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Genomic and genic sequence variation in synthetic hexaploid wheat (AABBDD) as compared to their parental species

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Abstract

In order to understand the genomic changes during the evolution of hexaploid wheat, two sets of synthetic hexaploid wheat from hybridization between maternal tetraploid wheat (AABB) and paternal diploid goat grass (DD) were used for DNA-AFLP and single strand conformation polymorphism (SSCP) analysis to determine the genomic and genic variation in the synthetic hexaploid wheat. Results indicated that more DNA sequences from paternal diploid species were eliminated in the synthetic hexaploid wheat than from maternal tetraploid wheat, suggesting that genome from parental species of lower ploidity tends to be eliminated preferentially. However, sequence variation detected by SSCP procedure was much lower than those detected by DNA-AFLP, which indicated that much less variation in the genic regions occurred in the synthetic hexaploid wheat, and sequence variations detected by DNA-AFLP could be derived mostly from non-coding regions and repetitive sequences. Our results also indicated that sequence variation in 4 genes can be detected in hybrid F_1, which suggested that this type of sequence variation could be resulted from distant hybridization. It was interesting to note that 3 out of the 4 genes were mapped and clustered on the long arm of chromosome 2D, which indicated that variation in genic sequences in synthetic hexaploid wheat might not be a randomized process.

Keywords: Synthetic hexaploid wheat; Polyploidy; Genome; Sequence variation

1. Introduction

Hybridization and polyploidization are important in the speciation and the evolution of higher plants. Up to 70% of all angiosperm species have experienced polyploidization during their evolution [1]. Although many established wild and cultivated allopolyploids are well adapted and genetically stable, newly synthesized allopolyploids commonly display genomic and phenotypic instabilities [2,3]. Genomic changes, such as sequence elimination, were observed for allopolyploids in *Triticum* [4–6] and *Brassica* [7]; however, synthetic *Arabidopsis* allotetraploids [3,8] and allopolyploid cotton (*Gossypium*) species [9] displayed a negligible amount of changes in the genomic sequences.

As one of most extensively cultivated crops worldwide, common wheat (*Triticum aestivum* L. $2n = 6x = 42$, AABBDD) is a classical allohexaploid plant species, and an excellent material to study polyploidization. Previous studies indicated that sequence elimination, being a general phenomenon during polyploidy, was initiated in F_1 hybrids [10], but polyploidy genomes were stabilized in the S3–S6 generations of the synthetic allopolyploids [4,5]. By using AFLP technology, Shaked et al. [6] found that in the synthetic allotetraploid wheat between *Ae. sharonensis* and *Ae. umbellulata*, 14% of the loci from *Ae. sharonensis* were eliminated, but only 0.5% from *Ae. umbellulata*, and alter-

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ations in cytosine methylation occurred in 13% of the loci, either in the F_1 hybrid or in the allopolyploid. Dong et al. [11] analyzed a 5–7 generations of synthetic allohexaploid wheat by AFLP and found that about 20% bands showed deviation from parental additivity. Sequence analysis indicated that a majority of the changed bands represented genes with known functions and transposable elements. Study also showed that activation of retrotransposons occurred in newly formed allopolyploid wheat [12]. Kashkush et al. [13] performed transposon display assay using DNA and cDNA as templates. They found no evidence for DNA rearrangements involving wis2-1A, and identified expression changes for 26 (7%) genes flanking wis2-1A, 10 of which were activated in the amphiploid and 16 of which were present in one or both parents but absent in the amphiploid. Liu et al. [14] found "loss and/or gain" of fragments in all nine amphiploids probed with 43 coding sequences, which were attributed to the changes in DNA methylation. Bottley et al. [15] developed 236 primer pairs corresponding to single-copy genes mapped to the three homoeologous chromosomes within groups 1, 2, 3 and 7 of wheat. Using the single strand conformation polymorphism (SSCP) technique allowed the identification of all three expected genomic copies for each set of homoeoalleles, which could be used to study coding sequence variations during polyploidy.

