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LOSS OF PARENTAL CODING SEQUENCES IN AN EARLY GENERATION OF WHEAT-RYE ALLOPOLYPLOID

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During wheat-rye allopolyploidization, the characteristics of the sequences in the bands that appear in both parents and progeny are still unclear. In this study, two different combinations of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.), including F_1 hybrids and the first and second allopolyploid generations, were analyzed by PCR and sequencing using 60 wheat expressed sequence tag (EST)–derived single-sequence repeat markers and EST-derived sequence-tagged site markers. Thirty markers produced the same bands from parental plants, F_1 plants, and amphiploids. Seven of the 30 markers amplified identical sequences from wheat and rye parents. Most of these sequences have high similarity between the two parental plants. The variation patterns of sequences in the bands produced by the seven markers were observed. In the F_1 hybrids and amphiploids, loss of parental sequences was observed and the frequency of losing rye sequences was higher than that of losing wheat sequences. In addition, a few sequences in these bands exhibited significant differences, indicating that parental sequences changed drastically during allopolyploidization. Therefore, the fact that the parents and progeny contained the same bands should not be regarded as conservation. The results in this study add to the investigations dealing with variation patterns of coding sequences during wheat-rye allopolyploidization.

Keywords: allopolyploid, coding sequence, elimination of DNA, eSSR markers, rye, wheat.

Introduction

Polyploidization plays an important role in plant evolution. A number of recent reports have documented genetic and epigenetic instability in newly synthesized allopolyploids (Kashkush et al. 2002; Madlung et al. 2002). Ma and Gustafson (2008) have indicated that among well-studied newly synthesized species such as wheat (*Triticum* spp.), *Brassica*, *Arabidopsis*, and cotton (*Gossypium* spp.), triticale is mostly characterized because of its genome complexity, different ploidy levels of parental genomes, and intergeneric hybridization. These features make the genomic changes of triticale more extensive than those of any of the other allopolyploid species studied. It has already been reported that the total DNA content of triticale was less than that expected for the combined genomes of its wheat and rye parents, and the DNA content decreased by approximately 9% in octoploid triticale (Boyko et al. 1984, 1988). A series of studies on newly synthesized triticale have revealed rapid genomic and epigenomic changes (Ma et al. 2004; Ma and Gustafson 2006, 2008; Bento et al. 2008). Amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and PCR-based molecular marker techniques have often been used to investigate the overall genomic variation of triticale (Ma et al. 2002, 2004; Ma and Gustafson 2006; Bento et al. 2008). A typical observation of genetic changes in newly synthesized wheat-rye allopolyploids was sequence elimination, with the main eliminated sequences being the repetitive, low-copy sequences and coding sequences (Ma et al. 2004; Ma and Gustafson 2006; Bento et al. 2008). AFLP and cDNA-probed RFLP analyses have indicated that the frequency of losing parental bands was much higher than the frequency of gaining novel bands, and parental band loss occurred as early as in the F_1 hybrids (Ma et al. 2004; Ma and Gustafson 2006). PCR-based molecular marker techniques involving retrotransposons and microsatellites have been used to uncover polyploidization-induced genetic restructuring in triticale, where parental sequence loss was also observed (Bento et al. 2008). In these previous studies, sequence elimination was determined by comparing the size of bands in wheat-rye allopolyploids and their corresponding wheat and rye parents. The bands, which appeared in parental plants but not in progeny, were regarded as eliminated. The bands, however, which appeared in both triticale and their parental plants, were considered unchanged. The characteristics of the sequences in the bands that appeared in parental plants, F_1 hybrids, and triticales are still unclear.

The purpose of this study is to discover the characteristics and variation patterns of the sequences, represented by the bands that appeared in newly synthesized octoploid triticales (amphiploids), F_1 hybrids, and their exact parents. The variation patterns of coding sequences that have a high similarity between two parental plants were observed and discussed.

