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ORIGINAL ARTICLE

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Agropyron elongatum chromatin localization on the wheat chromosomes in an introgression line

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Abstract The introgressed small-chromosome segment of *Agropyron elongatum* (Host.) Neviski (*Thinopyrum ponticum* Podp.) in F_5 line II-1-3 of somatic hybrid between common wheat (*Triticum aestivum* L.) and *A. elongatum* was localized by sequential fluorescence in situ hybridization (FISH), genomic in situ hybridization (GISH) and karyotype data. Karyotype analysis offered basic data of arm ratios and relative lengths of 21 pairs of chromosomes in parent wheat Jinan177 and hybrid II-1–3. Using special high repetitive sequences pSc119.2 and pAs1 for FISH, the entire B- and D-genome chromosomes were detected. The FISH pattern of hybrid II-1-3 was the same as that of parent wheat. GISH using whole genomic DNA from *A. elongatum* as probe determined the alien chromatin. Sequential GISH and FISH, in combination with some of the karyotype data, localized the small chromosome segments of *A. elongatum* on the specific sites of wheat chromosomes 2AL, 1BL, 5BS, 1DL, 2DL and 6DS. FISH with probe OPF-03_{1296} from randomly amplified polymorphic DNA (RAPD) detected E-genome chromatin of *A. elongatum*, which existed in all of the small chromosome segments introgressed. Microsatellite primers characteristic for the chromosome arms above were used to check the localization and reveal the genetic identity. These methods are complementary and provide comprehensive information about the genomic constitution of the hybrid. The relationship between hybrid traits and alien chromatin was discussed.

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Introduction

Relatives of wheat have some important traits beneficial for its quality and resistance improvement. Somatic hybridization provides a novel way for crop breeding (Waara and Glimelius 1995). It offers the possibility of getting over the barriers of sexual crossing and allows the transfer of superior genes from nuclear and cytoplasmic genome separately or simultaneously (Bijoya et al. 1999; Yue et al. 2001). UV irradiation could induce introgression of DNA or chromosome segments during protoplast fusion (Forsberg et al. 1998; Xiang et al. 2003). A small portion of DNA segments from donor integrated into the genome of receptor has some advantages over adding a complete set of chromosomes or intact chromosome(s), which could increase the regeneration ability, inherited stability and fertility of the hybrid (Xiang et al. 2003, 2004; Wang et al. 2003; Chen et al. 2004). With the development of molecular biology, the identification of alien chromosome and chromatin in somatic hybrid has greatly extended from traditional analysis of morphology, cytology and biochemistry (Xia et al. 1996) to verification with molecular markers, including randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR) and genomic in situ hybridization (GISH) etc. (Forsberg et al. 1998; Zhou et al. 2001; Xiang et al. 2003; Wang et al. 2003; Xu et al. 2003).

In wheat breeding programs, chromosome identification (Xu et al. 1994) and alien chromatin detection in wheat background is important (Gill 1987; Forsström et al. 2002; Ko et al. 2002). The localization of alien chromatin is particularly useful in connection with the

physical mapping of other DNA sequences to chromosomes (Pedersen and Langridge 1997), which can offer the feasibility for target-gene cloning and marker-assistant breeding. We have described the detection of alien chromosomes and chromatin in several wheat somatic hybrids (Zhou et al. 2001; Xia et al. 2003; Xiang et al. 2003; Xu et al. 2003). However, there were no reports about the precise localization of alien chromatin in wheat somatic hybrids (also any other somatic hybrids). This limited the application and development of somatic-hybridization technology.

The fluorescence in situ hybridization (FISH) patterns of wheat chromosomes are beneficial for rapid and intuitive physical localization of heterogeneric chromatin. Using high repetitive sequences pSc119.2 from rye and pAs1 including the inserted repetitive sequences of *Aegilops tauschii* (Cross.) Schmal for two-color FISH, Mukai et al. (1993) established the idiogram of B- and D-genome chromosomes of Chinese Spring wheat. Pedersen and Langridge (1997) presented a detailed complete wheat molecular karyotype based on pAs1 sequence and GAA-satellite sequence (pHvG38), permitting identification of all 21 wheat chromosomes by two-color FISH. In addition, the identification of *Aegilops elongatum* chromatin in wheat background has been reported using special repeat sequences of E-genome from RAPD as probes (Zhang et al. 1998). GISH and two-color FISH in combination with the use of genetically mapped barley SSR have been developed for the identification of wheat–barley translocations (Nagy et al. 2002). It was also reported that several pairs of wheat SSR primers from 1A and 1D were used to locate the recombined chromosomes and intercalary 1D chromosome segments in a durum wheat background (Blanco et al. 2002). This research provides important information for alien chromatin localization of wheat somatic hybrid.

Fertile hybrids and progenies have already been created via somatic hybridization between *Triticum aestivum* (cv. Jinan 177) and *Agropyron elongatum* treated by UV (Xia et al. 2003). Cytogenetical analysis of hybrids showed that the chromosome numbers varied in the range of 38∼44 (Wang et al. 2004). GISH analysis revealed the different hybrid lines with different sites of translocation or insertion of chromosome segments (Xia et al. 2003; Wang et al. 2004). Some F_1∼F_5 hybrid lines expressed valuable traits, such as high quality, short stalk, salt tolerance and disease resistance. These materials may have potential for wheat improvement. Of these, a hybrid line II-1-3 possessed salt tolerance (Chen et al. 2004) and high quality (Zhao et al. 2003). The stable phenotype from F_2 to F_5 and the normal PMC MI chromosome pairing in F_5 (Wang et al. 2004) imply that II-1-3 is an introgressed small-chromosome-fragment line. The goal of this study was to localize the alien chromatin of II-1-3 using cytological and molecular approaches, including identification of wheat chromosomes involved and determination of the relative distance of *A. elongatum* chromatin introgressed.

