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Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis

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Abstract Hybrids derived from wheat (*Triticum aestivum* L.) × rye (*Secale cereale* L.) have been widely studied because of their important roles in wheat cultivar improvement. Repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 are usually used as probes in fluorescence in situ hybridization (FISH) analysis of wheat, rye, and hybrids derived from wheat × rye. Usually, some of these repetitive sequences for FISH analysis were needed to be amplified from a bacterial plasmid, extracted from bacterial cells, and labeled by nick translation. Therefore, the conventional procedure of probe preparation using these repetitive sequences is time-consuming and labor-intensive. In this study, some appropriate oligonucleotide probes have been developed which can replace the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 in FISH analysis of wheat, rye, and hybrids derived from wheat × rye. These oligonucleotides can be synthesized easily and cheaply. Therefore, FISH analysis of wheat and hybrids derived from wheat × rye using these oligonucleotide probes becomes easier and more economical.

Keywords Chromosome identification · FISH · Oligonucleotide probe · Rye · Wheat

Allopolyploids or hybrids derived from wheat (*Triticum aestivum* L.) × rye (*Secale cereale* L.), especially wheat–rye 1BL.1RS translocation lines, have been widely studied because of their important roles in wheat cultivar improvement (Badaeva et al. 1986; Ko et al. 2002; Ma et al. 2004; Tang et al. 2008, 2009; Ren et al. 2009; Fu et al. 2010; Hao et al. 2013). Fluorescence in situ hybridization (FISH) is widely used to detect alterations of wheat and rye chromosomes in wheat–rye hybrids (Alkhimova et al. 1999; Fu et al. 2013a, b, c). FISH analysis is also used to characterize B chromosomes and minichromosomes in plants (Jones et al. 2008). Repetitive sequences pAs1, pSc119.2, and pTa71 are usually used as probes in FISH analysis to distinguish wheat A-, B-, and D-genome chromosomes and rye chromosomes (Cuadrado et al. 1997; Pedersen and Langridge 1997; Ribeiro-Carvalho et al. 2001; Cuadrado and Jouve 2002; Schneider et al. 2003; Schwarzacher 2003; Contento et al. 2005; Sepsi et al. 2008; Fu et al. 2013a, b; Fradkin et al. 2013; Hao et al. 2013). pTa71 was also used in research on the morphology and organization of chromosomes of *Luzula elegans* (Heckmann et al. 2013). Recently, some new tandemly repeated sequences, such as pTa-535, pTa-713, and pTa-86, were also obtained and used as FISH probes to identify wheat A-, B-, and D-genome chromosomes (Komuro et al. 2013). In addition, cereal centromere-specific repetitive sequence CCS1 (Aragón-Alcaide et al. 1996) and rye centromere-specific repetitive sequence pAWRC.1 (Francki 2001) were obtained. These

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centromeric repetitive sequences were used to investigate the neocentric activity of 5RL chromosome in wheat (Manzanero et al. 2000, 2002), the centromeric structure of wheat–rye 1BL.1RS translocation lines (Francki et al. 2002; Tang et al. 2009), and the meiotic behavior of chromosomes in wheat–rye hybrids (Lukaszewski 2008; Valenzuela et al. 2013). In these previous studies, these centromeric repetitive sequences for FISH analysis usually needed to be amplified from a bacterial plasmid, extracted from bacterial cells, and labeled by nick translation. Therefore, the procedure of the preparation of these probes is time-consuming. Synthetic oligonucleotides carrying a fluorescent label can also be used as a probe for FISH analysis (Cuadrado and Schwarzacher 1998; Cuadrado and Jouve 2002). It is convenient to use this kind of probe for FISH analysis because oligonucleotides labeled with fluorochrome can be purchased directly from commercial sources. Additionally, FISH probes can be generated by polymerase chain reaction (PCR) and it is also convenient (Ijdo et al. 1991; Houben et al. 1996). FISH probes pTa794, pSc119.2, and pAs1 can also be labeled by the method of PCR (Molnár-Láng et al. 2010; Molnár et al. 2011; Kwiatek et al. 2013). The appropriate pools of pTa-535, pTa71, CCS1, and pAWRC.1 for PCR amplification have not been developed. In fact, oligonucleotides, which were developed from pAs1 and pTa71, have already been used to distinguish wheat chromosomes (Danilova et al. 2012). However, the oligonucleotides that can replace the roles of pSc119.2, pTa535, CCS1, and pAWRC.1 have not been reported.

