Rapid Elimination of Low-Copy DNA Sequences in Polyploid Wheat: A Possible Mechanism for Differentiation of Homoeologous Chromosomes

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ABSTRACT

To study genome evolution in allopolyploid plants, we analyzed polyploid wheats and their diploid progenitors for the occurrence of 16 low-copy chromosome- or genome-specific sequences isolated from hexaploid wheat. Based on their occurrence in the diploid species, we classified the sequences into two groups: group I, found in only one of the three diploid progenitors of hexaploid wheat, and group II, found in all three diploid progenitors. The absence of group II sequences from one genome of tetraploid wheat and from two genomes of hexaploid wheat indicates their specific elimination from these genomes at the polyploid level. Analysis of a newly synthesized amphiploid, having a genomic constitution analogous to that of hexaploid wheat, revealed a pattern of sequence elimination similar to the one found in hexaploid wheat. Apparently, speciation through allopolyploidy is accompanied by a rapid, nonrandom elimination of specific, probably noncoding DNA sequences at the early stages of allopolyploidization, resulting in further divergence of homoeologous chromosomes (partially homologous chromosomes of different genomes carrying the same order of gene loci). We suggest that such genomic changes may provide the physical basis for the diploid-like meiotic behavior of polyploid wheat.

ALLOPOLYPLOIDY has played a major evolutionary role in the formation of many plant species (STEBBINS 1950, 1971; SOLTIS and SOLTIS 1993, 1995). Yet, little information exists about the nature of genomic changes that are required for successful speciation via allopolyploidy. In particular, the mechanism by which several genomes coexist in the same nucleus is largely unknown. This mechanism may involve adjustments in DNA sequences that differentiate between the constituent genomes of an allopolyploid plant. We have addressed this issue in wheat, which provides a classical example of evolution through allopolyploidy. Bread wheat is a hexaploid species (2n = 6x = 42; genomes AABBDD) that originated from hybridization events involving three different diploid progenitors classified in the genera Triticum and Aegilops (Figure 1) (for review see FELDMAN et al. 1995). On the basis of genetic similarities, the 21 pairs of homologous chromosomes of bread wheat (seven pairs of each genome) fall into seven homoeologous groups, each containing one pair of chromosomes from the A, B, and D genomes, respectively (SEARS 1954). Hence, homoeologous group 1, for example, contains the pairs 1A, 1B, and 1D. In each group, homoeologous chromosomes, being derived from a common ancestral chromosome, share a high level of gene synteny but differ from one another by a number of noncoding DNA sequences (FLAPELL 1982). On the basis of specificity and distribution in the constituent genomes of polyploid wheat, we classified the DNA sequences of these polyploid species into the following four categories: nonspecific sequences, group- or homoeologous-specific sequences, genome-specific sequences, and chromosome- or homologous-specific sequences (LIU et al. 1997). To study the nature of changes associated with the divergence of homoeologous chromosomes, we analyzed several low-copy chromosome-specific sequences (CSSs) and genome-specific sequences (GSSs) previously isolated from hexaploid wheat and studied their occurrence in different lines of tetraploid and hexaploid wheat, in lines of their diploid progenitors, as well as in a newly synthesized hexaploid wheat.

MATERIALS AND METHODS

Plant materials: To confirm the chromosome specificity of the studied DNA sequences, several kinds of aneuploid lines were used. Hexaploid nullisomic-tetrasomic lines, each deficient for a given pair of chromosomes (nullisomic) and carrying four doses of one of its homoeologous chromosomes (tetrasomic), were produced by the late E. R. SEARS. The tetraploid nullisomic 5B-diisomic 5D line carries 5D chromosomes from hexaploid wheat substituting for the 5B of the tetraploid. This line was produced and kindly provided by L. R. JOPPA (USDA-ARS Agronomy Department, North Dakota State University, Fargo, ND).

