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ORIGINAL PAPER

Effects of suppressing the DNA mismatch repair system on homeologous recombination in tomato

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Abstract In plant breeding, the ability to manipulate genetic (meiotic) recombination would be beneficial for facilitating gene transfer from wild relatives of crop plants. The DNA mismatch repair (MMR) system helps maintain genetic integrity by correcting base mismatches that arise via DNA synthesis or damage, and antagonizes recombination between homeologous (divergent) DNA sequences. Previous studies have established that the genomes of cultivated tomato (*Solanum lycopersicum*) and the wild relative *S. lycopersicoides* are substantially diverged (homeologous) such that recombination between their chromosomes is strongly reduced. Here, we report the effects on homeologous recombination of suppressing endogenous MMR genes in *S. lycopersicum* via RNAi-induced silencing of *SlMSH2* and *SlMSH7* or overexpressing dominant negatives of *Arabidopsis MSH2* (AtMSH2-DN) in an alien substitution line (SL-8) of *S. lycopersicoides* in tomato. We show that certain inhibitions of MMR (RNAi of *SlMSH7*, AtMSH2-DN) are associated with modest increases in homeologous recombination, ranging from 3.8 to 29.2% (average rate of 17.8%) compared to controls. Unexpectedly, only the AtMSH2-DN proteins but not RNAi-induced silencing of *MSH2* was found to increase homeologous recombination. The ratio of single to double crossovers (SCO:DCO ratio) decreased by approximately 50% in progeny of the AtMSH2-DN parents. An increase in the frequency of heterozygous SL-8 plants was also observed in the progeny of the *SlMSH7*-RNAi parents. Our findings may contribute to acceleration of introgression in cultivated tomato.

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Introduction

Meiotic recombination is a fundamental processes shared by most sexually reproducing organisms. In this complex cell division mechanism, homologous chromosomes are drawn into physical proximity and must recognize each other as homologous partners. Alignment of chromosomes occurs along their entire lengths to form synaptonemal complexes within which homologous recombination takes place. The products of recombination are gene conversion events associated with either a crossover (CO) or a non-crossover (NCO). Crossovers in particular reshuffle genetic information to generate genetic diversity and provide physical links (chiasmata) to ensure proper segregation of homologous chromosomes during meiosis I. The molecular mechanisms of meiotic recombination, including structural components, DNA-modifying enzymes and molecular pathways, are relatively well studied in the model organisms yeast, *Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Arabidopsis* (Baudat and de Massy 2007;

Edlinger and Schlohelhofer 2011; Hoffmann and Borts 2004; Mercier and Grelon 2008; Osman et al. 2011). However, studies on the regulation of anti-recombination mechanisms in plants have been relatively limited.

Two factors are known to strongly influence meiotic recombination: the pairing of homologous chromosomes and DNA sequence similarity. In wheat, the well-studied Pairing homeologous (*Ph*) loci, especially *Ph1* from the 5B genome, suppress pairing between homeologous (partially homologous) chromosomes. Mutants with *Ph* deletions exhibit a higher frequency of pairing between homeologous chromosomes, and multivalent formation at metaphase I results in homeologous recombination (Roberts et al. 1999; Sears 1977). Efforts to clone the *Ph1* locus reveal a complex structure, comprised of a segment of subtelomeric heterochromatin inserted into a cluster of *cdc2* (*cdk*)-related genes following polyploidization (Al-Kaff et al. 2008; Griffiths et al. 2006; Yousafzai et al. 2010). The *Ph1* region (5L0.5) contains other important meiotic genes (Sidhu et al. 2008), making it difficult to pursue similar research in other (diploid) organisms.

Sequence divergence between chromosomes (for example from related species) decreases the efficiency of homologous recombination because meiotic chromosome pairing is based on DNA homology (Belmaaza et al. 1994; Bozza and Pawlowski 2008). An important system shown to antagonize recombination between divergent/mismatched DNA sequences is the DNA mismatch repair (MMR) system. The MMR system functions to maintain genetic integrity by correcting mismatches that arise during DNA synthesis, genetic recombination and DNA damage (Jiricny 2006; Kimura and Sakaguchi 2006; Li 2008; Spampanito et al. 2009). Certain MMR proteins have an anti-recombination activity due to their ability to recognize mismatches and interfere with the formation and/or extension of heteroduplex intermediates, triggering either helicase-driven unwinding or immediate resolution of the heteroduplex intermediates (Chen and Jinks-Robertson 1999; Kolas and Cohen 2004; Surtees et al. 2004).

In the well-characterized *E. coli* MutHLS system, the MutS protein recognizes base–base mismatches and small insertion/deletion (ID) mispairs and interacts with MutL to enhance mismatch recognition, and together MutS and MutL in the presence of ATP, recruit and activate MutH. The MutH protein specifically incises the unmethylated daughter DNA strand, leading to initiation of mismatch provoked excision (Li 2008). Eukaryotes have multiple homologues of MutS (MSH1–MSH7) and MutL (MLH1-3, PMS1, 2), and these proteins (with the exception of MSH1) function as heterodimers. In eukaryotes, MSH2 and MLH1 are the core monomers that form heterodimers with other MMR proteins, including MSH2·MSH6 (MutSα), MSH2·MSH3 (MutSβ), MSH2·MSH7 (MutSγ), MLH1·PMS1 (MutLα, for humans, MLH1·PMS2), and MLH1·MLH3 (MutLγ) (Adé et al. 1999; Culligan and Hays 1997, 2000; Higgins et al. 2004; Li 2008).

MMR-deficient mutants of yeast showed increases in recombination frequency between DNA sequences that diverged by up to 10% (Chambers et al. 1996; Chen and Jinks-Robertson 1999; Hunter et al. 1996; Nicolson et al. 2000). Strains of *E. coli* lacking MutS activity are able to undergo interspecific recombination with *Salmonella typhimurium,* of which DNAs were about 20% divergent (Matic et al. 1994). Recently in *Arabidopsis thaliana,* the loss of MSH2 and PMS1 activities led to increases in homeologous somatic (mitotic) recombination between sequences of varying divergence (0.5–9%) (Li et al. 2006, 2009). Mutants of *MSH2* induced a threefold increase in intrachromosomal recombination between highly diverged sequences (13%) in germinal tissues of *A. thaliana* (Lafleuriel et al. 2007) and *MSH2* was reported to be involved in the suppression of somatic recombination between divergent direct repeats and between homologues from different *A. thaliana* ecotypes (Emmanuel et al. 2006). The MSH7 protein of *A. thaliana* possesses a drastically reduced DNA-binding clamp domain, which suggested a specialized role for meiotic recombination fidelity (Wu et al. 2003). Early expression of the *TaMSH7* gene during meiosis and its linkage to the *Ph2* locus (on 3DS) proposed *MSH7* as a candidate for *Ph2*, a minor suppressor of homoeologous pairing in wheat (Dong et al. 2002; Sears 1982). A subsequent study in transgenic barley showed that *TaMSH7* affects fertility and is functionally important during meiosis (Lloyd et al. 2007) but to date, the identity of *Ph2* has not been confirmed. Additional phenotypes associated with mutations in MMR genes (*MLH1, MLH3, MSH7, PMS1*) include reduced fertility and transmission bias of the transgene (Dion et al. 2007; Jackson et al. 2006; Lloyd et al. 2007).

A better understanding of how plants control meiotic recombination would facilitate to efforts to breed improved varieties, particularly if they involve transfers of genetic material from related species (Li et al. 2007; Martinez-Perez and Moore 2008; Qi et al. 2007; Wijnker and de Jong 2008). Because many crop plants have been depleted of genetic diversity, their wild relatives often constitute important sources of novel traits, such as disease or pest resistance, increased yield, etc. (Tanksley and McCouch 1997). Knowledge of recombination mechanisms is also fundamental to the understanding of plant evolution and speciation (Butlin 2005; Gaut et al. 2007; Schuermann et al. 2005). The cultivated tomato (*Solanum lycopersicum* L., formerly *Lycopersicon esculentum* Mill.) and its distant wild relative *Solanum lycopersicoides* Dun. provide a useful model system in which to study homeologous recombination in a diploid crop genome.

Previous studies have established that the genomes of tomato and *S. lycopersicoides* are homeologous. The two species have the same number of chromosomes ($2n = 24$), yet differ by at least one paracentric inversion (Pertuze et al. 2002). Diploid F_1 interspecific hybrids, easily obtained by embryo rescue, show reduced chromosome pairing, chiasma formation and pollen fertility (Chetelat et al. 1997; Menzel 1962, Rick et al. 1986). Allotetraploid hybrids exhibit nearly complete preferential pairing between homologous chromosome sets. Genetic recombination is reduced an average of 27% genome-wide in progeny of the diploid hybrid (Chetelat et al. 2000). In addition, heterozygous substitution lines containing a single *S. lycopersicoides* chromosome bred into the genetic background of *S. lycopersicum* recombine at less than 50% of the rate observed for the same chromosome in the F_1 hybrid. Chromosomes of both species are readily distinguished by genomic in situ hybridization (GISH), with detectable differences in chromosome size and timing of condensation (Ji and Chetelat 2003). Despite these differences, chromosomes of the F_1 hybrid synapse at pachytene (Menzel 1962), but at diakinesis and metaphase I, significant numbers of unpaired chromosomes are observed (Ji and Chetelat 2003; Rick 1951).