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Material and Methods

Plants and DNA Extraction

Plants used for this study consisted of two different combinations of newly synthesized octoploid triticales, F_1 hybrids, and their exact parental plants. The first combination was *Triticum aestivum* Mianyang11 (genome AABBDD) × *Secale cereale* Kustro (genome RR; MK combination), and the second combination was *T. aestivum* Chinese Spring (genome AABBDD) × *S. cereale* Jinzhou-heimai (genome RR; CJ combination). A single rye plant was selected as the pollen donor, and a single wheat plant was selected as the recipient. All parental plants were maintained by strict selfing. The parental plants (Kustro, Jinzhou-heimai, Mianyang11, and Chinese Spring) have already been demonstrated to be homozygous by Tang et al. (2008). Young F_1 seedlings were treated with 0.05% colchicine solution, and allopolyploids were obtained after chromosome doubling. The allopolyploids used in this study were the first and second allopolyploid generations. The names of the F_1 plants and amphiploids are listed in table 1. CJ1-2 (S1 generation) and MK7-17 (S1 generation) correspond to the F_2 generation derived from the F_1 plants that were treated with colchicine. After initial production, the amphiploids were maintained by strict selfing. CJ1-2-4 (S2 generation) was derived from CJ1-2, and MK7-17-7 (S2 generation) was derived from MK7-17. Some plants were lost as a result of low frequencies of fertility and germination failure of seeds. In addition, some partial amphiploids were not used in this study. Genomic DNA of materials was extracted according to the method described by Zhang et al. (1995).

Genomic In Situ Hybridization (GISH)

GISH analysis was used to identify the progeny of F_1 plants as amphiploid. For the first and second allopolyploid generations, only the octoploid triticales were used in this study. The GISH analysis was performed following the procedure of Tang et al. (2009).

PCR Amplification and Sequence Cloning

Sixty wheat expressed sequence tag (EST)–derived single-sequence repeat (eSSR) markers and EST-derived sequence-tagged site (eSTS) markers (Xue et al. 2008) were screened for amplification in the newly synthesized amphiploids, F_1 hybrids, and their exact parental plants. The PCR amplification was performed following the procedure of Tang et al. (2009). The PCR products were separated in 8% vertical nondenaturing polyacrylamide gels (60 cm × 30 cm × 0.4 mm) with 1 × TBE (Tris-borate-EDTA) buffer and visualized by silver staining.

The products of the markers, which produced the same bands from F_1 plants, amphiploids, and their exact parental plants, were first cloned from wheat and rye parents for sequencing. For each of these amplifications, three randomly selected clones from each of the target fragments were sequenced. The three sequences were then used for alignment analysis. Markers that could produce identical sequences from wheat and rye parental plants were used to investigate variation patterns of the sequences in subsequent progeny. The target bands were recovered from polyacrylamide gels, reamplified by PCR, and sequenced. The procedure was performed according to Tang et al. (2009). The second amplification products were cloned into pMD18-T Simple Vector (TaKaRa). Inserts were sequenced by the commercial company Invitrogen Biotechnology (Shanghai). In addition, two kinds of mixed genomic DNA were used as positive control DNA template for the PCR reaction to verify that the elimination of rye- or wheat-specific sequences in the F_1 hybrids and/or amphiploids were attributable to hybridization or allopolyploidization. The one mixed genomic DNA (CJ mixed parental genomic DNA) included 40 ng of Chinese Spring genomic DNA and 40 ng of Jinzhou-heimai genomic DNA, and the other mixed genomic DNA (MK mixed parental genomic DNA) contained 40 ng of Mianyang11 genomic DNA and 40 ng of Kustro genomic DNA. The nucleotide sequences were deposited in the GenBank database. Sequence analysis was performed with DNAMAN (ver. 4.0) and Clustal X (ver. 1.81) software.

Results

Identification of Amphiploids Using GISH

The root-tip preparations in which rye chromatin was present were distinguishable by fluorescing signals at metaphase. Chromosome counts indicated that the chromosome number of the seeds (CJ1-2, CJ1-2-4, MK7-17, and MK7-17-7) 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 CJ1-2, CJ1-2-4, MK7-17, and MK7-17-7 were amphiploids (octoploid triticales).

