

Cytological and Molecular Identification of Alien Chromatin in Giant Spike Wheat Germplasm

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Abstract: Alien chromosomes of twelve giant spike wheat germplasm lines were identified by C-banding, genomic *in situ* hybridization (GISH), sequence characterized amplified region (SCAR), and random amplified polymorphic DNA (RAPD). All lines showed a chromosome number of $2n = 42$, five of them carried both a pair of wheat-rye (*Triticum aestivum*-*Secale cereale*) 1BL/1RS translocation chromosomes and a pair of *Agropyron intermedium* (Ai) chromosomes, three carried a pair of Ai chromosomes only, three others carried a pair of 1BL/1RS chromosomes only, and one carried neither 1BL/1RS nor Ai chromosome. Further identification revealed that the identical Ai chromosome in these germplasm lines substituted the chromosome 2D of common wheat (*T. aestivum* L.), designated as 2Ai. The genetic implication and further utilization of 2Ai in wheat improvement were also discussed.

Key words: giant spike germplasm; 1BL/1RS; *Agropyron intermedium*; C-banding; genomic *in situ* hybridization (GISH); sequence characterized amplified region (SCAR); random amplified polymorphic DNA (RAPD)

Spike is the fertilizing organ of common wheat (*Triticum aestivum* L.), hence weighty spike, either by increasing the number of kernels per spike or by increasing the kernel weight, usually leads to a high yield. Long spike combined with a large number of spikelets was regarded as an ideal spike type for wheat high yield in breeding, as endeavored by wheat breeders and geneticists (Millet, 1983; Yen *et al.*, 1993).

Giant spike wheat is a kind of particular wheat germplasm (Xie *et al.*, 1994). Its spike is distinct from other genotypes of common wheat. A series of stable giant spike germplasm has been obtained. A few varieties, such as Plateau 175, Plateau 158, Plateau 913 and Plateau 363, were developed using these germplasm as basic parents and have been up to now released. The introduction of alien chromatin is a common strategy used in the early stage of giant spike germplasm development. Zaisheng No. 1 and Kavkaz are the main donors of alien chromatin. Zaisheng No. 1, derived from Zhong 5 (private correspondence with SUN Shan-Cheng, Shanxi Academy of Agricultural Sciences), is a partial amphiploid ($2n = 56$) selected from the hybrid progenies between common wheat and *Agropyron intermedium*. Kavkaz is a common wheat variety involving wheat-rye (*Secale cereale* L.) 1BL/1RS translocation chromosomes.

C-banding and genomic *in situ* hybridization (GISH), using biotin-labeled total genomic DNA of the donor

species as a probe and unlabeled total genomic of the recipient as blocking DNA, are now well established and highly efficient techniques for detecting alien chromatin in wheat (Lapitan *et al.*, 1986; Gill *et al.*, 1991; Mukai and Gill, 1991; Friebe *et al.*, 1992). Molecular biology techniques, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), and others are more and more popularly applied in detecting alien chromatin in wheat (Dellaport *et al.*, 1983; Francis *et al.*, 1995; Zhang *et al.*, 1998; Zhang *et al.*, 2000).

Detection and identification of wheat alien chromatin in giant spike germplasm will be helpful to understand its genetic basis and its utilization in wheat improvement.

1 Materials and Methods

1.1 Plant material

A series of giant spike wheat germplasm was obtained from XIE Jun-Feng, Northwest Plateau Institute of Biology, The Chinese Academy of Sciences. Dwarf stem and giant spike types I, II (designated as ZJ-1, ZJ-2), short stem and giant spike types I, II, III, IV, V, VI (designated as AJ-1, AJ-2, AJ-3, AJ-4, AJ-5, AJ-6), and weighty and giant spike types I, II, III, IV (designated as JZ-1, JZ-2, JZ-3, JZ-4) were classified mainly on the agronomic traits. The spike