Materials and methods

Plant materials

Seeds of *T. aestivum* L (cv. Jinan 177), *A. elongatum* (Host.) Neviski (synonym, *Thinopyrum ponticum* Podp.) and the F_5 hybrid line II-1-3 between *T. aestivum* and *A. elongatum* were saved in our laboratory. Seeds of the 4E/4D substitution line of wheat, *T. urartu* Thum, *Aegilops speltoides* Tausch and *Aegilops tauschii* (Coss) were kindly provided by Quality and Resource Institute of Agriculture Science Academy of China.

Root tip and chromosome preparation

Seeds of *T. aestivum*, *A. elongatum*, II-1-3 and 4E/4D substitution line were germinated on moist filter paper for a few days at 25°C in the dark. The root tips of 0.5–1 cm in length were excised and placed in ice water for 24 h, and then fixed in 1:3 acetic acid–ethanol for 2 days. For chromosome counting, the root-tip meristem was squashed under a cover slip in Carbol Fuchsin solution. For GISH and FISH analysis, the root-tip meristem was squashed in 45% acetic acid.

Karyotype analysis

More than 500 cells were counted for the parent wheat and hybrid, respectively. Karyotype classification followed the method of Sear (1969) and consulted the data of Chinese Spring from Gill (1987). The parameters of the karyotypes were based on ten metaphase cells spread. Student's *t*-test (Table 2) via the Statistical Package for the Social Sciences (SPSS) software was used to compare the karyotypes of Jinan177 with II-1-3.

DNA probes of repetitive sequence and genome

Genomic DNA of common wheat Jinan177, *A. elongatum*, *T. urartu*, *Aegilops tauschii*, *Aegilops speltoides* and II-1-3 were isolated by CTAB method according to Doyle and Doyle (1990). Total genomic DNA of *A. elongatum* was labeled as a probe for GISH.

pSc119.2 and pAs1 containing particular repetitive sequences of B and D genome, respectively, were offered by Dr. Zhang Xueyong (Quality and Resource Institute of Agriculture Science Academy of China). They were labeled as probes for FISH.

Three RAPD primers (Operon Technology, USA) were used to derive amplicons following Zhang et al. (1998). Annealing temperatures were 38°C for OPF-03, 41°C for OPB-08 and 48°C for OPN-01, respectively. The 1,296-bp segment of *A. elongatum* amplified with OPF-03 was retrieved by BioDev glassmilk kit (Boda Biocompany, Beijing) and labeled as a probe for E genome.

All probes were labeled with digoxigenin-11-dUTP using a nick translation kit following the manufacturer's instructions (976776 Boehringer Mannheim).

GISH and FISH

GISH and FISH were carried out following the method described by Xiang et al. (2003). The combinations and ratios of probes and blocking DNA are listed in Table 1.

Some combinations of GISH with FISH were tested on the same sides: I. GISH + B; II. GISH + D; III. GISH + B + D. For I and II, pSc119.2 and pAs1 were used for FISH, respectively, after the preparation of GISH was rinsed. For III, FISH/ pAs1 followed the FISH/ pSc119.2, which was performed after the GISH preparation was rinsed.

The 1,296-bp segment of E genome from *A. elongatum* was used for FISH on the chromosome plates of the hybrid II-1-3 and 4E/4D substitution line.

Microsatellite marker

DNA was extracted from leaves of the hybrid II-1-3 and parents by using the same method as above. Microsatellite loci mapping on particular wheat chromosomes (based on the locations from GISH/FISH/karyotype) were amplified using 52 pairs of primers identified according to the procedure described by Röder et al. (1998).

Results

Chromosome number and karyotype

Statistical data of chromosome numbers revealed that 77.25% of the cells in hybrid II-1-3 were $2n = 42$, near to the proportion of parent wheat Jinan 177. The karyotypes (Fig. 1) and basic data (Table 2) of arm ratios and relative lengths of 21 pairs of chromosomes from hybrid and parent wheat were similar at a global level. However, the arm ratios of 2A, 6B and 6D and the relative length of 3D in II-1-3 chromosomes were obviously greater than that of Jinan177($P < 0.05$) (Table 2). It was suggested that these differences were due to the introgression of *A. elongatum*. These data provide a reference for the location of *A. elongatum* chromatin.

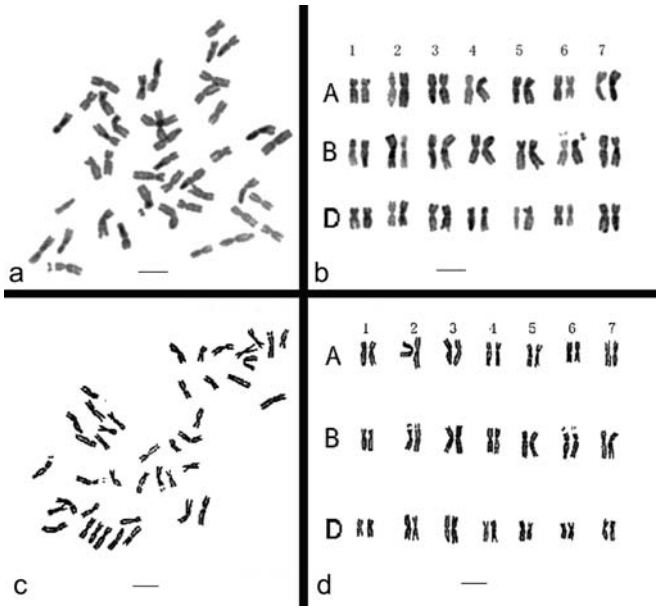

Fig. 1 Karyotype of the chromosomes of the hybrid F5 II-1-3 and parent wheat Jinan177. **a** The metaphase chromosomes of Jinan177. **b** Karotype of Jinan177. **c** Chromosomes of hybrid F_5 II-1-3. **d** Karotype of F_5 II-1-3. *Scale bars* 10 μm