PCR Amplification

Of the 60 eSTS and eSSR markers, 30 markers produced the same bands from F_1 plants, amphiploids, and their exact parental plants (fig. 2*a*). Another 17 markers produced the same bands from wheat parents, F_1 plants, and amphiploids but not from rye parents (fig. 2*b*). A third set of 13 markers amplified different bands from wheat and rye parents, and all the wheat bands and some rye bands appeared in F_1 plants and amphiploids (fig. 2*c*, 2*d*). However, some other rye parental bands

Table 1

Names of F_1 Plants and First and Second Allopolyploid Generations Derived from Two Wheat × Rye Crosses in This Study

Wheat × rye	F_1 plant	First amphiploid generation (S1)	Second amphiploid generation (S2)
Chinese Spring × Jinzhou-heimai	CJ1	CJ1-2	CJ1-2-4
Mianyang11 × Kustro	MK7	MK7-17	MK7-17-7

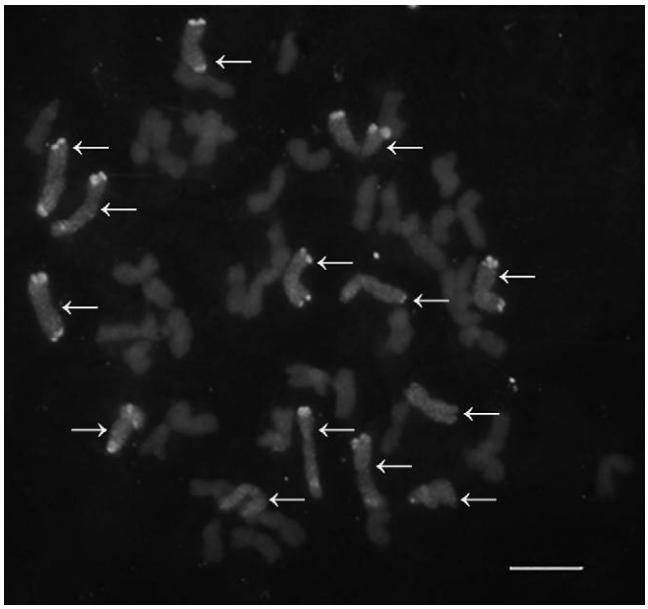

Fig. 1 Amphiploids CJ1-2, CJ1-2-4, MK7-17, and MK7-17-7 shown by genomic in situ hybridization analysis to contain 56 chromosomes including 14 *Secale cereale* chromosomes distinguishable by bright fluorescing domains. Arrows indicate *S. cereale* chromosomes. Scale bar = 10 μm.

disappeared in F_1 plants and amphiploids (fig. 2*d*). Therefore, only the products of the initial 30 markers were used.

Characteristics and Transmission Pattern of Sequences

A total of 360 DNA sequences were sequenced. Some of the 30 markers could produce identical sequences from parental plants. For example, the three sequences amplified by marker MAG681 from Chinese Spring have 99%–100% similarity with each other, and the three sequences amplified by marker MAG972 from Jinzhou-heimai have 100% similarity with each other. There were 12 markers that could produce identical sequences from parental plants. The names of the 12 markers and the names and GenBank accession numbers of the sequences amplified by the 12 markers are listed in the appendix. In the appendix, we note that markers MAG681, MAG972, MAG3030, and MAG3185 could amplify identical sequences only from Chinese Spring, Jinzhou-heimai, and Mianyang11. Marker MAG1036 could amplify identical sequences from wheat parental plants but not from rye parental plants. However, the other seven of the 12 markers could amplify identical sequences from both wheat and rye parents. For example, MAG299 produced identical sequences from Chinese Spring, Jinzhou-heimai, Mianyang11, and Kustro. MAG695 produced identical sequences from Chinese Spring and Jinzhou-heimai. Therefore, for CJ combinations (Chinese Spring × Jinzhou-heimai), only the sequences amplified by MAG299, MAG695, MAG1353, MAG1757, MAG1885, and STS-BCD348 were used for further analysis, while for MK combinations (Mianyang11 × Kustro), only the products of MAG299 and MAG1571 were used for further analysis.

Twelve clones from each of the target products were randomly selected for sequencing, and another 288 DNA sequences were sequenced. Sequences isolated from wheat and rye for markers MAG1885, MAG299, MAG1571, STS-BCD348, MAG1757, and MAG1353 had 89%–96% sequence identity (between genomes). The sequences for MAG695 had only 50% sequence identity. The similarity of the sequences cloned from F_1 and amphiploid plants with parental sequences are listed in table 2. For MK combinations, 17 sequences amplified by MAG299 had 99%–100% similarity with wheat parental sequences, 16 sequences of MAG299 had 98%–100% similarity with rye parental sequences, and

