The Psm Locus Controls Paternal Sorting of the Cucumber Mitochondrial Genome

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Abstract

The mitochondrial genome of cucumber shows paternal transmission and there are no reports of variation for mitochondrial transmission in cucumber. We used a mitochondrially encoded mosaic (MSC) phenotype to reveal phenotypic variation for mitochondrial-genome transmission in cucumber. At least 10 random plants from each of 71 cucumber plant introductions (PIs) were crossed as the female with an inbred line (MSC16) possessing the MSC phenotype. Nonmosaic F1 progenies were observed at high frequencies (greater than 50%) in F1 families from 10 PIs, with the greatest proportions being from PI 401734. Polymorphisms near the mitochondrial cox1 gene and JLV5 region revealed that nonmosaic hybrid progenies from crosses of PI 401734 with MSC16 as the male possessed the nonmosaic-inducing mitochondrial DNA (mtDNA) from the paternal parent. F2, F3, and backcross progenies from nonmosaic F1 plants from PI 401734 × MSC16 were testcrossed with MSC16 as the male parent to reveal segregation of a nuclear locus (Psm for Paternal sorting of mitochondria) controlling sorting of mtDNA from the paternal parent. Psm is a unique locus at which the maternal genotype affects sorting of paternally transmitted mtDNA.

Plants possess two organelles, plastids and mitochondria, both of which carry multiple copies of circular DNA that differ greatly in their sizes and structures. The chloroplast genomes of higher plants are of similar size, at approximately 155 kb, and show conserved gene order among distantly related species (Palmer and Stein 1986). In contrast, the mitochondrial genomes of higher plants vary greatly in size and structure among closely related species (Palmer and Herbon 1988; Ward et al. 1981). Brassica hirta has the smallest mitochondrial genome known among angiosperms, at 218 kb (Palmer and Herbon 1987). Watermelon and melon, both members of the Cucurbitaceae, possess great differences in mitochondrial genome sizes, at 330 kb and 2400 kb, respectively (Ward et al. 1981). Although mitochondrial coding sequences are highly conserved, the physical arrangements of mitochondrial genes can change rapidly due to recombination among direct and inverted repeats (Fauron et al. 1995; Small et al. 1989).

Plant mitochondrial-specific mutations include cytoplasmic male sterility in many species (Schnable and Wise 1998), the nonchromosomal stripe (NCS) mutants of maize (Newton and Coe 1986), chm-conditioned mutants of Arabidopsis (Martinez-Zapater et al. 1992), and the mosaic (MSC) mutants of cucumber (Lilly et al. 2001; Malepszy et al. 1996). With the exception of CMS, mitochondrial mutants often condition abnormal growth with striping or mosaic leaf colors due to poor chloroplast development (Malepszy et al. 1996; Newton et al. 1990). In cucumber, somaclonal variants have been identified that paternally transmit a strongly mosaic (MSC) phenotype (Malepszy et al. 1996). MSC plants develop small and distorted cotyledons and leaves with sectoring of green and chlorotic tissues, grow weakly, and produce flowers of reduced size as compared to wild type (Malepszy et al. 1996). The MSC phenotype has been repeatedly recovered after passing the highly inbred (>S18) cucumber line B through cell cultures (Ladyzynski et al. 2002). Independently generated MSC plants possess different rearrangements in their mitochondrial DNA (mtDNA) relative to the nonmosaic parental line B (Bartoszewski et al. 2004; Lilly et al. 2001). When MSC plants are crossed as
in cucumber. In this study we evaluated diverse cucumber paternal transmission, a salient question is whether there is paternal transmission of mtDNA (Mogensen 1996; Neale and Straight 1989). Although the mtDNA of cucumber shows paternal transmission of the chloroplast genome and maternal transmission of the mtDNA (Mogensen et al. 1994). Some gymnosperms show paternal transmission of mtDNA (Bartoszewski et al. 2004; Lilly et al. 2001). mtDNA (Bartoszewski et al. 2004; Lilly et al. 2001).