The occurrence of the various CSSs and GSSs was studied in lines of the following diploid and polyploid species of wheat (Triticum) and in lines of their closely related genus Aegilops (classification after VAN SLAGEREN 1994): T. urartu Tumanian
Another set of sequences, including eight CSSs and two GSSs, was isolated from a genomic library of bread wheat species related to the laboratory. This library was produced by amplification and cloning wheat, in this study (Table 1). Five CSSs and one GSS were isolated from our germplasm collection. Line); and five other chromosomes (five lines); and T. urartu (one line); \( \text{T. aestivum} \) (three lines); \( \text{T. turgidum} \) (four lines); and \( \text{T. turgidum} \) (two lines). All these lines were selected from the bread wheat cultivar Chinese Spring (\( \text{VEGA} \)) and diploid parents were kindly provided by the late \( \text{SEARS} \) in our greenhouse, and mapped to specific chromosomal regions (\( \text{LIU} \) et al. 1994, 1997). Evidently, group I sequences do not play an essential role. Their presence in only one diploid progenitor may indicate that they evolved at the diploid level during or after the divergence of the three diploid progenitors from one another. For this reason, additional studies on the distribution of group I sequences among a larger sample of lines from all the diploid species of the Triticeae may illuminate phylogenetic relationships among these species as well as between them and the polyploid species of \( \text{Triticum} \) and \( \text{Aegilops} \).

Group I sequences exhibit high polymorphism at the diploid and polyploid levels (Figure 2a and data not shown). In many sequence-species combinations these sequences are present only in some lines and absent from others (Table 1 and Figure 2a, lanes 7 and 8). Evidently, group II sequences do not play an essential role. Their presence in only one diploid progenitor may indicate that they evolved at the diploid level during or after the divergence of the three diploid progenitors from one another. For this reason, additional studies on the distribution of group I sequences among a larger sample of lines from all the diploid species of the Triticeae may illuminate phylogenetic relationships among these species as well as between them and the polyploid species of \( \text{Triticum} \) and \( \text{Aegilops} \).

The nine group II sequences appear to be relatively conserved, exhibiting little or no polymorphism at the diploid and polyploid levels (Figure 2b, and data not shown). One of these sequences, WPG15, was found non-polymorphic both in the present study as well as in a broader study that included 72 wild and domesticated lines of tetraploid wheat and 34 cultivars of \( \text{Triticum} \) (L. \( \text{HUANG} \), E. \( \text{MILLET} \) and M. \( \text{FELDMAN} \), unpublished data). Moreover, we also found the three group II CSSs isolated in our lab (WPG15, WPG79 and WPG90) in other species of \( \text{Aegilops} \) and in three closely related genera of the tribe Triticeae, \( \text{Secale cereale} \) (rye), \( \text{Aegilops intermedium} \) (wheat grass) and \( \text{Hordeum vulgare} \) (barley), as well as in the more remotely related grass species \( \text{Oryza sativa} \) (rice) (data not shown). The ubiquitous occurrence of these sequences strongly suggests an ancient origin, early in the development of...
Sequence Elimination in Polyploids

TABLE 1

Occurrence of chromosome-specific sequences and genome-specific sequences in hexaploid and tetraploid wheats and in their diploid progenitors

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Designation</th>
<th>Size (kb)</th>
<th>Chromosome-arm location in common wheat</th>
<th>Hexaploids</th>
<th>Tetraploids</th>
<th>Diploid progenitors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T. aestivum (AABBDD)</td>
<td>T. turgidum (AABB)</td>
<td>T. urartu (AA) Ae. speltoides (SS SS = BB) Ae. tawschii (DD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSR1202</td>
<td></td>
<td>0.500</td>
<td>5AL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>3BS</td>
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<td>+/−</td>
<td>+/−</td>
</tr>
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<td>+</td>
<td>+</td>
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<tr>
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<td>3BL</td>
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<td>+/−</td>
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<tr>
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<td>0.211</td>
<td>5BL</td>
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<td>PSR548</td>
<td></td>
<td>1.800</td>
<td>7DL</td>
<td>+/−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>PSR560</td>
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<td>2.700</td>
<td>7DL</td>
<td>+/−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>PSR743</td>
<td></td>
<td>2.200</td>
<td>7AX'</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td></td>
<td>2.800</td>
<td>5BS</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>WPG90</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>2.120</td>
<td>6BX'</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSR551</td>
<td></td>
<td>1.200</td>
<td>2BS, 6BS</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PSR593</td>
<td></td>
<td>2.500</td>
<td>2BS, 4BS, 7BL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WPG176</td>
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<td>0.206</td>
<td>3BL, 4BX', 5BL</td>
<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

+, occurrence in all lines; −, absence from all lines; and +/−, occurrence in some lines.

