Methodology of tetraploid induction and expression of microsatellite alleles in triploid watermelon

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Abstract

Methods to obtain tetraploid watermelon plants from germinating seeds by treatments with chromosome doubling chemicals have been assayed and some tetraploid plants obtained by such treatments were pollinated with a diploid to obtain triploid watermelons. Three concentrations each of dinitroaniline (12 to 15 mg.L⁻¹) and colchicine (300 to 500 mg.L⁻¹), using three exposure durations (12 to 36 h), were compared for efficiency of tetraploid induction on germinating seeds of five watermelon cultivars. In all five cultivars, the highest percentage of tetraploid plants was obtained using dinitroaniline at concentration of 12 mg.L⁻¹ for 24 hrs. The expression of the microsatellite alleles in a triploid obtained from crossing a tetraploid plant of ‘Sugar Baby’ with diploid ‘Crimson Sweet’ was clearly codominant in most cases.

INTRODUCTION

Tetraploid watermelons, Citrullus lanatus (Thunb.) Matsum. & Nakai, of diverse genetic backgrounds provide a basis for synthesizing a wide range of hybrid triploid watermelons (Zhang et al. 1995). Tetraploids can be induced by applying an aqueous colchicine solution to the growing apex of diploid seedlings (Kihara 1951) or soaking diploid seeds in colchicine solution. However, the frequency of tetraploids in treated populations is low, less than 5 % in most cases (Lower and Johnson 1969). Tetraploid watermelons can also be induced by treatment with dinitroaniline (Ying et al. 1999; Omran 2003).

Molecular markers have been developed in recent years for the purpose of distinguishing among watermelon genotypes (Guerra-Sanz 2002; Levi et al. 2004; Levi and Thomas 2007), but little is known concerning the inheritance or expression of microsatellite loci in hybrid polyploids. The purpose of our work was to find an optimal method for obtaining tetraploid watermelon plants from germinating diploid seeds. Furthermore, one of the tetraploids obtained was crossed with diploid pollen in order to obtain a triploid, for the purpose of observing SSR expression.

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MATERIALS AND METHODS

Plant material

The experiments were carried out in 2004 at the laboratory and farm of the Horticultural Research Institute, Agricultural Research Center in Egypt. Five diploid watermelon cultivars were self-pollinated for two seasons. These were ‘Giza 1’ from the Horticultural Research Institute in Egypt, ‘Sugar Baby’ and ‘Crimson Sweet’ from Seminis (U.S.A.), and ‘Tirg’ and ‘Luo’ from China (Chinese Academy of Tropical Agricultural Sciences). All of them were subjected to treatments for producing tetraploids.

Polyploid production

Germinated diploid seeds were soaked in a dinitroaniline solution (12, 14, or 15 mg/l) in water or in a colchicine solution (300, 400, or 500 mg/l) in water for 12, 24, or 36 hrs. Triploid plants were obtained by pollinating a tetraploid obtained from ‘Sugar Baby’ with pollen of ‘Crimson Sweet’ (Omran 2003).

Cytology

Identification of ploidy level was by chloroplast number, using the methodology of Fassuliotis and Nelson (1992). Ten guard cell pairs were examined per leaf for a total of 30 per regenerated plant (Compton et al. 1996).

Microsatellite analyses

Triploid seeds that were obtained as described above were sown in perlite trays and germinated in a greenhouse together with the parental lines, ‘Sugar Baby’, ‘Crimson Sweet’ and the tetraploid derived from ‘Sugar Baby’. True leaves from the triploid and the parental lines were excised and used for DNA extraction and purification following the method described in Guerra-Sanz (2002).

Primers to obtain polymorphisms of repeat sequence length were used with the PCR conditions described by Joobert et al. (2006). Primers were labeled with the fluorescent dyes 6-FAM, TET and HEX to allow running the samples in an ABI Prism/Applied Biosystems DNA Sequencer/Genetic Analyzer Model# 310.

RESULTS AND DISCUSSION

The percentages of diploid seedlings converted into tetraploids, assessed by the number of chloroplasts per guard cell, are presented for all treatments in Table 1. The optimal treatment for doubling of chromosomes was 12 mg.L\(^{-1}\) of dinitroaniline for 24 hours, in all five cultivars, with percent conversion among the cultivars ranging from 30.3 to 40.0 %. Colchicine was less effective, the optimal treatment being 400 mg.L\(^{-1}\) for 36 hours, with conversion ranging from 22.0 to 27.3 % among the cultivars.