Table 1. Percentage of nonmosaic F1 progenies from representative paired crosses using the same male flowers from MSC16 to individual female plants from USDA PIs 401734 or 525155 and Straight 8

<table>
<thead>
<tr>
<th>Female</th>
<th>Nonmosaic F1 progenies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 401734-1</td>
<td>54.2</td>
</tr>
<tr>
<td>Straight 8</td>
<td>0.0</td>
</tr>
<tr>
<td>PI 401734-4</td>
<td>64.0</td>
</tr>
<tr>
<td>Straight 8</td>
<td>0.0</td>
</tr>
<tr>
<td>PI 525155-2</td>
<td>38.1</td>
</tr>
<tr>
<td>Straight 8</td>
<td>4.3</td>
</tr>
<tr>
<td>PI 525155-3</td>
<td>29.4</td>
</tr>
<tr>
<td>Straight 8</td>
<td>0.0</td>
</tr>
<tr>
<td>PI 525155-6</td>
<td>20.0</td>
</tr>
<tr>
<td>Straight 8</td>
<td>0.0</td>
</tr>
<tr>
<td>PI 525165-3</td>
<td>72.0</td>
</tr>
<tr>
<td>Straight 8</td>
<td>4.0</td>
</tr>
<tr>
<td>PI 525165-5</td>
<td>20.8</td>
</tr>
<tr>
<td>Straight 8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Plants were randomly selected from plant introductions or Straight 8.

Nonmosaic F1 progenies are occasionally (<5%) produced that possess the mitochondrial polymorphisms of the nonmosaic parental line B, indicating that MSC plants are heteroplasmonic with rearranged mitochondrial genomes and substoichiometric levels of nonmosaic-inducing mtDNA (Bartoszewski et al. 2004; Lilly et al. 2001). Most angiosperms transmit their mitochondria and plastids predominantly from the female parent (Mogensen 1996; Sears 1980; Tilney-Bassett 1976). Occasional biparental transmission of chloroplast DNA has been reported (Corriveau and Coleman 1988; Mogensen 1996) and may be under genetic control (Chiu et al. 1988; Mason et al. 1994; Smith 1989; Tilney-Bassett et al. 1992). Biparental or paternal transmission of mtDNA has been reported for Brachysia napus (Erickson and Kemble 1990), Cucumis melo (Havae et al. 1998), Cucumis sativus (Havae 1997), and Musa acuminata (Faure et al. 1994). Some gymnosperms show paternal transmission of the chloroplast genome and maternal transmission of mtDNA (Mogensen 1996; Neale and Sederoff 1989). Although the mtDNA of cucumber shows paternal transmission, a salient question is whether there exists genetic variation for mitochondrial transmission in cucumber. In this study we evaluated diverse cucumber germplasms for phenotypic variation affecting mitochondrial transmission and studied the genetic basis of mtDNA sorting in cucumber.

Materials and Methods

Phenotypic Evaluations

We used resistance to scab (caused by Cladosporium cucumerinum Ell. & Arth.) as a phenotypic marker for hybridity. Scab resistance in cucumber is inherited as a dominant allele at the Ccm locus (Pierce and Wehner 1990). Seventy-one scab-susceptible U.S. Department of Agriculture (USDA) plant introductions (PIs) were selected with mean disease severity indices (DSIs) greater than 8.0 and no plant with a score lower than 5 (specific PIs listed in Park [2002]). Ten random plants from each scab-susceptible PI were hand-pollinated in the greenhouse with MSC16 as the pollen parent. MSC16 is scab resistant (mean disease severity index of 0.0), homozygous dominant at the Ccm locus (Park 2002), and possesses the paternally transmitted MSC phenotype conditioned by the mitochondrial genome (Lilly et al. 2001; Malepszy et al. 1996). Thirty to 40 days after pollination, cucumber fruits were harvested and the seeds extracted. F1 progenies were evaluated for scab resistance and the normal versus MSC phenotypes as described by Park and Havae (2004) and Lilly et al. (2001), respectively. Phenotypic classes were scored as scab-resistant nonmosaic progenies (from maternal or biparental transmission or paternal sorting of the mtDNA) versus scab-resistant MSC plants (from paternal mitochondrial transmission).