In the present study, we analyzed the sequence variation at the genomic level of the 9-generation-old synthetic hexaploid wheat and its parental species (kindly provided by CIMMYT) with DNA-AFLP, and the rate of coding sequence variation among the 208 single-copy genes was also determined. In addition, by using the newly synthesized hexaploid wheat and its parental species, the sequence variations were also investigated as compared to the CIMMYT synthetic hexaploid wheat.

2. Materials and methods

2.1. Plant materials

Two sets of synthetic hexaploid wheat were used in this study. The first set included one 9-generation-old synthetic allohexaploid wheat SCAUP/SQ523 and its parental species durum wheat SCAUP (*T. turgidum*, AABB, $2n = 28$), goat grass SQ523 (*Ae. tauschii*, DD, $2n = 14$), which were provided by International Maize and Wheat Improvement Center (CIMMYT). The second set included the parental species tetraploid wheat DM4 (*T. dicoccum*, AABB, $2n = 28$), goat grass Y199 (*Ae. tauschii*, DD, $2n = 14$), F_1 hybrid and newly synthesized allohexaploid wheat, which was produced by our laboratory. Briefly, hybrid between DM4 and Y199 was obtained with immature embryo rescue, and doubled by treating with 0.04% colchicine +1.5% DMSO for 20–22 h at 15–18 °C and then washed in tap water for 1 h. After treatment, plants were grown in the greenhouse, and all spikes were bagged for self-pollination.

2.2. DNA-AFLP analysis

Genomic DNA was extracted by the CTAB method. The AFLP procedure was performed according to Vos et al. [16] with minor modifications. Briefly, (1) Genomic DNA was digested with MseI and EcoRI in 40 μl reaction containing the extracted DNA 300 ng, 4 μl 10 × NE buffer, 0.3 μl EcoRl (20 U/μl), 0.3 μl MSEl (10 U/μl), and 0.4 μl BSA. After vortex, the mixture was incubated at 37 °C for 3 h. The reaction was stopped by incubating at 70 °C for 15 min. (2) Digested DNA fragments were ligated with MseI and EcoRI adaptors, respectively. Using the following ligation master mix containing 2 μl 5 pM EcoRl adaptors, 2 μl 50 pM MSEl adaptors, 1 μl T4 ligase and 5 μl 10× buffer, and 10 μl of ligation mix was added to 40 μl of digested DNA, mixed well and incubated at 16 °C overnight. After incubation, the digestion/ligation reaction mixture was 1:5 diluted with ddH_2O. (3) The pre-amplification reaction was performed in a 50 μl reaction containing 5 μl diluted digestion/ligation reaction mixture, 1 μl 10 mM dNTP, 5 μl 10× buffer, 1.6 μl E0 primer (20 ng/μl), 1.6 μl MSE0 primer (20 ng/μl), 1 μl *Taq* DNA polymerase. The PCR profile was 25 cycles of 30 s at 94 °C, 30 s at 56 °C, and 60 s at 72 °C. The PCR product was diluted to 1:50 with ddH_2O. (4) The selective amplification reaction was carried out in a 10 μl reaction containing 2.5 μl template

Table 1
Adaptor and primer sequences

	No.	EcoRI	No.	MSEI
Adaptor	EcoR-L	5′-CTCGTAGACTGCGTACC-3′	MSE-L	5′-GACGATGAGTCCTGAG-3′
	EcoR-R	3′-CATCTGACGCATGGTTAA-5′	MSE-R	3′-TACTCAGGACTCAT-5′
Pre-amp primer	E0	5′-GACTGCGTACCAATTC-3′	MSE0	5′-GATGAGTCCTGAGTAA-3′
Selective primer	E1	5′-GACTGCGTACCAATTC ACA-3′	MSE1	5′-GATGAGTCCTGAGTAA CAA-3′
	E2	5′-GACTGCGTACCAATTC ACC-3′	MSE2	5′-GATGAGTCCTGAGTAA CAC-3′
	E3	5′-GACTGCGTACCAATTC AGG-3′	MSE3	5′-GATGAGTCCTGAGTAA CAG-3′
	E4	5′-GACTGCGTACCAATTC ACG-3′	MSE4	5′-GATGAGTCCTGAGTAA CAT-3′
	E5	5′-GACTGCGTACCAATTC AGC-3′	MSE5	5′-GATGAGTCCTGAGTAA CTA-3′
	E11	5′-GACTGCGTACCAATTC AGT-3′	MSE6	5′-GATGAGTCCTGAGTAA CTC-3′
	E12	5′-GACTGCGTACCAATTC CAA-3′	MSE7	5′-GATGAGTCCTGAGTAA CTG-3′
	E13	5′-GACTGCGTACCAATTC CAT-3′	MSE8	5′-GATGAGTCCTGAGTAA CTT-3′

