MutS-Homolog2 silencing generates tetraploid meioocytes in tomato (Solanum lycopersicum)

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**SUMMARY**

MSH2 is the core protein of MutS-homolog family involved in recognition and repair of the errors in the DNA. While other members of MutS-homolog family reportedly regulate mitochondrial stability, meiosis, and fertility, MSH2 is believed to participate mainly in mismatch repair. The search for polymorphism in *MSH2* sequence in tomato accessions revealed both synonymous and nonsynonymous SNPs; however, SIFT algorithm predicted that none of the SNPs influenced MSH2 protein function. The silencing of *MSH2* gene expression by RNAi led to phenotypic abnormalities in highly-silenced lines, particularly in the stamens with highly reduced pollen formation. *MSH2* silencing exacerbated formation of UV-B induced thymine dimers and blocked light-induced repair of the dimers. The *MSH2* silencing also affected the progression of male meiosis to a varying degree with either halt of meiosis at zygotene stage or formation of diploid tetrads. The immunostaining of male meioocytes with centromere localized CENPC (Centromere protein C) protein antibody showed the presence of 48 univalent along with 24 bivalent chromosomes suggesting abnormal tetraploid meiosis. The mitotic cells of root tips of silenced lines showed diploid nuclei but lacked intervening cell plates leading to cells with syncytial nuclei. Thus we speculate that tetraploid pollen mother cells may have arisen due to the fusion of syncytial nuclei before the onset of meiosis. It is likely that in addition to Mismatch repair (MMR), MSH2 may have an additional role in regulating ploidy stability.
INTRODUCTION

The survival of the living organisms is dependent on the precise and error-free replication of DNA. In addition, normal metabolic activity and environmental factors such as radiation also cause damage to the DNA. The fidelity of the DNA is ensured by a cohort of mechanisms that detects and eliminates mistakes occurring during DNA replication. At least five major repair pathways are known to operate in higher plants: base excision repair, nucleotide excision repair, mismatch repair (MMR) and double-strand break repair comprising of homologous recombination repair and nonhomologous end joining (Takata et al., 1998; Guarné et al., 2004; Reardon and Sancar, 2005; Jacobs and Schär, 2012). Among these, MMR is a major pathway that corrects base–base and insertion–deletion mismatches generated during DNA replication or mutagenesis (Dowen et al., 2010; Srivatsan et al., 2014).

MMR is a highly conserved pathway that exists in all living organisms. The MMR process consists of three key steps: mismatch recognition, excision, and resynthesis (Ban et al., 1999; Antony and Hingorani, 2003; Guarné et al., 2004). The first step of the mismatch recognition in eukaryotes is carried out by the homologs of prokaryotic MutS proteins, namely MSH protein subunits. There are eight homologs of MutS in eukaryotes: MSH1 to MSH8, of which MSH7 is found only in plants (Culligan and Hays, 2000) and MSH8 in Euglenozoa (Sachadyn, 2010). The MSH proteins recognize mismatches as heterodimers, where MutSα (MSH2-MSH6) repairs base-base mismatches or 1-2 nucleotide insertion-deletion loops (Acharya et al., 1996; Genschel et al., 1998), while MutSβ (MSH2-MSH3) recognizes larger, up to 14 nucleotide insertion-deletion loops (Modrich, 1991; Marti et al., 2002). Plants form an additional heterodimeric complex known as MutSγ (MSH2–MSH7) (Culligan and Hays, 2000) which recognizes some base–base mismatches and reportedly plays a role in meiotic recombination (Lloyd et al., 2007). The binding of MutSα/β to mismatched DNA initiates conformational changes in MutS recruiting MutL complex followed by activation of exonuclease1/PCNA. The gap generated by DNA excision is filled by action of DNA polymerase and DNA ligase.

It is reported that the other members of MSH2 family function beyond MMR. MSH1 is required for mitochondrial stability (Reenan and Kolodner, 1992) and disruption of MSH1 can influence male sterility in several species (Sandhu et al., 2007;
The MSH4 and MSH5 form heterodimer between them without involving MSH2 and exclusively function in meiosis (Hollingsworth et al., 1995; Schofield and Hsieh, 2003, Lu et al., 2008; Wang et al., 2016). The expression of MSH7 is required for wild-type level of fertility in barley (Lloyd et al., 2007). MSH7 also plays a role in UV-B-induced DNA damage recognition and recombination in Arabidopsis (Lario et al., 2015).

Among the MMR proteins, MSH2 is a common protein in three of the heterodimers formed in the plants. In conformity of the role of MSH2 protein in repair of replication errors, an Arabidopsis mutant defective in MSH2 shows microsatellite instability (Depeiges et al., 2005). The msh2 mutants in Moss show strong developmental defects, are sterile and have a mutator phenotype (Trouiller et al., 2006). A similar phenotype was also observed in Arabidopsis thaliana msh2 mutant lines albeit only after five generations (Hoffman et al., 2004). Considering that suppression of MMR leads to an increase in natural mutation frequency, MSH2 gene was silenced in Nicotiana tabacum and N. plumbaginifolia using an artificial microRNA (amiRNA). Though the transformed lines showed developmental and morphological abnormality, the mutant phenotype was not transmitted to subsequent generations (van Marcke and Angenon, 2013).