Localization of *A. elongatum* chromatin on wheat chromosomes

FISH patterns of B- and D-genome chromosomes of hybrid and parent wheat

Using the clone pSc119.2 and pAs1 separately for FISH produced hybrid bands on all B- and D-genome chromosomes of II-1-3 and parent wheat (Fig. 2). According to the FISH patterns of Chinese Spring wheat (Mukai et al. 1993), seven pairs of chromosomes of B and D genome were paired and identified, respectively (Fig. 2). In addition, a pAs1 probe hybridized with a pair of 4A chromosomes, showing minor sites on the terminals of long arms (Fig. 2), the same as the result in Chinese Spring (Mukai et al. 1993). The FISH patterns of B and D genome of II-1-3 (Fig. 2a, c) were identical with parent wheat (Fig. 2b, d), and in general agreement with Mukai et al. (1993). The results indicate that pSc119.2 and pAs1 can be efficiently used for the detection of B- and D-genome chromosomes in wheat Jinan 177 and probably permit identification of related chromosomes in most wheat cultivars.

Table 1 Assemblies and ratios of probes and blocking DNA for GISH and FISH

Probe (P)	*A. elongatum* genomic DNA	pSc119.2	pAs1	$OPF\text{-}03_{1296}$
Blocking DNA (B)	Wheat genomic DNA	*T. urartu* and *Aegilops tauschii* DNA	*T. urartu* and *Aegilops speltoides* DNA	Wheat genomic DNA
Ratios (P:B)	1:200, 1:145	1:50 (25 + 25)	1:50 (25 + 25)	1:50

Table 2 Karyotype comparison of somatic hybrid F_5 II-1-3 with parent Jinan177

No	II-1-3			177			*P* value (*t* test)[b]	
	Relative length	Arm ratio	Pattern	Relative length	Arm ratio	Pattern	Arm ratio	Relative length
1A	4.273	1.691	M	4.252	1.790	SM	0.416	0.916
2A	5.944	1.263	M	5.746	1.172	M	0.044a	0.240
3A	5.369	1.292	M	5.184	1.560	M	0.097	0.200
4A	4.976	1.854	SM	4.876	1.634	M	0.179	0.304
5A	4.433	1.583	M	4.342	1.554	M	0.819	0.598
6A	4.108	1.240	M	4.004	1.214	M	0.797	0.398
7A	5.017	1.116	M	5.068	1.068	M	0.222	0.757
1B	4.582	1.765	SM	4.552	1.852	SM	0.572	0.884
2B	5.357	1.360	M	5.476	1.380	M	0.838	0.447
3B	5.864	1.408	M	6.028	1.440	M	0.493	0.392
4B	5.169	1.283	M	5.24	1.218	M	0.128	0.496
5B	5.41	1.881	SM	5.264	2.028	SM	0.295	0.367
6B	5.509	1.264	M	5.444	1.082	M	0.026a	0.763
7B	5.358	1.457	M	5.312	1.528	M	0.176	0.685
1D	3.553	1.698	M	3.656	1.610	M	0.356	0.361
2D	4.625	1.179	M	4.434	1.210	M	0.631	0.161
3D	4.952	1.170	M	4.612	1.236	M	0.333	0.013a
4D	4.001	1.908	SM	3.81	1.826	SM	0.496	0.218
5D	4.169	1.826	SM	4.03	1.790	SM	0.87	0.402
6D	3.67	1.211	M	3.666	1.078	M	0.029a	0.978
7D	4.589	1.400	M	4.924	1.430	M	0.756	0.092

M Mediocentric chromosome, *SM* submetacentric chromosome
[a]Data of relative length and arm ratio deriving from the average of ten samples
[b]Significant at 0.05

Distribution of A. elongatum chromatin on B-, D- and A-genome chromosomes

Sequential GISH and FISH were used to identify the chromatin of *A. elongatum* on the B-, D- and several A-genome chromosomes of wheat in the hybrids. The combinations of in situ hybridization include: GISH + B (I), GISH + D (II) and GISH + B + D (III).

GISH results from combination I, II and III detected the alien chromatin translocated or inserted into wheat chromosomes. Statistical data from 510 cells indicated that 67.73 % of them had six green hybridization signals (Figs. 3a, c, 4a), accounting for the highest proportion.

FISH results using pSc119.2 as a probe in combination I and III showed two GISH signals (Figs. 3a, 4a) on 1B and 5B chromosomes, respectively (Figs. 3b, 4b). This indicates that there are two alien segments in the B genome.

Using pAs1 for FISH in combination II and III showed three GISH signals (Figs. 3c, 4a) on 1D, 2D and 6D chromosomes, respectively (Figs. 3d, 4c). Therefore, the D genome contains three *A. elongatum* segments.

Integrating B-genome and D-genome signals of FISH in the same chromosome spread from combination III can differentiate parts of A-genome chromosomes including 1A, 4A and 5A (Mukai et al. 1993). In combination with karyotype data, 2A (the longest) and 6A (the shortest) could also be identified. Thus, a small *A. elongatum* chromosome segment has been located on the 2A chromosome (Fig 4c).

In sum, all *A. elongatum* small chromosome segments were localized on 2AL, 1BL, 5BS, 1DL, 2DL and 6DS of wheat chromosomes of the hybrid (Table 3; Fig. 4). In addition, the arm ratios of the chromosomes involved and the relative distances from centromeres to the breaking points were analyzed, based on the data from GISH. The relative distances from centromeres to the breaking points were 72.45 ± 11.12, 75.54 ± 10.12, 50.89 ± 12.45, 69.23 ± 15.33, 72.88 ± 14.02 and 20.63 ± 13.57, respectively (Table 3).