In the present study, the oligonucleotides that can replace the roles of repetitive sequences pAs1, pSc119.2, pTa71, pTa535, CCS1, and pAWRC.1 to distinguish wheat and rye chromosomes, and to investigate centromeric structure, were developed. These oligonucleotides were developed according to the repeat sequences available in public databases (Table 1). Oligonucleotide probes were synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China). The synthetic oligonucleotides were 5′ end-labelled with 6-carboxyfluorescein (6-FAM) or 6-carboxytetramethylrhodamine (Tamra) (Table 1). Common wheat *T. aestivum* L. Mianyang11, *T. aestivum* L. Chinese Spring, and octoploid triticales from crossing between Mianyang11 and

Table 1 Oligonucleotide probes for fluorescence in situ hybridization (FISH) analysis

Name of probe	Sequence and fluorochrome label	Amount applied to FISH analysis (ng/slide)	Sequence used to develop probes (GenBank accession no.)
Oligo-pAs1-1	Tamra-5′CCTTT CTGAC TTCAT TTGTT ATTTT TCATG CATTT ACTAA TTATT TTGAG CTATA AGAC3′	5.9	*Aegilops squarrosa* repetitive DNA sequence (D30736.1)
Oligo-pAs1-2	Tamra-5′CATTT CATCC ACATA GCATG TGCAA GAAAT TTGAG AGGGT TACGG CAAAA ACTGG AT3′	5.4	*Aegilops squarrosa* repetitive DNA sequence (D30736.1)
Oligo-pSc119.2-1	6-FAM-5′CCGTT TTGTG GACTA TTACT CACCG CTTTG GGGTC CCATA GCTAT3′	6.1	*Secale cereal* tandem repeat sequence (KF719093)
Oligo-pSc119.2-2	6-FAM-5′TTCCA CGATT GACGA TTCCG GGGGT GCGTT TACGT GTCCG TCGTC3′	6.2	*Secale cereal* tandem repeat sequence (KF719093)
Oligo-pTa71-2	Tamra-5′GGGCA AAACC ACGTA CGTGG CACAC GCCGC GTA3′	5.8	Wheat rDNA 25S-18S intergenic region EcoRI-BamHI fragment (X07841.1)
Oligo-pTa535-1	Tamra-5′AAAAA CTTGA CGCAC GTCAC GTACA AATTG GACAA ACTCT TTCGG AGTAT CAGGG TTTC3′	5.6	*Triticum aestivum* clone pTa-535 FISH-positive repetitive sequence (KC290894.1)
Oligo-pTa535-2	Tamra-5′GACGA GAACT CATCT GTTAC ATGGG CACTT CAATG TTTTT TAAAC TTATT TGAAC TCCA3′	5.7	*Triticum aestivum* clone pTa-535 FISH-positive repetitive sequence (KC290894.1)
Oligo-pAWRC.1	Tamra -5′ CGTAG GCGCC GATCT TGAAA GAGAC TTGCA CGGTG TGCTC GACTC GAAGA ATTCC GGCGT 3′	58	Rye centromeric repeat sequence (AF245032)
Oligo-CCS1	6-FAM-5′CCGTT TGATA GAGGC AAAGG TGTCC CGTCT TTTGA TGAGA3′	58	*Brachypodium sylvaticum* stem-loop sequence Hi-10 region (U52217)

S. cereale L. Kustro were used to test these oligonucleotide probes. These synthesized probes were diluted by using 1×TE solution and the amount applied to each slide is listed in Table 1. In addition, the genomic DNA of rye Kustro was also used as a probe for genomic in situ hybridization (GISH) analysis and was labeled with Texas Red-5-dUTP (Invitrogen) or Alexa Fluor 488-5-dUTP (Invitrogen). Probe labeling and in situ hybridization were operated according to Han et al. (2006). The chromosome spreads of materials were also prepared through the methods described by Han et al. (2006).