Group I sequences are defined by their occurrence in only one diploid progenitor; group II sequences are defined by their occurrence in all three diploid progenitors. Sequences PSR743 and PSR551 were found in all diploid species only when some restriction enzymes were used.

The sequence was not yet located to chromosome arm.

Although group II sequences occur in all three diploid progenitors, they hybridized to chromosomes of a single genome in hexaploid wheat: CSSs hybridized to only one chromosome pair (Figure 3, lanes 1–4) and GSSs to several chromosome pairs of the same genome,
indicating elimination from the homoeologous chromosomes of the other two genomes. Probing a newly synthesized hexaploid wheat and its two parental lines with four group II sequences (two chromosome-specific, WPG90 and PSR301, and two genome-specific, PSR551 and PSR593), we further investigated this intriguing phenomenon of occurrence in all three diploid progenitors vs. elimination in the polyploid species. Each of these sequences exhibited a unique restriction fragment length polymorphism pattern in the parental tetraploid and diploid lines that were used to produce the synthetic hexaploid wheat, thus allowing to study their existence in the synthetic line. As shown in Figure 4, all four sequences of the tetraploid parent (genomes AABB) exist in the three different amphiploid plants analyzed, whereas those of the diploid parent (genome DD) are absent.

As a control to the elimination of the CSSs and GSSs, 42 group (homoeologous)-specific sequences, i.e., sequences present in all three pairs of homologues of a given homoeologous group in hexaploid wheat, were hybridized to genomic DNA of the synthetic hexaploid wheat and of its parental lines. The 42 group-specific sequences were selected to represent the proximal and distal regions of the short and long arms of all seven homoeologous groups. It was found that some genomic changes, such as loss of parental hybridization fragment(s) and/or gain of novel fragment(s), occurred in the amphiploid with some of the sequence/ enzyme combinations. However, these changes were not brought about by elimination since for every studied sequence in at least one of the enzyme digests (e.g., Figure 5), the hybridization pattern of the synthetic hexaploid revealed the combination of its two parents. This finding confirmed the integrity of the chromosomal complement of the amphiploid. We thus concluded that the observed elimination of CSSs and GSSs did not result from loss of a chromosome arm or segment.

The data obtained from the newly synthesized amphiploid strongly suggests that further differentiation of homoeologous chromosomes via elimination of low-copy DNA sequences occurs rapidly, soon after the polyploid speciation event rather than by a slow evolutionary process. Moreover, the elimination of a particular sequence is nonrandom, consistently targeting the same genome(s) in the polyploids. This elimination has presumably happened twice in the evolution of bread wheat: initially, with the formation of AABBD tetraploid species, there has been an elimination of CSSs or GSSs from either the A or the B genomes at the tetraploid level; subsequently, with the formation of the hexaploid species, there has been an elimination of D or A/B sequences at the hexaploid level. Results with WPG15 (chromosomal arm 5BL) clearly demonstrate this phenomenon. Although present in all three diploid progenitors, WPG15 is specific to chromosome arm 5BL in polyploids, i.e., it is absent from chromosome arm 5AL in tetraploid wheat and from chromosome arms 5AL and 5DL in hexaploid wheat (Figure 3). (Unfortunately, WPG15 is not polymorphic and therefore could not be used for studying the newly synthesized hexaploid wheat). Such nonrandom elimination of DNA sequences may contribute to cytological diploidization by increasing the divergence of homoeologous chromosomes and might be an essential prerequisite for the successful formation of the new polyploid species.