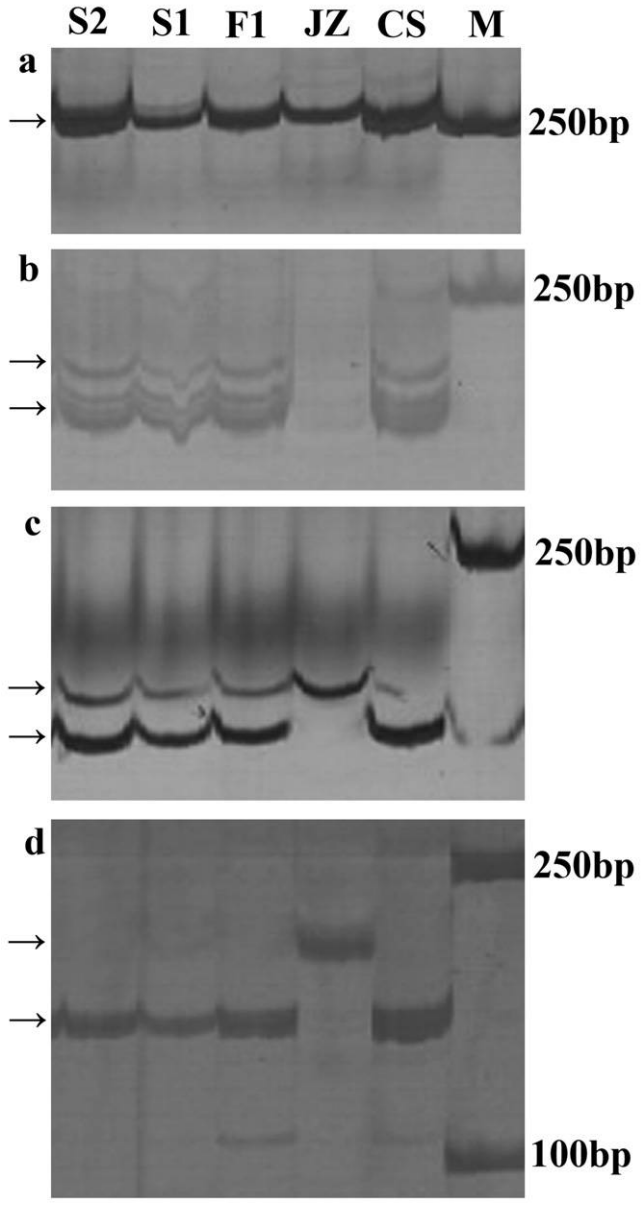

Fig. 2 Bands amplified by eSSR markers. *a*, Products of MAG1885; *b*, products of MAG1280; *c*, products of MAG2137; *d*, products of MAG3227. CS = Chinese Spring; JZ = Jinzhou-heimai; F1 = CJ1; S1 = CJ1-2; S2 = CJ1-2-4; M = DNA marker. Arrows indicate the target bands.

three sequences had 45%–56% similarity with both parental sequences. These results indicate that both the sequences of Mianyang11 and Kustro amplified by MAG299 were present in plants MK7, MK7-17, and MK7-17-7. However, in CJ combinations, all 36 sequences amplified by MAG299 had 98%–100% similarity with the sequences of Jinzhou-heimai. This result indicates that only the sequences of Jinzhou-heimai amplified by MAG299 were transmitted to plants CJ1, CJ1-2, and CJ1-2-4. The products amplified by markers MAG695 and MAG1757 indicated that plants CJ1, CJ1-2, and CJ1-2-4 inherited only the sequences of Chinese Spring. In the same way, plants MK7, MK7-17, and MK7-17-7 inherited only the sequences amplified by MAG1571 from Mianyang11. The CJ1 plant contained both the sequences produced by MAG1353 from Chinese Spring and Jinzhou-heimai; however, the CJ1-2 and CJ1-2-4 plants contained only the Chinese Spring sequences of MAG1353. For the STS-BCD348 marker, the CJ1 plant obtained both the wheat and rye parental sequences, whereas the CJ1-2 and CJ1-2-4 plants inherited only the rye parental sequences. The 36 sequences that were amplified by MAG1885 from CJ1, CJ1-2, and CJ1-2-4 plants displayed great differences from both the wheat and rye parental sequences. Similarly, the sequences amplified by MAG1885 from CJ1, CJ1-2, and CJ1-2-4 plants were also different from each other.

