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Rapid Evolution of Simple Sequence Repeat Induced by Allopolyploidization

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Abstract Microsatellite evolution normally occurs in diploids. Until now, there has been a lack of direct experimental evidence for microsatellite evolution following allopolyploidization. In the present study, F_1 hybrids and newly synthesized allopolyploids were derived from *Triticum aestivum* Chinese Spring × *Secale cereale* Jinzhou-heimai. One hundred and sixty-three wheat simple sequence repeat (SSR) markers were used to investigate the variation of wheat microsatellites after allopolyploidization and variation of the PCR products of 29 of the SSR markers was observed. Of these 29 SSR markers, 15 were unable to produce products from amphiploids. The other 14 SSR markers did produce products from parental wheat, F_1 hybrids and amphiploids. However, the length of the products amplified from amphiploids was different from the length of the products amplified from parental wheat and F_1 hybrids. Sequencing indicated that the length variation of the 14 microsatellites stemmed mainly from variation in the number of repeat units. The alteration of repeat units occurred in both perfect and compound repeats. In some compound SSR loci, one motif was observed to expand whereas another to contract. Almost all the microsatellite evolution observed in this study could be explained by the slipped-strand mispairing model. The results of this study seem to indicate that stress caused by allopolyploidization might be one of the factors that induce microsatellite evolution. In addition, the findings of present study provided an instance of how simple sequence repeats evolved after allopolyploidization.

Keywords Amphiploid · Allopolyploidization · Microsatellite · Evolution · Wheat · Rye

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Introduction

Polyploidization appears to be a significant cause of speciation in the plant kingdom. It has been estimated that at least 50%, and perhaps more than 70%, of angiosperms have experienced polyploidization in their evolutionary history (Masterson 1994; Wendel 2000). Many species of plants, which have been traditionally considered as diploid, have proven to be ancient allopolyploids (Shoemaker et al. 1996; Gaut and Doebley 1997; Gómez et al. 1998; Muravenko et al. 1998; Vision et al. 2000). From these reports, it can be concluded that polyploidization plays an important role in plant evolution. Revolutionary changes and evolutionary changes are two ways by which allopolyploidy advances genome evolution in wheat (Feldman and Levy 2005). A number of recent reports have documented genetic and epigenetic instability in newly synthesized allopolyploids (Madlung et al. 2002; Kashkush et al. 2002). It has been reported that allopolyploidization is attended by a nonrandom loss of specific, low-copy, probably noncoding DNA sequences and repetitive sequences at the early stages of allopolyploidization (Feldman et al. 1997; Shaked et al. 2001; Ma et al. 2004; Ma and Gustafson 2006). In allopolyploids, genomic sequence elimination and chromosome rearrangement are probably the major driving

forces for diploidization, and epigenetic modifications are most likely leading factors in genetic diploidization (Ma and Gustafson 2005). However, there is a lack of direct experimental evidence that diploid-like evolutionary processes are accelerated as a result of allopolyploidy. Feldman and Levy (2005) suggested that it might be interesting to investigate whether evolutionary processes that normally occur in diploids, such as microsatellite expansion, insertions, and point mutations, occur at a faster rate in allopolyploids compared to their diploid progenitors.

A microsatellite is a tandemly repeated DNA motif of 1–6 bp in length. It is also referred to as simple sequence repeat (SSR). These sequences are found throughout many genomes, and are highly polymorphic, even among closely related plant varieties (Beckmann and Soller 1990; Morgante and Olivieri 1993; Toth et al. 2000). Microsatellites are important tools for understanding evolution (Levinson et al. 1985; Harding et al. 1992; Primmer and Ellegren 1998; Makova et al. 2000; Barrier et al. 2000; Zhu et al. 2000; Zhivotovsky et al. 2003; Gáspári et al. 2007). Previous studies of microsatellite evolution have focused mainly on the relationship between microsatellite mutation and the number of repeat units (Zhu et al. 2000; Innan et al. 1997; Vigouroux et al. 2002; Lai and Sun 2003; Dettman and Taylor 2004; Azaiez et al. 2006), the patterns and mechanisms of microsatellite evolution (Levinson and Gutman 1987; Harding et al. 1992; Primmer and Ellegren 1998; Innan et al. 1997; Di Rienzo et al. 1994; Taylor and Breden 2000; López-Giráldez et al. 2007), the influence of the base composition on microsatellite variability (Bachtrog et al. 2000), the mutation rate of microsatellites (Wong and Weber 1993; Beck et al. 2003; Thuillet et al. 2005), the distribution of microsatellites in genomes (Kubik et al. 1999; Cardle et al. 2000; Katti et al. 2001; Karaoglu et al. 2005), and the polymorphism of microsatellites (Akkaya et al.1992). Information on the dynamics of microsatellite evolution has come mainly from studies within species or comparisons of closely related species. Previous reports have, for the most part, examined the historical mutation events of microsatellites. It is unclear whether speciation is accompanied by immediate microsatellite evolution and what kind of evolutionary pressures will accelerate microsatellite evolution. Hybridization is one of the evolutionary pressures (Madlung and Comai 2004). Allopolyploids are derived from interspecific or intergeneric hybridizations. Allopolyploidization is an important process through which new species may theoretically arise quickly. Hence, allopolyploids, especially new synthetic allopolyploids, are useful materials for studying microsatellite evolution. Until now, there has been a lack of direct experimental evidence for microsatellite evolution as a result of allopolyploidization.