That newly formed polyploids undergo genomic changes at the molecular level was recently reported by SONG et al. (1995) who found extensive intra- and intergenomic changes at the DNA level in the F2 to F5 generations of amphiploids of Brassica. These changes involved loss and/or gain of parental restriction fragments as well as the appearance of novel fragments. Our data, showing differential and nonrandom elimination of DNA sequences from two of the three homoeologous chromosome pairs, point to a different type of genome change. Unlike the differences between individual plants observed in synthetic Brassica tetraploids (SONG et al. 1995), we found a similar pattern of sequence elimination in all plants tested, both of wild and cultivated species, as well as in a newly synthesized hexaploid wheat. Hence, we conclude that this pattern of sequence elimination occurs through a precisely orchestrated mechanism and may be of adaptive value. According to SONG et al. (1995), the genomic changes observed in Brassica may have resulted either from chromosomal rearrangements due to intergenomic recombination or from DNA methylation. We can exclude the possibility of either intergenomic recombination or DNA methylation as an explanation for the hybridization patterns that we have found. Given the
Sequence Elimination in Polyploids

Figure 4.—Southern hybridization of chromosome- and genome-specific sequences to genomic DNA from a newly synthesized hexaploid wheat and from its two parents. (a) The chromosome-specific sequence WPG90 (located on chromosome arm 5BL) hybridized to BamHI-digested genomic DNA. (b) The genome-specific sequence PSR551 (located on chromosome arms 2BS and 6BS) hybridized to EcoRI-digested genomic DNA. (c) The chromosome-specific sequence PSR593 (located on chromosome arms 2BS, 4BS and 7BL) hybridized to EcoRI-digested genomic DNA. The genomic DNA was obtained from the amphiploid XX340 (genomes AABBDD) and from its two parental lines. The amphiploid (lanes 3–5) was produced by chromosome doubling of the F1 hybrid between tetraploid wheat T. turgidum var. dicocoides (TTD09; genomes AABB) (lane 2) and the diploid Ae. tauschii (TQ01; genome DD) (lane 1). Each of the lanes 3–5 represents a different amphiploid plant. The hybridization fragment(s) found in the D genome of the diploid parent that are marked by arrows are polymorphic to the corresponding fragments of the AB genomes and are absent from the amphiploid. The third fragment of Ae. tauschii in Figure 4a, and the sixth and seventh fragment of this species in Figure 4d (marked by asterisks) are polymorphic to the corresponding fragments of var. dicocoides. However, judging by the intensity of these bands in lanes 3–5, these D genome sequences appear to be absent in the amphiploid. Similar results were repeatedly obtained following digestion with other enzymes. Genomic formulae and size (kb) are indicated.

Figure 5.—Southern hybridization of a group (homoeologous)-specific sequence to genomic DNA from a newly synthesized hexaploid wheat and from its two parents. Dmal-digested genomic DNA from three individual plants of the newly synthesized hexaploid wheat (XX340) (lanes 3–5) and from its two parents, tetraploid T. turgidum ssp. dicocoides (TTD09) (lane 2) and diploid Ae. tauschii (TQ01) (lane 1) was hybridized to sequence PSR370. This group-specific sequence locates at distal regions of the long arm of chromosomes of group 5 (5AL, 5RL and 5DL). Arrow indicates the 5DL fragment. Genomic formulae and size (kb) are indicated.

Presence of the tetraploid parent (ssp. dicocoides) gene system, which suppresses homoeologous pairing (RILEY 1960; SEARS 1976), chromosome pairing leading to intergenomic recombinations would not have occurred in our synthetic amphiploid. With respect to methylation, we digested DNA of the newly synthesized amphiploid and its parental lines with the isoschizomers HpaII and MspI, which recognize a predominant DNA methylation site (CCCG) in plants but differ in methylation sensitivity (SAMBROOK et al. 1989). The blot was then probed with the four group II sequences mentioned above (WPG90, PSR301, PSR551 and PSR593). Only two of the four sequences, namely, WPG90 and PSR551, detected informative polymorphism between the two parental lines in MspI/HpaII digests, and for these two sequences the hybridization fragment(s) of the D genome parent (TQ01) was absent from the amphiploid in both of the enzyme digests (Figure 6). This finding implies that absence of parental hybridization fragment(s) in the amphiploid is not due to methylation changes in a CCGG site within or immediately adjacent to the group II sequences.

