Genomic shock induced genetic and epigenetic changes in homoeologs of class ABCDE MADS-box genes in hexaploid wheat

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GENOMIC SHOCK IN POLYPLOIDY

Polyploidy, the duplication of a single genome (autopolyploid) or the combination of two or more divergent genomes (allopolyploid), has occurred frequently during the evolutionary history of flowering plants [1]. It is known that the polyploid genome displays dynamic changes in DNA sequences and gene expression patterns, which are caused by "genomic shock" [2]. Bread wheat (*Triticum aestivum*) is a hexaploid species with a genomic constitution AABBDD that originated from three diploid ancestral species: the A genome came from *T. urartu*, the B genome from *Aegilops speltoides* or another species classified in the Sitopsis section, and the D genome from *Ae. tauschii*. In synthetic allopolyploid wheats, non-random and reproducible elimination of non-coding and low-copy DNA sequences was found in early generations after allopolyploidization [3]. Furthermore, genome-wide alteration of the DNA methylation pattern was also identified [4]. These observations indicate that genomic shock induces genetic and epigenetic changes in the polyploid wheat genome. Allopolyploidization leads to the generation of duplicated homoeologous genes (homoeologs) rather than of paralogous genes (paralogs). Consequently, the hexaploid wheat genome contains triplicated homoeologs derived from the ancestral diploid species. There are three possible evolutionary fates for homoeologs: functional diversification; gene silencing; or, retention of the original or similar function [5]. How does genomic shock affect homoeologs? Is there any general rule regarding which homoeologs of which ancestral genome are changeable? Moreover, what is the mechanism of homoeolog-specific regulation? To answer these questions, we are studying gene structures and expression profiles of three homoeologs of MADS-box genes, as they provide a good model system of genes associated with a particular biological program, in this case floral organ formation.

CLASS ABCDE MADS-BOX GENES IN WHEAT

The ABCDE model explains how floral organ identity is defined by five classes of homeotic genes, called A, B, C, D and E. Class A and E genes specify sepals in the first floral whorl, class A, B and E genes specify petals in the second whorl, class B, C and E genes specify stamens in the third whorl, class C and E genes specify carpels in the fourth whorl, and class D and E genes specify the ovule in the pistil [6]. In previous studies in wheat, we identified three class B genes, *WAP3* (*wheat APETALA3*) [7], *WPI-1* (*wheat PISTILLATA-1*) and *WPI-2* [8], two class C genes, *WAG-1* (*wheat AGAMOUS-1*) [9] and *WAG-2*, two class E genes, *WSEP* (*wheat SEPALLATA*) and *WLHS1* (*wheat Leafy Hull Sterile 1*) [10], and one class D gene, *WSTK* (*wheat SEEDSTICK*). The wheat *AP1*-like gene *WAP1* (*wheat AP1,* identical to *VRN1*) apparently does not have a class A function but acts during the phase transition from vegetative to reproductive growth in diploid [11] and hexaploid wheat [12,13,14].

DIFFERENTIAL EXPRESSION OF MADS-BOX HOMOEOLOGS

We used homoeolog-specific real-time PCR to examine the expression profiles of the three homoeologs of various MADS-box genes at different stages of inflorescence development and in different parts of the floral organ. We observed significant differences in the expression levels of the three homoeologs of class B MADS-box genes (Figure 1). For *WAP3* and *WPI-1*, the A genome homoeolog was predominantly expressed. However, for *WAP3*, the B genome homoeolog exhibited high expression restricted to the lodicule. For *WIP1-2,* there was no significant difference in the amounts of transcripts of A and D genome homoeologs, but that of the B genome homoeolog was clearly lower. Overall, these results indicate that expression of class B genes was differentially regulated. Homoeolog-specific expression patterns have also been observed for class C and class D genes.

GENETIC AND EPIGENETIC CHANGES IN A CLASS E MADS-BOX GENE

We investigated three homoeologs of two wheat class E type genes, *WSEP* (*wheat SEPALLATA*) and *WLHS1* (*wheat Leafy Hull Sterile 1*) [10]. Analyses of gene structure, expression patterns and protein functions showed no alterations were present in the *WSEP* homoeologs. In contrast, the three *WLHS1* homoeologs showed genetic and epigenetic alterations. The A genome homoeolog of *WLHS1* (*WLHS1-A*) contained a large novel sequence in place of the K domain sequence (Figure 2). A yeast two-hybrid analysis and a transgenic

experiment indicated that the WLHS1-A protein had no function. *WLHS1-B* and *WLHS1-D*, located in the B and D genomes, respectively, had a complete MADS-box gene structure, but *WLHS1-B* was predominantly silenced by cytosine methylation. Consequently, of the three homoeologs, only *WLHS1-D* functioned in hexaploid wheat. To obtain an insight into the origin of the sequence change, we compared the *WLHS1-A* locus in diploid, tetraploid and hexaploid species of *Triticum*. We detected the variant *WLHS1-A* in the tetraploid wheat ssp. *dicoccum* (AABB) and the hexaploid wheat ssp. *macha* (AABBDD) and ssp. *aestivum* (AABBDD) (Figure 3). These findings indicate that the sequence change in *WLHS1-A* occurred in a lineage of ssp. *dicoccum* (cultivated tetraploid), and that hexaploid wheats originated on multiple occasions from crosses of the domesticated tetraploid wheats and the D genome donor, *Ae. tauschii*.