In this study, we investigated the effects of *MSH2* and *MSH7* deficiencies on homeologous recombination in tomato. Suppression of these key MMR genes was achieved using RNAi gene silencing and dominant-negative transgenes. A heterozygous substitution line containing chromosome 8 from *S. lycopersicoides* (SL-8) in the background of cultivated tomato was used to assay homeologous recombination.

Materials and methods

Synthesis of MMR-suppression constructs

RNAi gene silencing constructs

Molecular cloning and characterization of the full length *MSH2* and partial *MSH7* cDNA sequences from tomato were described earlier (Tam et al. 2009). The primers MSH5′RNAi (5′-CCTCGAGTCTAGAGGCAAGCTCAA GGGTTTC-3′) and MSH3′RNAi (5′-GGAATTCGGATCC GCAACAATCACTGGAGAA-3′) were used to amplify a 400-bp *MSH2* cDNA fragment from the full length *MSH2* cDNA clone. These PCR primers generated a PCR product that contained XhoI and XbaI restriction sites on the 5′ end and BamHI and EcoRI restriction sites on the 3′ end to facilitate subcloning. This same 400 bp *MSH2* fragment was then directionally ligated in forward and reverse orientations flanking the *Arabidopsis thaliana* Yabby5 5th intron downstream of the 35S promoter in the RNA interference vector pRNA69. In order to facilitate *Agrobacterium tumefaciens* mediated plant transformation, a NotI fragment containing the 35S promoter, the cloned inverted repeat, and octopine synthase termination of transcription sequences was sub-cloned into the pART27 binary vector (Gleave 1992).

The primers msh7attB1 (5′-GGGGACAAGTTTGTAC AAAAAAGCAGGCT TTGCCGTCAAGGAGACTT-3′) and msh7attB2 (5′-GGGGACCACTTTGTACAAGAAAG CTGGGT ACAAGTGTCTGTCCATCC-3′) amplified a 510-bp *MSH7* cDNA fragment. PCR products were visualized on 2% agarose gels and bands purified using the Qiagen gel extraction kit (Qiagen, USA). Using the BP clonase recombination reaction (Gateway® BP Clonase Enzyme Mix, Invitrogen USA), the *MSH7* PCR product was then directionally cloned, in forward and reverse orientations flanking the *Pdk* intron and downstream of the CaMV 35S promoter using a modified RNA interference vector, MW11 derived from pCambia 1300. This construct was then transformed into One Shot® OmniMAX™ 2 T1 Phage-Resistant cells (Invitrogen USA) following manufacturer's protocol and positive colonies isolated using ampicillin as a selective agent (100 μg/ml).

Dominant negative constructs

The two dominant negative constructs used in this study incorporated mutations in either the ATPase site (Gly671Asp, construct AtMSH2-DN1) or the helix–turn–helix domain (Gly815Asp, construct AtMSH2-DN2) of the *Arabidopsis* MSH2 protein. Expression of mutant mRNAs from these transgenes was driven by the super-promoter in the MSP-2 Gelvin vector pE1803 (Lee et al. 2007). Though not demonstrated, it is expected that AtMSH2 (dominant-negative) proteins over-expressed in tomato would dimerize with the tomato mismatch repair proteins as MMR genes are evolutionary conserved (Tam et al. 2009).

Plant transformations

The resulting four different MMR transgenes, which included two RNAi (*SlMSH2*- and *SlMSH7*-RNAi) and two dominant negative (AtMSH2-DN1 and -DN2) constructs, were transformed into *Agrobacterium tumefaciens* cells (ElectroMAX™ *Agrobacterium tumifaciens* LBA4404, Invitrogen, USA) following the manufacturer's protocol, and further transformed into tomato *cv.* Gold Nugget. Plant transformations were carried out by the Ralph M. Parsons Foundation Plant Transformation Facility at UC-Davis.

Analysis of gene expression by semi-quantitative RT-PCR

Tissues excised from plants were immediately frozen in liquid nitrogen. Young leaves (approximately 5 mm in length) and/or young floral buds (approximately 2–4 mm in length) were used. Total RNA was extracted from 200 to 300 mg of frozen tissues using Trizol Reagent (Invitrogen, USA) according to the manufacturer's specifications. First-strand cDNA synthesis was carried out using up to 5 µg of template RNA per reaction, 0.5 µg of Oligo$(dT)_{18}$ primer (Fermentas, USA) and 200 units of M-MLV Reverse Transcriptase enzyme (Promega, USA) according to manufacturer's instructions.

Semi-quantitative RT-PCR was used to compare expression levels of *SlMSH2*, *SlMSH7*, AtMSH2-DN1 and AtMSH2-DN2. The *MSH2* primers U1732 (5′-GTAGTTC AAACAGTTGCGAGTT-3′) and L2146 (5′-ATAAAA GTAGAAACCCCCTTC-3′) produce a 434 bp amplicon from cDNA. The *MSH7* primers msh7RNAiF (5′-CCT CGAGTCCTAGATCTTGCCGTCAAGGAGAC-3′) and msh7RNAiR (5′-GGAATTCGGATCCACAAGTGTCTGT CCATCC-3′) amplifies a 510 bp product of the *MSH7* gene. For the two *AtMSH2* transgenes, species-specific primer pairs AtMSH2DN1f (5′-GCCAGTTGCCCTACT CCATA-3′), AtMSH2DN1r (5′-CCAGCTCTGCAGCTT TCTCT-3′) and AtMSH2DN2f (5′-GCTGATGGCTCAA GTTGGTT-3′), AtMSH2DN2r (5′-ATCTCACGAACC CGTTGAAG-3′) were designed to flank the mutated nucleotides in *A. thaliana*, and produced 745 and 708 bp amplicons, respectively. The PCR protocols used to amplify *MSH2* and *MSH7* were described previously (Tam et al. 2009). For PCR reactions, 300–500 ng of cDNA were used for PCR following these conditions—AtMSH2-DN1 and -DN2: one cycle of 95°C for 6 min; then 32 cycles of 94°C denaturation for 30 s, 55°C annealing for 30 s and 72°C extension for 45 s, with a final extension cycle of 72°C for 5 min. PCR products were analyzed using 2% agarose gel electrophoresis to verify size and expression levels.

Homeologous recombination assay

Transgenic tomato *cv*. Gold Nugget plants were checked for the presence of transgene insertions using the selectable marker genes NPTII (primers NPTIIF 5′-CGCTCAGAAG AACTCGTCAA-3′ and NPTIIR 5′-AGACAATCGGCT GCTCTGAT-3′) or HPT (HPTf 5′-CGCTCCCGATTC CGGAAGTG-3′ and HPTr 5′-ACGATTGCGTCGCATCG ACC-3′). Transgenic plants were crossed as male parents to tomato alien substitution lines heterozygous for *S. lycopersicoides* chromosome 8 (SL-8, accession LA4307). This stock was used because it exhibited about 3.3-fold less recombination compared to the tomato EXPEN 2000 map (Fulton et al. 2002; Ji and Chetelat 2003). The SL-8 line also showed a lower rate of pairing failure than similar substitution lines for chromosomes 7 and 10 (Ji and Chetelat 2003). SL-8 is nearly isogenic in the genetic background of *cv*. VF-36 (LA0490) (Canady et al. 2005). The F_1 progeny from these crosses were screened both for the presence of the transgene using selectable markers and the substituted *S. lycopersicoides* chromosome using two molecular markers (T1123 and CT68) and the dominant monogenic morphological mutant *Wa* (*White anthers*, Rick 1988). F_1 progenies that carried both the transgenes and an intact *S. lycopersicoides* chromosome 8 were selected to characterize MMR expression levels by semi-quantitative RT-PCR. Three F_1 individuals per transgenic cross with significantly lower levels of gene expression (RNAi lines) or strong expression of the heterologous genes (dominant negative lines) were self-fertilized to generate 12 F_2 populations. Two control F_2 populations were generated using crosses between wild-type *cv*. Gold Nugget and SL-8.