DNA from the diluted pre-amplification reaction, 20 ng EcoRI selective primer, 20 ng MseI selective primer, 0.2 μl dNTP (10 mM), 0.1 μl *Taq* DNA polymerase, 1 μl 10× buffer. The PCR conditions were one cycle of 30 s at 94 °C, 30 s at 65 °C, and 60 s at 72 °C, followed by 12 cycles with each cycle reducing the annealing temperature by 0.7 °C, and 24 cycles of 30 s at 94 °C, 30 s at 56 °C, and 60 s at 72 °C. (5) The PCR products were separated on 5% denaturing polyacrylamide gel at 80 W for 2.5 h. Gels were visualized using silver staining and dried at room temperature. The adaptor and primer sequences are listed in Table 1.

2.3. SSCP analysis

SSCP analysis was performed according to Bottley et al. [15]. We selected and synthesized 208 primer pairs from 236 primer pairs developed by Bottley et al. [15]. Each primer pair can amplify intron-free wheat sequence of size 200–250 bp mapped exclusively on one of three homoeologous chromosomes belonging to groups 1, 2, 3 or 7 chromosomes. PCR conditions were 35 cycles of 95 °C for 30 s, 59 °C for 45 s and 72 °C for 60 s. The PCR products were denatured in formamide loading buffer before separated on 12% polyacrylamide gel for 18 h at 8 W. Band profiles were visualized using silver staining.

3. Results

3.1. DNA-AFLP polymorphisms between synthetic hexaploid wheat and their parental species

DNA-AFLP band patterns were compared between the 9-generation-old synthetic hexaploid wheat SCAUP/SQ523 (AABBDD) and its two parents SCAUP (AABB) and SQ523 (DD) (Fig. 1). Using 49 primer pairs, 4393 clear bands were obtained (Table 2), among which 3305 bands were polymorphic between two parental lines, 2271 were specific to SCAUP and 1034 specific to SQ523. Further analysis indicated that 30 bands from SCAUP and 280 bands from SQ523 were absent in the synthetic hexaploid wheat (Table 2), or in other words, only 1.32% (30 of 2271) bands from maternal parent SCAUP were disappeared in synthetic stable hexaploid wheat SCA/SQ, but up to 27.08% (280 of 1034) bands from paternal parents SQ523 were disappeared.

Genomic sequence variations in F_1 hybrid (ABD) and newly synthesized hexaploid wheat (AABBDD) S_1 generation between tetraploid wheat DM4 (AABBB) and diploid goat grass Y199 (DD) were also investigated (Fig. 2) by using DNA-AFLP procedure. Using 18 primer pairs, 1639 clear bands were obtained (Table 2), among which 1194 were polymorphic and 445 were monomorphic between two parents. For those polymorphic bands, 782 were specific to maternal parent DM4, and 412 specific to paternal parent Y199. When comparing the synthetic hexaploid wheat S_1 generation with their parents, we found that

