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ORIGINAL PAPER

Exploiting synteny in *Cucumis* for mapping of *Psm*: a unique locus controlling paternal mitochondrial sorting

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Abstract The three genomes of cucumber show different modes of transmission, nuclear DNA bi-parentally, plastid DNA maternally, and mitochondrial DNA paternally. The mosaic (MSC) phenotype of cucumber is associated with mitochondrial DNA rearrangements and is a valuable tool for studying mitochondrial transmission. A nuclear locus (*Psm*) has been identified in cucumber that controls sorting of paternally transmitted mitochondrial DNA. Comparative sequencing and mapping of cucumber and melon revealed extensive synteny on the recombinational and sequence levels near *Psm* and placed this locus on linkage group R of cucumber and G10 of melon. However, the cucumber genomic region near *Psm* was surprisingly monomorphic with an average of one SNP every 25 kb, requiring that a family from a more diverse cross is produced for fine mapping and eventual cloning of *Psm*. The cucumber ortholog of *Arabidopsis* mismatch repair (MSH1) was cloned and it segregated independently of *Psm*, revealing that this candidate gene is not *Psm*.

Names are necessary to report factually on available data; however, the US 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. This work was supported by USDA-ARS and a graduate fellowship from King Saud University to SAF.

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Introduction

Mitochondrially encoded phenotypes in plants include cytoplasmic male sterility (Hanson 1991), *chm*-induced mutants of *Arabidopsis* (Martinez-Zapater et al. 1992; Sakamoto et al. 1996), non-chromosomal stripe (NCS) of maize (Newton and Coe 1986), and mosaic (MSC) of cucumber (Lilly et al. 2001). These phenotypes are often associated with mitochondrial rearrangements that produce chimeric reading frames (Conley and Hanson 1995). Plants carrying mitochondrial mutations possess both mutant and non-mutant mitochondria (heteroplasmy), which reduces the severity of the mutation or rearrangement (Yamato and Newton 1999). The *chm* locus of *Arabidopsis* has been cloned and encodes a protein (MSH1) important for mismatch repair (Abdelnoor et al. 2003). The mutant allele for MSH1 presumably does not maintain a predominant mitochondrial genome and allows relatively rare sublimons to become more prevalent (Abdelnoor et al. 2003, 2006).

The MSC phenotypes of cucumber are somaclonal variants that appear after passage through cell cultures, are strongly mosaic on cotyledons and leaves, and show paternal transmission (Malepszy et al. 1996). The MSC phenotypes are associated with rearrangements in the paternally transmitted mitochondrial DNA (Havey 1997), presumably

negatively affecting mitochondrial function (Lilly et al. 2001; Bartoszewski et al. 2004). In crosses involving MSC as the pollen parent, relatively few (<5%) progenies show the wild-type phenotype (Malepszy et al. 1996). Restriction fragment length polymorphisms (RFLPs) in the mitochondrial DNA from these relatively rare wild-type progenies confirmed they carry normal mitochondrial DNA of the paternal parent (Lilly et al. 2001). A nuclear locus, named Paternal Sorting of Mitochondria (*Psm*), has been identified that controls sorting of wild-type mitochondrial DNA from paternal parent (Havey et al. 2004). When a maternal plant homozygous for the wild-type (*Psm+*) allele is crossed with MSC16 as the pollen parent, progenies almost exclusively (>95%) show the MSC phenotype. A plant homozygous for the rarer *Psm-* allele crossed with MSC16 as the pollen parent produces almost all wild-type progenies. A maternal plant heterozygous at *Psm* produces approximately equal numbers of MSC and wild-type progenies in crosses with MSC16 as the pollen parent (Havey et al. 2004).

Significant synteny among orthologous genes and markers has been demonstrated in many plant families, including the Solanaceae (Bonierbale et al. 1988), Rosaceae (Dirlewanger et al. 2004), Poaceae (Devos and Gale 1997), and Fabaceae (Cannon et al. 2006). Synteny is useful for construction of comparative genetic linkage maps (Dominguez et al. 2003) and identification of candidate genes after fine mapping (Ku et al. 2001). In *Cucumis*, Katzir et al. (1996) and Danin-Poleg et al. (2000) showed that genetic markers were transferable between melon and cucumber. Danin-Poleg et al. (2000) observed linkage between one pair of markers in cucumber and melon and Park et al. (2004) showed that physically linked genomic regions in melon co-segregate in cucumber, offering the first indications of synteny between these two important *Cucumis* species. In this study, a cucumber family segregating at *Psm* was produced and synteny with melon exploited to map this unique locus. We also cloned and mapped the cucumber ortholog of MSH1 as a candidate gene for *Psm*.