The F_2 progenies were genotyped using PCR-based cleaved amplified polymorphic sequence (CAPS) and simple sequence repeats (SSR) markers, modified from the protocol described previously (Canady et al. 2005). Five molecular markers (T1123, C2_At2g25950 or SSR327, TG302, C2_At5g47010 and CT68) were chosen to span the entire length of chromosome 8. Primer sequences for these markers were obtained from the Solanaceae Genomics Network (SGN, http://solgenomics.net), or were designed from the marker sequences available there. Amplified DNA fragments were digested with the following restriction enzymes to reveal polymorphisms between *S. lycopersicum* and *S. lycopersicoides*: AluI for At2g25950 and TG302, RsaI for At5g47010, HinfI for CT68. Certain double crossovers and all higher order crossovers were re-genotyped to validate these events.

Each of these markers was co-dominant, thus the three possible F_2 genotypic classes were observed. MAPMAKER version 2.0 (for Macintosh) was used to estimate recombination fractions using a threshold LOD score of 3.0 for detecting linkage. Map distances were calculated using the Kosambi mapping function. Results from different lines (homeologous wild type control and transgenic populations) were compared for significant differences using the *t* test statistic for independent samples with the assumption of equal variances for the two samples. Deviations from expected Mendelian inheritance were determined using the Chi-square goodness-of-fit statistic. Statistical tests were carried out online at http://faculty.vassar.edu/lowry/VassarStats.html.

Results

Production of MMR-impaired tomato lines

Most of the transgenic plants displayed normal morphology, vigor, development and fertility both in vitro (i.e. grown on kanamycin containing media) and under greenhouse conditions. The only exception was that a significant proportion of plants (up to 20% from each transformation) appeared to be polyploid, which is a common occurrence in tomato plants regenerated from tissue culture (Evans and Sharp 1983; Jacobs and Yoder 1989). Only the likely diploid regenerants were used for further analysis.

Four groups of transgenic cv. Gold Nugget plants were studied, two containing knockdowns of *MSH2* and *MSH7* expressions by RNAi, and two containing dominant-negative constructs of AtMSH2 with mutations at two highly conserved domains, namely the ATPase and helix-turn-helix domains (AtMSH2-DN1 and -DN2, respectively; for a summary of the structural domains and functions of the MutS family, please refer to Spampanito et al. 2009). The ATPase domain has been shown to be involved in ATP hydrolysis in the MSH2 protein complex whereby mutants retained normal mismatch binding activity but were resistant to ATP-mediated mismatch release and lost the ability to signal mismatch repair, thus resulting in mismatch deficiency (Alani et al. 1997; Lin et al. 2004). It was reported that the helix-turn-helix domain plays a role in modulating mismatch recognition by responding to conformational changes in MSH2 and its dimer proteins, induced by ATP hydrolysis (Alani et al. 1997). For the RNAi lines, semi-quantitative RT-PCR was used to verify reduced gene expression in the F_1 plants (Fig. 1). For the dominant-negative lines, semi-quantitative RT-PCRs were performed using species specific primers to detect expression of the transgenes (Fig. 2). Three F_1 individuals with intact SL-8 chromosomes were selected for each transgenic group. Self-fertilization of the corresponding MMR-deficient F_1 double heterozygotes containing both the transgene and SL-8 chromosome resulted in the F_2 populations that were analyzed for recombination estimates. 12 independent F_2 families comprised of 96–271 (mean 157) plants each were genotyped. Two control populations were also analyzed, with an average of 128 plants per population.

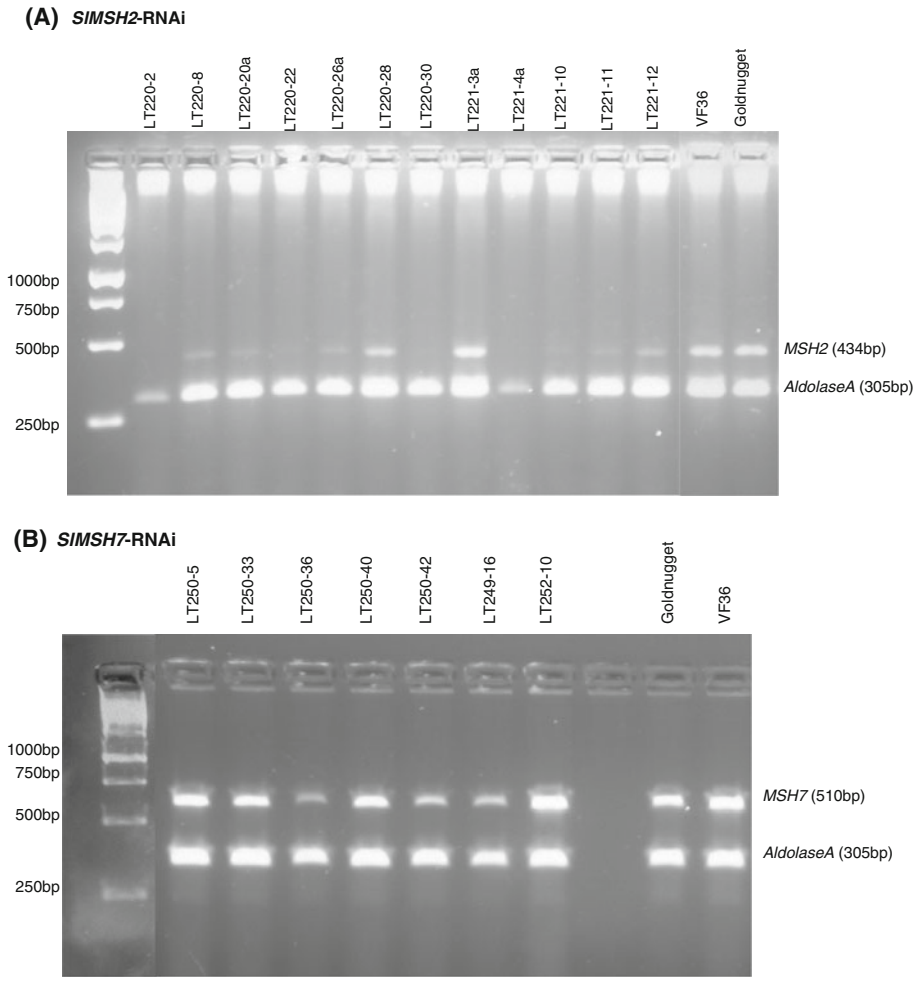

Fig. 1 Expression analysis of transgenic MMR-suppressed tomatoes by RT-PCR. MMR gene expression was assayed by semi-quantitative RT-PCR using mRNA from young leaves or floral buds of F_1 plants to select individuals with reduced *MSH2* and *MSH7* gene expression. Non-transgenic VF36 and Gold Nugget were used as controls. **a** Gene expression of *SlMSH2*. *Upper band* is *MSH2* (434 bp), *lower band* is *Aldolase A* as control (305 bp). Three selected transgenic *MSH2*-SL-8 F_1 individuals are LT220-30, LT221-11 and LT220-20a (**b**) Gene expression of *SlMSH7*. *Upper band* is *MSH7* (510 bp), lower band is *Aldolase A* control (305 bp). Three selected transgenic *MSH7*-SL-8 F_1 individuals are LT249-16, LT250-36 and LT250-42