Additionally, the markers MAG299, MAG695, MAG1353, MAG1571, MAG1757, MAG1885, and STS-BCD348 were used to amplify the mixed parental genomic DNA. Thirteen sequences were randomly selected from each of the target products for sequencing. The similarities of the sequences amplified from mixed genomic DNA with wheat and rye parental sequences are listed in table 2. The result in table 2 indicates that there was no competition of the six primer annealing sites between genomic DNA of wheat and rye.

Table 2

Numbers of Sequences That Have the Indicated Range of Similarity between the F_1 and Amphiploid Lines with the Indicated Parent

Marker and DNA plate	97%–100% similarity with wheat parental sequences	97%–100% similarity with rye parental sequences	40%–60% similarity with both parental sequences	30%–40% similarity with both parental sequences
MAG299:				
CJ1	0	12	0	0
CJ1-2	0	12	0	0
CJ1-2-4	0	12	0	0
CJ mixed parental genomic DNA	6	7	0	0
MK7	5	6	1	0
MK7-17	6	5	1	0
Mk7-17-7	6	5	1	0
MK mixed parental genomic DNA	7	6	0	0
MAG695:				
CJ1	12	0	0	0
CJ1-2	11	0	1	0
CJ1-2-4	11	0	1	0
CJ mixed parental genomic DNA	5	8	0	0
MAG1757:				
CJ1	12	0	0	0
CJ1-2	11	0	1	0
CJ1-2-4	11	0	1	0
CJ mixed parental genomic DNA	6	7	0	0
MAG1571:				
MK7	12	0	0	0
MK7-17	12	0	0	0
MK7-17-7	12	0	0	0
MK mixed parental genomic DNA	6	6	1	0
MAG1353:				
CJ1	5	7	0	0
CJ1-2	10	0	2	0
CJ1-2-4	9	0	3	0
CJ mixed parental genomic DNA	5	8	0	0
STS-BCD348:				
CJ1	8	4	0	0
CJ1-2	0	12	0	0
CJ1-2-4	0	10	2	0
CJ mixed parental genomic DNA	7	6	0	0
MAG1885:				
CJ1	0	1	5	6
CJ1-2	0	0	6	6
CJ1-2-4	0	0	5	7
CJ mixed parental genomic DNA	6	7	0	0

Discussion

A series of studies on newly synthesized allopolyploid species of wheat (*Triticum* spp.) have been particularly revealing of rapid sequence elimination and gene expression changes after allopolyploid formation (Feldman et al. 1997; Liu et al. 1998*a*, 1998*b*; Ozkan et al. 2001; Shaked et al. 2001; Kashkush et al. 2002; Han et al. 2005). So far, few researchers have paid attention to the genomic variation of newly synthesized wheat-rye allopolyploidy, although the genetic restructuring of triticale has been investigated by AFLP, RFLP, and PCR methods (Ma et al. 2004; Ma and Gustafson 2006; Bento et al. 2008). These studies focused mainly on the loss of parental bands or the appearance of new bands in triticales. It has been reported that, when a band was present in both wheat and rye parents, the frequency of that band being conserved in triticale was much higher than if it appeared in only one parent (Ma et al. 2004; Ma and Gustafson 2008). The bands that were present in each of the two parental plants and progeny were generally regarded as unchanged or conserved; therefore, the precise characterization and variation pattern of the sequences in these bands were often ignored. The results in this study indicated that some sequences in the bands that appeared in both wheat and rye parents have high similarity between the two parental plants. These bands that contained highly similar sequences also appeared in the parents, F_1 hybrids, and amphiploids. Some of the bands in F_1 hybrids and amphiploids contained only single parental sequences, indicating the loss of the other parental sequences. In addition, a few band sequences exhibited significant differences, indicating that the parental sequences changed drastically during allopolyploidization. Therefore, the fact that the parents and progeny contained the same bands should not be regarded as conservation.