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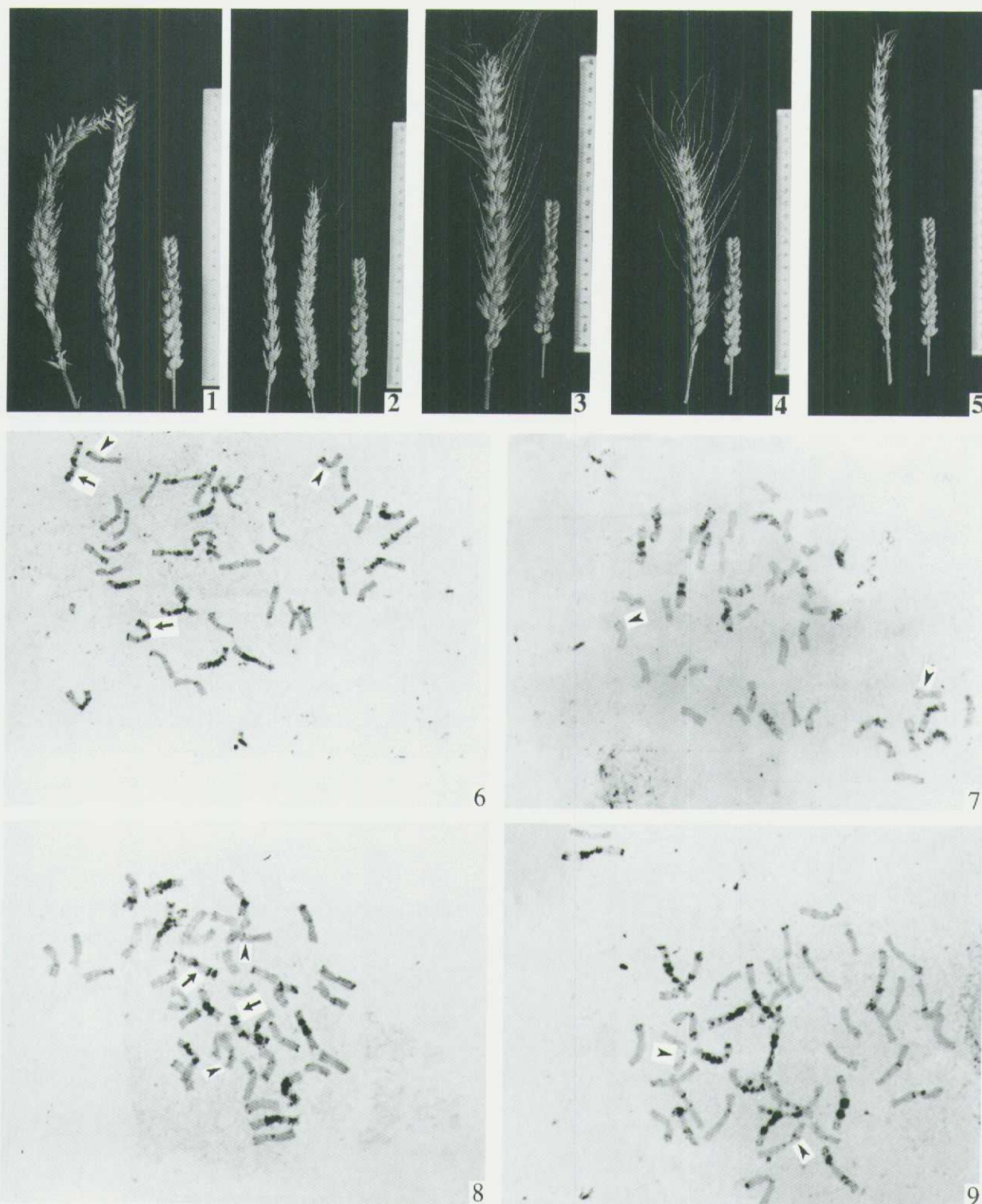
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morphology of giant spike wheat germplasm representatives, investigated in Nanjing, China was photographed (Figs.1–5), and main agronomic traits investigated

in Xining, China are listed in Table 1.

1.2 Methods

1.2.1 C-banding and GISH analysis C-banding and



Figs.1–5. Spike morphology. **1.** From left to right: AJ-3; (CS × AJ-3) F₁; CS (Chinese Spring). **2.** From left to right: AJ-4; (CS × AJ-4) F₁; CS. **3.** Left, JZ-2; right, CS. **4.** Left, JZ-4; right, CS. **5.** Left, “Plateau 913”; right, CS. **Figs.6–9.** Mitotic metaphases after C-banding. **6.** C-banded mitotic metaphases of AJ-3, arrows indicate 1BL/1RS, arrowheads indicate 2Ai. **7.** C-banded mitotic metaphases of AJ-4, arrowheads indicate 2Ai. **8.** C-banded mitotic metaphases of JZ-2, arrows indicate 1BL/1RS, arrowheads indicate 2D (2Ai absent). **9.** C-banded mitotic metaphases of JZ-4 (both 1BL/1RS and 2Ai absent).