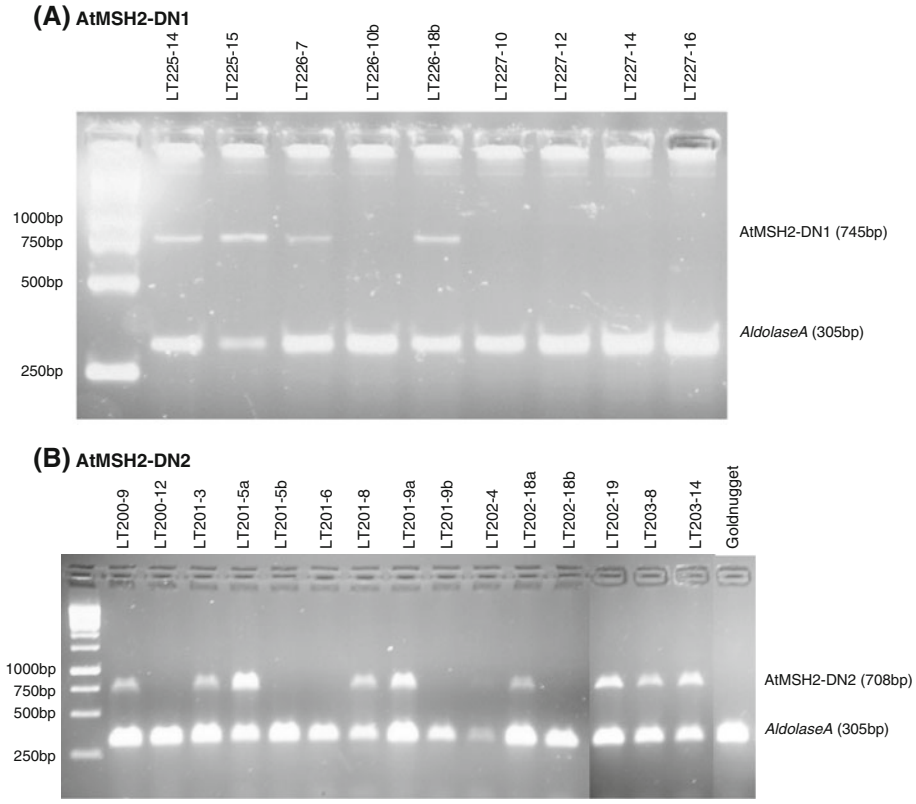

Fig. 2 Analysis of transgene expressions of AtMSH2-DN1 and AtMSH2-DN2 (dominant negative constructs) in transgenic tomato lines studied by semi-quantitative RT-PCR using mRNA from young leaves or floral buds of F_1 plants. Species specific primers were used to distinguish transgene from endogenous gene expression. Control is non-transgenic Gold Nugget. **a** Expression in AtMSH2-DN1 lines. *Upper band* is AtMSH2-DN1 (745 bp), *lower band* is *Aldolase A* control (305 bp). Three selected transgenic SL-8 individuals include LT225-14, LT225-15 and LT226-18b. **b** Expression in AtMSH2-DN2 lines. *Upper band* is AtMSH2-DN2 (708 bp), *lower band* is *Aldolase A* control (305 bp). Three selected transgenic SL-8 F_1 individuals include LT201-9a, LT202-18a and LT203-8

Effects on homeologous recombination

The average total map length of chromosome 8 in the control populations was 34.7 cM (±1.7 cM). Homeologous recombination was found to be increased in three of the four different transgenic F_2 populations when compared to the control populations, i.e. AtMSH2-DN1 ($P < 0.001$), *SlMSH7*-RNAi ($P = 0.016$) and AtMSH2-DN2 ($P = 0.084$) (Table 1). The exception is the *SlMSH2*-RNAi transgenic group of which all three F_2 populations recorded lower (though not significant, $P = 0.11$) recombination rates, averaging a 7.8% decrease in total map length (Fig. 3). One transgene construct AtMSH2-DN1, showed a 1.9-fold increase in recombination frequency, accounted for mainly by recombination in the C2_At5g47010 and CT68 marker interval on chromosome 8 (Fig. 3). However, genotyping with additional markers (C2_At1g63770, T1522 and C2_At5g41350) located between the markers C2_At5g 47010 and CT68 revealed an unexpected prior double crossover event in this line. Therefore, the increase in recombination rate could be due to a chromosome segment (<31.3 cM) homozygous for *S. lycopersicum* in this region; this line was therefore excluded from further analysis.

The largest increases in average recombination rates were observed in the F_2 populations encoding AtMSH2-DN2, which averaged 41.4 cM, representing about a 19.5% gain in total map length (Fig. 3). For this transgenic group, the average increase in the total percentage of crossover events compared to the control populations was about 10.5% (Table 2). The *SlMSH7*-RNAi transgenic group displayed an average map length of 40.2 cM, which is about a 16.1% increase in recombination rate above the controls. In terms of crossover events, this transgenic group demonstrated only a 2.5% average increase in the percentage of total crossovers compared to controls (Table 2). Overall increases in recombination rates for individual transgenic populations ranged from the lowest gain shown by LT308 (AtMSH2-DN2) which recorded 36.03 cM, an increase of 3.8%, to the highest total map length observed from LT309 (AtMSH2-DN2) at 44.84 cM (29.2% increase) compared to the control populations (Table 1; Fig. 3). Analysis of transgene expression levels and rate of recombination show that there is no clear relationship between transgene mRNA abundance (lower expression for RNAi lines or strong expression for the dominant negative lines) and the magnitude of change in homeologous recombination. For example, the mRNA abundance in the F_1 parent (LT230-8) was not higher than the other two AtMSH2-DN2 populations (Fig. 2).

Increased average recombination rates were only consistently observed for one marker interval, namely between C2_At5g47010 and CT68, located on the long arm of chromosome 8, which varied in the transgenic F_2 populations from 23.9 to 30.5 cM (the control average value was

Table 1 Homeologous recombination in control and MMR-impaired lines of tomato, heterozygous for a chromosome 8 (SL-8) from *S. lycopersicoides*. Recombination rates were estimated in F_2 progenies of each cross

F_1 plant	F_2 line	Construct	T1123-C2At2g25950	C2At2g25950 or SSR327-TG302	TG302-C2At5g47010	C2At5g 47010-CT68	Total map length (cM)	Average map length (cM)	% Change from control
Marker interval (cM)									
LT233-8	LT310	Control	1.4	4.3	5.4	21.9	32.99	34.7	
LT234-2	LT311	Control	5.1	2.3	8	21	36.31		
LT220-30	LT300	*SlMSH2*-RNAi	1.5	3.6	5.1	20	30.22	32.0	−7.8
LT221-11	LT301	*SlMSH2*-RNAi	1.4	2.2	8	20.4	31.96	$P = 0.113$	
LT220-20a	LT306	*SlMSH2*-RNAi	1.8	3	7.8	21	33.66		
LT202-18a	LT308	AtMSH2-DN2	4.8	7.1	5.4	18.7	36.03	41.4*	19.5
LT203-8	LT309	AtMSH2-DN2	3.2	2.5	10.8	28.3	44.84	$P = 0.084$	
LT201-9a	LT312	AtMSH2-DN2	4.3	7.1	7.1	24.8	43.32		
LT249-16	LT316	*SlMSH7*-RNAi	1	0	1.9	38.2	41.13	40.2**	16.1
LT250-36	LT317	*SlMSH7*-RNAi	3.4	2.6	7.7	25.3	39.01	$P = 0.016$	
LT250-42	LT318	*SlMSH7*-RNAi	4.8	3.7	4.1	28	40.55		
LT255-14	LT302	AtMSH2-DN1	0.8	0.2	1.2	66.9	69.05	65.4***	88.9
LT226-18b	LT303	AtMSH2-DN1	1.9	1.9	2	56	61.79	$P < 0.001$	
LT225-15	LT307	AtMSH2-DN1	2	2	0.4	61.1	65.47		

Level of statistical significance * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$

P values given are for one-tailed test

21.5 cM). One population, LT316 (*SlMSH7*-RNAi) recorded a total of 38.2 cM, representing an increase of 78% of the map distance in this particular interval over the control map distance. For the remaining three marker intervals, no particular trend was apparent with some populations recording increases while others showing decreases in map distances compared to the controls (Table 1). Generally, the AtMSH2-DN2 F_2 populations showed increases in map distances for all marker intervals compared to controls. In contrast, the *SlMSH7*-RNAi group exhibited a significant increase in map length for the fourth marker interval, but lower recombination rates for the other three marker intervals (Fig. 3).

Number and classes of meiotic crossovers

The ratio of single crossovers (SCO) to double crossovers (DCO) in the recombinant individuals was skewed in favor of SCOs in the control and *SlMSH2*-RNAi populations, ranging from 4.5 (LT306) to 10 (LT301). However, significantly lower SCO:DCO ratios (i.e. more DCO events) were detected in the other transgenic populations. All three populations of AtMSH2-DN2 ($P < 0.05$) and one population from *SlMSH7*-RNAi (LT317) showed approximately twofold decreases in the ratio of SCO to DCO, ranging from 2.25 (LT309) to 3.8 (LT312). The remainder two populations for *SlMSH7*-RNAi were within the control ranges (Table 2). In addition, appearances of three and four crossovers events were observed in the AtMSH2-DN2 and *SlMSH7*-RNAi groups, while none was recorded from the control and *SlMSH2*-RNAi F_2 populations (these higher-order crossover events were not included in determining the SCO:DCO ratios).

Though significant increases in recombination rates were detected in the AtMSH2-DN2 and *SlMSH7*-RNAi groups, no significant differences were seen when the proportion of recombinant individuals per population was analyzed between the transgenic and control populations. Only two F_2 transgenic populations (LT312 and LT318) showed slightly higher number of recombinant individuals (Table 2). In addition, for the recombinant individuals, no apparent trend was observed between transgenic and control populations in the distribution of total crossover classes in any of the intervals measured. As expected, plants with a single crossover event (i.e. from one gamete) were more abundant than 'double' types (i.e. crossovers in both gametes) in every F_2 population (Supplementary material 1).