Origin of *A. elongatum* chromosome segments

RAPD profiles of wheat, *A. elongatum* and II-1-3 showed specific segments of 525, 1,296 and 817 bp in *A. elongatum*. There was no evidence for any introgressed segment homologous with 525 and 817 bp in II-1-3. In contrast, II-1-3 displayed an allele of *A. elongatum* 1,296-bp sequence amplified by prime OPF-03(CCTGATCACC) (Fig. 5) (Zhang et al. 1998). It implies the introgression of *A. elongatum* DNA via asymmetric somatic hybridization.

The $OPF\text{-}03_{1296}$ segment was hybridized with II-1-3 and 4E/4D substitution line which was used as control. According to expectations, typical hybridized signals localized on a pair of chromosome terminals of 4E/4D substitution in the FISH pattern (Fig. 6a). Among 77 cells in hybrid II-1-3 observed, 48 (62.33%) have six green hybridized sites (Fig. 6b), coinciding with the re-

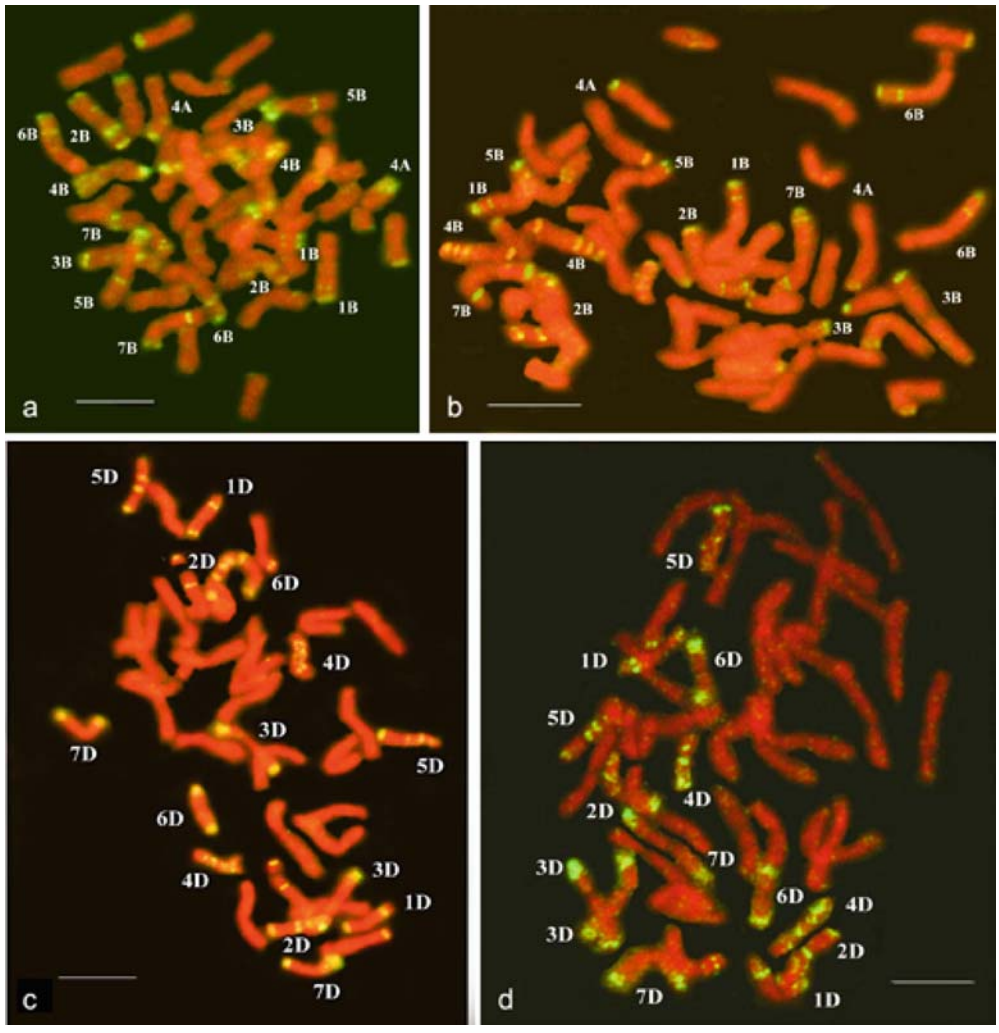

Fig. 2 FISH karyotype of metaphase chromosomes of the hybrid II-1-3 and parent Jinan 177. The probes were labeled using digoxigenin and detected with FITC (*yellow–green*). The chromosomes counterstained with PI (*red*). **a**, **b** B-genome patterns of II-1-3 (**a**) and Jinan 177 (**b**) with rye repetitive DNA probe (pSc119.2) on metaphase plates of the root tips. **c**, **d** D-genome patterns of II-1-3 (**c**) and Jinan 177 (**d**) with *Aegilops tauschii* repetitive (pAsI) probe on metaphase plates of the root tips. *Scale bars* 10 μm

sult from GISH. This indicated that E-genome DNA was involved in *A. elongatum* chromatin in hybrid II-1-3.

SSR analysis of special sites on hybrid chromosomes

To test the result from GISH/ FISH, a total of 52 pairs of microsatellite primers located on 2AL, 1BL, 5BS, 1DL, 2DL and 6DS of wheat were used to amplify the genomic DNA of the hybrid and parents. Sixteen markers (Table 4; Fig. 7) showed clear polymorphism among different genotypes. The SSR profiles of II-1-3 (Table 4; Fig. 7) carried (1) the fragments originated from both parents (P); (2) the fragments derived from *A. elongatum*(A); (3) the fragments from *A. elongatum*/ novel fragment(s) (A, N) and (4) the biparental/novel fragment(s) (P, N). Markers P, A, N (Fig. 7) indicated the integration and rearrangement between biparental DNA. The relative distance (%) from the centromeres to SSR loci checked were counted. Several SSR bands were located on 2AL, 1BL and 2DL and the relative distances [relative distance = (distance from centromere to SSR locus checked/length of the arm involved) × 100%] of most fragments ranged from 63.79% to 77.59% (Table 4), in agreement with the results of sequential GISH and FISH (Table 3). The alien segments, 2AL, 1BL, 5BS, 2DL and 6DS, have also been primarily positioned on the macrosatellite genetic map of wheat with centiMorgans according to the results of Röder et al. (1998) (Fig. 8).