Materials and methods

Development of family segregating for *Psm*

A single plant (#3) from USDA Plant Introduction (PI) 401734 was selected that produced a high frequency of wild-type progenies when crossed as the female with MSC16. This plant was crossed with a single plant (#9) from "Straight 8" (ST8) which produced almost exclusively MSC progenies after crossing with MSC16 as the male. Both parental plants were also self-pollinated. F1 plants were crossed as the female with MSC16 and a single F1 plant (C10037C) was propagated by cuttings and the F2 progenies produced after self-pollination. F2 progenies were self-pollinated and testcrossed with MSC16 as the male. Resistance to scab (*Cladosporium cucumerinum* Ell. & Arth.) was used to confirm hybridity of testcross progenies as described by Havey et al. (2004). Segregations at the *Psm* locus were scored as *Psm+/Psm+* (>95% mosaic testcross progenies), *Psm+/Psm−* (similar numbers of mosaic and wild-type testcross progenies), or *Psm−/Psm−* (>95% wild-type testcross progenies).

Mapping of *Psm*

DNA was isolated from parental plants and 92 F2 progenies from the *Psm* mapping family as previously described (Kennard et al. 1994). We screened for amplified fragment length polymorphisms (AFLPs) using DNAs of 5 individuals each of *Psm+/Psm+* and *Psm−/Psm−*. DNAs (200–500 ng) were digested with *Eco*RI (Promega, Madison,WI) and *Mse*I (New England Biolabs, Ipswich, MA) at 37ºC for 3 h, followed by incubation at 70ºC for 5 min to inactivate the restriction enzymes. *Eco*RI (5′-CTGGTAGACTGCGTACC and 5′-AATTGGTACGCAGAGTCTAC) and *Mse*I (5′-GACGATGAGTCCTGAG and 5′-TACTCAGGACTCAT) adaptors were created by treatments of 95ºC for 5 min; 85ºC for 5 min; 75ºC for 5 min; 65ºC for 10 min; and cooling to room temperature. The adaptors were ligated overnight to the digested DNAs at 16ºC using T4 DNA ligase (Promega). Pre-selective amplification was carried out using pre-amplification primers (Primers reported in Al-Faifi 2007), 1.25 units of Taq polymerase (Promega) with commercial buffer, 0.25 mM dNTPs, and initial extensions at 72ºC for 1 min, then 20 cycles of 94ºC for 40 s, 65ºC for 2 min, 72ºC for 2 min, and final extension at 72ºC for 7 min. The pre-amplification mixture was diluted 1:15 in water before selective amplifications with 37 combinations of fluorescently labeled *Eco*RI primers and non-labeled *Mse*I primers (primer sequences listed in Al-Faifi 2007) at 20 μM. Selective amplification conditions used the same buffer as described above with one cycle at 94ºC for 40 s; 65ºC for 1 min; and 72ºC for 2 min with annealing temperature lowered by 0.7ºC for each cycle over the next 12 cycles. Twenty-three cycles were carried out at 94ºC for 40 s; 56ºC for 1 min; and 72ºC for 2 min, and a final extension at 72ºC for 7 min. Samples were cleaned using CleanSEQ magnetic beads (Agencourt, Beverly MA) and fractionated on an automatic sequencing (ABI3730) machine. Peaks were visualized using GeneScan (ABI, Foster City, CA) software and polymorphisms scored. Linkages between AFLPs and *Psm* were assessed using Mapmanager (Manly et al. 2001) at $P = 0.00001$ allowing for segregation distortion (Lorieux et al. 1995) and the Kosambi mapping function. AFLPs showing linkage to *Psm* were placed on the cucumber map developed by Park et al. (2000).