Alien chromosome and allele transmission frequencies

For non-recombinant F_2 individuals, transmission of the entire *S. lycopersicoides* chromosome 8 in the control and transgenic groups was severely distorted from Mendelian expectations (1:2:1), with an excess of *S. lycopersicum* homozygotes and a deficiency of heterozygotes in most groups. However, an excess of heterozygotes was observed for the *SlMSH7*-RNAi transgenic group ($P = 0.09$)

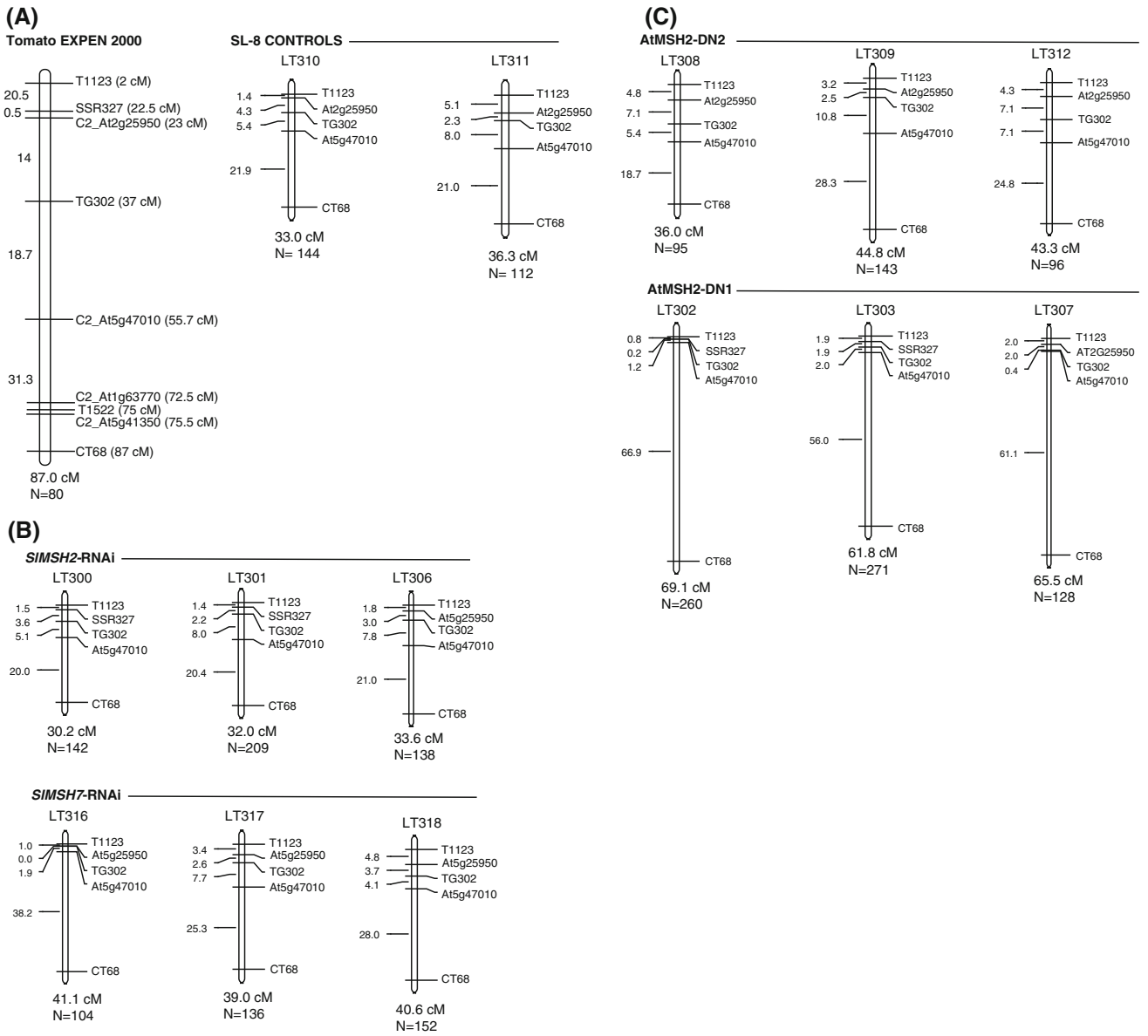

Fig. 3 Genetic linkage maps of control and MMR-impaired lines of tomato, heterozygous for chromosome 8 (SL-8) from *S. lycopersicoides* based on recombination in F_2 progeny. Genetic distances are in Kosambi map units, with total genetic length (cM) and the total number of individuals (N) in each F_2 mapping population represented below the chromosome. **a** The tomato reference map (EXPEN 2000) is from Fulton et al. (2002), control maps are LT310 and LT311 (**b**) LT300, LT301 and LT306 are from *SlMSH2*-RNAi lines; LT316, LT317 and LT318 are from *SlMSH7*-RNAi lines (**c**) LT308, LT309 and LT312 are from AtMSH2-DN2 lines, LT302, LT303 and LT307 are from AtMSH2-DN1 lines

(Supplementary material 2). No individuals homozygous for the entire *S. lycopersicoides* (*S/S*) chromosome were recovered from any of the F_2 populations, suggesting that either these genotypes are inviable or that pollen bearing an intact SL-8 chromosome are eliminated (or at least outcompeted).

A similar trend was observed amongst the recombinant individuals, wherein the segregation of alleles was skewed towards *S. lycopersicum* for all five markers. A deficiency of alleles from *S. lycopersicoides* (*S*) compared to *S lycopersicum* (+) was apparent, with most regions showing severe distortions from the 1:1 normal Mendelian segregation. Among the five markers, allele frequency from CT68 consistently showed the lowest level of segregation distortion, corresponding to the C2_At5g47010-CT68 interval having the highest recombination rate compared to the other intervals. For AtMSH2-DN1 populations, which recorded the highest recombination rates for this interval due to an unexpected short region of homology, the CT68 allele frequency almost approached a 1:1 segregation ratio

Table 2 Distribution of crossovers (single crossovers SCO, double crossovers DCO and other crossovers) and proportion of recombinant progenies in the control and MMR-impaired tomato x SL-8 F_2 populations

F_2 line	Construct	Map length (cM)	# SCOs	# DCOs	SCO:DCO ratio	Other COs	Total COs	% CO in total # of F_2 progeny	Total # recombinants	% recombinants in F_2 progeny	Total # non-recombinants	Total # of F_2 progeny
LT310	Control	32.99	63	10	6.3	0	83	57.6	73	50.7	71	144
LT311	Control	36.31	52	10	5.2	0	72	64.3	62	55.4	50	112
LT300	*SlMSH2*-RNAi	30.22	60	9	6.6	0	78	54.9	69	48.6	73	142
LT301	*SlMSH2*-RNAi	31.96	100	10	10	0	120	57.4	110	52.6	99	209
LT306	*SlMSH2*-RNAi	33.66	58	13	4.5	0	84	60.9	71	51.4	67	138
LT308	AtMSH2-DN2	36.03	37	12	3.1	0	61	64.2	49	51.6	46	95
LT309	AtMSH2-DN2	44.84	54	24	2.3	1 (4CO)	106	74.1	79	55.2	64	143
LT312	AtMSH2-DN2	43.32	46	12	3.8	1 (3CO)	73	76.0	59	61.5	37	96
LT316	*SlMSH7*-RNAi	41.13	46	7	6.6	0	60	57.7	53	51.0	51	104
LT317	*SlMSH7*-RNAi	39.01	50	19	2.6	1(3CO)	91	66.9	70	51.5	66	136
LT318	*SlMSH7*-RNAi	40.55	74	13	5.7	0	100	65.8	87	57.2	65	152

between *S. lycopersicum* and *S. lycopersicoides* (Supplementary material 3).

Discussion

Suppression of MMR genes increases homeologous recombination rates

The recombination results showed that suppression of the MMR genes *MSH7* by gene silencing and *MSH2* via dominant-negative overexpression is associated with small but significant increases in homeologous recombination rates. Interestingly, *MSH7*, an MMR gene unique to plants whose possible role in homeologous recombination has not been tested, is shown here to exhibit anti-recombination activity in tomato. RNAi induced gene silencing of *MSH7* resulted in significantly increased recombination rates (approx. 16% greater than controls). Modest increases in recombination rates observed in our study suggest that *SlMSH7* is a minor suppressor of homeologous recombination.

Our findings that dominant-negative suppression of MMR genes such as *MSH2* can affect homeologous recombination rates is consistent with other studies in plants, such as *Physcomitrella patens* (Trouiller et al. 2006) and *A. thaliana* (Emmanuel et al. 2006; Lafleuriel et al. 2007; Li et al. 2006, 2009). However, we did not observe much difference in recombination rates among AtMSH2-DN and *SlMSH7*-RNAi transgenic populations. This contrasts with findings in yeast that showed higher anti-recombination activity for *MSH2* compared to *MSH3*, *MSH6* and *MLH1* (Chambers et al. 1996; Nicholson et al. 2000) or *PMS1* whose effects were highly dependent on the amount of sequence divergence (Datta et al. 1996; Selva et al. 1995). Indeed, our result (with the exception of the *SlMSH2*-RNAi lines) agrees more with observations in *Arabidopsis*, in which similar increases in homeologous (mitotic) recombination were detected in mutants of *AtMSH2*, *AtMLH1* and *AtPMS1* (Dion et al. 2007, Li et al. 2006, 2009).