Discussion

It was clear that UV induced both donor chromosome elimination and fragmentation (Hall et al. *1992*; Xia et al. 2003; Wang et al. 2003; Xiang et al. 2003, 2004). Novel chromosomes, including intercalary translocations (Figs. 3, 4; Tables 2, 3), could produce when the instant break points of receptor DNA linked with donor DNA segments. Such a chromotype is particularly interesting in the context of alien introgression (Xia et al. 2003), as it is superior to big-segment translocation. e.g. it can largely exclude the interference of disadvantage genes; it benefits heredity and localization of alien target genes. Therefore, it is very important to both genetic theory research and genetic breeding of wheat.

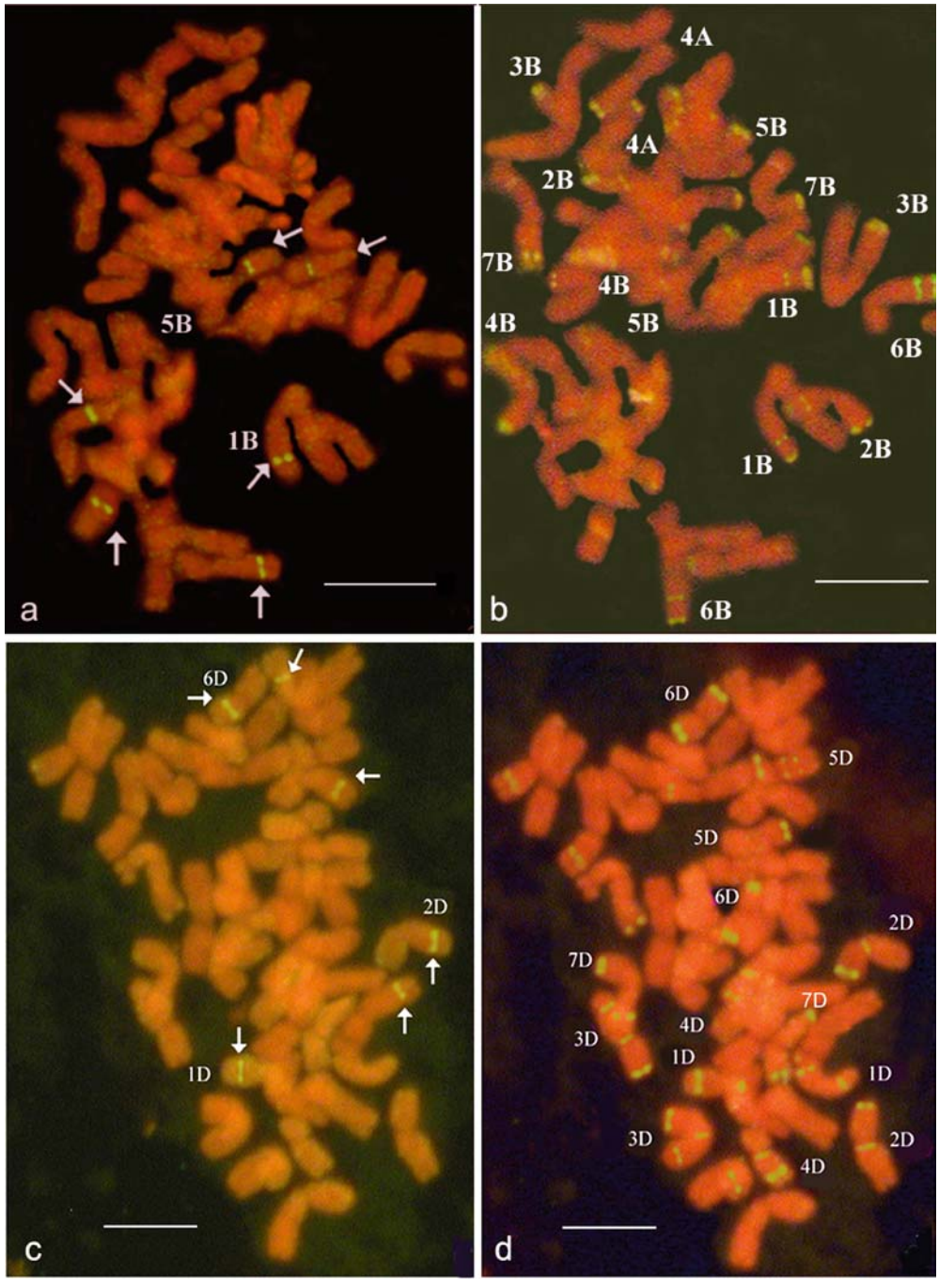

Fig. 3 Sequencial GISH and FISH (**a**/**b** and **c**/**d**) on the same spread of metaphase chromosomes of root tip in hybrid II-1-3. The probes were labeled using digoxigenin and detected with FITC (*yellow–green*). The chromosomes were counterstained with PI (*red*). *Right arrow* Hybridized signal. **a**, **c** GISH patterns of II-1-3 with total genome DNA of *A. elongatum*. **b**, **d** B- and D-genome patterns of II-1-3 with pSc119.2 and pAsI probes on the same plates of the hybrid, respectively. *Scale bars* 10 μm

The amount of chromatin introgressed in translocated wheat lines can be evaluated using GISH (Blanco et al. 2002). In combination with other cytogenetic and molecular genetic approaches, the translocation breakpoint and the physical size of introgressed chromosome segment in recombined wheat could be determined. For example, Jiang et al. (1993) detected wheat–rye substitution using GISH/C-banding/N-banding for the first time. Zhang et al. (2001) localized the Yellow dwarf disease resistant gene of *Thinopyrum intermedium* (Host) to 7DL using GISH/RFLP/RAPD. Malysheva et al. (2003) identified barley chromosomes and chromosome segments in wheat–barley hybrids via GISH/SSR. Wei et al. (1999) detected rye chromatin in the new wheat germplasm 10-A with FISH/RFLP/A-PAGE. However, most of the translocation lines of wheat reported contained alien whole arm or big segment created by sexual cross/chromosome engineering (Ren and Zhang (1997). Only a few small-segment-translocation lines were produced (Blanco et al. 2002; Malysheva et al. 2003).