A RFLP revealed by clone CsP483 (Genbank accession CC144388) was linked to an AFLP that showed linkage to *Psm* (Park et al. 2000). CsP483 was hybridized to high-density filters of a melon BAC library (Luo et al. 2001) and positive BAC clones were selected. These BACs were end sequenced and primers were designed to amplify from genomic DNAs of the parents of the melon mapping family Piel de Sapo and PI 161375 (Gonzalo et al. 2005). Melon BAC ends were mapped in melon using a single nucleotide polymorphism (SNP) amplified with primers (F-ACCAA AGTGGGATTTGGTGA and R-CGGGAAAAGGTAAG ACCGTA). Melon markers from the Gonzalo et al. (2005) map that showed linkage to the CsP483-carrying BAC ends were used to amplify genomic regions from cucumber populations PI 401734-3, ST8-9, TMG1, and *C. sativus var. hardwickii*. In the case that no amplicons were produced from cucumber, melon amplicons were used to select additional melon BACs. Cucumber amplicons or melon BAC ends were sequenced and used to generated genomic amplicons from cucumber and melon, which were screened for polymorphisms.

Melon markers HS_07-C10, A_23-C03, P6.89, and MU27 were derived from MELOGEN sequences (Gonzalez-Ibeas et al. 2007). AEST25, MC219, MC99a, and MC60 were markers derived from RFLPs (Gonzalo et al. 2005) and their sequences are available through MELOGEN (Gonzalez-Ibeas et al. 2007). CMTC168 is an SSR marker (Gonzalo et al. 2005). Primers designed from these melon markers (Table 1) were used to produce genomic amplicons from cucumber, which were sequenced to confirm high (>90%) similarities. These cucumber amplicons were hybridized to high-density filters of a cucumber fosmid library (Meyer et al. 2008) and positive clones selected. Fosmids were mini-preped using Qiagen (Valencia CA) kit and sequenced using transposon insertions following the manufacturer's protocols (Epicenter, Madison WI). Contigs were built from overlapping sequences using Sequencher (Genecodes, Ann Arbor, MI). Primers were designed from the fosmid sequences, genomic amplicons produced, and SNPs identified that segregated in the mapping families TMG1 × ST8 (Park et al. 2000) or PI 401734-3 × ST8-9. Segregations of SNPs were scored after gel purification and direct sequencing of amplicons as previously described (Martin et al. 2005). All linkages were assessed using Mapmanager as previously described.

Cloning and mapping of cucumber mismatch repair

A cDNA library of cucumber was synthesized from leaf tissues of PI 401734 using the SMART cDNA kit (Clontech, Mountain View CA). Primers were designed specific to the carboxyl (5′-TCCACTCACTTGCATGGAATATTT and 5′-AAAATTCCTATGTTTACCA TCAGC) and amino (5′-TGTGGTGGAAAGAGAGAATGG and 5′-ACAAG-TAT GCAAGCATCTATT) ends of MSH1 based on Genbank sequences from bean (AY795558), soybean (AY856369), tomato (AY866434), and *Arabidopsis thaliana* (AY1919303). Gradient PCR (94°C for 30 s, 45–65°C for 1 min, 72°C final extension for 1 min) was used to produce amplicons from the cucumber cDNAs, followed by cloning using the T-Easy (Promega) vector. Cloned amplicons were sequenced and primers designed to amplify the MSH1 genomic region from cucumber, which was sequenced to confirm high similarities with MSH1 and hybridized to a DNA-gel blot (Kennard et al. 1994) of cucumber inbred line B (Malepszy et al. 1996) to estimate copy-number. The genomic amplicon was used to select cucumber fosmids, which were sequenced by transposon insertions as described above. SNPs in genomic regions near MSH1 were mapped in PI 401734-3 × ST8-9 and TMG1 × ST8 (Park et al. 2000) families.

Results

Exploiting synteny with melon to map *Psm*

PI 401734-3 was a single plant that produced a high frequency of wild-type progenies (44 wild-type and 12 MSC = 79% wild-type) in crosses with MSC16 as the male

Table 1 Primer sequences designed from melon markers on linkage group G10 and sizes of cucumber amplicons