The magnitude of increase in homeologous recombination observed in our study (3.8–29.2%) is also much lower than seen in other studies, which used reporter systems to analyze homeologous recombination during somatic recombination (sequence divergence range 0–9%; Dion et al. 2007; Li et al. 2006, 2009) or intrachromosomal recombination (13% divergence; Lafleuriel et al. 2007). Inactivation of MMR genes in *A. thaliana* increased recombination rates from 2 to 7-fold for *MSH2* (Li et al. 2006), 3-fold for *MSH2* (Lafleuriel et al. 2007), 2.3 to 2.6-fold for *MLH1* (Dion et al. 2007) and 1.7 to 4.8-fold for *PMS1* (Li et al. 2009). The smaller effects seen in

MMR-impaired tomato lines could be caused by incomplete gene silencing (vs. knockout mutants in *Arabidopsis*), differences in the degree of DNA divergence, and/or the type of recombination examined in each system (i.e. mitotic vs. meiotic).

The strong recombination suppression observed between tomato and *S. lycopersicoides* is likely due to sequence divergence, as no structural rearrangements have been found on this chromosome (Pertuze et al. 2002). An RFLP analysis using single- or low copy probes indicated around 75% average polymorphism rate between the two species (Chetelat et al. 2000). GISH cytology readily distinguished chromosomes of the two species, generally interpreted as the genomes having 85% or less sequence identity (Ji et al. 2004; Parokonny et al. 1997). Estimation of sequence identity between *S. lycopersicum* and *S. lycopersicoides* using *blastn* of 45 currently available accessions of the latter species in GENBANK which included COS (conserved ortholog sequences), coding and non-coding regions, showed an average sequence divergence of approximately 11% (total sequence analyzed 41,072 bp).

Though both species can be hybridized and introgressed during breeding programs, the moderately high estimation (11%) of genome-wide average sequence divergence between tomato and *S. lycopersicoides* might be enough to preclude the effects of MMR-deficiency on recombination, a phenomenon reported in other systems. Studies in yeast found that *MSH2* mutations had only small stimulatory effects on meiotic recombination between highly diverged (10–15%) sequences (Bailis and Rothstein 1990; Priebe et al. 1994; Selva et al. 1995). When sequence divergence exceeded this limit, recombination rarely occurred even in mismatch repair-deficient strains, as there is minimal chance of escaping the anti-recombination activity and/or the requirement of the minimum efficient processing segment is not met such as to efficiently initiate recombination (Chambers et al. 1996; Chen and Jinks-Robertson 1999; Datta et al. 1996, 1997; Hunter et al. 1996).

Part of the rate difference can also be accounted for by genetic background. Substitution lines containing alien chromosomes in a uniform tomato genome tend to recombine at lower rates than the same chromosomes in the original F_1 hybrid, a trend observed for both *S. lycopersicoides* and *S. pennellii* substitutions (Ji and Chetelat 2003; Rick 1969). MMR proteins are primarily involved in assessing the degree of homology, therefore, inhibiting *MHS2* or *MSH7* genes would not alter recombination rates if other factors were limiting. Other possible factors include chromatin conformation and/or the timing of events in meiosis. In the interspecific hybrid, condensation of the *S. lycopersicoides* chromosomes is noticeably delayed relative to the *S. lycopersicum* chromosomes. It is known that disruption of meiotic chromosome pairing in F_1 *S. lycopersicum* × *S. lycopersicoides* did not commensurate with the dramatic reduction in recombination, suggesting additional contributing factors, such as distorted segregation ratios, and/or differential pre- and/or post-zygotic lethality (Chetelat and Meglic 2000; Ji and Chetelat 2003). Therefore, selection against *S. lycopersicoides* alleles might have eliminated recombinant gametes (or those with higher number of crossovers), which could partially explain the modest increase in meiotic recombination rates seen here.

Last, MMR antagonism of homeologous recombination is still not well understood; in particular, not much is known about the effects of silencing individual genes on the function of other MMR or recombination proteins. One example is the RecQ helicases, which are involved in processing DNA structures arising during replication, recombination, and repair. It was reported that both *Arabidopsis* and rice contains seven copies of RecQ-like genes, whilst five copies were identified in *Physcomitrella patens*, thus the specific functions and roles of these genes in the DNA repair and recombination pathways are not yet fully understood (Hartung and Puchta 2006; Hartung et al. 2007). The Rad51 and DMC1 proteins are also important in recombination, specifically in initiating homology searches and interhomologue repair respectively in *Arabidopsis* (Mercier and Grelon 2008). Another important group is the ZMM proteins, which comprises at least seven interacting proteins (Zip1–Zip4, MSH4, MSH5 and Mer3) involved in recombination, synaptonemal complex assembly and crossover formation (class I COs) (Lynn et al. 2007). Therefore, it remains a possibility that other DNA repair or recombination proteins might compensate for the lack of MSH2 or MSH7 activity.

Comparison of dominant negative and RNAi constructs

In the MMR system, the *MSH2* gene codes for the major subunit that heterodimerizes with other MMR proteins such as MSH3, MSH6 and MSH7 (Harfe and Jinks-Robertson 2000; Wu et al. 2003). Comparison of two different gene silencing methods, namely the dominant negative construct (with mutation in the helix-turn-helix region) and the RNAi method for suppressing expression of *MSH2* should permit selection for lines with stable and efficient reduction of MSH2 activity. However, *SlMSH2*-RNAi lines of tomato generated in this study unexpectedly did not show any increase in homeologous recombination. Instead, the genetic map lengths shrank (albeit not significantly), as opposed to results shown by the dominant negative AtMSH2-DN protein construct. This finding suggests either the three chosen RNAi lines were incompletely silenced, or perhaps a large reduction in mRNA levels might not strongly affect protein levels, or the presence of

the MSH2 protein is required for homeologous recombination in tomato. Li et al. (2006) have previously reported that inactivation of MSH2 could significantly impair homologous recombination by about 22%, presumably interfering with the normal recombination pathway.

Due to an unexpected region of homology in some SL-8 lines, the recombination results from the first dominant-negative construct, AtMSH2-DN1, which carried a mutation at the ATPase could not be compared with the AtMSH2–DN2 construct that contained a helix-turn-helix mutation. However, the AtMSH2–DN1 transgenic lines achieved an almost twofold increase in recombination frequency ascribed to homologous recombination, whereby the average genetic map length of these transgenic populations recovered to about 80% of the genetic map length for chromosome 8 of the tomato EXPEN 2000 map. Therefore, a deficiency of the ATPase site of the MSH2 protein presumably did not severely impede the process of homologous recombination. However, in this system, chromosomes with crossovers in the region of homology might be favored and were more abundant in the F_2 progeny.

More frequent double crossovers in AtMSH2-DN transgenic lines

A complete absence of multiple crossovers has been observed in several studies of homeologous recombination between chromosomes from wheat and related species (Dubcovsky et al. 1995; Lukaszewski 1995, 2000; Luo et al. 1996, 2000), in contrast to homologous recombination. The restriction to essentially single crossovers per arm and absence of multiple exchanges among recovered recombinants showed that the reduction of multiple crossovers was not in proportion to the reduction in overall recombination frequency relative to homologues (Lukaszewski et al. 2004). Therefore, it is interesting to note that in tomato, disruption of the *MSH2* gene can have an effect on the unequal recovery of second- and higher-order crossover events.

A previous study by Ji and Chetelat (2003) showed that for homologous recombination in tomato, average genome wide chromosome pairing consisted of 6.2 ring and 5.8 rod bivalents per cell, giving an approximate 1:1 ratio of SCO:DCO events. For the homeologous chromosome pair in SL-8, a decrease in ring bivalents relative to rod bivalents (5.18 and 6.72 per cell, respectively) and substantial numbers of unpaired chromosomes (0.2 univalents per cell) were observed. GISH studies of SL-8 revealed over twice as many rod bivalents as ring bivalents (62.6 vs. 27.6%, respectively), indicating a marked decrease in double crossover events. In our study, suppression of MSH2 by dominant negative overexpression resulted in the recovery of more DCOs relative to SCO events. In addition, the occurrences of three and four crossover events at very low frequency were also detected in the MSH2-DN transgenic F_2 progenies. The probability of a crossover influencing additional crossovers is determined by crossover interference, a process imposed sequentially at two different stages in the development of crossovers, from the formation of DSBs early during Prophase I and later at the maturation of crossovers (de Boer et al. 2006). Our findings suggest that MSH2 may influence crossover interference during homeologous recombination in tomato. However, as our study is unable to distinguish the types of crossovers, it could be that the additional crossovers observed arose from an increase in class II crossovers (which are not sensitive to interference) and formed in the Mus81–Mms4 pathway (Mercier and Grelon, 2008).