Table 1 Main agronomic traits of giant spike wheat germplasm lines

Lines	Plant height (cm)	Spike length (cm)	Spikelets per spike	Kernels per spike	A thousand kernels weight (g)	Spike morphological variation*
ZJ-1	41	20	28	70	38	+
ZJ-2	70	28	30	160	38	+
AJ-1	65	25	30	90	40	+
AJ-2	72	26	30	160	45	+
AJ-3	72	26	30	150	55	+
AJ-4	75	24	32	90	55	+
AJ-5	100	23	27	165	60	+
AJ-6	80	32	32	150	60	+
JZ-1	110	19	25	136	68	—
JZ-2	125	22	25	85	81	—
JZ-3	125	20	24	115	80	—
JZ-4	100	20	22	125	65	—

* Morphological variation of spike including ramified spike, four-rowed spikelets. +, present; —, absent.

GISH were used for chromosome identification. Chromosome C-banding was according to Gill *et al.* (1991), and GISH analysis was inspired from Mukai and Gill (1991) with minor modifications. Genomic DNA was extracted by CTAB method (Dallaport *et al.*, 1983). *Ag. intermedium* and *S. cereal* genomic DNAs were respectively labeled by biotin-11-dUTP and digoxigenin-11-dUTP, using nick translation (Enzo Diagnostics, Inc.). Hybridization was carried out at 37 °C for 6 h or over night in 10 µL per slide of a mixture containing 10–15 ng of labeled probe DNA, 0.5–1 µg of sheared wheat genomic DNA as blocker, 5–10 µg of sheared salmon sperm DNA, 50% formamide, 2 × SSC, and 10% dextran sulfate. For double-color GISH, both labeled *Ag. intermedium* and *S. cereal* genomic DNAs were added to the mixture. After the post-hybridization wash, signal detection mixture was applied to chromosome slide preparation. Fluorescein anti-biotin was used for detection of biotin labeled *Ag. intermedium* DNA. Both Anti-Digoxigen-Rodamin Fab fragments and Fluorescein anti-biotin were simultaneously used in double-color GISH. After an incubation at 37 °C for 50 min, slides were washed three times with 1 × PBS (5 min each), and dehydrated in 70%, 95%, and 100% Ethanol (5 min each). Observation of Fluorescein was carried out by adding a thin layer of antifade solution containing propidium iodide (PI) with a cover slip, then Fluorescein and PI were excited by light at 460–490 nm wavelength using an Olympus-reflected light fluorescence attachment. For double-color GISH, added antifade solution containing 4, 6-diamidino-2-phenylindole (DAPI) all over the observation of Fluorescein and Rhodamin in one preparation. Fluorescein, Rhodamin and DAPI were excited by light at 460–490 nm, 520–550 nm, and 330–350 nm respectively. GISH patterns were photographed with Fuji 400 ASA films.

1.2.2 SCAR and RAPD analyses SCAR and RAPD reaction were all carried out in a volume of 25 µL. The reaction mixture contained 0.2 µmol/L of each primer, 0.2 mmol/L of each deoxynucleotide, 1.5 mmol/L MgCl₂, 1 U *Taq* polymerase, and 50–100 ng of template DNA, 1 × PCR buffer (10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl).

For SCAR, the amplifications were carried out in PE 9 600 DNA Thermal Cycler. Rye genome-specific primers AF₁/AF₄ (Dellaport *et al.*, 1983) were adopted: AF₁ (5'-GGAGACATCATGAAACATTTG-3') and AF₄ (5'-CTGTTGTTGGGCAGAAAG-3'). PCR conditions were 35 cycles of 94 °C 30 s, 55 °C 1 min, and 72 °C 1 min, with a 5 min extension at 72 °C following the final cycle. The amplified products were fractionated on a 1.2 % agarose gel and observed under a UV lamp after stained by ethidium bromide.