Because eSSRs are components of the transcribed genes, the sequence loss in this study might reflect the coding sequence variation during wheat-rye allopolyploidization. The frequency of losing rye coding sequences was higher than that of losing wheat coding sequences, and the sequence loss occurred as early as the F_1 generation. Similar results have also been reported by Ma et al. (2004) and Ma and Gustafson (2006), who found that the rye genome was less well conserved than wheat genomes in octoploid triticales. These results lead us to think that the genome of the diploid parent undergoes most of the changes while those of the polyploid parent change relatively little. Ma et al. (2004) have argued that the intergeneric relationship between wheat and rye and the wheat cytoplasmic background may be the factors that determine the direction and amount of sequence elimination. In addition, it is reasonable to assume that wheat is an allohexaploid species, in which three different ancestral genomes have already harmoniously coexisted in the wheat nucleolus, and the alien rye chromosomes could be excluded for greater stability. Therefore, the loss of single parental coding sequences might contribute to the genetic diploidization of newly formed wheat-rye allopolyploids.

In this study, sequence loss was not caused by chromosome loss because plants were checked cytologically and found to be octoploid. In addition, the PCR reaction using mixed wheat-rye genomic DNAs as template indicated that sequence elimination in some F_1 hybrids and amphiploids was not attributable to the competition of primer annealing sites between genomic DNA of wheat and rye.

The transferability of eSSR markers from wheat (*Triticum aestivum*) to related species (*Triticum durum*, *Triticum monococcum*, *Aegilops speltoides*, *Aegilops tauschii*, rye [*Secale cereale*], barley [*Hordeum vulgare*], *Aropyron elongatum*, and rice [*Oryza sativa*]) has been reported (Zhang et al. 2005). In *Cerasua jamasakura* and Japanese white birch, eSSRs were developed and their transferability to closely aligned taxa was evaluated (Tsuda et al. 2009*a*, 2009*b*). Cross-species transferability of EST-SSR markers for cereal crops (Tang et al. 2006) and rubber trees (Feng et al. 2009) has also been reported. These results indicate that eSSR markers developed from one species can be used to enlarge the source of molecular markers for use in other species. The eSSR markers available for rye breeding and genetics are less abundant than for wheat. In this study, 43 of the 60 markers could amplify products from rye, suggesting the high transferability of wheat eSSR and eSTS markers to rye (71.67%). These markers can be used for rye breeding and genetics.

In conclusion, the loss and variation of parental sequences have occurred in the bands that appeared in both parents and progeny during wheat-rye allopolyploidization. Although parents and progeny often contain the same bands, this should not be regarded as conservation because of sequence differences that do not alter the size of the amplified product. There was a high transferability of wheat eSSR and eSTS markers to rye. The results in this study are an addition to the investigations dealing with the variation patterns of coding sequences that have high similarity between wheat and rye parental plants during wheat-rye allopolyploidization.

Acknowledgments

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Appendix

Information about Markers That Produced Identical Sequences from Parental Plants

The following information is given after marker name: parental plant, sequence name, GenBank accession number. Only one sequence was named and deposited in the GenBank database because the three sequences amplified by each marker from each parental plant were identical.

MAG299: Chinese Spring, 299CS, HN176586; Mianyang11, 299MY11, HN176595; Kustro, 299Kustro, HN176602; Jinzhouheimai, 299JZ, HN176604.

MAG681: Chinese Spring, 681CS, HN176587.
MAG695: Chinese Spring, 695CS, HN176588; Jinzhou-heimai, 695JZ, HN176605.
MAG972: Jinzhou-heimai, 972JZ, HN176606.
MAG1036: Chinese Spring, 1036CS, HN176589; Mianyang11, 1036MY11, HN176596.
MAG1353: Chinese Spring, 1353CS, HN176590; Mianyang11, 1353MY11, HN176597; Jinzhou-heimai, 1353JZ, HN176607.
MAG1571: Chinese Spring, 1571CS, HN176591; Mianyang11, 1571MY11, HN176598; Kustro, 1571Kustro, HN176603.
MAG1757: Chinese Spring, 1757CS, HN176592; Mianyang11, 1757MY11, HN176599; Jinzhou-heimai, 1757JZ, HN176608.
MAG1885: Chinese Spring, 1885CS, HN176593; Jinzhou-heimai, 1885JZ, HN176609.
MAG3030: Mianyang11, 3030MY11, HN176600.
MAG3185: Mianyang11, 3185MY11, HN176601.
STS-BCD348: Chinese Spring, 348CS, HN176610; Jinzhou-heimai, 348JZ, HN176594.