Small segment translocation was more complex in the somatic hybrid line than in sexual hybrid of wheat. The karyotype analysis offered an assistant means for identification of A-genome chromosome in the hybrid

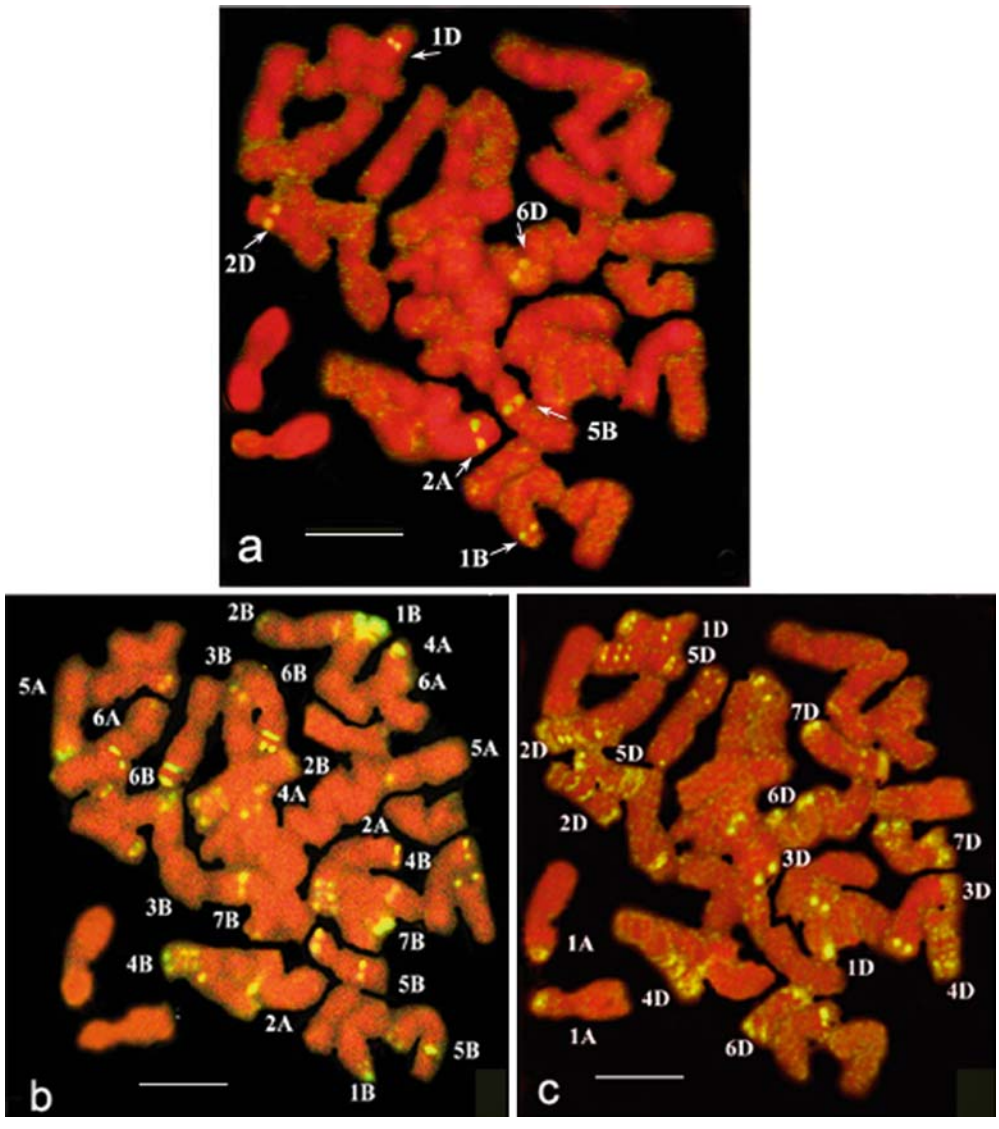

Fig. 4 Sequencial GISH and FISH (**a**/**b**/**c**) on the same spread of metaphase chromosomes of the hybrid II-1-3 root tip. The probes were labeled using digoxigenin and detected with FITC (*yellow–green*). The chromosomes were counterstained with PI (*red*). *Right arrow* Hybridized signal. **a** GISH patterns of II-1-3 with total genome DNA of *A. elongatum*. **b**, **c** FISH patterns of B- and D-genome chromosomes of II-1-3 with pSc119.2 or pAsI probes on the same plates, respectively. *Scale bars* 10 μm

(Fig. 4) and some information on the hybrid genome variation (Table 2, Fig. 1). However, the chromosomes with distinct differences in the karyotype between the hybrid and parent wheat are not all in correspondence with the chromosomes involved in the introgression. Out of four chromosomes detected by karyotype analysis (Table 2), only 2A and 6D related to the introgression of alien chromosome segments detected by GISH. So, the karyotype change was likely derived from not only alien chromatin introgression but also somaclonal variation. Through single-color FISH with pSc119.2 and pAs1, consulting some of the karyotype data, we detected the