For RAPD, the amplification was carried out by PE 480 DNA Thermal Cycler with the following program: the first five cycles were at 96 °C 1 min, 35 °C 1 min, and 72 °C 1.5 min, followed by a further 40 cycles of 94 °C 45 s, 36 °C 1 min, 72 °C 1 min, and ending with 10 min at 72 °C. Observation of amplified products was as that of SCAR.

2 Results

2.1 C-banding and GISH

C-banded mitotic metaphases of 12 lines showed euploid chromosome number of 2n = 42. Wheat-rye 1BL/1RS translocation chromosome with typical C-band of 1RS could be easily identified in lines ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3, JZ-1, JZ-2, JZ-3 (Figs. 6, 8). The 1BL/1RS translocation chromosome was not observed in lines AJ-4, AJ-5, AJ-6 and JZ-4 (Figs. 7, 9).

GISH using biotin-labeled total genomic DNA of *Ag. intermedium* as probe and unlabeled genomic DNA of

wheat as block were carried out for the identification of *Ag. intermedium* chromatin in these giant spike wheat germplasm lines. In lines ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3, one pair of chromosomes showed the hybridization signal over the entire chromosome, but the other pair exhibited this hybridization signal on the terminus of the short arms (Fig.10A). In

lines AJ-4, AJ-5, and AJ-6 only one pair of chromosomes displayed the signal over the entire length of both arms, whereas all the other chromosomes of the complement showed no hybridization (Fig.10D). In lines JZ-1, JZ-2, and JZ-3, one pair of chromosomes showed the hybridization signal on the terminus of the short arms (Fig.10E). No