Altered transmission of SL-8 in *SlMSH7*-RNAi transgenic lines

Segregation distortion, which is a disturbance of Mendelian inheritance, is commonly observed in progeny of interspecific hybrids in plants (Song et al. 2006). In the present study, we observed strong deficiencies in the transmission of *S. lycopersicoides* alleles to the progeny of the SL-8 substitution lines, including a complete absence of homozygous SL-8 genotypes. These observations agree with similar trends reported in other interspecific tomato crosses (Canady et al. 2005; Chetelat and Meglic 2000; Gadish and Zamir 1986; Ji and Chetelat 2003). However, reduced expression of *MSH7* increased the transmission rate of parental *S. lycopersicoides* chromosomes, implicating this MMR gene in recombination or other processes influencing transmission, such as selective elimination of alien genotype during male gametogenesis presumably due to pollen competition (Ji and Chetelat 2003) or fertilization and/or post-syngamic processes (Chetelat and Meglic 2000). In addition, results from our AtMSH2-DN1 populations, which contained a region of homology on the SL-8 chromosome, showed that high recombination (presumably homologous in this case) rates can overcome selective barriers, as transmission ratios for the flanking loci returned to almost normal (1:1 ratio of alleles).

MSH2 and *MSH7* gene suppressions did not affect seed set or fertility in tomato

It was reported that *MSH7* suppression affected fertility (seed set and weight) in transgenic barley (Lloyd et al. 2007). *AtMLH1* and *AtPMS1* mutants also exhibited reduced seed set, as well as biased transmission of the mutant alleles (Dion et al. 2007; Li et al. 2009). Our experiments using *SlMSH2*- and *SlMSH7*-deficient lines

did not display marked reduction in fertility or selection against the relevant transgenes. However, Li et al. (2009) further reported that RNAi lines generated to confirm resultant phenotypes of *AtPMS1* did not show decreased fertility or distorted transgene transmission ratios. The authors suggest that incomplete gene silencing methods such as RNAi and dominant negative proteins may not sufficiently reduce gene expression to impact fertility and/or transgene segregation. This is an important point to consider for plant breeding, because genetic manipulations that negatively affect fertility or gene transmission are generally undesirable.

Recent published findings from *Arabidopsis* have revealed significant differences in the impact of MMR function on homeologous recombination between *E. coli,* yeast and higher plants, and our study demonstrates further that differences can be observed between tomato and *Arabidopsis*. It may be that recovery of homologous recombination rates for tomato will require manipulation of additional gene systems besides the MMR system, thus, future work should take into consideration other factors that can influence observed recombination rates measured from the progeny such as transmission ratio bias, if we are to significantly increase the accessibility of a wider array of genetic resources for tomato breeding.

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References

Adé J, Belzile F, Philippe H, Doutriaux MP (1999) Four mismatch repair paralogues coexist in Arabidopsis thaliana: AtMSH2, AtMSH3, AtMSH6–1 and AtMSH6–2. Mol Genet Genom 262:239–249

Alani E, Sokolsky T, Studamire B, Miret JJ, Lahue RS (1997) Genetic and biochemical analysis of Msh2p-Msh6p: Role of ATP hydrolysis and Msh2p-Msh6p subunit interactions in mismatch base recognition. Mol Cell Biol 17:2436–2447

Al-Kaff N, Knight E, Bertin I, Foote T, Hart N, Griffiths S, Moore G (2008) Detailed dissection of the chromosomal region containing the Ph1 locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling. Ann Bot 101:863–872

Bailis AM, Rothstein R (1990) A defect in mismatch repair in *Saccharomyces cerevisiae* stimulates ectopic recombination between homeologous genes by an excision repair dependent process. Genetics 126:535–547

Baudat F, de Massy B (2007) Regulating double stranded DNA break repair towards crossover or non-crossover during mammalian meiosis. Chromosome Res 15:565–577

Belmaaza A, Milot E, Villemure JF, Chartrand P (1994) Interference of DNA sequence divergence with precise recombinational DNA repair in mammalian cells. EMBO J 13:5355–5360

Bozza CG, Pawlowski WP (2008) The cytogenetics of homologous chromosome pairing in meiosis in plants. Cytogenet Genome Res 120:313–319

Butlin RK (2005) Recombination and speciation. Mol Ecol 14:2621–2635

Canady MA, Meglic V, Chetelat RT (2005) A library of *Solanum lycopersicoides* introgression lines in cultivated tomato. Genome 48:685–697

Chambers SR, Hunter N, Louis EJ, Borts RH (1996) The mismatch repair system reduces meiotic homeologous recombination and stimulates recombination-dependent chromosome loss. Mol Cell Biol 16:6110–6120

Chen W, Jinks-Robertson S (1999) The role of the mismatch repair machinery in regulating mitotic and meiotic recombination between diverged sequences in yeast. Genetics 151:1299–1313

Chetelat RT, Meglic V (2000) Molecular mapping of chromosome segments introgressed from *Solanum lycopersicoides* into cultivated tomato (*Lycopersicon esculentum*). Theor Appl Genet 100:232–241

Chetelat RT, Cisneros P, Stamova L, Rick CM (1997) A male fertile *Lycopersicon esculentum* × *Solanum lycopersicoides* hybrid enables direct backcrossing to tomato at the diploid level. Euphytica 95:99–108

Chetelat RT, Meglic V, Cisneros P (2000) A genetic map of tomato based on BC(1) *Lycopersicon esculentum x Solanum lycopersicoides* reveals overall synteny but suppressed recombination between these homeologous genomes. Genetics 154:857–867

Culligan KM, Hays JB (1997) DNA mismatch repair in plants. An *Arabidopsis thaliana* gene that predicts a protein belonging to the *MSH2* subfamily of eukaryotic muts homologs. Plant Physiol 115:833–839

Culligan KM, Hays JB (2000) *Arabidopsis* MutS homologs-At*MSH2*, AtMSH3, AtMSH6, and a novel AtMSH7-form three distinct protein heterodimers with different specificities for mismatched DNA. Plant Cell 12:991–1002

Datta A, Adjiri A, New L, Crouse GF, Jinks-Robertson S (1996) Mitotic crossovers between diverged sequences are regulated by mismatch repair proteins in *Saccharomyces cerevisiae*. Mol Cell Biol 16:1085–1093

Datta A, Hendrix M, Lipsitch M, Jinks-Robertson S (1997) Dual roles for DNA sequence identity and the mismatch repair system in the regulation of mitotic crossing-over in yeast. Proc Natl Acad Sci USA 94:9757–9762

de Boer E, Stam P, Dietrich AJ, Pastink A, Heyting C (2006) Two levels of interference in mouse meiotic recombination. Proc Natl Acad Sci USA 103:9607–9612

Dion E, Li L, Jean M, Belzile F (2007) An Arabidopsis MLH1 mutant exhibits reproductive defects and reveals a dual role for this gene in mitotic recombination. Plant J 51:431–440

Dong C, Whitford R, Langridge P (2002) A DNA mismatch repair gene links to the *Ph2* locus in wheat. Genome 45:116–124

Dubcovsky J, Luo M-C, Dvorak J (1995) Differentiation between homoeologous chromosomes 1A of wheat and 1Am of *Triticum monococcum* and recognition of homology by the Ph1 locus of wheat. Proc Natl Acad Sci USA 92:6645–6649

Edlinger B, Schlohelhofer P (2011) Have a break: detreminants of meiotic DNA double strand break (DSB) formation and processing in plants. J Exp Bot 62:1545–1563

Emmanuel E, Yehuda E, Melamed-Bessudo C, Avivi-Ragolsky N, Levy AA (2006) The role of AtMSH2 in homologous recombination in *Arabidopsis thaliana*. EMBO Rep 7:100–105

Fulton T, van der Hoeven R, Eannetta N, Tanksley S (2002) Identification, analysis and utilization of a conserved ortholog set (COS) markers for comparative genomics in higher plants. Plant Cell 14:1457–1467

Gadish I, Zamir D (1986) Differential zygotic abortion in an interspecific *Lycopersicum* cross. Genome 29:156–159

Gaut BS, Wright SI, Rizzon C, Dvorak J, Anderson LK (2007) Recombination: an underappreciated factor in the evolution of plant genomes. Nat Rev Genet 8:77–84

Gleave AP (1992) A versatile binary vector system with a T-DNA organizational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Mol Biol 20:1203–1207

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752

Harfe BD, Jinks-Robertson S (2000) DNA mismatch repair and genetic instability. Annu Rev Genet 34:359–399

Hartung F, Suer S, Puchta H (2007) Two closely related RecQ helicases have antagonistic roles in homologous recombination and DNA repair in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 140:18836–18841

Higgins JD, Armstrong SJ, Christopher F, Franklin H, Jones GH (2004) The *Arabidopsis MutS* homolog *AtMSH4* functions at an early step in recombination: evidence for two classes of recombination in *Arabidopsis*. Genes Dev 18:2557–2570