Marker	Forward	Reverse	Size (bp)
AEST25	CGACATGGATGAAAGCAAAA	TGGGACATCATCGTTTCTGA	956
MU27	CCAGTGACAAGATTGCAGGA	GAGGTTCATCAGGGACAACAA	2,155
MC60	GATCCCGATTTCCTCTGAATC	TGCAATCTGCAAAATCCTG	2,221
CMTC168	ATCATTGGATGTGGGATTCT	ACAGATGGATGAAACCTTAGG	213
MC219	TTCAGGGATTGGACCCTCTA	TCCAAATCTCTGGCAATCGT	416
MC99a	CAACGACATCACCACTTTG	ATGGTCCAGTAGCGTCCATC	420
P6.89	GCCGCCATATTCTTCACTTC	CGGAAGTTGTGCTTTGTTGA	410
A_23-C03	AGGGCGAGGTCTGATCTGTA	CCCATGGAAAATCCAACAAC	2,301
HS_07-C10	CGATTCCTAGCCCGTGATAA	TTTGTTCAACGCCAAATTGA	601

and was scored as *Psm−/Psm+*. A single plant from the cucumber cultivar "Straight 8" (ST8-9) produced almost exclusively MSC progenies (1 wild-type and 46 MSC = 2% wild-type) after crossing with MSC16 and was scored as *Psm+/Psm+*. These two plants were crossed to produce the F1 family. Testcrosses of F1 progenies with MSC16 revealed 11 heterozygotes (*Psm−/Psm+*) and 8 homozygotes (*Psm+/Psm+*), fitting the expected 1:1 ratio ($P = 0.491$) for a heterozygous genotype for PI 401734-3. F2 progenies were derived from a single F1 plant and were scored as 13 *Psm+/Psm+* (>95% mosaic testcross progenies), 34 as *Psm+/Psm−* (similar numbers of mosaic and wild-type testcross progenies), and 45 as *Psm−/Psm−* (>95% wild-type testcross progenies) (Testcross segregations are reported in Al-Faifi 2007). These F2 segregations did not fit the expected 1:2:1 ratio ($P < 0.001$) because of too many *Psm−/Psm−* progenies (Table 2). We used resistance to scab to confirm hybridity of testcross progenies to eliminate the possibility of pollination errors. Scab resistance is inherited as a dominant allele at the *Ccu* locus (Pierce and Wehner 1990). Both PI 401734 and ST8 are susceptible to scab; MSC16 is resistant (Havey et al. 2004). We inoculated all testcross progenies with *C. cucumerinum* as a phenotypic marker and observed no susceptible progenies, revealing that no pollination errors occurred and all testcross progenies were hybrids with MSC16. In order to further investigate the skewed F2 segregation, we crossed MSC16 as the male to F3 progenies from F2 plants previously scored as *Psm−/Psm−*, *Psm−/Psm+*, and *Psm+/Psm+*. As expected, testcross progenies from F3 plants scored as homozygous at *Psm* showed almost exclusively wild-type or MSC phenotypes (Segregations reported by Al-Faifi 2007). For F3 families from parental F2 plants that were heterozygous at *Psm*, we established homogeneity of errors, pooled segregations, and observed 25:54:22 segregations, which fit ($P = 0.718$) the expected 1:2:1 ratio consistent with segregation of a single locus (Table 2).

AFLPs were identified between parents and F2 progenies scored as *Psm−/Psm−* or *Psm+/Psm+*. A 215-bp fragment amplified by primers E18 (5′-GACTGCGT ACCAATT CCT) and M62 (5′-GATGAGTCCTGAGTA-ACTT) was polymorphic between ST8-9 and PI 401734-3 and segregated 80:12 (presence versus absence) of the fragment, yielding a relatively poor fit to the expected 3:1 ratio (Table 2). This AFLP showed linkage to *Psm* at 4.9 cM ($P < 0.0001$). A similarly sized (219 bp) AFLP revealed by the same primer combination had been previously assigned to linkage group R of cucumber (Park et al. 2000). A RFLP revealed by probe CsP483 and *Eco*RI was linked at 4.8 cM to the E18/M62 AFLP in the TMG1 × ST8 family (Park et al. 2000). This RFLP segregated 30:48:13 in the PI 401734-3 × ST8-9 population, yielding a relatively poor fit to the expected 1:2:1 ratio (Table 2). CsP483 was linked to *Psm* (Fig. 1), confirming that these loci are on linkage group R of cucumber (Park et al. 2000). The size difference for the E18/M62 AFLP (215 bp in PI 401734 and 219 bp in TMG1) was likely caused by different fragment-analysis platforms.