In this study, we crossed *Triticum aestivum* Chinese Spring and *Secale cereale* Jinzhou-heimai, and produced several F_1 hybrids and first generation allopolyploids (amphiploids). Changes of microsatellites in these progeny derived from Chinese Spring × Jinzhou-heimai were investigated using wheat SSR markers. Microsatellite evolution following allopolyploidization was observed.

Materials and Methods

Plant Materials

The parental wheat plant was Chinese Spring (*Triticum aestivum* L.) and the parental rye plant was Jinzhou-heimai (*Secale cereale* L.). The F_1 hybrids were derived from Chinese Spring (genome AABBDD) × Jinzhou-heimai (Chinese rye;genome RR) and the synthetic amphiploids were obtained by treating young seedlings of F_1 hybrids with 0.05% colchicine. Four F_1 hybrids and four amphiploids were used for studying. The four F_1 plants were named CJF_1-1, CJF_1-2, CJF_1-3, and CJF_1-4; and the four amphiploids were named CJS_1-1, CJS_1-2, CJS_1-3, and CJS_1-4. CJS_1-1, CJS_1-2, CJS_1-3, and CJS_1-4 were derived from the respective F_1 hybrids. All the parental plants were maintained by strict selfing. The parental rye plant was inbred for 10 generations. The parental wheat plant was selfed for 15 generations to maximize homozygosity.

Genomic In Situ Hybridization

Genomic in situ hybridization (GISH) analysis was used to identify CJS_1-1, CJS_1-2, CJS_1-3, and CJS_1-4 as amphiploids. The total genomic DNA from *S. cereale* Jinzhou-heimai was labeled with digoxigenin-11-dUTP according to the manufacturer's instruction (Roche). A 15-μl hybridization mixture, consisting of 20-ng probe DNA, 0.5 μg of sheared wheat cv. Chinese Spring genomic DNA as blocker, 10-μg sheared salmon sperm DNA, 50% formamide, 2× SSC, 10% dextran sulfate, was denatured at 80°C for 8 min, chilled on ice for 5 min, and added to each slide. For hybridization, slides were placed in a moist box at 37°C overnight. A post-hybridization wash was carried out with 2× SSC (including 2% Tween-20) at 25°C (5 min), 42°C (10 min), 25°C (5 min), respectively, and 1× phosphate-buffered saline (PBS) at room temperature (5 min). Fifty microliters of fluorescein-conjugated anti-digoxigenin antibody (1:100 dilutions) were applied to each slide. After incubation for 1 h at 37°C, the slides were washed 3–4 times in 1× PBS at room temperature (5-min each), and then rinsed briefly with deionized water and quickly dried using a puffer. A thin layer of antifade solution containing 1-μg/ml propidium iodide (PI) was added to each slide. Photographs were taken with a cooled CCD camera system (DP70) on an Olympus BX-51

fluorescence microscope. At least 25 metaphase cells were examined for each seed.

DNA Extraction

Genomic DNA of newly synthesized amphiploids, F_1 hybrids (before treatment with colchicine) and their exact parents (the individual pollen donor plant and the individual recipient plant) were extracted according to the method described by Zhang et al. (1995).

PCR Amplification and Sequence Cloning

One hundred and sixty-three wheat SSR markers (Röder et al. 1998) were screened for amplification in the newly synthesized amphiploids, F_1 hybrids, and their exact parental plants. These SSR markers were listed in Table 1. The PCR reaction mixture (25-μl total) consisted of 50-mM KCl and 10-mM Tris–HCl (pH 8.8), 1.5-mM $MgCl_2$, 0.2 mM of dNTP, 0.2 mM of each primer, 1.0 unit of *Taq* polymerase (Promega), and 80 ng of genomic DNA. Amplification was carried out in an MJ Research PTC-200 (Programmable Thermal Controller, MJ Research), using a program that consisted of initial denaturation for 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 30 s at annealing temperature, 2 min at 72°C, and final extension for 10 min at 72°C. The annealing temperature of SSR markers was according to Röder et al. (1998). After PCR amplification, 8-μL loading buffer (98% formamide, 2% dextran blue, and 0.25 Mm EDTA) were added to each tube. Samples were denatured at 90°C for 5 min and then immediately placed on ice. For each sample, 4 μL were loaded onto a 6% vertical polyacrylamide gel (60 cm × 30 cm × 0.4 mm), run for 3 h at 60 W, and then scanned using the Genomyx system (Beckman Coulter Corporation, CA). The products amplified by the SSR markers, which displayed length polymorphism among F_1 plants, amphiploids and parental wheat plant were recovered from polyacrylamide gels, reamplified by PCR and sequenced. To accomplish this, the polymorphic bands were isolated from the gel, and the DNA was extracted by boiling in 100 μL of water for 5 min. The eluted DNA was used as template for PCR amplification again using corresponding SSR markers. The second amplification products amplified by these SSR markers were separated in a 2% high-resolution agarose gel (FMC brand, Spain), prepared with 0.5 × TBE (pH 8.0). Gels were stained with ethidium bromide and visualized with a UVP Gel Documentation System (Bio-Rad). The second amplification products were recovered using Gel Extraction Kit (Omega E. Z. N.A. USA), and were cloned into pMD18-T Simple Vector (TaKaRa, Japan). Inserts were sequenced by the commercial company Invitrogen Biotechnology (Shanghai) Co., Ltd. In addition, the products amplified by five SSR markers, X*gwm*44, X*gwm*191, X*gwm*268, X*gwm*340, and X*gwm*666, which are adjacent to some of the SSR markers that displayed variation after allopolyploidization, were also sequenced. At least two separate clones from each individual were sequenced for the products of each SSR markers. The nucleotide sequences were deposited in the GenBank Database. Sequence analysis was performed with the software DNAMAN (Version 4.0).