Hoffmann ER, Borts RH (2004) Meiotic recombination intermediates and mismatch repair proteins. Cytogenet Genome Res 107:232–248

Hunter N, Chambers SR, Louis EJ, Borts RH (1996) The mismatch repair system contributes to meiotic sterility in an interspecific yeast hybrid. EMBO J 15:1726–1733

Jackson N, Sanchez-Moran E, Buckling E, Armstrong SJ, Jones GH, Franklin FC (2006) Reduced meiotic crossovers and delayed prophase I progression in AtMLH3-deficient *Arabidopsis*. EMBO J 25:1315–1323

Ji Y, Chetelat RT (2003) Homoeologous pairing and recombination in *Solanum lycopersicoides* monosomic addition and substitution lines of tomato. Theor Appl Genet 106:979–989

Ji Y, Pertuzé R, Chetelat RT (2004) Genome differentiation by GISH in interspecific and intergeneric hybrids of tomato and related nightshades. Chromosome Res 12:107–116

Jiricny J (2006) The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol 7:335–346

Kimura S, Sakaguchi K (2006) DNA repair in plants. Chem Rev 106:753–766

Kolas NK, Cohen PE (2004) Novel and diverse function of the DNA mismatch repair family in mammalian meiosis and recombination. Cytogenet Genome Res 107:216–231

Lafleuriel J, Degroote F, Depeiges A, Picard G (2007) Impact of the loss of AtMSH2 on double-strand break-induced recombination between highly diverged homeologous sequences in *Arabidopsis thaliana* germinal tissues. Plant Mol Biol 63:833–846

Lee L-Y, Kononov ME, Bassuner B, Frame BR, Wang K, Gelvin SB (2007) Novel plant transformation vectors containing the superpromoter. Plant Physio 145:1294–1300

Li GM (2008) Mechanisms and functions of DNA mismatch repair. Cell Res 18:85–98

Li L, Jean M, Belzile F (2006) The impact of sequence divergence and DNA mismatch repair on homeologous recombination in *Arabidopsis*. Plant J 45:908–916

Li J, Hisa A-P, Schnable PS (2007) Recent advances in plant recombination. Curr Opin Plant Biol 10:131–135

Li L, Dion E, Richard G, Domingue O, Jean M, Belzile FJ (2009) The Arabidopsis DNA mismatch repair gene PMS1 restricts somatic recombination between homeologous sequences. Plant Mol Biol 69:675–684

Lin DP, Wang Y, Scherer SJ, Clark AB, Yang K, Avdievich E, Jin B, Werling U, Parris T, Kurihara N, Umar A, Kucherlapati R, Lipkin M (2004) An Msh2 point mutation uncouples DNA mismatch repair and apoptosis. Cancer Res 64:517–522

Lloyd AH, Milligan AS, Langridge P, Able JA (2007) TaMSH7: a cereal mismatch repair gene that affects fertility in transgenic barley (*Hordeum vulgare* L.). BMC Plant Biol 20:7–67

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90:714–719

Lukaszewski AJ (2000) Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. Crop Sci 40:216–225

Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R (2004) Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome 47:36–45

Luo M-C, Dubcovsky J, Goyal S, Dvorak J (1996) Engineering of interstitial foreign chromosome segments containing the K^+/NA^+ selectivity gene *Kna*1 by sequential homoeologous recombination in durum wheat. Theor Appl Genet 93:1180–1184

Luo M-C, Yang ZL, Kota RS, Dvorak J (2000) Recombination of chromosomes 3A*m* and 5A*m* of *Triticum monococcum* with homoeologous chromosomes 3A and 5A of wheat: the distribution of recombination across chromosomes. Genetics 154:1301–1308

Lynn A, Soucek R, Borner GV (2007) ZMM proteins during meiosis: crossover artists at work. Chromosome Res 15:591–605

Martinez-Perez E, Moore G (2008) To check or not to check? The application of meiotic studies to plant breeding. Curr Opin Plant Biol 11:222–227

Matic I, Radman M, Rayssiguier C (1994) Structure of recombinants from conjugational crosses between *Escherichia coli* donor and mismatch repair deficient *Salmonella typhimurium* recipients. Genetics 136:17–26

Menzel MY (1962) Pachytene chromosomes of the intergeneric hybrid *Lycopersicon esculentum* × *Solanum lycopersicoides*. Am J Bot 49:605–615

Mercier R, Grelon M (2008) Meiosis in plants: ten years of gene discovery. Cytogenet Genome Res 120:281–290

Nicholson A, Hendrix M, Jinks-Robertson S, Crouse GF (2000) Regulation of mitotic homeologous recombination in yeast. Functions of mismatch repair and nucleotide excision repair genes. Genetics 154:133–146

Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FCH (2011) Pathways to meiotic recombination in *Arabidopsis thaliana*. New Phytol 190:523–544

Parokonny AS, Marshall JA, Bennett MD, Cocking EC, Davey MR, Power JB (1997) Homeologous pairing and recombination in backcross derivatives of tomato somatic hybrids [*Lycopersicon esculentum* (+) *L. peruvianum*]. Theor Appl Genet 94:713–723

Pertuze RA, Ji Y, Chetelat RT (2002) Comparative linkage map of the *Solanum lycopersicoides* and *S. sitiens* genomes and their differentiation from tomato. Genome 45:1003–1012

Priebe SD, Westmoreland J, Nilsson-Tillgren T, Resnick MA (1994) Induction of recombination between homologous and diverged DNAs by double-strand gaps and breaks and role of mismatch repair. Mol Cell Biol 14:4802–4814

Qi L, Friebe B, Zhang P, Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res 15:3–19

Rick CM (1951) Hybrids between *Lycopersicon esculentum* Mill. And *Solanum lycopersicoides* Dun. Proc Natl Acad Sci USA 37:741–744

Rick CM (1969) Controlled introgression of chromosomes of *Solanum pennellii* into *Lycopersicon esculentum*: segregation and recombination. Gene 62:753–768

Rick CM (1988) Tomato like nightshades: affinities, autecology and breeders opportunities. Econ Bot 42:145–154

Rick CM, deVerna JW, Chetelat RT, Stevens MA (1986) Meiosis in sesquidiploid hybrids of *Lycopersicon esculentum* and *Solanum lycopersicoides*. Proc Natl Acad Sci USA 83:3580–3583

Roberts MA, Reader SM, Dalgliesh C, Miller TE, Foote TN, Fish LJ, Snape JW, Moore G (1999) Induction and characterization of *Ph1* wheat mutants. Genetics 153:1909–1918

Schuermann D, Molinier J, Fritsch J, Hohn B (2005) The dual nature of homologous recombination in plants. Trends Genet 21:172–181

Sears ER (1977) An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Sears ER (1982) A wheat mutation conditioning an intermediate level of homeologous chromosome pairing. Can J Genet Cytol 24:715–719

Selva EM, New L, Crouse GF, Lahue RS (1995) Mismatch correction acts as a barrier to homeologous recombination in *Saccharomyces cerevisiae*. Genetics 139:1175–1188

Sidhu GK, Rustgi S, Shafqat MN, von Wettstein D, Gill KS (2008) Fine structure mapping of a gene-rich region of wheat carrying Ph1, a suppressor of crossing over between homoeologous chromosomes. Proc Natl Acad Sci USA 105:5815–5820

Song XL, Sun XZ, Zhang TZ (2006) Segregation distortion and its effect on genetic mapping in plants. Chin J Agr Biotechnol 3:163–169

Spampanito CP, Gomez RL, Galles C, Lario LD (2009) From bacteria to plants: a compendium of mismatch repair essays. Mutat Res 682:110–128

Surtees JA, Argueso JL, Alani E (2004) Mismatch repair proteins: key regulators of genetic recombination. Cytogenet Genome Res 107:146–159

Tam SM, Samipak S, Britt A, Chetelat RT (2009) Characterization and comparative sequence analysis of the DNA mismatch repair MSH2 and MSH7 genes from tomato. Genetica 137:341–354

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Trouiller B, Schaefer DG, Charlot F, Nogué F (2006) *MSH2* is essential for the preservation of genome integrity and prevents homeologous recombination in the moss *Physcomitrella patens*. Nucl Acids Res 34:232–242

Wijnker E, de Jong H (2008) Managing meiotic recombination in plant breeding. Trends Plant Sci 13:640–646

Wu SY, Culligan K, Lamers M, Hays JB (2003) Dissimilar mispair-recognition spectra of *Arabidopsis* DNA-mismatch-repair proteins MSH2*MSH6 (MutSα) and MSH2*MSH7 (MutSγ). Nucl Acids Res 31:6027–6034

Yousafzai FK, Al-Kaff N, Moore G (2010) Structural and functional relationship between the *Ph1* locus protein 5B2 in wheat and CDK2 in mammals. Funct Integr Genom 10:157–166