We exploited the genetic map of melon (Gonzalo et al. 2005) to identify additional markers linked to *Psm* in cucumber. CsP483 was used to select three (5B18, 13G21, and 34C4) melon BACs (Luo et al. 2001) carrying strongly hybridizing regions. Primers designed from the melon BAC end sequences (Genbank accessions ER935954 through ER935967) produced genomic amplicons from cucumber (PI 401734-3, TMG1, and ST8-9) and melon (Piel de Sapo and PI 161375), which were sequenced to verify high similarities (>90%). We chose a C/T SNP at position 223 bp in melon amplicons and used DNAs from 14 doubled haploids from the Piel de Sapo by PI 161375 mapping family (Gonzalo et al. 2005) to assign this SNP linkage group G10 of melon

Table 2 Segregations and goodness-of-fit to the expected 1:2:1 or 3:1 ratios for the *Psm* locus of cucumber and linked genetic markers segregating among F2 or F3 progenies from the cross of plant 401734-3 crossed as the female with plant ST8-9

Marker	Gen.	Observed			Expected	
		4[a]	H[a]	S[a]	Ratio	P
Psm	F2	45	34	13	1:2:1	0.000
Psm	F3[b]	25	54	22	1:2:1	0.718
E18/M62-F-215	F2	--80--		12	3:1	0.008
CsP483/E1	F2	30	48	13	1:2:1	0.036
HS_07-C10	F2	33	43	12	1:2:1	0.007
MC60	F2	30	40	14	1:2:1	0.043
MSH1	F2	33	35	19	1:2:1	0.020

[a] *4* allele from PI 401723-3, *S* allele from ST8-5, and *H* heterozygous

[b] Testcross segregations from heterozygous F3 progenies were combined after establishing homogeneity of errors

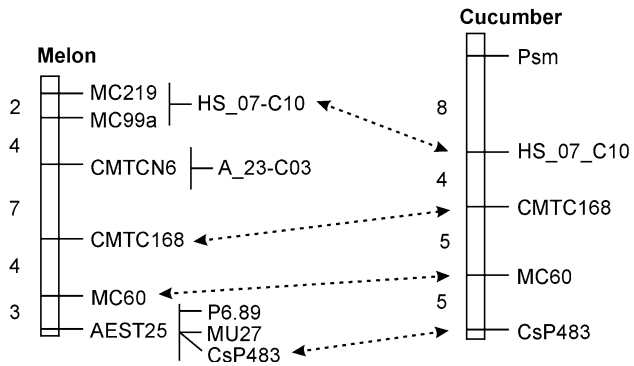

Fig. 1 Comparative linkage arrangements among codominant markers from linkage group G10 of melon and linkage group R of cucumber. Kosambi distances are shown on *left*; markers on *right*. All melon markers shown were evaluated for polymorphisms in cucumber amplicons and flanking genomic regions

(Howad et al. 2005). We selected melon markers spanning approximately 20 cM on linkage group G10 (AEST25, MC60, MU27, P6.89, CMTC168, CMTCN6, A_23-C03, MC99a, HS_07-C10, and MC219) near CsP483 (Fig. 1) and attempted to map these markers in cucumber. All primers designed from the melon markers (Table 1) produced cucumber amplicons except CMTCN6. Cucumber amplicons were sequenced and we observed high sequence similarities (91–94%) between genomic amplicons from melon and cucumber for markers on melon linkage group G10 (Table 3).