Oligo-pAs1-1 and Oligo-pAs1-2 mainly hybridized to D-genome chromosomes of common wheat Mianyang11 and Chinese Spring (Fig. 1a, b, e, f). The Oligo-pAs1-1 and Oligo-pAs1-2 signals to D-genome chromosomes are agreement with the pAs1 signals to D-genome chromosomes (Schneider et al. 2003). Oligo-pAs1-1 and Oligo-pAs1-2 also gave apparent signals to 1A, 2A, 3A, 4A,

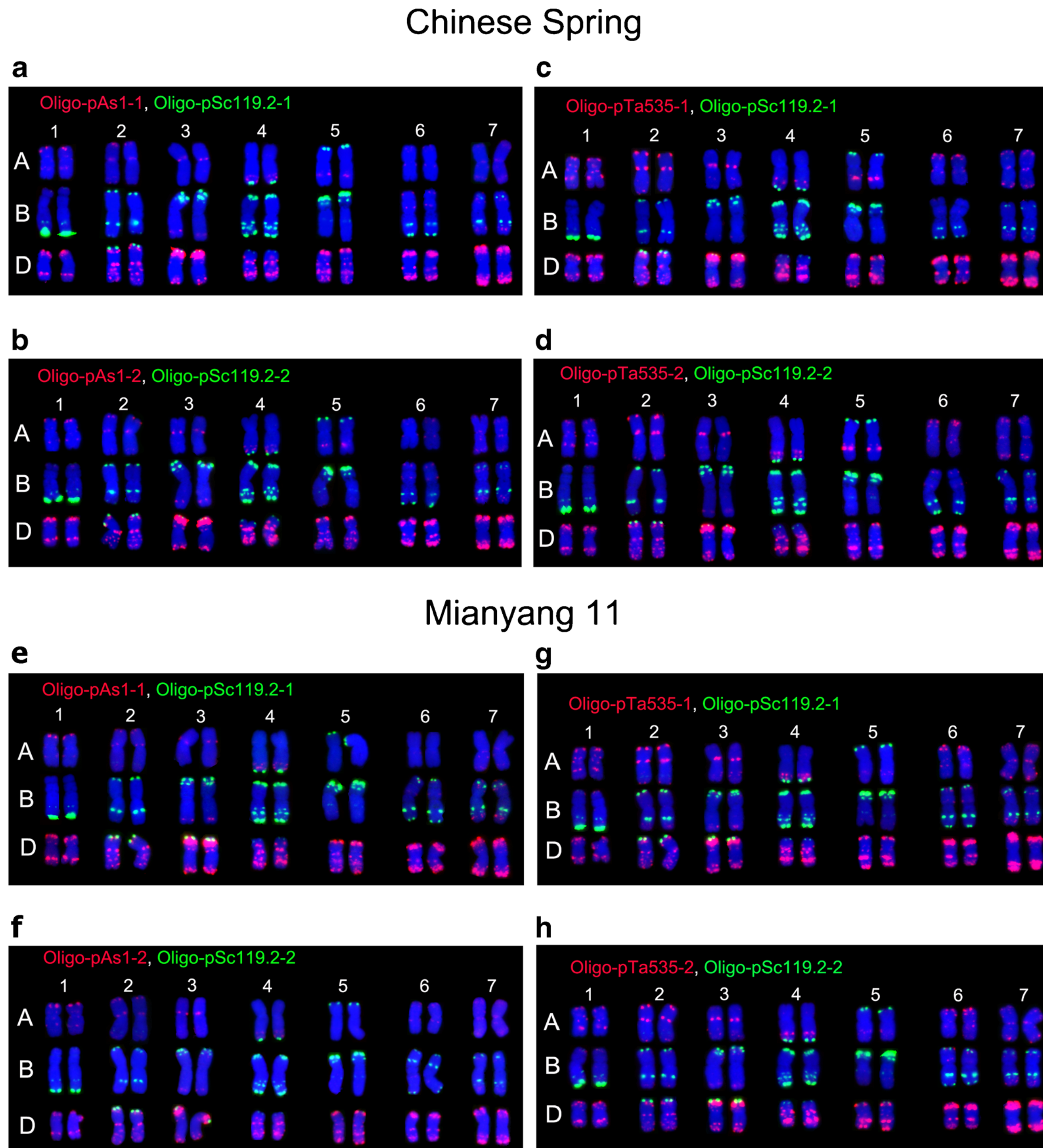

Fig. 1 Fluorescence in situ hybridization (FISH) analysis using Oligo-pAs1-1 (*red*), Oligo-pAs1-2 (*red*), Oligo-pTa535-1 (*red*), Oligo-pTa535-2 (*red*), Oligo-pSc119.2-1 (*green*), and Oligo-pSc119.2-2 (*green*) as probes on root tip metaphase chromosomes of Chinese Spring (**a**, **b**, **c**, **d**) and Mianyang11 (**e**, **f**, **g**, **h**). Chromosomes were counterstained with DAPI (*blue*)