Table 1 Wheat SSR markers used in analysis of parental wheat, F_1 hybrids and amphiploid

Chromosome	Markers
1A	X*gwm*: 33, 99, 135, 136, 164, 357, 497, 666
2A	X*gwm*: 95, 265, 275, 294, 359, 382, 425, 558, 614, 636
3A	X*gwm*: 2, 5, 30, 155, 162, 369, 391, 480
4A	X*gwm*: 4, 160, 397, 601, 610, 637
5A	X*gwm*: 126, 129, 156, 186, 205, 293, 304, 595
6A	X*gwm*: 169, 334, 427, 459, 494, 570, 617
7A	X*gwm*: 60, 63, 233, 276, 260, 282, 350, 471
1B	X*gwm*: 18, 124, 140, 153, 259, 268, 413, 498, 550
2B	X*gwm*: 47,120, 148, 210, 257, 374, 429, 526, 630
3B	X*gwm*: 77, 108, 131, 181, 264, 376, 299, 340
4B	X*gwm*: 6, 107, 149, 251, 368, 495, 513, 538
5B	X*gwm*: 67, 68, 159, 191, 234, 335, 371, 408, 443, 604
6B	X*gwm*: 70, 132, 133, 193, 219, 361, 508, 644
7B	X*gwm*: 16, 112, 274, 333, 557, 569, 573
1D	X*gwm*: 106, 232, 337, 458, 642
2D	X*gwm*: 157, 261, 296, 301, 311, 320, 349, 484, 539
3D	X*gwm*: 3, 71, 114, 183, 314, 341, 383, 456, 640, 645, 664
4D	X*gwm*: 165, 194, 608, 609, 624
5D	X*gwm*: 121, 174, 182, 192, 269, 271, 272, 358, 639
6D	X*gwm*: 55, 325, 469
7D	X*gwm*: 37, 44, 111, 295, 428, 437, 635

Results

Identification of Amphiploids Using GISH

Seeds (CJS_1-1, CJS_1-2, CJS_1-3, and CJS_1-4) derived from F_1 plants (CJF_1-1, CJF_1-2, CJF_1-3, and CJF_1-4) were used for GISH analysis. The root-tip preparations in which rye chromatin was present were distinguishable by fluorescing signals at interphase and metaphase. Chromosome counts indicated that the chromosome number of each seed was 56. Among the chromosomes of these seeds, 14 exhibited strong hybridization signals (Fig. 1) and were identified as rye chromosomes. The results confirmed that CJS_1-1, CJS_1-2, CJS_1-3, and CJS_1-4 were amphiploids (octoploid triticales).

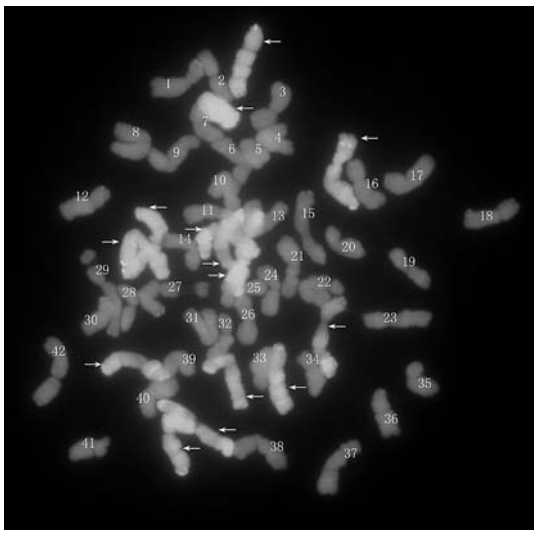

Fig. 1 CJS_1-1, CJS_1-2, CJS_1-3, and CJS_1-4 are confirmed to be amphiploids using GISH analysis. *Arabic numerals* indicate wheat chromosomes. *Arrows* indicate rye chromosomes

Table 2 The length of the abnormal SSR in Chinese Spring, F_1 hybrids, and amphiploids in which each variant was independently observed

SSR	Length in Chinese Spring and F_1 hybrids (bp)	Length in amphiploids (bp)
X*gwm*124	211	189
X*gwm*136	297	245
X*gwm*156	283	266/264
X*gwm*186	136	122
X*gwm*282	220	196
X*gwm*337	185	173
X*gwm*437	105	91
X*gwm*497	167	127
X*gwm*644	163	151
X*gwm*18	184	196
X*gwm*120	135	153
X*gwm*140	214	317
X*gwm*153	180	231
X*gwm*539	139	161