In order to assess environmental effects on mitochondrial transmission in cucumber, we repeated crosses in the greenhouse for at least seven random plants from the five PIs showing the highest frequencies of scab-resistant nonmosaic F1 progenies in initial crosses with MSC16 (Table 1). For each cross, two to three male flowers from one MSC16 plant were used to pollinate a single female flower on individual plants from each PI, and then the exact same male flowers were subsequently used to pollinate a female flower on the scab-susceptible (Park and Havae 2004) nonmosaic cultivar Straight 8. Evaluation of F1 progenies for scab resistance and the MSC phenotype were performed as previously described (Lilly et al. 2001; Park and Havae 2004).

Identification of Mitochondrial Polymorphisms

MSC16 was selected after passage of the nonmosaic, highly inbred (>S18) line B through cell culture (Malepszy et al. 1996). The mtDNA of MSC16 possesses a major deletion relative to B revealed by hybridization of cucumber mitochondrial clone V5 to XhoI digests (Lilly et al. 2001). Hybridization of cox1 (Isaia et al. 1985) to HaeIII digests revealed a restriction fragment length polymorphism (RFLP) between B and MSC16 (2532 bp fragment) versus cucumber population PI 401734 (2042 bp fragment). Scab-resistant nonmosaic F1 progenies from crosses of plants of PI 401734 × MSC16 were evaluated for the V5 and cox1 polymorphisms by DNA blot hybridizations (Bartoszewski et al. 2004). Cotyledon tissues were collected from the seedlings and genomic DNA extracted with the DNeasy miniprep kit (Qiagen, Valencia, CA) following the manufacturer’s instructions.

Genetic Evaluations

We self-pollinated two randomly selected scab-resistant nonmosaic F1 progenies from PI 401734-4 × MSC16 (Table 1). F2 plants were crossed as females with MSC16, and progenies were scored for the frequencies of nonmosaic versus nonmosaic F1 progenies in initial crosses with MSC16 (Table 1). For each cross, two to three male flowers from one MSC16 plant were used to pollinate a single female flower on individual plants from each PI, and then the exact same male flowers were subsequently used to pollinate a female flower on the scab-susceptible (Park and Havae 2004) nonmosaic cultivar Straight 8. Evaluation of F1 progenies for scab resistance and the MSC phenotype were performed as previously described (Lilly et al. 2001; Park and Havae 2004).
MSC plants. F2 progenies were also scored for segregations of spine color on fruits; black spines are conditioned by a dominant allele at the \( B \) locus and white by the homozygous recessive genotype (Pierce and Wehner 1990). MSC16 is \( BB \) and PI 401734 is \( bb \). F2 plants were chosen that showed segregations for MSC versus nonmosaic testcross progenies (two F2 plants) or because they showed only MSC or nonmosaic progenies in the testcrosses (one F2 plant each). These F2 plants were self-pollinated to produce F3 families. One BC family was generated by backcrossing a nonmosaic F1 plant from PI 401734-4 × MSC16 to MSC16. F3 and BC progenies were testcrossed as females with MSC16 and the phenotypes of testcross progenies scored. Goodness-of-fit to genetic models was tested using chi-square analyses.