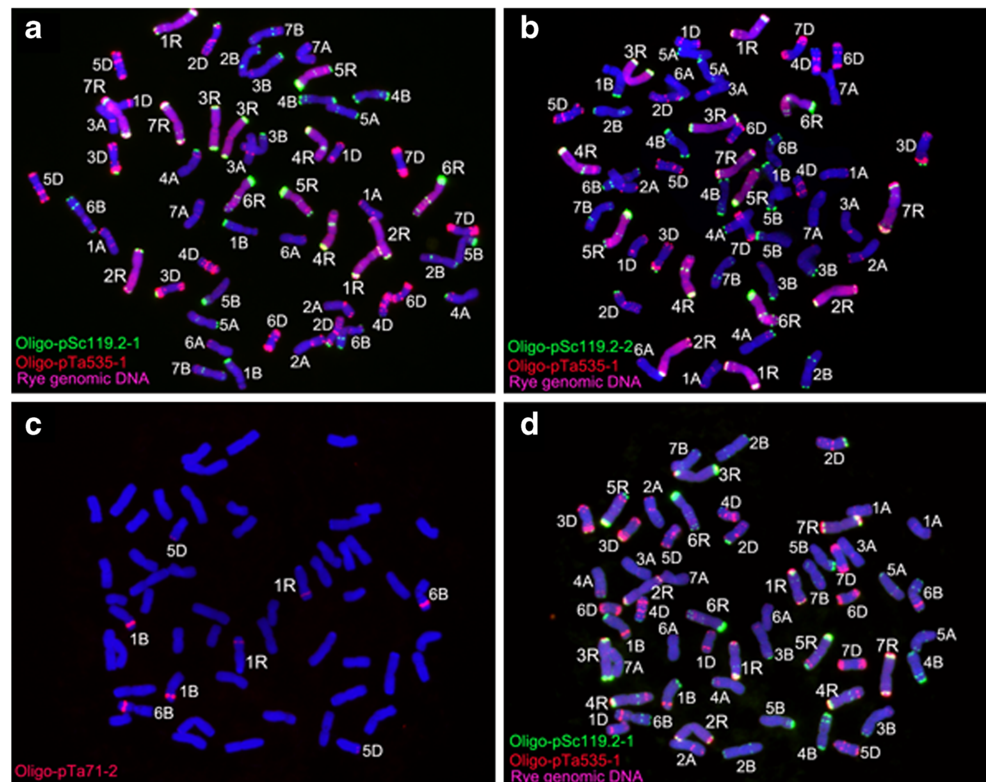

Fig. 2 **a** FISH and genomic in situ hybridization (GISH) analyses using Oligo-pSc119.2-1 (*green*), Oligo-pTa535-1 (*red*), and rye genomic DNA (*red*) as probes on root tip metaphase chromosomes of triticale. **b** FISH and GISH analyses using Oligo-pSc119.2-2 (*green*), Oligo-pTa535-1 (*red*), and rye genomic DNA (*red*) as probes on root tip metaphase chromosomes of triticale. **c** FISH analysis using Oligo-pTa71-2 as a probe on root tip metaphase chromosomes of triticale. **d** FISH and GISH analyses using Oligo-pSc119.2-1 (*green*), Oligo-pTa535-1 (*red*), and rye genomic DNA (*red*) as probes on the same cell as in **c**. Each rye chromosome can be discriminated by Oligo-pSc119.2-1 or Oligo-pSc119.2-2 signals. Chromosomes were counterstained with DAPI (*blue*)

6A, 7A, 3B, 6B, and 7B chromosomes of Mianyang11 and Chinese Spring (Fig. 1a, b, e, f). In addition, Oligo-pAs1-1 and Oligo-pAs1-2 can produce