Quantitative variation of Revolver transposon-like genes in synthetic wheat and their structural relationship with the LARD element

Motonori Tomita*1), Tatsurou Noguchi1) and Taihachi Kawahara2)

1) Molecular Genetics Laboratory, Faculty of Agriculture, Tottori University, 4-101 Minami, Koyama, Tottori 680-8553, Japan
2) Plant Germ-plasm Institute, Graduate School of Agriculture, Kyoto University, 1 Nakajyo, Muko, Kyoto 617-0001, Japan

Revolver is a multi-gene family dispersed through Triticeae genomes like transposons. Revolver is similar to class II transposable elements and shows considerable quantitative variation in wheat and its relatives. The highest copy number of Revolver is found in Secale cereale (RR) and the lowest in hexaploid wheat Triticum aestivum (AABBDD). In this study, Revolver copy numbers were determined in synthetic hexaploid wheat lines from crosses between Aegilops tauschii (DD) and T. turgidum tetraploid wheat species (T. dicoccoides, T. dicoccum, T. carthlicum and T. durum, AABB). Eight out of 18 lines showed significantly lower copies than the sum of their parents and seven lines were equal to the sum, suggesting that polyploidy caused loss of Revolver. Members of the Revolver family also showed structural variation in the 5′ region, especially in length. Revolver did not share any similarity with autonomous transposable elements. However, the long terminal repeats (LTRs) of the non-autonomous large retrotransposon derivative (LARD) in barley, showed 60% homology to both 5′ and 3′ ends of some variants of Revolver. LARD LTRs lack the Revolver region from the first exon to the middle of the first intron resulting in non-coding sequences. Evolutionary relationships between Revolver and LARD are discussed.

Key Words: Revolver, transposon, copy number, synthetic wheat, gene structure, LARD element.

Introduction

In higher eukaryotes, genes required for cellular function can comprise as little as 20% of the genome (Flavell et al. 1977, Grandbastien 1992, Harrow et al. 2009) and occur in islands separated by repetitive DNA sequences, which comprise >80% of the genomes (Barakat et al. 1997, Prak and Kazazian Jr. 2000). With the advent of large-scale DNA sequencing, it has become apparent that transposable elements constitute a large proportion of the repetitive DNA component of eukaryote genomes, that is, 45% of the human genome (Panstruga et al. 1998) and 50–80% of some grass genomes (SanMiguel and Bennetzen 1998, Meyers et al. 2001). Moreover, a significant proportion of an organism’s genome is expressed as regulatory non-coding RNAs, some of which are reconstructed from transposable elements (Feschotte 2008, Siomi and Siomi 2008). Despite the vast DNA databases that exist for higher eukaryotes, most repetitive genomic components other than homologues of known transposable elements or their derivatives, still need to be annotated (Stein 2007, Wicker et al. 2008). We have sought novel genomic components that might be key tools for use as molecular tags to aid the comprehension of huge and complex genomes.

Revolver is a new multi-gene family dispersedly present like transposons in Triticeae genomes (Tomita et al. 2008). An 89bp segment of Revolver that is enriched in the genome of rye was isolated via a genomic subtraction method, which eliminated the DNA sequences common to rye and wheat (Tomita et al. 2009). The entire structure of Revolver was determined using rye genomic clones, which were screened with the 89bp probe (Tomita et al. 2008). Revolver is 2929–3041 bp in length, has 20 bp of terminal inverted-repeat sequences at both ends and contains a transcriptionally active gene encoding a DNA binding-like protein. A putative TATA box is located at base 221, with a cap site at base 261 and a possible polyadenylation signal AATAAA at base 2918. Revolver is similar to class II transposable elements (Bennetzen 1996, Feschotte et al. 2002a, 2002b, Kunze and Well 2002). It is abundant in the genome of the wheat relative, Secale cereale (RR), and is present at intermediate levels in the wheat ancestral species Triticum monococcum (AA), T. turgidum (AABB), and Aegilops tauschii (DD). However, Revolver is rare in the genome of bread wheat, T. aestivum (AABBDD), indicating that Revolver has existed since the diploid and tetraploid progenitors of wheat, and then was probably lost from bread wheat or increased in copy number in the other species (Tomita et al. 2008). The considerable quantitative variation of Revolver among wheat and its relatives strongly indicates that Revolver is transcriptionally active, as it has been either propagated, or differentially lost in recent evolutionary time.
In this paper, we report quantitative variation of Revolver in synthetic hexaploid wheat varieties derived from crosses between tetraploid wheat species T. turgidum (AABB) and Ae. tauschii (DD): T. dicoccoides (AABB) × Ae. tauschii (DD), T. dicoccum (AABB) × Ae. tauschii (DD), T. carthlicum (AABB) × Ae. tauschii (DD), and T. durum (AABB) × Ae. tauschii (DD). Structural divergences of Revolver in genomic DNA were also analyzed, and length variants of the Revolver family were compared with large retrotransposon derivative (LARD) elements of barley. In addition, an anchored amplified fragment length polymorphism (AFLP) approach, called Sequence-Specific Amplification Polymorphism (SSAP) (Waugh et al. 1997), or transposon display, was applied to exploit the chromosome-specific variation related to Revolver.