**Results and Discussion**

**Phenotypic Evaluations**

Plants from 71 scab-susceptible nonmosaic PIs (Park 2002) were crossed as females with scab-resistant MSC16 to produce 212 F1 families from 53 PIs. Crosses were not possible for some PIs because plants were highly androecious. Two replications of 25 randomly selected seedlings from each F1 family were screened for scab resistance and the MSC phenotype. For 58 of the 212 F1 families, all progenies were scab-resistant and mosaic, as expected given paternal transmission of the cucumber mtDNA (Havey 1997; Lilly et al. 2001; Malepszy et al. 1996). For 141 of the 212 F1 families, scab-resistant nonmosaic progenies occurred at relatively low frequencies (≤10%), likely due to sorting of nonmosaic mtDNA from MSC16 (Bartoszewski et al. 2004; Lilly et al. 2001; Malepszy et al. 1996). However, relatively high frequencies (>50%) of scab-resistant nonmosaic progenies were observed in 13 F1 families from randomly selected female plants from PIs 181910, 181942, 220338, 223841, 342950, 401734, 525155, 525161, 525162, 525165, and Ames 3947. Scab-resistant nonmosaic F1 progenies could result from maternal or biparental transmission of the mtDNA, from paternal sorting of nonmosaic-inducing mitochondrial sublimons from MSC16 (Lilly et al. 2001; Malepszy et al. 1996), or from environmental conditions that unfavorably affected pollen-carrying MSC mitochondria. We selected five PIs (220338, 401734, 525155, 525165, and Ames 3947) having at least one plant showing 50% scab-resistant nonmosaic progenies in the initial set of crosses with MSC16 as the male. Random plants from each of these five PIs were hand-pollinated with MSC16 as the pollen parent and, at the same time and with the same male flowers, nonmosaic cultivar Straight 8 was pollinated as the control. For 23 plants (3 from Ames 3947, 5 from 220338, 5 from 401734, 4 from 525155, and 6 from 525165), F1 families were produced both on the PI and Straight 8 plants. Relatively high frequencies of scab-resistant nonmosaic progenies were observed in F1 families from the same PI as in the first set of crossings. These 23 F1 families showed higher percentages of scab-resistant nonmosaic plants in crosses with MSC16 than the testcrosses with Straight 8 (Table 1). This experiment confirmed over two greenhouse environments that phenotypic variation exists in cucumber for frequencies of nonmosaic F1 plants from crosses with MSC16 as the pollen parent.

**Mitochondrial Polymorphisms**

A restriction fragment length polymorphism (RFLP) revealed by \( ace1 \) hybridized to \( HaeIII \) digests distinguishes the mitochondrial genomes of B and MSC16 (2532 bp fragment) from PI 401734 (2042 bp fragment). Ten randomly selected plants from PI 401734 were evaluated for this polymorphism and all possessed the 2042 bp \( HaeIII \) fragment (autoradiogram not shown). All plants from inbred B and MSC16 possess the 2532 bp \( HaeIII \) fragment (Bartoszewski et al. 2004). All 20 scab-resistant nonmosaic F1 progenies from PI 401734 crossed as the female with MSC16 possessed the 2532 bp \( HaeIII \) fragment (10 plants shown in Figure 1A). A major deletion in the mtDNA of MSC16 relative to B is revealed by hybridization of mitochondrial clone V5 to \( XhoI \) digests (Lilly et al. 2001). All but one of 20 scab-resistant nonmosaic F1 progenies from PI 401734 crossed as the female with MSC16 possessed the JLV5 region (Figure 1B). We previously reported that nonmosaic sorters from MSC16 do not always possess the JLV5 region because this deletion is associated with, but not directly responsible for, the MSC phenotype (Lilly et al. 2001). These experiments strongly support the conclusion that normal F1 progenies from PI 401734 × MSC16 resulted from sorting of nonmosaic-inducing mitochondrial sublimons from MSC16 (Lilly et al. 2001).
2001; Malepszy et al. 1996); however, it is also possible that rare nonmosaic-inducing sublimons with the same mitochondrial polymorphisms as line B exist in and were transferred from the maternal parent.

Genetics of Mitochondrial Sorting in Cucumber

F₂ progenies were generated from two random nonmosaic F₁ plants from PI 401734-4 × MSC16. These F₂ progenies were testcrossed as females with MSC16 to score segregations for spine color (B) and frequencies of MSC versus nonmosaic testcross progenies. For both F₂ families, segregations of black (B⁻) versus white (bb) spines fit the expected 3:1 ratio (P = .530 and .505), demonstrating that progenies were sexually derived. Frequencies of nonmosaic versus MSC testcross progenies from the F₂, F₃, and BC families clearly supported a single locus controlling sorting of paternally transmitted mitochondrial sublimons in cucumber (Table 2). We named this locus Psm for Paternal sorting of mitochondria. The most common allele in cucumber (Psm⁺) conditions more than 90% MSC progenies. The allele from 401734 (Psm⁻) conditions more than 90% nonmosaic progenies (Table 2). Crosses of MSC16 to female plants heterozygous at Psm produced both MSC and nonmosaic progenies (Figures 2 and 3). Although this model predicts equal numbers of MSC and nonmosaic testcross progenies from heterozygous female parents, we consistently observed more nonmosaic than MSC progenies (Table 2 and Figure 3). The paucity of MSC progenies from heterozygous female parents was likely due to reduced viability of MSC progenies as reported by Malepszy et al. (1996).