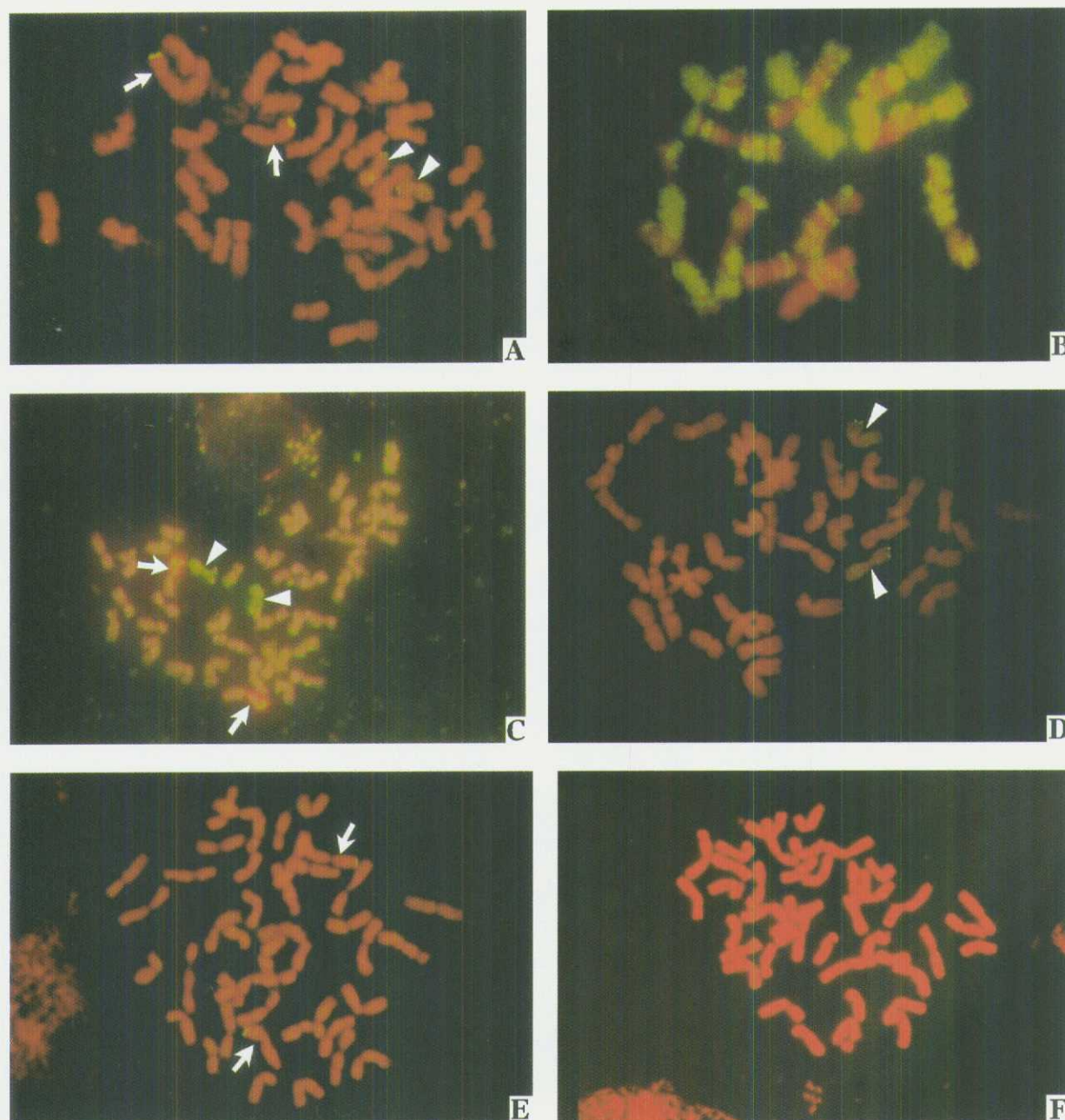


Fig.10. Mitotic metaphases after GISH. **A.** AJ-3, using labeled gDNA of *Agropyron intermedium* as probe and gDNA of CS as block, arrows indicate a pair of 1BL/1RS with signal on the terminus of the short arms, arrowheads indicate a pair of *Ag. intermedium* chromosomes. **B.** GISH pattern of a rye cultivar using labeled gDNA of *Ag. intermedium* as probe only, strong hybridization signal on seven pairs of chromosomes. **C.** AJ-3, using biotin-labeled gDNA of *Ag. intermedium* and digoxigenin-labeled gDNA of *Secale cereal* as probe, and gDNA of CS as block. Arrows indicate a pair of 1BL/1RS (red signal on 1RS), arrowheads indicate a pair of *Ag. intermedium* chromosomes (green signal over entire chromosome). **D.** AJ-4, using labeled gDNA of *Ag. intermedium* as probe and gDNA of CS as block, arrowheads indicate a pair of *Ag. intermedium* chromosomes. **E.** JZ-2, using labeled gDNA of *Ag. intermedium* as probe and gDNA of CS as block, arrows indicate a pair of 1BL/1RS. **F.** JZ-4, using labeled gDNA of *Ag. intermedium* as probe and gDNA of CS as block, no hybridization signal was observed.

hybridization signal was observed in line JZ-4 (Fig.10F).

IBL/IRS were revealed by C-banding in lines ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3, JZ-1, JZ-2, JZ-3, in which one pair of chromosomes showed hybridization signal on the terminus of the short arms. So it is necessary to verify the terminal hybridization signal further. Strong hybridization signal on the seven pairs of chromosomes of rye using biotin-labeled *Ag. intermedium* DNA as a probe implies that terminal hybridization signal probed by *Ag. intermedium* DNA in these lines could be IRS of rye (Fig.10B). Double-color *in situ* hybridization using both biotin-labeled total genomic *Ag. intermedium* DNA and Digoxin-labeled total genomic *S. cereal* DNA as probes blocked with unlabeled genomic wheat DNA were carried out to detect *S. cereal* and *Ag. intermedium* chromatin simultaneously in lines ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3. GISH pattern revealed that one pair of chromosomes presented green hybridization signal over the entire chromosomes and one pair of chromosomes presented red hybridization signal over the short arms detected by Florescein and Rodamin respectively (Fig.10C).

GISH patterns of 12 lines showed that ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3 involved both one pair of *Ag. intermedium* chromosomes and one pair of IBL/IRS; AJ-4, AJ-5, AJ-6 involved only one pair of *Ag. intermedium* chromosomes; JZ-1, JZ-2, JZ-3 involved only one pair of IBL/IRS; and JZ-4 involved neither IBL/IRS nor *Ag. intermedium* chromosome.