Materials and Methods

Plant materials

Ae. tauschii (DD) and T. turgidum tetraploid wheat species (AABB) (T. dicoccoides, T. dicoccum, T. carthlicum and T. durum) were used in this study. Ae. tauschii was crossed as a female parent with the tetraploid wheat species and then the F1 genomes were doubled as the result of colchicine treatment to generate 18 synthetic hexaploid wheat lines. The synthetic wheat lines were maintained by self-pollination through 7 generations prior to use in this study. Details of the crosses are listed in Table 1. In addition, a rye (Secale cereale) inbred pure line (IR27) developed in Tottori University, a cultivar of bread wheat, Triticum aestivum Chinese Spring (AABBDD, 2n=42), and a rye 1R-7R chromosome-addition wheat Chinese spring line (2n=44), were used.

Quantitative measurement of Revolver in synthetic hexaploid wheat

In this study, we examined the copy number of Revolver by slot blot analysis in 18 synthetic hexaploid wheat genotypes. Genomic DNA was extracted from fresh leaves by the cetlytrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). Total DNA isolated from 33 genotypes including 18 synthetic wheat lines and their parents was blotted onto hybond-N+ membranes (Amersham). The non-radioactive chemiluminescence method (Gene Images, Amersham) was used for probe labeling, hybridization, and detection of hybridization sites. The Revolver cDNA subfamily I (693 bp, GenBank Accession AB124645), which is conserved in Triticaceae species (Tomita et al. 2008), was used as a probe. The membranes were incubated at 60 °C for 30 min in hybridization buffer and then hybridized with a labeled probe at 60 °C overnight. The membranes were washed in 2×SSC, 0.1% SDS at 60 °C for 15 min, then in 0.1×SSC, 0.1% SDS for 15 min, followed by incubation for 60 min at room temperature in a 10% (w/v) blocking reagent in an antibody wash buffer. The membrane was then incubated in the presence of an anti-fluorescein antibody–alkaline phosphatase (AP) conjugate. The unbound conjugate was removed by three washes in 0.3% (v/v) Tween 20 in an antibody wash buffer at room temperature. Hybridization sites were detected using the CDP-Star detection reagent (Roche). Decomposition of the stabilized dioxetane was

Table 1. Comparison of Revolver copy numbers between synthetic wheat lines and their parental lines