Testcrosses of normal plants with MSC as the pollen parent produce relatively rare (<5%) nonmosaic progenies, due to sorting of nonmosaic-inducing mitochondrial sublimons (Lilly et al. 2001; Malepszy et al. 1996) (Table 1). However, it was not clear whether the occasional appearance of normal progenies was due to low levels of maternal or biparental mitochondrial transmission or sorting of nonmosaic mtDNA from the pollen parent. In this study we selected cucumber plants showing high frequencies of nonmosaic progenies in testcrosses with MSC16 (Table 1) and identified an RFLP distinguishing maternal nonmosaic and MSC16 mtDNA (cox1 polymorphism in Figure 1A). Our genetic studies revealed that a nuclear locus (Psm) in cucumber controls sorting of paternally transmitted nonmosaic mtDNA from MSC16 (Figure 1 and Table 2). We

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Table 2. Segregations and goodness-of-fit to expected ratios for nonmosaic versus mosaic (MSC) phenotypes for segregating progenies testcrossed as females with MSC16

<table>
<thead>
<tr>
<th>Family Pedigree</th>
<th>Generation</th>
<th>Expected</th>
<th>Nonmosaic</th>
<th>Nonmosaic + MSC</th>
<th>MSC</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10029 [(PI401734-4 × MSC16) ⊘]</td>
<td>F₂</td>
<td>1:2:1</td>
<td>10</td>
<td>15</td>
<td>11</td>
<td>.590</td>
</tr>
<tr>
<td>C10030 [(PI401734-4 × MSC16) ⊘]</td>
<td>F₂</td>
<td>1:2:1</td>
<td>10</td>
<td>24</td>
<td>6</td>
<td>.301</td>
</tr>
<tr>
<td>C10078 [(PI401734-4 × MSC16) ⊘]</td>
<td>F₃</td>
<td>1:2:1</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>.233</td>
</tr>
<tr>
<td>C10079 [(PI401734-4 × MSC16) ⊘]</td>
<td>F₃</td>
<td>1:2:1</td>
<td>8</td>
<td>11</td>
<td>7</td>
<td>.707</td>
</tr>
<tr>
<td>C10080 [(PI401734-4 × MSC16) ⊘]</td>
<td>F₃</td>
<td>0:1</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>.707</td>
</tr>
<tr>
<td>C10081 (PI401734-4 × MSC16) × MSC16</td>
<td>BC</td>
<td>1:1</td>
<td>0</td>
<td>9</td>
<td>6</td>
<td>.439</td>
</tr>
</tbody>
</table>

* Numbers of families showing testcross progenies predominately (>90%) MSC, predominately (>90%) nonmosaic, or approximately equal numbers of nonmosaic and MSC phenotypes (see Figure 3 for a representative distribution).
Figure 3. Numbers of mosaic (y-axis) and nonmosaic (x-axis) progenies in families produced by crossing individual F2 plants from [(401734-4 × MSC16) ×] with MSC16 as the male.

have not yet determined if selection of nonmosaic-inducing mtDNA occurs at pre- (Vitart et al. 1992) or postzygotic (Janska et al. 1998) stages. The phenotype conditioned by the \textit{Psm} locus is similar to the nuclear \textit{Ft} locus of common bean (Janska et al. 1998) and the dsm locus of \textit{Arabidopsis} (Abdelnoor et al. 2003), which affect substoichiometric shifting of maternally transmitted mtDNA. However, to our knowledge, the \textit{Psm} locus of cucumber is the first nuclear locus to control sorting of paternally transmitted mtDNA.

Acknowledgments

Names are necessary to report factually on available data, however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. We acknowledge the technical help of Mark Petrashek and funding support of Y. H. Park by the Pickle Seed Research Foundation and G. Bartoszewski by the NATO Advanced Fellowship Programme.

References


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