New karyotypes of an interspecific hybrid of *Torenia fournieri* and *Torenia baillonii* and its progenies

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Abstract

Although *Torenia fournieri* (2n=2x=18) and *Torenia baillonii* (2n=2x=16) have different chromosome numbers, almost all of the parental chromosomes form bivalents by interspecific pairing during meiosis in interspecific hybrids. Here, we produced another hybrid between the two species and its six BC1F1 progenies (F1 hybrid × *T. baillonii*). These plants had previously unreported chromosome compositions: the total chromosome number was 34, as expected for allotetraploids, but some *T. fournieri* chromosomes were gained and some *T. baillonii* chromosomes were lost. Plants with these new karyotypes grew well and showed different morphologies. This study indicates that two parental genomes in interspecific hybrids share several interchangeable homoeologous chromosomes.

Keywords: chromosome compensation, homoeologous chromosome, *Torenia*

Introduction

*Torenia fournieri* Linden ex E. Fourn. (2n=2x=18) and *Torenia baillonii* Godefroy ex André (2n=2x=16) have different chromosome numbers and different centromere repetitive sequences (Kikuchi et al. 2006). During meiosis, an F1 hybrid between the two species form eight bivalents by interspecific pairing and one univalent from *T. fournieri* (Kikuchi et al. 2006), suggesting that *T. fournieri* and *T. baillonii* share homoeologous chromosomes and *T. fournieri* possesses an extra chromosome.

Chromosome compensation is thought to restore gene dosage balance; it has been reported in several plants such as tomato (Khush and Rick 1967), Tragopogon (Chester et al. 2012) and pearl millet (Saini and Minocha 1981). In an allotetraploid, a missing chromosome is compensated for by the homoeologous chromosome present in the other genome. Xiong et al. (2011) reported that resynthesized allopolyploid *Brassica napus* (AACC, 2n=38) showed homoeologous paring and homoeologous chromosome compensation in the progeny.

In this study, we report the maintenance of chromosome number and aneuploidy for parental chromosomes in all six progeny derived from an aneuploid near the allotetraploid (2n=34) of *T. baillonii* and *T. fournieri*. The results suggest that allotetraploid hybrids of the two *Torenia* species have interchangeable homoeologous chromosomes.

Materials and methods

Plant materials

An interspecific hybrid with 34 chromosomes was developed spontaneously by crossing *T. baillonii* (female) and *T. fournieri* Thai-1 (male) followed by ovule culture. Six backcrossed plants obtained by using *T. baillonii* pollen were grown on ½ Murashige and Skoog medium (50% MS, 1.5% sucrose, 0.2% gelite, pH 5.7) at 25°C under 16-h fluorescent light.

Chromosome slide preparation

To observe mitotic chromosomes, immature flower buds were pretreated in 0.2 mM 8-hydroxyquinoline for 2 h at room temperature and then fixed in acetic acid–alcohol (1:3) for 3–5 days at 4 °C. Chromosome slides were prepared by the enzymatic maceration and squash methods as described by Kikuchi et al. (2006).

Fluorescence in situ hybridization (FISH)

Two species-specific centromere repeats, TCEN in *T. fournieri* and BCEN in *T. baillonii* (Kikuchi et al. 2005) were used as FISH probes to distinguish the parental genomes. TCEN was labeled with digoxigenin-dUTP and BCEN with biotin-dUTP; nick translation mix (Roche) was used for labeling. Hybridization and signal detection were performed according to Kikuchi et al. (2006).

Results and Discussion

An F1 plant produced by interspecific hybridization...
between *T. fournieri* and *T. baillonii* contained 34 chromosomes, as expected for an allotetraploid. However, FISH analysis showed that this plant possessed 19 *T. fournieri* chromosomes and 15 *T. baillonii* chromosomes (Fig. 1A). Thus, the F₁ plant was aneuploid for parental chromosomes. Although phenotypic changes were observed in the plant in comparison with an allotetraploid produced by Jiranapapan et al. (2011), it flowered (Fig. 1A);
phenotypic variation, similar to variation of flowering time by FISH analysis (Fig. 1B–1D and Fig. 1F–G). Such differences in plant shape and flower color, but not in leaf size, were observed among these plants (Fig. 1B–1G, Table 1). However, we could not find such differences among plants with karyotypes indistinguishable by FISH analysis (Fig. 1B–1D and Fig. 1F–G). Such phenotypic variation, similar to variation of flowering time in Brassica (Pires 2004; Schranz and Osborn 2000) and changes in morphological and quantitative characteristics in maize (Lee et al. 1996), may have resulted from different combinations of exchanged homoeologous chromosomes. In F1 hybrids, chromosomes of the two species can pair and form chiasmata during meiosis (Kikuchi et al. 2006). Our FISH analysis with centromere repeat probes could not form chiasmata during meiosis (Kikuchi 2006). However, we could not find such differences among plants with karyotypes indistinguishable by FISH analysis (Fig. 1B–1D and Fig. 1F–G).

Changing in gene dosage in aneuploids might have deleterious effects (Antonarakis et al. 2004), whereas the effects may not be considered lethality by in allopolyploid plant (Sears 1954), diploid plant (Weber 1991), animal (Gearhart et al. 1986) and yeast (Torres et al. 2010). The stability of chromosome number and aneuploidy for parental chromosomes in BC1F1, Torenia plants imply chromosome compensation, even though we could not distinguish the homoeologous chromosomes. Using FISH markers for each homoeologous chromosome, Xiong et al. (2011) demonstrated that the loss of a chromosome in resynthesized Brassica napus was compensated by the gain of the homoeologous chromosome from the other genome. Tragopogon miscellus has two genomes (Dn and Dn0), and the loss of a Dn chromosome is compensated by the gain of the homoeologous chromosome from Dn0 (Chester et al. 2012). Mis-separation of homologs and homoeologs during meiosis is the assumed mechanism that produces karyotypic variation in allotetraploids (Santaguida and Amon 2015; Vousafzai et al. 2010).

In conclusion, our results suggest that homoeologous chromosomes of T. baillonii and T. fournieri can compensate for each other in allotetraploids. Development of homoeologous FISH markers may help to understand homoeologous chromosome compensation in the two Torenia species.

Table 1. Flower color, growth habit, and leaf size of F1 hybrid and six BC1F1 plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>Flower color</th>
<th>Growth form</th>
<th>Leaf size</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 hybrid</td>
<td>Bright yellow-purple</td>
<td>drooping</td>
<td>2.8±0.3ns</td>
</tr>
<tr>
<td>BC1F1-#1</td>
<td>Yellow-purple</td>
<td>semi uprigh</td>
<td>2.8±0.4ns</td>
</tr>
<tr>
<td>BC1F1-#2</td>
<td>Yellow-dark-purple</td>
<td>semi uprigh</td>
<td>3.2±0.2ns</td>
</tr>
<tr>
<td>BC1F1-#3</td>
<td>Yellow-purple</td>
<td>semi uprigh</td>
<td>2.8±0.3ns</td>
</tr>
<tr>
<td>BC1F1-#4</td>
<td>Yellow-purple</td>
<td>drooping</td>
<td>2.5±0.4ns</td>
</tr>
<tr>
<td>BC1F1-#5</td>
<td>Yellow-purple</td>
<td>horizontal</td>
<td>3.5±0.5ns</td>
</tr>
<tr>
<td>BC1F1-#6</td>
<td>Bright yellow-maroon</td>
<td>horizontal</td>
<td>3.3±1.0ns</td>
</tr>
</tbody>
</table>

*ns* No significant differences by Turkey’s test (P>0.05) among F1 and six BC1F1 plants.

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References