signals on 5AL arms of Chinese Spring (Fig. 1a, b). The FISH signal patterns of Oligo-pTa535-1 and Oligo-pTa535-2 on wheat chromosomes are similar to those of probes Oligo-pAs1-1 and Oligo-pAs1-2 (Fig. 1). The FISH signals on A-genome chromosomes produced by Oligo-pTa535-1 and Oligo-pTa535-2 are stronger than those generated by Oligo-pAs1-1 and Oligo-pAs1-2 (Fig. 1). Oligo-pSc119.2-1 and Oligo-pSc119.2-2 have the same signal pattern and they especially hybridize to wheat B-genome chromosomes and rye chromosomes (Figs. 1 and 2a, b, d). The Oligo-pSc119.2-1 and Oligo-pSc119.2-2

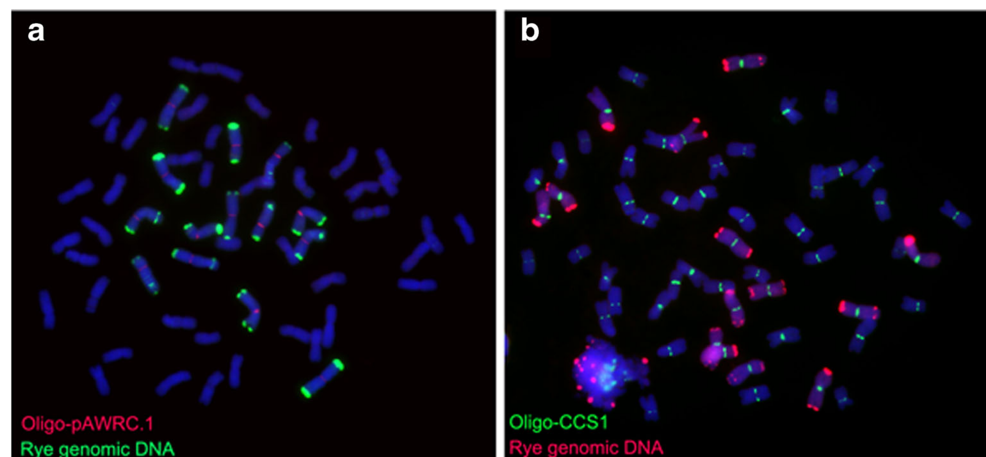

Fig. 3 **a** FISH and GISH analyses using Oligo-pAWRC.1 (*red*) and rye genomic DNA (*green*) as probes on root tip metaphase chromosomes of triticale. **b** FISH and GISH analyses using Oligo-CCS1 (*green*) and rye genomic DNA (*red*) as probes on root tip metaphase chromosomes of triticale. Chromosomes were counterstained with DAPI (*blue*)