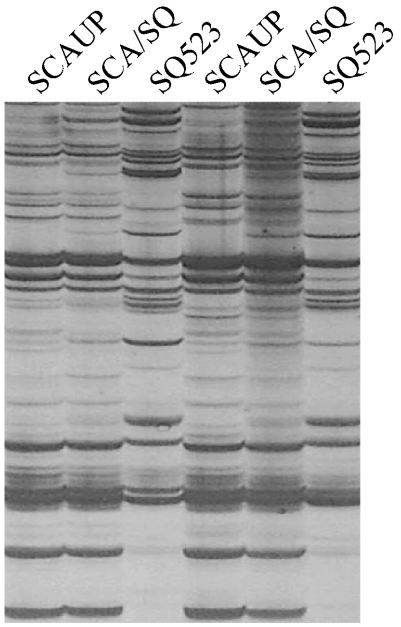

Fig. 1. AFLP pattern of SCAUP/SQ523 and its parents (primer combination E13/MSE6).

9 (1.15%) of 782 from DM4 were disappeared in synthetic hexaploid and 71 (17.23%) of 412 from Y199 were disappeared in synthetic hexaploid. To further determine whether sequence elimination occurred in triploid F_1 hybrid, we compared sequence variation between synthetic hexaploid S_1 generation with the triploid F_1 hybrid. It was found that the majority of the disappeared bands in hexaploid S_1 generation were also absent in the triploid F_1 hybrid, only one bands from Y199 which was present in triploid F_1 hybrid but was absent in synthetic hexaploid S_1 generation.

3.2. SSCP polymorphisms in the genic sequences between synthetic hexaploid wheat and their parents

To determine the sequence changes in the coding regions of the genome during wheat polyploidization, 208 primer pairs developed by Bottley et al. [15] were used to amplify the DNA from the synthetic hexaploid wheat and their parental species for SSCP analysis. Using genomic DNA of synthetic hexaploid SCAUP/SQ523 and its parental lines as templates, we analyzed each homoeoallele from three genomes. The results showed that all the homoeoalleles from D genome can be amplified by these 208 primer pairs from the DNA template of diploid parental species SQ523 (DD genome), but 5 (2.4%) alleles were absent in the synthetic hexaploid wheat SCAUP/SQ523, which included BG313738 (Fig. 3), BF483382, BG274576, BF145580 and BE489901. However, all the homoeoalleles from the maternal parental species SCAUP (AABB genome) could be detected in the synthetic hexaploid wheat.

The newly synthesized hexaploid wheat (DM4/Y199) S_1 generation, F_1 hybrid and their parental species lines were

Table 2
AFLP banding pattern of the synthetic hexaploid and their parental lines

Hybrids	Total bands[a]	Polymorphic bands in parents[b]		The number (rate) of disappeared bands in synthetic hexaploid	
		♀	♂	♀ (%)	♂ (%)
SCAUP × SQ523	4393	2271	1034	30(1.32)	280(27.08)
DM4 × Y199	1639	782	412	9(1.15)	71(17.23)

[a] Total bands were the sum of the bands amplified from both parents.
[b] Polymorphic bands indicate the number of band specific to each parent.

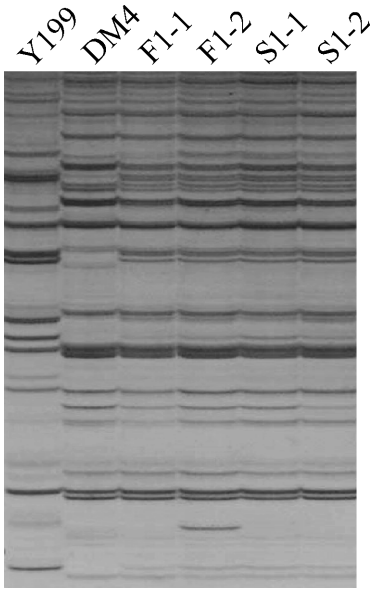

Fig. 2. AFLP pattern of DM4/Y199 and its parents (primer combination E4/MSE2).

