, Zhu LH (1999) RFLP variations of common wheat doubled haploid progenies from wheat × maize crosses. Acta Bot Sin 41:55–59

Doğramaci-Altuntepe M, Peterson TS, Jauhar PP (2001) Anther culture-derived regenerants of durum wheat and their cytological characterization. J Hered 92:56–64

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Gill BS (1987) Chromosome banding methods standard chromosome nomenclature and applications in cytogenetic analysis. In: Heyne EG (ed) Wheat and wheat improvement, 2nd edn. American Society of Agronomy, Madison, pp 243–254

Inagaki M, Tahir M (1990) Comparison of haploid production frequency in wheat varieties crossed with Hordeum bulbosum L. and maize. Jpn J Breed 40:209–216

James AB, Reiner AV (2007) The gene balance hypothesis: from reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13:1749–1759

Liu JH, Xu XY, Deng XX (2005) Intergeneric somatic hybridization and its application to crop genetic improvement. Plant Cell Tissue Organ Cult 82:19–44

Ma XF, Gustafson JP (2008) Allopolyploidization-accommodated genomic sequence changes in triticale. Ann Bot 101:825–832

Malyshева L, Sjakste T, Matzf K, Röder M, Ganal M (2003) Molecular cytogenetic analysis of wheat-barley hybrids using genomic in situ hybridization and barley microsatellite markers. Genome 46:314–322

Mukai Y, Nakahara Y, Yamamoto M (1993) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome 36:489–494

Nagy ED, Molnár-Láng M, Linc G, Láng L (2002) Identification of wheat-barley translocations by sequential GISH and two colour FISH in combination with the use of genetically mapped barley SSR markers. Genome 45:1238–1247

Pershina LA, Dobrovil’skaia OB, Rakovtseva TS, Kravtsova LA, Shchhapova AI, Shumnvy VK (2003) The effect of rye chromosomes on callus induction and regeneration in callus cultures of immature embryos of wheat-rye substitution lines, Triticum aestivum L. cultivar Saratovskaia 29/Secale cereale L. cultivar Onokoishkaia. Genetika 39:1073–1080

Polanco C, Ruiz ML (2002) AFLP analysis of somaclonal variation in Arabidopsis thaliana regenerated plants. Plant Sci 162:817–824

Röder MS, Korzun V, Wendehake K (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Sears ER (1969) Wheat cytologenetics. Annu Rev Genet 3:451–468

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13:1749–1759

Silikova OG, Dobrovil’skaia OB, Dubovets NI, Adonina IG, Kravtsova LA, Roeder MS, Salina EA, Shchhapova AI, Shumnvy VK (2006) Production of wheat-rye substitution lines and identification of chromosome composition of karyotypes using C-banding, GISH, and SSR markers. Genetika 42:793–802

Song XQ, Xia GM, Chen HM (2000) Chromosomal variation in long-term cultures of several related plants in Triticinae. Acta Phytophysiol Sin 26:33–38

Wang J, Xiang FN, Xia GM, Chen HM (2004) Transfer of small chromosome addition lines. Genome 45:434–441

Xia GM, Chen HM (1996) Plant regeneration from intergeneric asymmetric hybridization between Triticum aestivum and Leymus chinesis. Plant Sci 120:197–203

Xia GM, Wang H, Chen HM (1996) Plant regeneration from intergeneric asymmetric hybridization between wheat (Triticum aestivum L.) and Russian wildrye (Psathyrostachys juncea (Fisch.) Nevski) and wheat grass (Agropyron elongatum (Host) Nevski). Chin Sci Bull 41:1382–1386

Xia GM, Xiang FN, Zhou AF, Wang H, Chen HM (2003) Asymmetric somatic hybridization between wheat (Triticum aestivum L.) and Agropyron elongatum (Host) Nevishi. Theor Appl Genet 107:299–305

Xiang FN, Xia GM, Chen HM (1999) Asymmetric somatic hybridization between wheat (Triticum aestivum) and Bromus inermis. Acta Bot Sin 41:458–462

Xiang FN, Xia GM, Chen HM (2003a) Asymmetric somatic hybridization between wheat (Triticum aestivum) and Avena sativa L. Sci Chin Ser C 49:243–252
Xiang FN, Xia GM, Chen HM (2003b) Effect of UV dosage on somatic hybridization between common wheat (*Triticum aestivum* L.) and *Avena sativa* L. Plant Sci 164:697–707

Xiang FN, Xia GM, Zhi DY, Wang J, Nie H, Chen HM (2004) Regeneration of somatic hybrids in relation to the nuclear and cytoplasmic genomes of wheat and *Setaria italica*. Genome 47:680–688

Xu CH, Xia GM, Zhi DY, Xiang FN, Chen HM (2003) Integration of maize nuclear and mitochondrial DNA into the wheat genome through somatic hybridization. Plant Sci 165:1001–1008

Yue W, Xia GM, Zhi DY, Chen HM (2001) Transfer of salt tolerance from *Aeleuropus littoralis* sinensis to wheat (*Triticum aestivum* L.) via asymmetric somatic hybridization. Plant Sci 161:259–266

Zhou AF, Xia GM (2005) Introgression of the *Haynaldia villosa* genome into γ-ray-induced asymmetric somatic hybrids of wheat. Plant Cell Rep 24:289–296