<table>
<thead>
<tr>
<th>Line No.</th>
<th>Synthetic hexaploid wheat crosses</th>
<th>Synthetic wheat lines</th>
<th>Sum of each Tetraploid parents</th>
<th>Diploid parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T. dicoccoides (KU109) × Ae. tauschii (KU2080)</td>
<td>10500 = 10400</td>
<td>8800 1600</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>T. dicoccoides (KU109) × Ae. tauschii (KU20-2)</td>
<td>8600 &lt; 13100</td>
<td>8800 4300</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>T. dicoccoides (KU109) × Ae. tauschii (KU20-2)</td>
<td>16500 &gt; 13100</td>
<td>8800 4300</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>T. dicoccoides (KU109) × Ae. tauschii (KU2083)</td>
<td>5900 &lt; 10400</td>
<td>8800 1600</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>T. dicoccoides (KU109) × Ae. tauschii (KU2074)</td>
<td>7700 &lt; 11000</td>
<td>8800 2200</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>T. dicoccum (KU124) × Ae. tauschii (KU2074)</td>
<td>7300 = 6800</td>
<td>4600 2200</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>T. durum (KU125) × Ae. tauschii (KU2135)</td>
<td>10600 &gt; 6900</td>
<td>5900 1000</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>T. durum (KU125) × Ae. tauschii (KU2135)</td>
<td>7300 = 6900</td>
<td>5900 1000</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>T. durum (KU126) × Ae. tauschii (KU20-1 × KU20-2)</td>
<td>18200 &lt; 21100</td>
<td>16500 4600</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>T. durum (KU134) × Ae. tauschii (KU2076)</td>
<td>7500 &lt; 12900</td>
<td>11000 1900</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2135)</td>
<td>8900 = 8800</td>
<td>7800 1000</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2074)</td>
<td>9200 = 10200</td>
<td>8000 2200</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2074)</td>
<td>8700 &lt; 12100</td>
<td>7800 4300</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2074)</td>
<td>8200 &lt; 10000</td>
<td>7800 2200</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2083)</td>
<td>5600 &lt; 9400</td>
<td>7800 1600</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2083)</td>
<td>5800 &lt; 8900</td>
<td>7800 1100</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2084)</td>
<td>6500 &lt; 9600</td>
<td>7800 1800</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>T. carthlicum (KU138) × Ae. tauschii (KU2135)</td>
<td>14300 &gt; 8800</td>
<td>7800 1000</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>9300 10600 8900 2200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>18200 21100 16500 4600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>5600 6900 5900 1000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
catalyzed by probe-bound AP, and the light emission was exposed on X-ray film and then recorded by a fluoro-image analyzer (Fujifilm FLA-5000). The copy number was calculated based on the most approximate function, which Image Gauge software computed from control slot blot hybridization to a series of measured amounts of Revolver cDNA (0, 10, 100, 1000, 5000, 10 000, 15 000, 20 000, 30 000, 50 000, and 100 000 copies).

**Structural diversity of Revolver**

A single primer for amplification of Revolver genomic DNA was designed from the 3'-flanking region including an inverted repeated sequence of a typical clone of Revolver (Revolver-2). Reaction mixtures contained 10 ng of template genomic DNA, 50 pmole of primer (5'-GTAGTCTCAG GAGTCCCTACCA-3'), 0.4 mM dNTPs, 1× LA PCR buffer II, 2.5 mM MgCl₂, and 0.5 U of LA Taq polymerase (Takara) in a volume of 50 μl. The PCR reaction program consisted of 30 cycles of 30 s at 95°C, 1 min at 70°C, and 3 min at 72°C. The two types of PCR products (3.3 kb, and 4.3 kb) amplified from the rye genome were purified, ligated into the pGEM-T vector (Promega), and then sequenced.

**Revolver Sequence-Specific Amplification Polymorphism (SSAP)**

Three rye-specific Revolver sequences were used to design SSAP primers. These primers and restriction site specific adaptor primers were used to establish SSAP markers for the rye chromosome in a wheat background. Revolver-associated polymorphisms were assigned to rye chromosomes by scoring rye chromosome addition lines. In order to selectively amplify and label fragments containing Revolver and the adjacent host sequence, two rounds of consecutive PCRs were performed.

Genomic DNA was restricted with EcoRI and ligated with the EcoRI adaptor. Pre-selective amplification was carried out using the 17 bp EcoRI adaptor primers containing one selective nucleotide. The total 25 μl volume of reaction mixture contained 30 ng of template DNA, 0.8 μM adaptor primer, 200 μM dNTPs, 1× LA PCR buffer II, and 1 U LA Taq polymerase. Touch-down PCR was applied using the following conditions: 2 min at 95°C, 24 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C. The final elongation step was performed at 72°C for 5 min. Selective amplification was carried out using the pre-selective PCR product as a template. The pre-selective amplification product was diluted 1:10 with distilled water. The selective Revolver primer 5'-AAAGCAGGCCCTAAAAACTCTC-3', which was designed from the second exon, was labeled at the 5' end with the fluorescent dye HEX. The labeled primers were combined with EcoRI adaptor primers extended with two selective nucleotides. The 10 μl mixture included 2 μl of template DNA, 1 μM labeled and 1 μM unlabeled primer, 200 μM dNTPs, 1× LA PCR buffer II, and 1 U LA Taq polymerase. The PCR conditions were 37 cycles of 30 s at 94°C, 30 s at 70°C (−1°C per cycle until 60°C), and 1 min at 72°C. After PCR, the samples were loaded onto a 6% Long Ranger sequencing gel. Electrophoresis was carried out in 1×TBE at a constant power of 40 W.