The Variation of Simple Sequence Repeat

The products of 29 of the 163 SSR markers exhibited obvious variation after allopolyploidization. Three types of variation of the products of the 29 SSR markers were observed. First, the length of the products produced by nine SSR markers (X*gwm*124, X*gwm*136, X*gwm*156, X*gwm* 186, X*gwm*282, X*gwm*337, X*gwm*437, X*gwm*497, and X*gwm*644) from the four amphiploids were shorter than that of the products produced from Chinese Spring and the four F_1 hybrids (Fig. 2a). Second, the length of the products produced by five SSR markers (X*gwm*18, X*gwm*120, X*gwm*140, X*gwm*153, and X*gwm*539) from the four amphiploids were longer than that of the products produced from Chinese Spring and the four F_1 hybrids (Fig. 2b). The lengths of these 14 abnormal SSR in Chinese Spring, F_1 hybrids and amphiploids are listed in Table 2. Third, the microsatellite products of 15 SSR markers (X*gwm*33, X*gwm*47, X*gwm*77, X*gwm*106, X*gwm*126, X*gwm*299, X*gwm*260, X*gwm*265, X*gwm*314, X*gwm*359, X*gwm*371, X*gwm*573, X*gwm*595, X*gwm*610, and X*gwm*636) disappeared from the four amphiploids (Fig. 2c). However, the band patterns of Chinese Spring and the four F_1 plants produced by the 29 SSR markers were identical and they were different from those of amphiploids. The band patterns of the amphiploids produced by the 29 SSR markers were identical (Fig. 2). In addition, the other 134 SSR markers produced identical band patterns among the four F_1 hybrids, the four amphiploids and their parental wheat (Fig. 2d).

Furthermore, six SSR markers (X*gwm*219, X*gwm*232, X*gwm*259, X*gwm*268, X*gwm*408, and X*gwm*644) amplified rye-specific bands whose sizes ranged from 469 to 747 bp from the genomic DNA of parental rye (Tang et al. 2008). However, the six SSR markers did not amplify products whose sizes were less than 400 bp from parental

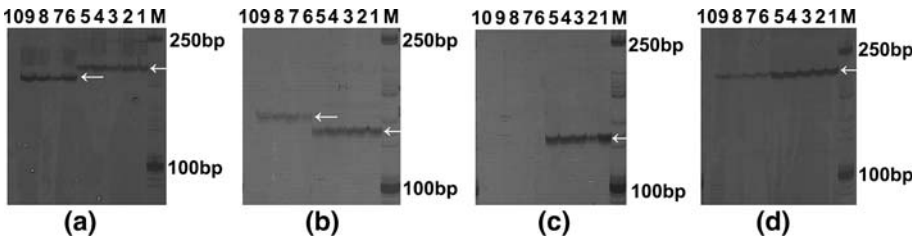

Fig. 2 Length variation of microsatellite after allopolyploidization. **a** Products amplified by X*gwm*124 represent contraction of microsatellite after allopolyploidization. **b** Products amplified by X*gwm*539 represent expansion of microsatellite after allopolyploidization. **c** Products amplified by X*gwm*106 represent disappearance of products in amphiploids. **d** Products amplified by X*gwm*349 represent identical band pattern among amphiploids, F_1 hybrids, and parental wheat plant. *1* Chinese Spring, *2* CJF_1-1, *3* CJF_1-2, *4* CJF_1-3, *5* CJF1-4, *6* CJS_1-1, *7* CJS_1-2, *8* CJS_1-3, *9* CJS_1-4, *10* Jinzhou-heimai, *M* DNA marker. *Arrows* indicate target fragments