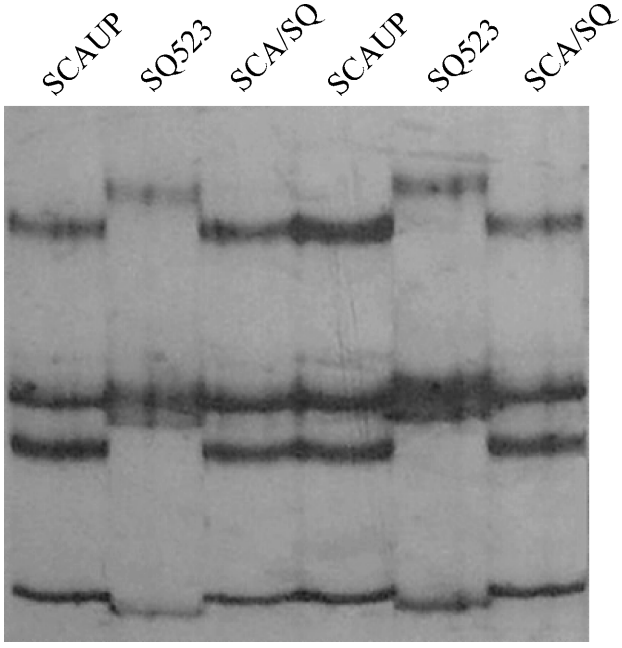

Fig. 3. SSCP analysis in SCAUP/SQ523 and its parents by primer pair BG313738.

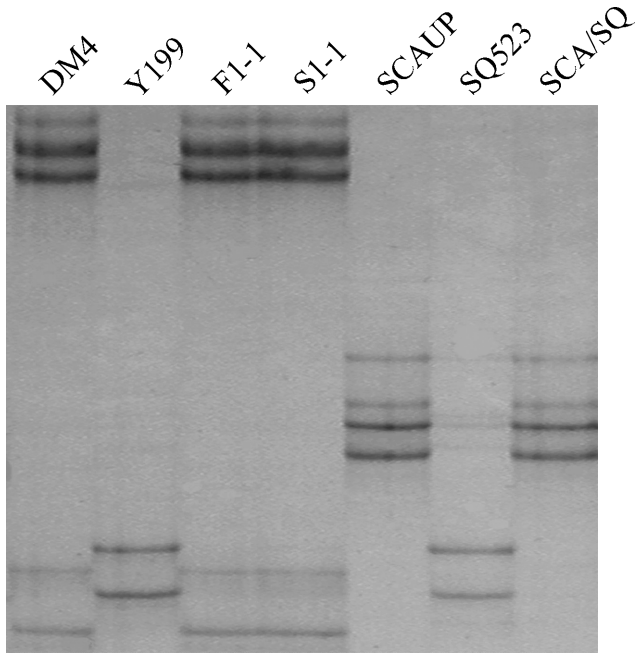

Fig. 4. SSCP analysis in two different synthetic hexaploid and their parents by primer pair BE489901.

also used for SSCP analysis. We found that among the five alleles derived from D genome parent which were absent in the synthetic hexaploid wheat SCAUP/SQ523, four alleles (BF483382, BG274576, BF145580, BE489901) derived from D genome of parent Y199 were also absent in the synthetic hexaploid wheat DM4/Y199 S_1 generation and F_1 hybrid (See Fig. 4, showing the SSCP analysis for primer pair BE489901). However, the allele amplified by primer pair BG313738 from D genome parent was present in the newly synthesized hexaploid wheat DM4/Y199 S_1 generation and F_1 hybrid, but absent in the stable synthetic hexaploid wheat SCAUP/SQ523.

4. Discussion

Allopolyploidization is an important process of speciation in plant kingdom, which generate two forms of genomic "shocks" in the newly formed allopolyploid species: hybridity, in which two divergent genomes are joined together to form one nucleus; and polyploidy which results in duplicated genomes [10]. In response to these two genomic shocks, the genomes of the newly formed allopoly-