The identical pattern of the *Ag. intermedium* chromosome in different germplasm lines suggests that the same *Ag. intermedium* chromosome was involved in these lines. GISH pattern of strong hybridization signal on the terminus of the short arm of this chromosome implies that there is a terminal C-band on the short arm. This could be identified out from the wheat background by combining the GISH data with detailed C-banding (Figs. 6, 7). Further identification revealed that this *Ag. intermedium* chromosome substituted the 2D chromosome of wheat. Since lines involved the *Ag. intermedium* chromosome are fertile and stable for a long time, the *Ag. intermedium* chromosome may well compensate for wheat chromosome 2D. The good compensation of the *Ag. intermedium* chromosome for wheat chromosome 2D implies that the alien chromosome is homoeologous to the group 2. Thus, the *Ag. intermedium* chromosome was designated as 2Ai.

2.2 Rye genome-specific SCAR analysis

AF₁ and AF₄ are rye genome-specific SCAR primers. No amplification occurs from the wheat (lacking IBL/IRS) template when AF₁ and AF₄ are used as primers, but clearly a single 1.5 kb band can be amplified whenever the tem-

plate DNA including rye chromatin. Thus, it is very efficient to screen the carriers of IRS (Francis *et al.*, 1995). The results of rye genome-specific SCAR analysis in these germplasm lines showed that except AJ-4, AJ-5, AJ-6, and JZ-4, all other eight lines could detect the specific amplification product of rye (Fig.11). The IRS revealed by SCAR in these lines is in agreement with the result in cytology.

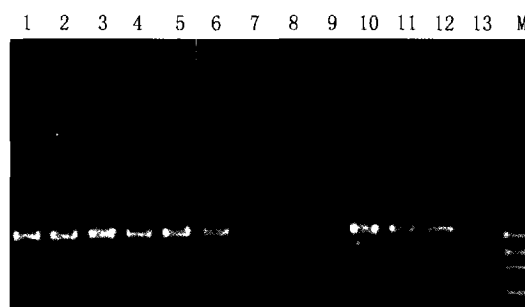


Fig.11. Rye genome-specific SCAR profiles using AF₁ and AF₄ as primers. 1, rye; 2, ZJ-1; 3, ZJ-2; 4, AJ-1; 5, AJ-2; 6, AJ-3; 7, AJ-4; 8, AJ-5; 9, AJ-6; 10, JZ-1; 11, JZ-2; 12, JZ-3; 13, JZ-4; M, marker.

2.3 RAPD analysis

Most RAPD products are repetitive sequences (Zhang *et al.*, 1998), and repetitive sequences usually distribute all over the genome. So using RAPD primers which amplified specific band of *Ag. intermedium* by other researchers could be helpful to find RAPD marker about 2Ai in giant spike wheat germplasm lines. OPR-16, OPH-09, OPA-07, OPA-08, OPA-09 and OPA-13 primers, which tagged *Ag. intermedium* chromatin (Zhang *et al.*, 2000; Tang *et al.*, 2000), were screened in these germplasm lines. Among these primers, OPR16 could repeatedly amplify the specific band presented in ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3, AJ-4, AJ-5, AJ-6 carrying 2Ai but absent in JZ-1, JZ-2, JZ-3, JZ-4 carrying no 2Ai (Fig.12).

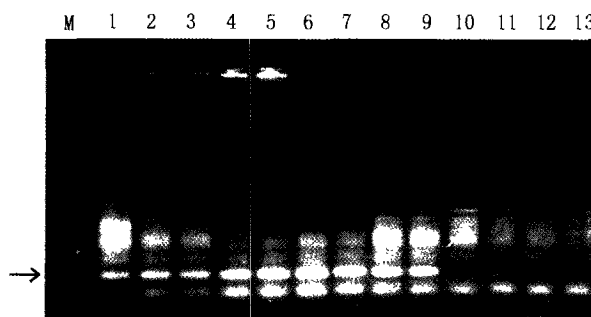


Fig.12. RAPD profiles generated from the primer OPR-16. 1, Zaisheng No.1; 2, ZJ-1; 3, ZJ-2; 4, AJ-1; 5, AJ-2; 6, AJ-3; 7, AJ-4; 8, AJ-5; 9, AJ-6; 10, JZ-1; 11, JZ-2; 12, JZ-3; 13, JZ-4. M, marker. Arrow indicates 2Ai specific band.