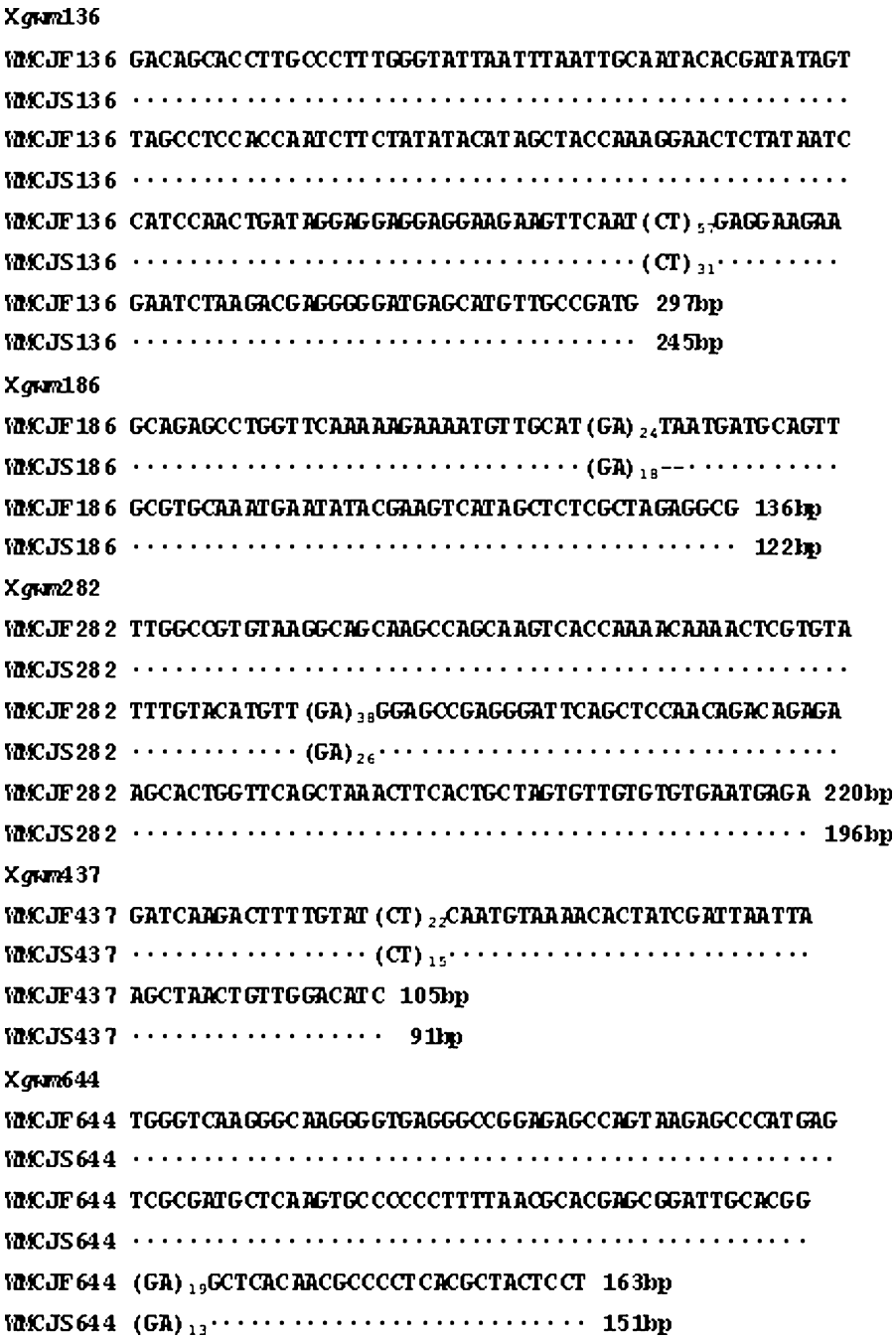

Fig. 3 Comparison of sequences amplified by X*gwm*136, X*gwm*186, X*gwm*282, X*gwm*437, and X*gwm*644 from F_1 plants and amphiploids. *WMCJF* indicates the sequences cloned from F_1 plants. *WMCJS* indicates the sequences cloned from amphiploids. *Dots* (·) show identical nucleotides. *Dashes* (–) show deletion of nucleotides

rye. The other 157 SSR markers (including the 29 SSR markers which have mutational changes) did not amplify products from parental rye (Fig. 2).

Changes in Repeat Composition

The products of the 14 markers which displayed length polymorphism were cloned from F_1 plants, amphiploids, and Chinese Spring for sequencing (GenBank accession numbers: EU294026, EU289296–EU289336, and GQ144325–GQ144330, respectively). For the SSR markers X*gwm*18, X*gwm*120, X*gwm*124, X*gwm*136, X*gwm*140, X*gwm*153, X*gwm*186, X*gwm*282, X*gwm*337, X*gwm*437, X*gwm*497, X*gwm*539, and X*gwm*644, the sequences amplified from the four F_1 plants and Chinese Spring were identical and they were different from those of amphiploids; and the sequences amplified from the four amphiploids were identical (Figs. 3, 4, 5, 6). For the SSR marker X*gwm*156, the sequences amplified from F_1 plants and Chinese Spring were identical and they were different from those of amphiploids, the sequences amplified from CJS_1-4 were identical and they were slightly different from the sequences amplified from