Melon marker HS_07-C10 (Gonzalo et al. 2005) produced amplicons from PI 401734-3 and ST8-9 that carried a G/T SNP (Genbank accessions BV725466 and BV725465), which mapped 8.3 cM from *Psm* (Fig. 1). For all other melon markers, the cucumber amplicons were monomorphic among PI 401734-3, ST8-9, and TMG1. In order to screen for polymorphisms in genomic regions adjacent to the monomorphic regions, cucumber amplicons were used to screen the cucumber fosmid library (Meyer et al. 2008). MC60 is a melon unigene from a seedling cDNA library and was mapped in melon as an RFLP (Gonzalo et al. 2005). We used MC60 to select cucumber fosmid 243O18, which was end sequenced. Primers (5′-CAATCTTTTAGGCTGGGAAGC and 5′-TGAGCTTA-CATGGC AACGAC) produced from the fosmid end revealed a C/G SNP between ST8-9 and PI401734-3 (Genbank accessions BV724001 and BV723997), which poorly fit the expected 1:2:1 ratio (Table 2). This SNP mapped 5.5 cM from CsP483 and 18.1 cM from *Psm*. This SNP also segregated in the TMG1 (Genbank accession BV724007) by ST8 family and was linked to CsP483 (Fig. 1). Melon marker CMTC168 is a simple sequence repeat (SSR) (Gonzalo et al. 2005) and the cucumber amplicon was used to select two overlapping cucumber fosmids (56C11 and 75L23). These fosmids were shot-gun sequenced (Genbank accessions EU500869 and EU500870) and primers designed from the fosmid contigs produced amplicons; however, no polymorphisms were identified between ST8-9 and the PI401734-3. A T/C SNP revealed between TMG1 and ST8 (Genbank accessions BV724006 and BV724003) mapped 7.6 cM from CsP483 and 5.1 cM from MC60 (Fig. 1). Primers designed from A_23-C03 (Fig. 1) produced genomic amplicons with no polymorphisms between PI 401734-3 and ST8-9 or TMG1 and ST8. The cucumber amplicon was hybridized to the cucumber-fosmid library and two overlapping fosmids (168G1 and 194J7) were identified. These fosmids were sequenced (Genbank accessions EU500868 and EU500866); however, no polymorphisms were revealed across this genomic region between ST8-9 and the PI 401734-3, nor between TMG1 and ST8.

Table 3 Percent similarities between genomic amplicons from cucumber and melon derived from molecular markers or regions adjacent to these markers on linkage group G10 of melon

Marker	Cucumber	Melon	Similarity (%)
AEST25	1,420	1,450	92
CsP483	823	826	94
MU27	1,956	2,084	92
HS_07_C10	724	742	94
CMTC168 region	5,987	6,012	93
A23 region	4,240	4,296	91
MC219	369	367	92
Mean ± SD[a]			92.6 ± 1.1

[a] *SD* standard deviation

Cloning and mapping of MSH1 as a candidate gene for *Psm*

Amplicons from a cDNA library from PI 401734 were highly similar to the amino and carboxyl ends (81 and 55%, respectively) of MSH1 (Genbank accessions EX150320 and EX150321). Genomic amplicons from cucumber were used to select one cucumber fosmid (173I20), which was sequenced and carried the entire MSH1 coding region (Genbank accession EU500865). Hybridization of the cucumber MSH1 region to DNA-gel blot of cucumber established low-copy number (Fig. 2). A SNP (C/T) was identified at position 350 bp between ST8-9 (BV724002) and PI 401734-3 (BV723996) and segregated independently of *Psm* and the other markers on cucumber linkage group R. In the TMG1 × ST8 family, a G/C SNP was identified at position 196 bp (BV725464 and BV725463, respectively) and mapped 1.5 cM from marker E18/M59-F-145 at the end of linkage group G (Park et al. 2000), independently of the genomic region carrying *Psm*.

Discussion

Because families used to identify the *Psm* locus had MSC in their pedigree (Havey et al. 2004), a new segregating family was developed that did not have MSC as a parent. Single plants from PI 401734 and ST8 were selected that produced high frequencies of wild-type progenies and mosaic progenies, respectively, when crossed as females with MSC16. Although we observed aberrant segregations in the F2 family, results from F1, F2, and F3 segregations supported *Psm* as a single locus (Table 2). Deviations from expected Mendelian ratios have been reported in many studies (Zamir and Tadmor 1986) and these aberrant segregation ratios may result from competition among gametes or differential survival of zygotes or embryos (Lyttle 1991).

Linkages to the AFLP E18/M62-215 and to an RFLP revealed by CsP483 placed *Psm* on cucumber linkage

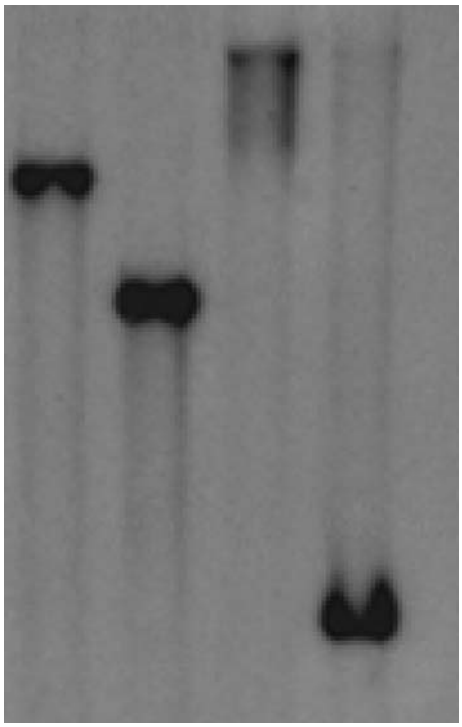

Fig. 2 Autoradiogram from hybridization of the cucumber amplicon of mismatch repair 1 (MSH1) to *Eco*RI, *Eco*RV, and *Hin*dIII, and *Pst*I digests (*left* to *right*) of DNA from inbred line B of cucumber