**Results**

**Quantitative variation of Revolver in synthetic wheat**

Among the Triticeae species, Revolver shows the highest copy number (19 000) in Secale cereale, and the lowest copy number (1000) in T. aestivum (Tomita et al. 2008). In this study, the copy numbers of Revolver in synthetic wheat lines were estimated by slot blot hybridization analysis (Table 1). The Revolver cDNA sub-family I (693 bp), which is conserved in Triticeae species (Tomita et al, 2008), was used as a probe. Synthetic hexaploid wheat lines were obtained from the crosses between Ae. tauschii (DD) and the tetraploid wheat species T. dicoccoides, T. dicoccum, T. carthlicum, and T. durum (AABB). The average copy number for the 18 synthetic wheat lines was 9300 (Table 1). This was significantly higher than the copy number of 2200 for the bread wheat (T. aestivum) cultivar Chinese Spring. The average copy number for the seven tetraploid wheat species (AABB) was 8900, with average copy numbers of 8800 for T. dicoccoides, 4600 for T. dicoccum, 11 100 for T. durum, and 7900 for T. carthlicum. The average copy number for eight lines of Ae. tauschii (DD) was 2200. Several genotypes had a low copy number of less than 1000. The average copy number of Revolver in the 18 synthetic wheat lines (i.e., 9300) was 1300 less than the average of the sum of Revolver copy numbers in their parental lines (i.e., 10 600). This suggests that the hybridization event resulting in polyploidy negatively influenced the maintenance of Revolver copy numbers. Revolver showed considerable variation in copy number in the synthetic wheat lines. Among the synthetic wheat lines, eight out of 18 lines had significantly lower copies of Revolver (by more than 3000 copies) than the sum of copy numbers in their parental plants. These lines were derived from the following crosses: T. dicoccoides (KU109)× Ae. tauschi (KU20-2), T. dicoccoides (KU109)× Ae. tauschi (KU2083), T. dicoccoides (KU109)× Ae. tauschi (KU2074), T. durum (KU134)× Ae. tauschi (KU2076), T. carthlicum (KU138)× Ae. tauschi (KU2083), T. carthlicum (KU138)× Ae. tauschi (KU2083), and T. carthlicum (KU138)× Ae. tauschi (KU2084). Seven lines (T. dicoccoides (KU109)× Ae. tauschi (KU2083), T. dicoccoides (KU124)× Ae. tauschi (KU2074), T. durum (KU125)× Ae. tauschi (KU2135), T. carthlicum (KU138)× Ae. tauschi (KU2135), and T. carthlicum (KU137)× Ae. tauschi (KU2074)), had almost equal copy numbers of Revolver as the sum of the copy numbers in their parental plants, and three other lines (T. dicoccoides (KU109)× Ae. tauschi (KU20-2), T. durum (KU125)× Ae. tauschi (KU2135), and T. carthlicum (KU138)× Ae. tauschi (KU2135)) showed significantly higher copy numbers of Revolver (by more than 3000 copies) than the sum of copy numbers in their parental plants. In
two synthetic wheat lines derived from the same parents (T. dicoccoides (KU109) \* Ae. tauschii (KU20-2)), one synthetic wheat showed a significant increase in Revolver copy number compared with the sum of copy numbers in the parents, while another synthetic wheat showed a significant decrease. The different mode of copy-mobilization among the same cross combinations might be attributed to potential differences in Revolver’s mobility or to unequal in-strand recombinations between adjacent Revolver copies in each synthetic wheat line that is generated.

Structural relationship with LARD element
The 3′-flanking region of a typical genomic clone of Revolver-2 (Tomita et al. 2008) was used as a single primer and it amplified four DNA fragments by PCR (2.3 kb, 2.8 kb, 3.3 kb, and 4.3 kb) from rye, but none from the wheat genome. The genomes of rye and wheat can therefore be easily distinguished using this primer. The four variants were found to contain the region downstream of the second intron, but they have structural modifications in the 5′ region of the first exon (Fig. 1).