Table 2 Costing of synthetic oligonucleotide probes used in this study

Name of probe	Amount of synthetic probe (μg)	Costing of synthetic probe ($)[a]	Number of slides that can be detected using the synthetic probe (piece)
Oligo-pAs1-1	147.95	107.82	25,000.00
Oligo-pAs1-2	137.3	107.34	25,000.00
Oligo-pSc119.2-1	154.5	67.34	25,000.00
Oligo-pSc119.2-2	156.05	67.34	25,000.00
Oligo-pTa71-2	147.05	101.53	25,000.00
Oligo-pTa535-1	140.35	107.82	25,000.00
Oligo-pTa535-2	143.55	107.82	25,000.00
Oligo-pAWRC.1	145.90	107.82	2,500.00
Oligo-CCS1	145.90	66.13	2,500.00

[a] The costing is valued at present market prices in China

signals are in agreement with the pSc119.2 signals to B-genome chromosomes and rye chromosomes (Schneider et al. 2003; Contento et al. 2005). Oligo-pSc119.2-1 and Oligo-pSc119.2-2 also generated obvious signals on 4A, 5A, 2D, 3D, and 4D chromosomes of common wheat (Figs. 1 and 2a, b, d). Oligo-pSc119.2-1 or Oligo-pSc119.2-2 combined with Oligo-pAs1-1, Oligo-pAs1-2, Oligo-pTa535-1, or Oligo-pTa535-2 can successfully discriminate the whole set of 42 common wheat chromosomes (Figs. 1 and 2a, b, d).

Oligo-pTa71-2 can produce strong signals on wheat 1B, 6B, and rye 1R chromosomes, and weak signals on wheat 5D chromosomes (Fig. 2c, d). Therefore, Oligo-pTa71-2 can replace the role of pTa71. Oligo-pAWRC.1 produces clear signals at centromeres of just rye chromosomes (Fig. 3a) and Oligo-CCS1 can generate clear signals at centromeres of both rye and wheat chromosomes (Fig. 3b). Therefore, Oligo-pAWRC.1 and Oligo-CCS1 can replace the roles of pAWRC.1 and CCS1 to investigate the centromeric structure of wheat and rye chromosomes.

Although some synthetic oligonucleotides such as (AAG)n, (AAC)n, and (GACA)n, which were end-labeled with biotin-11-dUTP, have been used to distinguish wheat A-, B-, and D-genome chromosomes and rye chromosomes (Cuadrado and Schwarzacher 1998), repetitive sequences pAs1, pSc119.2, and pTa71 are still widely used. The newly discovered repetitive sequence pTa-535 can also be used as a probe to distinguish wheat chromosomes (Komuro et al. 2013). In previous studies, the procedures of the preparation and labeling of some of these repetitive sequences were time-consuming and labor-intensive. To use oligonucleotides as probes for FISH analysis is relatively convenient. Although FISH probes can be prepared by PCR amplification (Ijdo et al. 1991; Molnár-Láng et al. 2010), the appropriate pools of pTa-535, pTa71, CCS1, and pAWRC.1 for PCR amplification have not been developed. Therefore, in this case, it is convenient to use synthetic oligonucleotide probes for FISH analysis because oligonucleotides labeled with fluorochrome can be purchased directly from commercial sources. The oligonucleotides developed in this study provide an easy path for FISH analysis of wheat and rye. Furthermore, synthesized oligonucleotide probes are cheap (Table 2).

The oligonucleotides developed from pAs1 and pTa71 in this study are different from those reported by Danilova et al. (2012) and the oligonucleotides in the present study offer further options for researchers. It is better to use Oligo-pTa535-1 and Oligo-pTa535-2 to distinguish wheat A-genome chromosomes because their signals were stronger and clearer than those of Oligo-pAs1-1 and Oligo-pAs1-2 on A-genome chromosomes (Fig. 1).

In conclusion, Oligo-pAs1-1, Oligo-pAs1-2, Oligo-pTa535-1, Oligo-pTa535-2, Oligo-pSc119.2-1, Oligo-pSc119.2-2, Oligo-pTa71-2, Oligo-pAWRC.1, and Oligo-CCS1 can replace the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. Additionally, Oligo-pSc119.2-1 and Oligo-pSc119.2-2 display the structural variation of 5A chromosomes because their signals appeared on 5AL arms in triticales but did not appear on 5AL arms of parental wheat Mianyang11 (Figs. 1e and 2a, b, d).

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