The number of chloroplasts per guard cell in each cultivar before (2\(x\)) and after (4\(x\)) chromosome doubling is presented in Table 2. Apart from these numbers, other anatomical and physiological traits were observed and compared for certifying ploidy level (Omran 2003). The cultivars did not differ significantly among themselves in number of chloroplasts per guard cell at each respective ploidy level.
Table 1. Production of tetraploid watermelon plants (%) using several concentrations of dinitroaniline and colchicine, for 12, 24, and 36 hours.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Soaking period for germinated seeds</th>
<th>Soaking period for germinated seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td>Dinitroaniline concentration (mg.L⁻¹)</td>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td>Colchicine concentration (mg.L⁻¹)</td>
<td>151</td>
<td>141</td>
</tr>
<tr>
<td>1</td>
<td>21.2</td>
<td>22.3</td>
</tr>
<tr>
<td>2</td>
<td>23.2</td>
<td>24.3</td>
</tr>
<tr>
<td>3</td>
<td>22.1</td>
<td>23.2</td>
</tr>
<tr>
<td>4</td>
<td>24.1</td>
<td>25.1</td>
</tr>
<tr>
<td>5</td>
<td>23.2</td>
<td>24.0</td>
</tr>
<tr>
<td>SD</td>
<td>1.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>


Table 2. Number of chloroplasts per guard cell of the diploid cultivars and their autotetraploids obtained from chromosome doubling treatments. Means followed by the same letter are not significantly different according to Duncan’s multiple range test.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of chloroplasts per guard cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2x</td>
</tr>
<tr>
<td>Giza 1</td>
<td>13.3a</td>
</tr>
<tr>
<td>Sugar Baby</td>
<td>12.1a</td>
</tr>
<tr>
<td>Crimson Sweet</td>
<td>12.6a</td>
</tr>
<tr>
<td>Tirg</td>
<td>9.3a</td>
</tr>
<tr>
<td>Luo</td>
<td>10.1a</td>
</tr>
</tbody>
</table>

The SSR alleles obtained from the DNA of the two parents of the triploid, ‘Crimson Sweet’ and the tetraploid of ‘Sugar Baby’, are listed in Table 3. The expression of these alleles, which are expected to behave as codominant, is clear for most of the primers used. MCPI 33, though, is a little difficult for discriminating between the parents. Interestingly, some of these primers (namely CMPI 21 and CMPI 33) gave only one allele in the tetraploid plant, whereas in the diploid ‘Sugar Baby’ two alleles were observed.

Table 3. Alleles of SSR loci amplified for each primer pair of the genotypes assayed. Alleles are expressed in molecular weight (bp).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sugar Baby diploid</th>
<th>Sugar Baby tetraploid</th>
<th>Crimson Sweet</th>
<th>Triploid (^z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCPI 05</td>
<td>205/207</td>
<td>205/207</td>
<td>188</td>
<td>188/207</td>
</tr>
<tr>
<td>MCPI 11</td>
<td>240/256</td>
<td>240/256</td>
<td>256</td>
<td>240/256</td>
</tr>
<tr>
<td>MCPI 12</td>
<td>193/206</td>
<td>193/206</td>
<td>188/191</td>
<td>191/193</td>
</tr>
<tr>
<td>MCPI 21</td>
<td>193/207</td>
<td>193</td>
<td>188/191</td>
<td>191/193</td>
</tr>
<tr>
<td>MCPI 33</td>
<td>272/274</td>
<td>274</td>
<td>272</td>
<td>272/274</td>
</tr>
</tbody>
</table>

\(^z\) F, Crimson Sweet diploid x Sugar Baby tetraploid
In polyploid species, the relationship between the parental genotype and the phenotype as shown by the gel band pattern is less clear-cut than in diploid species, due to the possibilities of different dosages of alleles, and this complicates the situation, as explained by Luo et al. (2000). Moreover, there have been a number of cases in which difficulty was encountered in the use of codominant markers such as SSRs with polyploids (Rodzen and May 2002). Even greater difficulty can occur in autotetraploid plants, such as artificially induced tetraploids of watermelon, a crop for which only limited knowledge on genomics has been obtained so far.

**Literature cited**