Homoeologous chromosomes of the different diploid species of Triticum and Aegilops retain a relatively
Thus, to prevent intergenomic pairing and ensure proper chromosome segregation and, hence, full fertility and disomic inheritance in the polyploid species, an exclusive bivalent pairing of homologous chromosomes is essential. We propose here that such bivalent pairing in polyploid wheat is brought about by two independent systems that complement each other. The first system is based on nonrandom elimination of DNA sequences from one of the two pairs of homoeologous chromosomes in the initial tetraploid wheat and, subsequently, from the third pair of homoeologous chromosomes in the initial hexaploid wheat. These sequences are assumed to be noncoding because sequencing data of clones WPG15, WPG79, WPG90 and WPG176 (not shown) indicate several stop codons and relatively short open reading frames. These elimination events occur instantaneously or soon after the formation of the polyploids and result in accentuating the differentiation among homoeologues, and thus provide the physical basis for the diploid-like meiotic behavior of these polyploids, a critical trait that has contributed to the successful establishment of the polyploids as new species. Preliminary data (Liu et al. 1997 and unpublished data) indicate that the CSSs and GSSs on chromosome arm 5BL are clustered in several regions, interstitial, subterminal and terminal (subtelomeric), which exhibit high homologous specificity (in contrast to other regions that contain group-specific and nonspecific DNA sequences, e.g., retrotransposons). Hence, it is tempting to suggest that these highly chromosome-specific regions may correspond to the chromosomal sites (pairing initiation sites) that play a critical role in homology search and initiation of pairing at first meiotic prophase (Hawley and Arbel 1993). The second system involved in the exclusive bivalent pairing in polyploid wheat is a genomic one consisting of the genes Ph1 and Ph2, which suppress pairing of homoeologues while allowing homoeologues to pair regularly (Riley 1960; Sears 1976; Feldman 1993). This system has probably evolved later, superimposing itself on and thereby reinforcing the already existing system of homoeologous differentiation.

The multiplication of genetic material in polyploid species can lead to overexpression and inefficiency. To reduce redundancy of gene expression, many coding DNA sequences undergo dosage compensation (Galili et al. 1986) or genetic diploidization (Feldman et al. 1986; Soltis and Soltis 1993); the latter is brought about mainly by a slow process of gene inactivation (Soltis and Soltis 1993) (via mutations and/or methylations). Our findings, assumed to be concerned with noncoding DNA sequences, suggest another type of diploidization: rapid elimination soon after the formation of the polyploid. CSSs and GSSs that are targeted for elimination might exert a regulatory control on the physical behavior of chromosomes.

As proposed by others (Song et al. 1995; Soltis and Soltis 1993, 1995; Teutonico and Osborn 1994; Wendel et al. 1995; Rieseberg et al. 1995, 1996; Walbot and Cullis 1985), our work supports the idea of considerable plasticity of plant genomes. It suggests that production of new polyploid species often is accompanied by extensive genomic modifications in a short period of time and that polyploid speciation per se may act as an accelerating evolutionary factor. Based on the example offered by the wheat model, rapid sequence elimination may be a mechanism promoting successful polyploid speciation events. Since several amphiploids that were produced by reciprocal crosses exhibited similar pattern of sequence elimination (unpublished data), and since we found in hexaploid wheat also CSSs and GSSs that are located on chromosome 5AL and 5DL (unpublished data), a possible major role for the cytoplasm in this specific elimination is ruled out. Un-
doubtedly, more studies are required to elucidate the mechanism by which nonrandom elimination of DNA sequences occurs in a short period of time.

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