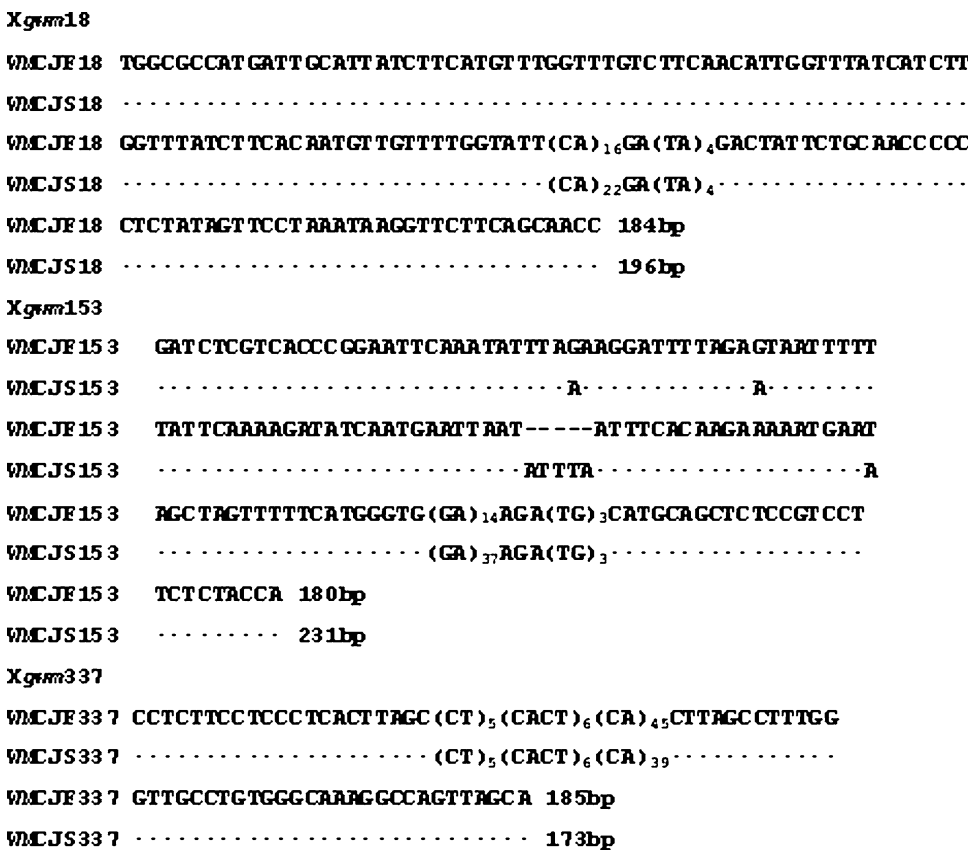

Fig. 4 Comparison of sequences amplified by X*gwm*18, X*gwm*153, and X*gwm*337 from F_1 plants and amphiploids. *WMCJF* indicates the sequences cloned from F_1 plants. *WMCJS* indicates the sequences cloned from amphiploids. *Dots* (·) show identical nucleotides. *Dashes* (–) show deletion of nucleotides

Fig. 5 Comparison of sequences amplified by X*gwm*124, X*gwm*140, and X*gwm*539 from F_1 plants and amphiploids. *WMCJF* indicates the sequences cloned from F_1 plants. *WMCJS* indicates the sequences cloned from amphiploids. *Dots* (·) show identical nucleotides

Fig. 6 Comparison of sequences amplified by X*gwm*120, X*gwm*156, and X*gwm*497 from F_1 plants and amphiploids. *WMCJF* indicates the sequences cloned from F_1 plants. *WMCJS* indicates the sequences cloned from amphiploids. *WMCJS156.4* indicates the sequences cloned from CJS_1-4. *Dots* (·) show identical nucleotides. *Dashes* (–) show deletion of nucleotides

amphiploids CJS_1-1, CJS_1-2, and CJS_1-3, and the sequences amplified from the three amphiploids were identical (Fig. 6). Therefore, the sequence alignment analysis was carried out only between F_1 plants and amphiploids. The variation in number of repeats was observed between sequences from F_1 plants and amphiploids. The variation in PCR product size of X*gwm*18, X*gwm*120, X*gwm*124, X*gwm*136, X*gwm*282, X*gwm*337, X*gwm*437, X*gwm*497, X*gwm*539, and X*gwm*644 stemmed from variation in number of repeats in the microsatellite; however, the flanking sequences of these microsatellite alleles are identical between F_1 plants and amphiploids (Figs. 3, 4, 5, 6). The variation in PCR product size of X*gwm*153, X*gwm*156, X*gwm*140, and X*gwm*186 stemmed mainly from variation in the number of repeats in the microsatellite. The flanking sequences of these microsatellite alleles are slightly different between F_1 plants and amphiploids (Figs. 3, 4, 5, 6).

The sequences amplified by X*gwm*136, X*gwm*186, X*gwm*282, X*gwm*437, and X*gwm*644 from F_1 plants and Chinese Spring contain perfect repeats $(CT)_{57}$, $(GA)_{24}$, $(GA)_{38}$, $(CT)_{22}$, and $(GA)_{19}$, respectively (Fig. 3). However, the sequences amplified by X*gwm*136, X*gwm*186, X*gwm*282, X*gwm*437, and X*gwm*644 from amphiploids contain perfect repeats $(CT)_{31}$, $(GA)_{18}$, $(GA)_{26}$, $(CT)_{15}$, and $(GA)_{13}$, respectively (Fig. 3). The sequences amplified by X*gwm*18, X*gwm*153, and X*gwm*337 from F_1 plants and Chinese Spring contain repeat structures $(CA)_{16}$ GA $(TA)_4$, $(GA)_{14}$ AGA $(TG)_3$ and $(CT)_5$ $(CACT)_6$ $(CA)_{45}$, respectively (Fig. 4). And yet the sequences amplified by X*gwm*18, X*gwm*153, and X*gwm*337 from amphiploids contain repeat structures $(CA)_{22}$ GA $(TA)_4$, $(GA)_{37}$ AGA $(TG)_3$, and $(CT)_5$ $(CACT)_6$ $(CA)_{39}$, respectively (Fig. 4). The sequences amplified by X*gwm*124, X*gwm*140, and X*gwm*539 from F_1 plants and Chinese Spring contain repeat