ploids react in a burst of genomic reorganizations and modifications. These changes include structural rearrangements in both chromosome [17] and sequence levels [7,18], changes of gene expression [3,12,19], activation of transposons [12,13,20], and amplification, reassortment or elimination of highly repetitive sequences [21] and low-copy sequences [4,5,10]. Previous studies [4–7] indicated that significant sequence elimination occurred during polyploidization in wheat and *Brassica*. Liu et al. [5] found that, in the synthetic amphiploids, the sequences were eliminated from the genome of the lower ploidy parent. In the present study, by using DNA-AFLP analysis it was shown that about 1% of the loci from the maternal genome (AABB) were eliminated in the stable and the newly formed hexaploid wheat; however, up to 27.08% and 17.23% from paternal genome (DD) were eliminated in 9-generation-old and newly synthesized hexaploid wheat, respectively, indicating that the genome from the lower ploidy parent showed much higher levels of change during the allopolyploid formation, which was consistent with previous studies [5]. The possible explanation could be that since tetraploid parent (AABB) has already experienced polyploidization process which was accompanied by genomic reorganizations and modifications of two different genome A and B, they can acclimate itself to the second genomic shocks of allopolyploidization during hexaploid formation.

We also analyzed the sequence changes in the coding regions of the genome during wheat polyploidization by using 208 primer pairs developed by Bottley et al. [15], each corresponding to single-copy gene. It was found that four (1.92%) homoeoalleles (BF483382, BG274576, BF145580 and BE489901) from paternal genome (D genome) were absent in two different synthetic hexaploid wheat SCAUP/SQ523 and DM4/Y199; however, all the alleles derived from maternal tetraploid parent could be detected in synthetic hexaploid wheat SCA/SQ. This provided further evidence that the genome from the lower ploidy parent (DD) showed much higher levels of change during allopolyploidization. In contrast to the results by using DNA-AFLP analysis, the rate of sequence variations detected by SSCP analysis was much lower, 2.4% in stable synthetic hexaploid and 1.92% in newly synthesized hexaploid, suggesting that the frequency of sequence variation in the coding regions were much lower than in the genomic sequences, and the most eliminated sequences detected by DNA-AFLP analysis might represent non-coding sequences, especially in repetitive DNA sequences.

Liu et al. [5] indicated that allopolyploid formation in wheat was accompanied by rapid nonrandom elimination of specific low-copy noncoding DNA sequences. Subsequently, by using 43 coding sequences as probes, Liu et al. [14] found that "loss and/or gain" of fragments in the nine amphiploids were attributed to DNA methylation. SSCP analysis in the present study showed that four homoeoalleles from paternal diploid genome were absent in two different synthetic hexaploid wheat SCA/SQ and DM4/Y199, suggesting that changes in the intron-free DNA sequences during polyploidization also occurred. Moreover, bioinformatic analysis indicated that three of the four above-mentioned genes were located in one cluster on the chromosome 2DL (data not shown). Therefore, we concluded that the changes in intron-free DNA sequence during hexaploid wheat evolution might be selective but not a randomized process, which is worthy for further investigation.

Based on the specificity and distribution in the three homoeologous genomes of hexaploid wheat, the DNA sequences can be classified into the following four categories [22]: (1) Non-specific sequences, which map to several or to all chromosomes of the A, B and D genomes; (2) group- or homoeolog-specific sequences, which map to the homoeologous chromosomes of the A, B and D genomes; (3) genome-specific sequences (GSS), which map to several or to all chromosomes of only one of the three genomes; (4) chromosome-specific sequences (CSS), which are located on one of the specific chromosomes. During allopolyploidization, elimination of different sequence could occur in different generation: eliminations of GSSs start in F_1 hybrid and finish in the first and second generations of the allopolyploids, which could be resulted from hybridity. Eliminations of CSSs start in S_1 and could be resulted from allopolyploidy [10]. In the present study, by using gene-specific primers for SSCP analysis, it was found that sequence changes in four genes started in F_1 hybrid, suggesting that these changes could be resulted from hybridity. However, it is worth to note that one gene BG313738 was present in the newly synthesized hexaploid wheat DM4/Y199 S_1 generation and F_1 hybrid, but absent in the 9-generation-old synthetic hexaploid wheat SCAUP/SQ523, suggesting that the elimination of gene BG313738 occurred during the stabilization of synthetic hexaploid wheat.

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