One of the length variants, Revolver-3 (Tomita et al. 2008), comprises a total length of 4269 bp, and at the 3′-end it has a region of 2112 bp from the middle of the first intron of Revolver through the third exon and reaching to the 3′ terminal region (Fig. 1). At the 5′ end, Revolver-3 has a homologous region of 149 bp including the inverted repeat sequence. In the 2 kb region between these sequences, Revolver-3 lacks the region from the first exon to the middle of the first intron. However, Revolver-3 includes a region of 370 bp showing 65% homology to the sequence of the LARD LTR-1 transposon (4960 bp), which is regarded as a solo-LTR (long terminal repeat) of the non-autonomous retrotransposon element LARD (large retrotransposon derivative) in barley (Kalander et al. 2004). In the region from 612 to 2157 bp at the 5′ end of Revolver-3 and the region from 599 to 2814 bp of the LARD LTR-1, there exist short repetitive sequences and both sequences exhibit 53% homology, but no homology was observed with a typical Revolver consensus sequence (Fig. 2). Revolver-3 and LARD LTR-1 have a unique consensus region at the 5′ end, and overall they exhibited high homology.

The LARD LTRs include the region homologous to both 5′ and 3′ ends of Revolver (Fig. 2). At the 5′ terminus, LARD LTRs have the region of 109–123 bp homologous to the region upstream of the transcription initiation site of Revolver. At the 3′ terminus, LARD LTR-1 has a region of 2146 bp showing 60% homology to Revolver, from the middle of the first intron to the 3′ terminus of Revolver. However, no homology to Revolver was observed in the region from 599 to 2630 bp of LARD LTR-1. As for the region of about 2 kb between these end regions, LARD LTRs lack the region from the first exon to the middle of the first intron. LARD LTRs have sequences not present in Revolver in place of the first exon of Revolver that result in non-coding sequences.

Revolver-4 (Tomita et al. 2008) consists of 3219 bp, and at the 3′ end it has a region of 1806 bp extending from immediately before the second exon to the 3′ terminus of Revolver (Fig. 1). However, at the 5′ end, the region homologous to Revolver is limited to only 101 bp at the 5′ terminal. As mentioned above, the members of the Revolver family showed considerable length variation, which was attributed to structural changes at the first exon.

Revolver SSAP
SSAP is an anchored AFLP approach that amplifies the

![Fig. 1. Structural relationship between non-autonomous elements of Revolver and the LARD element. Non-autonomous elements of Revolver (Revolver-3 and Revolver-4) and LARD share the second intron of Revolver and its downstream region, but have considerable structural changes at the 5′-end. Boxes and lines represent exons and introns, respectively, for Revolver, Revolver cDNA, Revolver-3, Revolver-4 and LARD are indicated. Dashed lines in Revolver-3, Revolver-4 and LARD represent structural variations that result in non-coding sequences. Numbers represent base pairs.](image-url)
Quantitative variation of *Revolver* transposon-like genes in synthetic wheat

Revolver transposon-like genes in synthetic wheat region between a transposon-specific primer and a nearby cleaved restriction site to which an oligonucleotide adaptor has been added (Waugh et al. 1997). SSAP or transposon display, was applied to exploit the variation in the sequences flanking the insertion site of *Revolver*. The regions between the *Revolver* family and adjacent EcoRI-cleaved host sites were amplified by a *Revolver* primer labeled with fluorescent dye and an EcoRI adaptor primer. The SSAP procedure based on *Revolver* amplified numerous genomic DNA fragments from an inbred rye line and callus derived from it. The SSAP banding patterns included several callus-specific SSAP fragments in the genome of rye but not in that of wheat. It also amplified numerous DNA fragments from the rye chromosome addition wheat lines including rye chromosome-specific SSAP products (Fig. 3). Since these amplified fragments contained *Revolver* sequences at one end and a host restriction site at the other, the callus-specific or chromosome-specific SSAPs were determined to be related to *Revolver* sequences. The callus-specific SSAPs driven by *Revolver* suggest mobilization of *Revolver* in the rye genome.