In addition, we examined gene-specific silencing of *WLHS1-B* homoeologs in diploid and tetraploid species by expression analysis of *WLHS1* genes in the tetraploid wheat ssp. *durum*, ssp. *dicoccoides*, and ssp. *dicoccum* (all AABB), and in diploid *Ae. speltoides* (SS, possibly modified BB). Silencing of *WLHS1-B* was not observed in the tetraploid species with a B genome or in *Ae. speltoides*, which belongs to the same Sitopsis section as the putative B genome donor of tetraploid and hexaploid wheat. This suggests that the epigenetic down-regulation of *WLHS1-B* occurred at the origin of the hexaploid genome, in which the AB genome was combined with the D genome.

CONCLUSION

Genomic shock induced genetic and epigenetic changes in homoeologs of MADS-box genes in wheat, which resulted in the differential expression of homoeologs. The expression patterns of the homoeologs of MADS-box genes indicated there was no rule of choice, i.e. no rule that determined which homoeologs from which genomes were chosen for altered expression. As to the meaning of the alteration of expression patterns of homoeologs in the determination of floral organ identity, this remains the biggest puzzle of the wheat ABCDE model. In this study, we used hexaploid wheat cv. Chinese Spring. Investigations of other cultivars would provide useful information about this subject.

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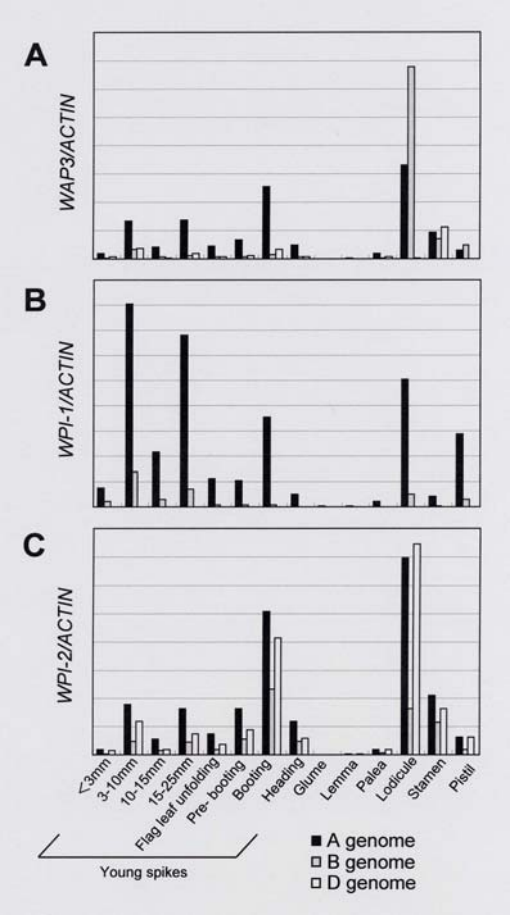

Figure 1. Expression analysis using real-time PCR of the three homoeologs of the wheat class B MADS-box genes *WAP3* (A), *WPI-1* (B) and *WPI-2* (C). *ACTIN* was used as the endogenous control. Total RNAs were isolated from spikes of CS plants at various developmental stages and from various floral organs at the booting stage.

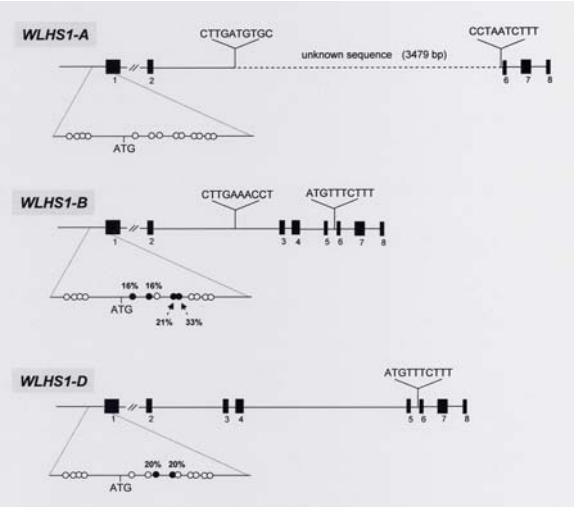

Figure 2. Gene structures and distributions of methylated cytosine in the 5'-region of the three homoeologs of *WLHS1*. Numbered black boxes indicate exons and lines indicate introns. The location of the 'unknown' sequence is indicated by the broken line. The relative methylation status of each CpG/CpNpG site in the 5'-regions of the *WLHS1* homoeologs is indicated by the percentage of methylated cytosines. ●, methylated; ○, unmethylated

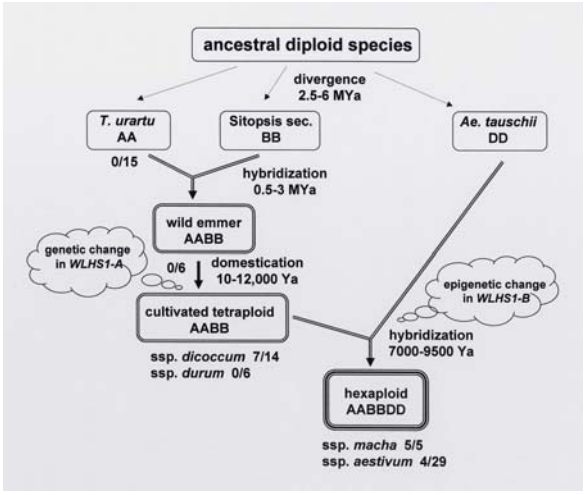

Figure 3. Evolutionary history of polyploid wheat.

A genetic change to *WLHS1-A* occurred in a lineage of ssp. *dicoccum* (cultivated tetraploid), and an epigenetic change in *WLHS1-B* occurred at the origin of hexaploidy. The frequencies of lines with variant *WLHS1-A* are indicated by the fractions after the ssp. name.