structures $(CT)_{25}$ TCT $(GT)_{10}$ AT $(GT)_7$, $(CT)_{40}$ C $(GC)_6$, and $(GA)_{24}$, respectively (Fig. 5), and the sequences amplified by X*gwm*124, X*gwm*140, and X*gwm*539 from amphiploids contain repeat structures $(CT)_7$ CC $(CT)_2$ TT $(CT)_6$ T $(CT)_2$ $(GT)_8$ AT $(GT)_5$, $(CT)_{29}$ CA $(CT)_{64}$ C $(GC)_4$, and $(GA)_6$ GG $(GA)_{28}$, respectively (Fig. 5). The sequences amplified by X*gwm*120, X*gwm*156, and X*gwm*497 from F_1 plants and Chinese Spring contain repeat structures $(CT)_{10}$ $(CA)_{16}$, $(GT)_{16}$, and $(GT)_{12}$ $(GC)_2$ $(GTGTGC)_3$ $(GT)_2$ $(GGGCGT)_2$ $(GC)_8$ $(GT)_6$, respectively (Fig. 6), whereas the sequences amplified by X*gwm*120, X*gwm*156, and X*gwm*497 from amphiploids contain repeat structure $(CT)_{20}$ $(CA)_{15}$, $(AT)_4$ $(GT)_4$, and $(GT)_{28}$, respectively (Fig. 6).

In addition, the products of X*gwm*44, X*gwm*191, X*gwm*268, X*gwm*340, and X*gwm*666 amplified from F_1 hybrids, amphiploids, and parental wheat were sequenced. The loci of X*gwm*44, X*gwm*340, and X*gwm*666 are adjacent to the loci of X*gwm*437, X*gwm*299, and X*gwm*497, respectively (Röder et al. 1998). The locus of X*gwm*191 is adjacent to the loci of X*gwm*120 and X*gwm*644 (Röder et al. 1998). The locus of X*gwm*268 is adjacent to the loci of X*gwm*124 and X*gwm*153 (Röder et al. 1998). For X*gwm*44, X*gwm*268, X*gwm*340, and X*gwm*666, the sequences amplified from F_1 plants, amphiploids, and Chinese Spring were identical (Fig. 7). Sequences amplified by X*gwm*44, X*gwm*268, X*gwm*340, and X*gwm*666 contain repeat structures $(GA)_{26}$, $(GA)_{16}$ TA $(GA)_{30}$, $(GA)_{25}$, and $(CA)_7$ TA $(CA)_{10}$, respectively (Fig. 7). For X*gwm*191, the sequences of F_1 plants and Chinese Spring were identical and contain repeat structure $(CT)_{12}$, the sequences of amphiploids were identical and contain repeat structure $(CT)_{13}$ (Fig. 7). The difference of the sequences amplified by X*gwm*191 between F_1 plants and amphiploids is also found in the flanking areas (Fig. 7).

Discussion

Allopolyploidization can Induce Microsatellite Evolution

The evolution of microsatellite has been studied extensively. So far, information on the dynamics of microsatellite evolution has come mainly from studies within species or comparisons of closely related species (Makova et al. 2000; Karhu et al. 2000). The factors that induce microsatellite evolution are unclear. Because allopolyploid is a hybrid containing two or more different genomes, it creates a considerable stress on the plant. McClintock (1978) has suggested that wide species crosses are among the stresses that might trigger reorganization of the parental genomes. Therefore, the stress caused by allopolyploidization can be considered as one of the factors which induce microsatellite evolution. Genomic changes in allopolyploids synthesized by colchicine treatment have been reported (Song et al. 1995; Liu et al. 1998; Shaked et al. 2001; Kashkush et al. 2002). These previous studies indicate that allopolyploids synthesized by colchicine treatment can be used to illustrate variations of genomic DNA sequences during allopolyploidization. Therefore, the results obtained here may indicate that allopolyploidization can induce rapid microsatellite evolution. However, so far, it is not clear whether allopolyploidization in nature can cause rapid microsatellite evolution. In the present study, the mutation rate of wheat microsatellite locus is 17.8% (29/163) and the mutation loci were dispersed on almost all the seven homeologous groups' chromosomes of wheat.

Although, the sequences of CJS_1-4 produced by X*gwm*156 were slightly different from the sequences of the other three amphiploids, the same mutational changes of microsatellites occurred in the four amphiploids. Because the four amphiploids are independent allopolyploidization events, it is surprising and interesting that the same mutational changes have occurred independently. Feldman et al. (1997) have reported that speciation through allopolyploidy is accompanied by a rapid, nonrandom elimination of specific, low-copy, probably noncoding DNA sequences at the early stages of allopolyploidization. Ozkan et al. (2001) have observed an identical pattern of changes in natural and newly synthesized allopolyploids with the same genomic constitution. The nonrandom and reproducible variation of genomic DNA sequences in allopolyploids synthesized by colchicine treatment have been reported (Song et al. 1995; Liu et al. 1998; Shaked et al. 2001; Kashkush et al. 2002). According to previous reports, it is possible that the same mutational changes of microsatellites occur in independent events of allopolyploidization. In present study, the variation of the microsatellites was not attributable to the heterozygosity of the parents or to PCR artifacts because the parent wheat and rye were single plants and selfed. The parents of the amphiploids are homozygous (Tang et al. 2008). In addition, the amplification products were not attributable to the possibility of PCR primers annealing to alternate sites. If PCR primers annealed to alternate sites, the sequences amplified from the four amphiploids should not be identical. However, it was striking that not only the mutational patterns of the 29 markers, but also the mutational patterns of the markers around the 29 markers are exactly the same among the four amphiploids. Possible reasons to explain this phenomenon might be (1) the parent wheat and rye were single plants and homozygous and (2) the microsatellite evolution induced by allopolyploidization is not random. Because the frequency of mutation of microsatellites in allopolyploids induced by colchicine is very low (Wanlong Li, personal communication), patterns of microsatellite variation induced by colchicine treatment