Discussion

**Quantitative variation of *Revolver* in wheat polyploidy**

*Revolver* is enriched in the genomes of wild wheat relatives but rare in the genome of hexaploid bread wheat (*T. aestivum*; 2*n* = 42, AABBDD). *T. aestivum* is an allohexaploid carrying three different subgenomes—A, B, and D—resulting from two independent hybridization events (Dvorak et al. 1988). The first combined the A genome of the wild diploid wheat *T. urartu* (AA) and the B genome, which has the highest genetic similarity to the S genome of *Ae. speltoides* (Dvorak and Zhang 1992, Friebe and Gill 1996, Tsunewaki 1996). This resulted in the tetraploid ancestor of modern *Triticum* species *T. dicoccum*, which hybridized with *Ae. tauschii*, the diploid donor of the D genome, over 8000 years ago, resulting in hexaploid wheat (Feldman et al. 1995). *Revolver* exists as $3 \times 10^4 - 8 \times 10^4$ copies in the wheat ancestral species *T. dicoccum* (AABB), *Ae. tauschii* (DD) and *T. monococcum* (AA), which are closely related to *T. urartu*. However, it is considerably less frequent in *T. aestivum* ($10^3$ copies). In this study, the

![Structural relationship between *Revolver* and the LARD element. LARD LTRs, showed 60% homology to both 5' and 3' ends of some of *Revolver*. LARD LTRs lack the *Revolver* region from the first exon to the middle of the first intron resulting in non-coding sequences.](image-url)

Fig. 2. Structural relationship between *Revolver* and the LARD element. LARD LTRs, showed 60% homology to both 5' and 3' ends of some of *Revolver*. LARD LTRs lack the *Revolver* region from the first exon to the middle of the first intron resulting in non-coding sequences.
average copy number in 18 synthetic wheat lines was significantly higher than the copy number in the bread wheat cultivar Chinese Spring. However, the average copy number in the synthetic wheat lines was less than the sum of their parental lines, suggesting that the polyploidy caused loss of Revolver. Eight lines out of the 18 synthetic wheat lines showed significantly lower copy numbers than the sum of their parental plants. The polyploidy that arises either by duplication of a single genome or by the acquisition of a few genomes from related species (alloploidy) is a major force in the evolution of plants; 50–70% of angiosperms have experienced at least one episode of polyploidy in their history (Leitch and Bennett 1997, Wendel 2000). The combination of A, B and D genomes into a single nucleus may generate more incompatibility than harmony for the propagation of some genes (Feldman. et al. 1997, Ozkan et al. 2001, SanMiguel et al. 2002). Revolver contains a single open reading frame (ORF) encoding a 139 amino acid residue protein, which features a transcriptional regulator able to bind DNA, suggesting that the Revolver element can transpose in a cut-and-paste fashion, like class II transposable elements. In wheat, no transcript of the Revolver gene is observed on a Northern blot (Tomita et al. 2008), and an expressed sequence tag (EST) homologue of Revolver is highly degenerated compared with those of other wheat related species (Tomita et al. 2008). If the Revolver gene activity is related to its ability to reintegrate into the host genome, Revolver may have been eliminated from the wheat genome through a loss of this gene activity after polyploidization, which may have induced incompatibility among the three genomes.

Tetraploid wheat T. durum had a large variation in Revolver copy number, from 5900 to 16500, despite the predominantly selfing nature of this species. Such a large variation has also been observed in wild emmer wheat, T. dicoccoides (AABB) in Israel (Tomita et al., unpublished). Wild emmer wheat is a tetraploid progenitor from which modern tetraploid and hexaploid cultivated wheat varieties are derived. T. dicoccoides deserves to be considered as a potential genetic resource for cereal improvement, because it harbors a rich genetic gene pool for multiple disease resistances, agronomic traits of economic significance, and environmental adaptations (Nevo et al. 2002). The center of distribution and genetic diversity of T. dicoccoides is found in the catchment area of the upper Jordan Valley in Israel and its vicinity (Nevo and Beiles 1989, Peng et al. 2003), where semi-humid and warm climatic conditions prevail. In barley, the selective pressures under hot, dry desert conditions significantly correlate with increasing copy number of the class I transposable element BARE-1 (Vicient et al. 1999, Kalander et al. 2000) and microsatellite sequences (Nevo 2001). Such a correlation between environmental stresses and copy number has not been reported for class II transposable elements. However, these examples suggest the possibility that the extensive quantitative diversity of Revolver in the single species T. durum may be associated with environmental stresses, which influence the propagative activity of Revolver.

Usefulness of Revolver’s biodiversity

Widely distributed transposable elements are the most rapidly evolving fraction of the eukaryotic genome (von Sternberg et al. 1992), because the methylated and heterochromatic states of most highly repetitive elements are more susceptible to sequence changes than genes (Marillonnet and Wessler 1998, SanMiguel et al. 1998). In general, the genomes of higher eukaryotes contain thousands, even millions, of seemingly inactive transposable elements, which have been suggested as a source of interspecific sequence divergence. Species specific repetitive elements serve as genetic tools for developing DNA markers, and PCR entry points are dispersed throughout the genome (Kumar et al. 1997).