Table 3 Statistic data of GISH hybridization sites in the hybrid F_5 II-1-3

Chromosome		Cell number	Relative distance (%) from centromere to the breaking point a	Arm ratio
2A	L	43	72.45 ± 11.12	1.24 ± 0.12
1B	L	16	75.54 ± 10.12	1.25 ± 0.11
5B	S	36	50.89 ± 12.45	1.86 ± 0.087
1D	L	8	69.23 ± 15.33	1.88 ± 0.09
2D	L	45	72.88 ± 14.02	1.37 ± 0.13
6D	S	52	20.63 ± 13.57	1.11 ± 0.098

L Long arm of chromosome, *S* short arm of chromosome
[a](The distance from centromere to breaking point / the length of the arm involved) ×100%

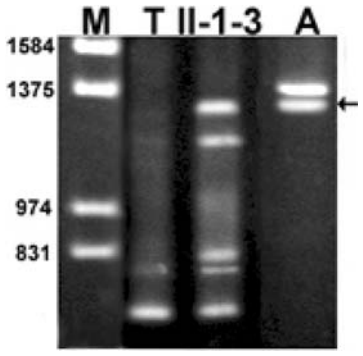

Fig. 5 RAPD profiles of the repeat sequences of *A. elongatum*. *A* *A. elongatum*, *T* Jinan177, *II-1-3* hybrid F_5 line. Specific fragment of *A. elongatum* amplified from OPF-03

chromosomes of B, D, 1A, 4A and 5A (Figs. 2, 3b, d, 4b, c), 2A and 6A (Table 2, Fig. 4a, b). In combination with GISH, we have successfully localized the small chromosome segments of *A. elongatum* on wheat chromosomes (Figs. 2, 3, 4; Table 3). The reliability of the location was confirmed further by using SSR markers mapped on the specific sites of wheat chromosomes (Figs. 7, 8; Table 4). It is noted from SSR data that Xgwm311, Xgwm265 and Xgwm382 far from the centromere were located on the long arm of 2A. They represent the small fragment on the 2AL (Fig. 8; Table 4). But the other locus Xgwm448 close to the centromere is also determined. This information indicates that chromosome 2A has two small interstitial translocations and one of them is too small to be visible with GISH. Therefore, more SSR data are needed to identify the size and break points of the hybrid in further studies.

Table 4 SSR position on the wheat chromosome of the hybrid and the amplification marker

Primer	Location	Band pattern	Relative distance (%) from the centromere[a]
Xgwm265	2AL	P	74.14
Xgwm311	2AL	P	68.97
Xgwm382	2AL	P	77.59
Xgwm448	2AL	P	25.86
Xgwm124	1BL	P	68.97
Xgwm153	1BL	P	55.17
Xgwm268	1BL	A	67.24
Xgwm274	1BL	P	63.79
Xgwm443	5BS	A	66.67
Xgwm544	5BS	A	48.48
Xgdm126	1DL	P, N	58.62
Xgwm301	2DL	P, N	68.97
Xgwm311	2DL	P	77.59
Xgwm349	2DL	A, N	70.59
Xgwm382	2DL	P	72.41
Xgwm325	6DS	P, N	24.24

P Both parental characteristic bands, *A* *A. elongatum* characteristic band, *N* new band, *T* wheat characteristic band
[a](The distance from centromere to SSR locus checked / the length of the arm involved)×100%

It is reported that most of the substitution and translocation occurred on D-genome chromosomes of wheat in the sexual hybrids of wheat and *Th. intermedium* (Zhang et al. 1991). Another experiment indicated that the *Th. intermedium* chromosome segment controlling blue-grain character was translocated to D-genome chromosomes of wheat (Ying et al. *2001*). In our experiment, four of six *A. elongatum* chromosome segments were localized on the similar sites of group 1 and 2 chromosomes, and three of six hybridization signals were positioned on D-genome chromosomes (Figs. 3, 4). So, maybe the introgression of the small-chromosome fragment of *A. elongatum* also prefers some special chromosomes and loci in wheat somatic hybridization.

St, E^e and E^b, the three basic genomes of *A. elongatum*, were much related to wheat A-, B-, D-genome (Zhang et al. 1999). *Thinopyrum bessarabicum* (Savul.

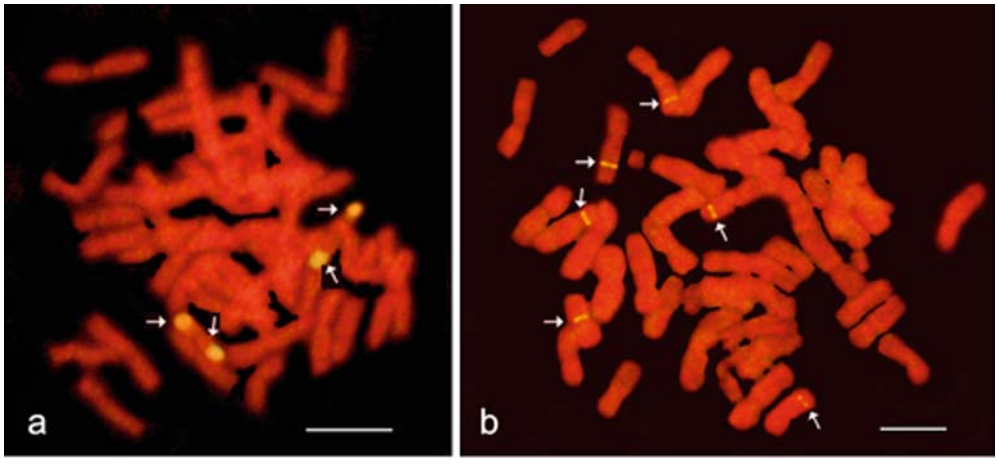

Fig. 6 FISH to metaphase chromosomes of hybrid II-1-3 and 4E/4D substitution line. The 1,296-bp segment of E genome amplified from *A. elongatum* with OPF-03 primer was labeled using digoxigenin and detected with FITC (*yellow–green*). The chromosomes were counterstained with PI (*red*). *Right arrow* Hybridized signal. **a** FISH patterns of 4E/4D substitution line with 1,296-bp segment probe on metaphase plate of the root tips. **b** FISH patterns of II-1-3 with 1296-bp segment probe on metaphase plate of the root tip. *Scale bars* 10 μm