group R and melon linkage group G10 (Fig. 1). To find additional markers close to *Psm*, we selected melon markers from G10 (Gonzalo et al. 2005) near CsP483 and attempted to map them in cucumber. Sequence similarities between genomic regions from cucumber and melon genomes averaged 92%, in agreement with previous results showing strong cross hybridizations between melon and cucumber DNAs (Neuhausen 1992) and amplicons from SSR markers (Katzir et al. 1996; Danin-Poleg et al. 2000). High sequence conservation between genomic regions from cucumber and melon are indicative of recent divergence and may explain the lack of polymorphism among the parents of our cucumber mapping families (TMG1 × ST8 and PI 401734-3 × ST8-9). In spite of the very low polymorphism rates in cucumber, we were able to scan physically linked genomic regions for polymorphisms for mapping. We placed three markers from melon linkage group G10 onto the linkage map of cucumber and observed the same order and similar genetic distances (Fig. 1). These results confirm synteny between cucumber and melon both on the sequence and recombinational levels, at least across this genomic region. Importantly, this result demonstrates that the detailed genetic maps independently developed for cucumber (Park et al. 2000; Bradeen et al. 2001) and melon (Gonzalo et al. 2005) can be exploited as sources of additional markers for the other species.

MSH1 of *Arabidopsis* encodes a protein similar to the mismatch repair proteins of yeast and *E. coli* and may suppress relatively rare mitochondrial sublimons (Abdelnoor et al. 2006, Shedge et al. 2007). We hypothesized that the cucumber *Psm* locus may work similarly because this locus controls the predominance of specific mitochondrial DNAs. We cloned a cucumber cDNA highly similar to MSH1 and used it to select a cucumber fosmid that carried the entire MSH1 coding region (Genbank accession EU500865). SNPs were identified in genomic regions near MSH1, which segregated independently of *Psm* and other markers on cucumber linkage group R. These results demonstrate that *Psm* is a unique locus that affects the sorting of mitochondrial DNAs independently of MSH1.

The main challenge to mapping of *Psm* was the relatively few polymorphisms in cucumber in general (Kennard et al. 1994; Dijkhuizen et al. 1996) and specifically in this cross. We generated 142,749 bp of genomic sequence from cucumber, from which we produced and sequenced amplicons from parents of the cucumber mapping families. Across 86,377 bp of genomic sequence, only three polymorphisms near MC60, HS_07-C10, and MSH1 were identified between PI 401734-3 and ST8-9 (1 SNP per 28.8 kb). Across 64,272 bp of genomic DNA, only three polymorphisms near the MC60, CMTC168, and MSH1 genomic regions were revealed between TMG1 and ST8 (1 SNP per 21.4 kb). This low level of polymorphism was surprising because ST8 and TMG1 are relatively diverse accessions within *C. sativus* var. *sativus* (Dijkhuizen et al. 1996) and indicates that the genomic region carrying *Psm* may be genetically uniform in cultivated cucumber, restricting the fine mapping of *Psm*. Therefore, a more diverse family must be generated, such as crossing between PI 401734 with *C. sativus* var. *hardwickii* (Csh). Csh is a wild or feral form of cucumber and is the most divergent for molecular markers within *C. sativus* (Dijkhuizen et al. 1996). Genomic amplicons from Csh totaling 3,503 bp were sequenced and revealed 19 polymorphisms with cultivated cucumber (1 polymorphism per 184 base pairs). Csh shows paternal transmission of the mitochondrial DNA (Matsuura 1995) and possesses the *Psm* + allele (Al-Faifi 2007). The significantly greater diversity between PI 401734 and Csh should provide adequate numbers of molecular markers for fine mapping and eventual cloning of *Psm*, a unique locus controlling sorting of paternally transmitted mitochondrial DNAs.

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