Table 2 Chromosome constitution of alien chromatin in the lines of giant spike wheat germplasm

Lines		ZJ-1	ZJ-2	AJ-1	AJ-2	AJ-3	AJ-4	AJ-5	AJ-6	JZ-1	JZ-2	JZ-3	JZ-4
1BL/1RS	C-banding	+	+	+	+	+	-	-	-	+	+	+	-
	GISH	+	+	+	+	+	-	-	-	+	+	+	-
	SCAR	+	+	+	+	+	-	-	-	+	+	+	-
2Ai	C-banding	+	+	+	+	+	+	+	+	-	-	-	-
	GISH	+	+	+	+	+	+	+	+	-	-	-	-
	RAPD	+	+	+	+	+	+	+	+	-	-	-	-

+, present; -, absent.

Cytological and molecular identification showed chromosome constitutions of twelve giant spike wheat lines, five of which carry both 1BL/1RS and 2Ai, three of which carry 2Ai only, three of which carry 1BL/1RS only, and one of which carry neither 1BL/1RS nor 2Ai. The results are summarized in Table 2.

3 Discussion

Eight of 12 giant spike germplasm lines carried 1BL/1RS. 1BL/1RS was existed high frequently in giant spike germplasm (Dou and Xie, 1999). Further cytological observation showed that varieties such as Plateau 175, Plateau 158, and Plateau 363 derived from giant spike germplasm carried 1BL/1RS (unpublished data). High frequent 1BL/1RS occurrence in these materials suggests that 1RS carries genes to control giant spike character. 1BL/1RS, involved in contribution to spike character, was reported previously. Zheng *et al.* (1992) found that the spike length of normal multispikelet line "10-A" with rather long spike was controlled by six pairs of genes, among which two major effect genes were located on the chromosomes of 1B and 2D. Analyzed by Fluorescence *in situ* hybridization (FISH) and RFLP markers, "10-A" was identified as a wheat-rye 1BL/1RS translocation line (Wei *et al.*, 1999).

In giant spike germplasm lines ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3, AJ-4, AJ-5, AJ-6, spike morphological variation including ramified spike, four-rowed spike, and long spike often high frequently occurred, and can be easily observed by morphology. Klindworth *et al.* (1990a; 1990b) reported that there are genes controlling ramified spike and four-rowed spike on the chromosomes 2A and 2B, and an inhibitor of spike morphological variation on the chromosome 2D. Chen *et al.* (1990) observed spike variation of ramified spike and long spike in nullsomic 2D-tetrasomic 2B derived from the progenies of wheat-*Haynaldia villosa*. In this study, we observed spike morphological variation including ramified spike, four-rowed spike and long spike in lines ZJ-1, ZJ-2, AJ-1, AJ-2, AJ-3, AJ-4, AJ-5, and AJ-6 in which a pair of chromosome 2Ai substituted a pair of chromosome 2D. This result is coincident with the findings of Klindworth

et al. (1990a; 1990b) and Chen *et al.* (1990). It suggested that there is gene promoting spike growth on chromosome 2Ai.

Line JZ-4 including neither 1BL/1RS translocation chromosome nor chromosome 2Ai also showed giant spike character (Figs. 4, 9 and 10F). It implied that there are other genes controlling the spike morphology. In common wheat, exploring and pyramiding the genes of spike morphology should be very useful to develop giant spike and increase the wheat yield (Fig. 9).

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(Managing editor: ZHAO Li-Hui)

巨穗小麦种质中外源遗传物质的细胞遗传学和分子生物学鉴定

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摘要: 利用C分带、基因组原位杂交并结合分子生物学手段, 对12份巨穗小麦种质材料中的外源遗传物质进行了检测。结果表明, 12份材料染色体数均为42, 其中5份材料均具有一对小麦-黑麦(*Triticum aestivum*-*Secale cereal*) 1BL/1RS易位染色体和一对中间偃麦草(*Agropyron intermedium* Garten)染色体、3份材料只具有一对中间偃麦草染色体、3份材料只具有一对1BL/1RS染色体、1份材料无1BL/1RS和中间偃麦草染色体。进一步细胞学分析表明, 此中间偃麦草染色体代换了普通小麦(*Triticum aestivum* L.)中的2D染色体, 因其良好的同源补偿性, 表示为2Ai。同时对2Ai在巨穗小麦种质中存在的遗传学意义及小麦遗传改良中的应用进行了讨论。

关键词: 巨穗小麦种质; 1BL/1RS; 中间偃麦草; C-分带; 基因组原位杂交; 特异序列扩增区域; 随机扩增多态DNA

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