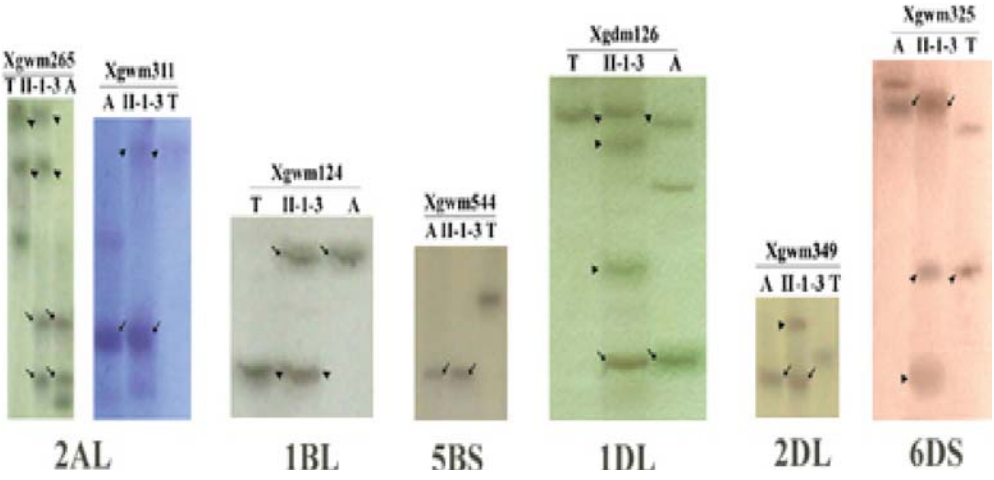

Fig. 7 SSR loci amplified from hybrid II-1-3 and both parents, using special primers on wheat chromosomes of 2AL, 1BL, 5BS, 1DL, 2DL and 6DS published. *A A. elongatum*. *T* Jinan177. *Right arrow* Characteristic segment of *A. elongatum*. *Arrowhead* Characteristic segment of wheat. *Solid triangle* Novel segment

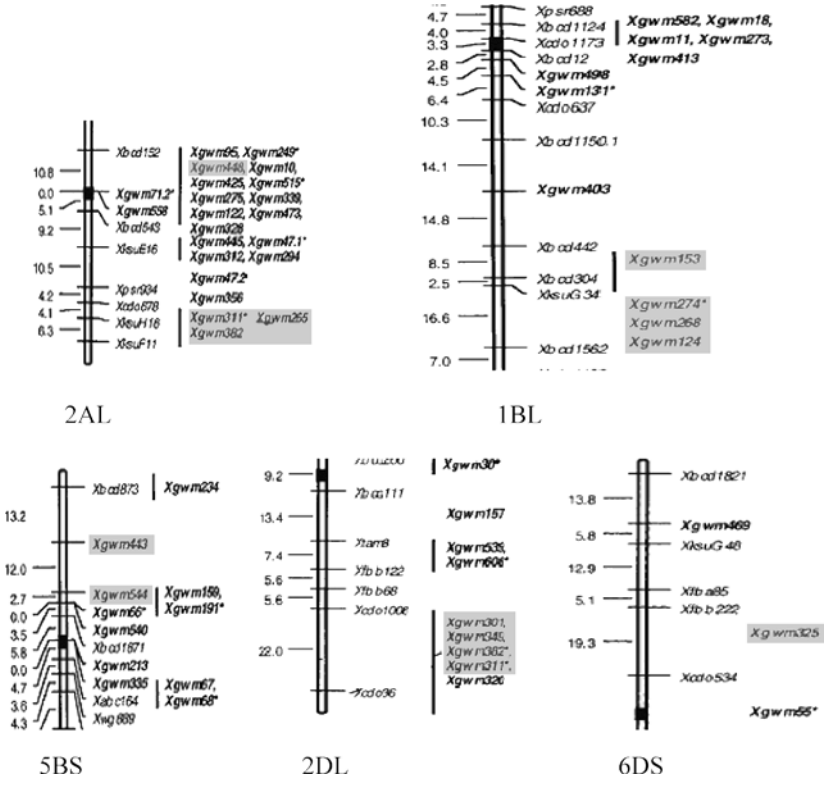

Fig. 8 The SSR genetic map of chromosomes 2AL, 1BL, 5BS, 2DL and 6DS and genetic distances (cM) from Röder et al. (1998). *Shadows* represent the special SSRs on relative chromosomes of II-1-3

and Rayss), the donor of E^b genome, has a high salt-tolerant trait (Zhuang et al. 2003). In our experiment, the involvement of E-genome chromatin in the small chromosome segments of *A. elongatum* was proved by FISH using the peculiar repetitive sequence from E genome (Zhang et al. 1998) as a probe (Figs. 5, 6). It is deduced that the salt tolerance of II-1-3 related to E^b chromatin from *A. elongatum*. In addition, II-1-3 contained special high molecular weight (HMW) glutenin subunits with the same mobility as *A. elongatum* and had a high quality (Zhao el al. 2003). It has been reported that HMW glutenin subunit genes localized on long arms of wheat group-1 chromosomes (Blasnco et al. 2002). It is worth studying whether the small chromosome segments and the SSR loci of *A. elongatum* on 1BL and 1DL (Figs. 3, 4, 7, 8 and Tables 3, 4) are related with the novel subunits.

This study indicates that the introgressed alien small fragment in somatic hybrid of wheat can be localized with GHSH/FISH/SSR etc., which provides a new mode of practice for wheat marker-assistant breeding.

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