Fig. 7 Comparison of sequences amplified by X*gwm*44, X*gwm*191, X*gwm*268, X*gwm*340, and X*gwm*666 from Chinese Spring, F_1 plants, and amphiploids. *WMCS* indicates the sequences cloned from Chinese Spring. *WMCJF* indicates the sequences cloned from F_1 plants. *WMCJS* indicates the sequences cloned from amphiploids. *Dots* (·) show identical nucleotides. *Dashes* (–) show deletion of nucleotides

is seldom found. Tang et al. (2008) have investigated the microsatellite variation among F_1 hybrids and amphiploids derived from four different combinations between wheat and rye including Chinese Spring × Jinzhou-heimai, *Triticum aestivum* Mianyang11 × *S. cereale* 'Kustro', Mianyang11 × *S. cereale* AR106BONE and Chinese Spring × AR106BONE. Their results indicated that some of the wheat SSR markers used in their study displayed microsatellite variation only in progenies derived from Chinese Spring × Jinzhou-heimai, but none of the SSR markers displayed microsatellite variation in the progeny derived from the other three combinations. Thus, it is cannot be concluded that microsatellite evolution was a general response to allopolyploidization. The well-regulated variation of microsatellite in synthesized allopolyploids discovered by chance in this study might represent one microsatellite variation model, which seldom occurs in synthesized allopolyploids. The nonrandom variation of microsatellites observed here might occur through a precisely orchestrated mechanism of some presently unknown. More data are needed to confirm whether the well-regulated variation of microsatellite we observed represents a general, directed event associated with allopolyploidy, or merely a stochastic anomalous incident caused by the specific parental combinations.

Mechanism of Microsatellite Evolution Caused by Allopolyploidization

The results of this study indicated that variation of microsatellites caused by allopolyploidization can occur at repeat regions and flanking regions. The disappearance of microsatellite products of 15 SSR markers from the amphiploids indicated the variation of primer sites at the flanking regions. The sequences amplified by X*gwm*140, X*gwm*153, X*gwm*156, X*gwm*186, and X*gwm*191 also displayed variation at the flanking regions. However, the length variation of microsatellites in the present study stemmed mainly from the variation in the number of repeat units.

The wheat microsatellite loci X*gwm*136, X*gwm*186, X*gwm*282, X*gwm*437, and X*gwm*644 contain perfect dinucleotide repeats (Fig. 3). The dinucleotide repeat units of all the five microsatellite loci were reduced after allopolyploidization (Fig. 3). The size alteration pattern in repeat regions of the five microsatellite loci completely accord with the slipped-strand mispairing (SSM) model (Levinson and Gutman 1987). The three microsatellite loci X*gwm*18, X*gwm*153, and X*gwm*337 contain complex compound repeats (Fig. 4). For each of the three microsatellite loci, only the longer dinucleotide repeat has changed and the variation patterns of repeat units of the three microsatellite loci can also be explained by the SSM model (Levinson and Gutman 1987).

The variation of microsatellite loci X*gwm*124, X*gwm*140, and X*gwm*539 may be caused by both base-substitution (Calabrese et al. 2001) and SSM, because their microsatellites were interrupted after allopolyploidization (Fig. 5). Although C–T transitions, T–A transversion, and A–G transitions occurred in the microsatellite of loci X*gwm*124, X*gwm*140, and X*gwm*539, respectively, the length variation of repeat unit of the three loci can still be explained by the SSM model. Furthermore, microsatellite loci X*gwm*120, X*gwm*124, and X*gwm*140 also contain complex compound repeats (Fig. 5, 6). The shorter repeat of the three microsatellite loci also changed. The sequences amplified by X*gwm*120 contain (CT)n and (CA)n dinucleotide repeats. However, the number of CT units increased but CA units decreased in the amphiploids (Fig. 6). This case indicated that expansion and contraction of the repeat unit could occur at the same time at the same microsatellite locus that contains the compound repeat. The variation of locus X*gwm*156 may be caused by a G-to-A mutation. That is, a G-to-A mutation produced an $(AT)_4$ repeat and led to a loss of GT repeats (Fig. 6). Levinson and Gutman (1987) have referred to tandem repeats produced by chance mutations as the "raw material" for repeat expansion by SSM. The variation of the locus X*gwm*156 mutation can not only provide "raw material" for repeat expansion, but also lead a reduction of repeats. The microsatellite variation of locus X*gwm*497 indicated that expansion of perfect dinucleotide repeats might be accompanied by a deletion of hexanucleotide and dinucleotide repeats. In addition, the loci that did not display length variation may be more stable.

In conclusion, allopolyploidization can induce immediate microsatellite evolution and almost all the variations of microsatellites observed in this study can be explained by the SSM model. In addition, the findings of the present study furnish an example of how microsatellites evolve after allopolyploidization.

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