Literature Cited

Bento M, HS Pereira, M Rocheta, JP Gustafson, W Viegas, M Silva 2008 Polyploidization as a retraction force in plant genome evolution: sequence rearrangements in Triticale. PLoS ONE 3: e1402.

Boyko EV, NS Badaev, NG Maximov, AV Zelenin 1984 Does DNA content change in the course of triticale breeding? Cereal Res Commun 12:99–100.

——— 1988 Regularities of genome formation and organization in cereals. I. DNA quantitative changes in the process of allopolyploidization. Genetika 24:89–97.

Feldman M, B Liu, G Segal, S Abbo, AA Levy, JM Vega 1997 Rapid elimination of low-copy DNA sequences in polyploidy wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147:1381–1387.

Feng SP, WG Li, HS Huang, JY Wang, YT Wu 2009 Development, characterization and cross-species/genera transferability of EST-SSR markers for rubber tree (*Hevea brasiliensis*). Mol Breed 23:85–97.

Han FP, G Fedak, W Guo, B Liu 2005 Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics 170:1230–1245.

Kashkush K, M Feldman, AA Levy 2002 Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. Genetics 160:1651–1659.

Liu B, JM Vega, M Feldman 1998*a* Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. II. Changes in low-copy coding DNA sequences. Genome 41:535–542.

Liu B, JM Vega, G Segal, S Abbo, M Rodova, M Feldman 1998*b* Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy noncoding DNA sequences. Genome 41:272–277.

Ma XF, P Fang, JP Gustafson 2004 Polyploidization-induced genome variation in triticale. Genome 47:839–848.

Ma XF, JP Gustafson 2006 Timing and rate of genome variation in triticale following allopolyploidization. Genome 49:950–958.

——— 2008 Allopolyploidization-accommodated genomic sequence changes in triticale. Ann Bot 101:825–832.

Ma XF, MA Rodriguez Milla, JP Gustafson 2002 AFLP-based genome studies of triticale following polyploidization. Pages 89–94 *in* A Aniol, E Arseniuk, K Cooper, N Darvey, JP Gustafson, R Jessop, eds. Proceedings of the 5th International Triticale Symposium. Plant Breeding and Acclimatization Institute (IHAR), Radzików, Poland.

Madlung A, RW Masuelli, B Watson, SH Reynolds, J Davison, L Comai 2002 Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. Plant Physiol 129:733–746.

Ozkan H, AA Levy, M Feldman 2001 Allopolyploidy-induced rapid genome evolution in the wheat (*Aegilops-Triticum*) group. Plant Cell 13:1735–1747.

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

Tang JF, LF Gao, YS Cao, JZ Jia 2006 Homologous analysis of SSR-ESTs and transferability of wheat SSR-EST markers across barley, rice and maize. Euphytica 151:87–93.

Tang ZX, SL Fu, ZL Ren, JP Zhou, BJ Yan, HQ Zhang 2008 Variations of tandem repeat, regulatory element and promoter regions revealed by wheat-rye amphiploids. Genome 51:399–408.

Tang ZX, SL Fu, ZL Ren, YT Zou 2009 Rapid evolution of simple sequence repeat induced by allopolyploidization. J Mol Evol 69: 217–228.

Tsuda Y, S Ueno, S Kato, T Katsuki, Y Mukai, Y Tsumura 2009*a* Development of 13 EST-SSRs for *Cerasus jamasakura* and their transferability for Japanese flowering cherries. Conserv Genet 10: 685–688.

Tsuda Y, S Ueno, J Ranta, K Salminen, Y Ide, K Shinohara, Y Tsumura 2009*b* Development of 11 EST-SSRs for Japanese white birch, *Betula platyphylla* var. *japonica* and their transferability to related species. Conserv Genet 10:1385–1388.

Xue SL, ZZ Zhang, F Lin, ZX Kong, Y Cao, CJ Li, HY Yi, et al 2008 A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor Appl Genet 117:181–189.

Zhang HB, XP Zhao, X Ding, AH Paterson, RA Wing 1995 Preparation of megabase-sized DNA from plant nuclei. Plant J 7: 175–184.

Zhang LY, M Bernard, P Leroy, C Feuillet, P Sourdille 2005 High transferability of bread wheat EST-derived SSRs to other cereals. Theor Appl Genet 111:677–687.