Cultivated rye (Secale cereale) has been used as a gene source for wheat and triticale breeding by interspecific chromosome introgression and rearrangement (translocation or substitution). A representative achievement of this type of manipulation is the introduction of a stem rust resistance gene into wheat. The rye genome has potential as a gene resource for future improvement of wheat. Rye-specific repeated sequences have been used as probes to determine alien chromatin and chromosome constitutions in wheat–rye cross-breeding (Guidet et al. 1991, Cuadrado et al. 1997). In this study, the SSAP procedure based on Revolver
amplified numerous genomic DNA fragments from inbred rye and rye chromosome addition wheat (Fig. 3). The SSAP banding patterns included chromosome-specific SSAP products, which are useful chromosome markers. Revolver is an effective resource for developing molecular tags for transferring useful germplasm from rye into the wheat genome. Revolver can be used for fluorescent in situ hybridization (FISH), Southern probing for genotyping, and as a dispersed PCR entry point to amplify multiple rye-specific genomic fragments (Tomita 2008).

Revolver family showed extreme length variation, which was attributed to structural diversities especially around the first exon (Fig. 1). Such a divergence in length is also found in some families of transposons, such as the CACTA family (Wicker et al. 2007), the Mutator family (Lisch 2002) and the MITE family (Zhang et al. 2004), but no homology was detected between Revolver and each of these families. If Revolver is a transposable element, the sequence variants are assumed to be non-autonomous elements. Revolver variants were found to have considerable variations in length. For example, there was a 22% (1050 bp) difference between Revolver-3 and Revolver-4. One of the non-autonomous variants, Revolver-3 showed structural resemblance with LARD element in barley. Revolver and LARD LTRs may be evolutionarily related, but the LARD element is a structural part of the LTR retrotransposon, whereas Revolver is a single gene consisting of an exon-intron structure. Therefore, Revolver is distinguished from LARD LTRs, in which exon 1 and exon 2 of Revolver are replaced by different sequences and the coding region is not present, and whose autonomous element has never been reported. Revolver is attractive as an index of genomic evolution and as a useful chromosome marker for evaluating evolutionary relationships among the Triticeae tribe.

The rye haploid genome contains 3.9 Gb of DNA and is the largest among the Triticeae genomes. Highly repetitive Revolver was visualized over the entire length of rye chromosomes using FISH (Tomita et al. 2008). The copy number of Revolver, calculated by slot blot hybridization with a Revolver probe, was found to be 1.9 × 10^4 per rye genome but only 2 × 10^3 copies in the wheat genome. Generally, angiosperm genomes vary tremendously in size; some species having less than 50 Mb of DNA per haploid genome and others having more than 85 000 Mb (Bennett and Leitch 1995). There is great variability in genome size among the grass family Poaceae: 450 Mb for rice, 2500 Mb for maize, 5000 Mb for barley, and 16 000 Mb for hexaploid wheat (Arunuganathan and Earle 1991). Most of the variation in genome size is attributable to the amount of repetitive DNA, which comprises 80% of cereal genomes (Flavell et al. 1977, Barakat et al. 1997). Cot analysis estimated that repetitive sequences comprise 92% of the rye genome (Flavell et al. 1974), and 24% of these were differentiated in a rye-specific array (Flavell et al. 1977). The accumulation of Revolver has contributed to the enlargement of the rye genome by 1.5%. If Revolver is a mobile element, it is reasonable to suggest that as many as 1.9 × 10^4 copies of the family have been generated and spread throughout the rye genome since the evolutionary event that separated rye and wheat. In this study, Revolver showed considerable variation in copy number in the synthetic wheat lines (Table 1). Of particular note, three synthetic wheat lines had higher Revolver copy numbers than the sum of the copy numbers of their parents.

The considerable variation in Revolver copy number resulting from genome hybridization events suggests the possibility that Revolver propagation activity might therefore be responsive to polyploidization events during the evolution of species. The novel transposon-like gene Revolver may be a dynamic element that has influenced the composition of the Triticeae genomes throughout their evolution.

Acknowledgments

The authors acknowledge the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) for the Grant-in-Aid for Scientific Research (No. 16580004) to M. Tomita that supported this work.

Literature Cited