Among the interacting partners for MSH2; mutants are reported only for MSH6 and MSH7 in Arabidopsis. The msh6 mutant does not display a visible phenotype (Lario et al., 2011) however, msh7 mutant shows an increase in meiotic recombination frequency (Lario et al., 2015). The role of MSH7 in meiotic recombination is also reported in tomato, where RNAi silencing of MSH7 improves homeologous meiotic recombination with a chromosome from S. lycopersicoides (Tam et al., 2011). Most likely the high increase in meiotic recombination is mediated by the MSH2-MSH7 complex as Arabidopsis msh2 mutant also shows 40% increase in the meiotic crossover rates (Emmanuel et al., 2006).

In this study, our aim was to isolate MSH2-defective mutants to obtain a hypermutable line to increase the efficiency of EMS-mutagenesis in tomato. We screened natural accessions of tomato and EMS-mutagenized lines for SNPs/mutation in tomato MSH2 by EcoTILLING and TILLING respectively. Considering that little polymorphism was observed in MSH2 in tomato accessions, we silenced MSH2 gene
using MSH2-RNAi construct. We report that silencing of MSH2 gene led to a developmental defect in tomato lines with abnormal flowers bearing tetraploid meiocytes in the anthers. The examination of root cells showed the formation of syncytial nuclei and defects in the cell plate formation. Our study indicates that MSH2 may also have a role in the regulation of meiotic and mitotic cell divisions.
RESULTS

EcoTILLING of MSH2 gene showed little polymorphism in tomato accessions

Given the pivotal role of MSH2 in MMR, we screened a total of 391 tomato accessions for polymorphism in the gene. A 2.7 Kb region (4-9 exon) of MSH2 predicted to be deleterious by CODDLE software was chosen for EcoTILLING. Consistent with the essential nature of the gene, only 7 accessions showed polymorphism with a total of 13 SNPs with a frequency of 1.23 SNP/100 Kb. (Figure S1). Among the identified SNPs, 6 were non-synonymous, and 7 were synonymous (Table 1). The likelihood of non-synonymous SNPs affecting protein function was examined by SIFT software. Since none of the SNP showed a SIFT score <0.05, these SNPs were predicted to be non-deleterious in nature. A parallel screening of 1243 bp region of MSH2 gene covering 4th exon of 11304 tomato EMS-mutagenized lines by TILLING did not yield any mutation.

Generation of MSH2-RNAi lines

Considering recalcitrance to the mutagenesis and low polymorphism in MSH2 gene, we silenced the gene by RNAi. A 301 bp region of MSH2 gene with no significant homology to any other tomato gene was amplified and cloned into pHELLSGATE 12 vector (Figure S2). A total of ten independent transgenic lines bearing MSH2-RNAi construct were raised using Agrobacterium-mediated transformation. Southern blot analysis of T0 lines revealed the presence of the transgene in five lines (Figure S3). In T2 generation out of three lines, the lines 1T21-11 and 2T27-5 with strong MSH2 silencing showed a single transgene copy (Figure S4).

MMR transcript analysis in MSH2-RNAi lines using real-time PCR

To ascertain the magnitude of gene silencing, the level of MSH2 transcript in transgenic lines was quantified by real-time PCR. Out of five Southern positive lines, the line 1T21-11 showed ~80% reduction of MSH2 transcript level followed by line 2T25-5 and 2T27-5 compared to wild-type (WT) control (Figure 1). Line 3T21-4 showed moderate, and 5T23-3 showed no reduction in MSH2 transcript levels. The reduction in the transcript level in line1T21-11 was not restricted to MSH2 alone; it also
reduced the transcript levels of \textit{MSH5} and \textit{MSH7} albeit to a moderate but a significant extent. Since, \textit{MSH2}, \textit{MSH5}, and \textit{MSH7} genes bear no significant sequence homology, the reduction in \textit{MSH5} and \textit{MSH7} transcript is most probably due to some indirect influence of \textit{MSH2} transcript reduction.

\textbf{\textit{MSH2} silencing exacerbates UV-B-mediated thymine dimer formation}

In mammalian cells, the contribution of the MMR pathway to the UVB-induced DNA damage is well established (Narine \textit{et al.}, 2007; Seifert \textit{et al.}, 2008). Similarly, Arabidopsis \textit{MSH2} T-DNA insertion lines show higher levels of Cyclobutane Pyrimidine Dimer (CPD) on exposure to UV-B (Lario \textit{et al.}, 2011, 2015). We used UV-B induced thymine dimer formation as an indirect assay to monitor the activity of \textit{MSH2} in transgenic plants in comparison with WT. Pre- and post-UV-B exposure, the thymine dimers were quantified in genomic DNA of above plants by using a thymine dimer-specific monoclonal antibody (Figure S5, Figure 2). Prior to UV-B exposure, the steady-state levels of thymine dimers in control and \textit{MSH2} silenced lines were nearly similar. However, post-6 h UV-B exposure, \textit{MSH2} silenced lines showed a high level of dimers than the control plants. The thymine dimer level in \textit{MSH2}-RNAi lines was higher than the control even after 24 h dark or white light incubation (Figure 2). It appears that high accumulation of thymine dimer in \textit{MSH2} transgenic lines may have resulted from low \textit{in-vivo} MMR activity.

\textbf{\textit{MSH2-RNAi} lines display abnormal pollen formation}

The lines 1T\textsubscript{2}1-11 and 2T\textsubscript{2}7-5 with a higher reduction in \textit{MSH2} transcript also showed phenotypic abnormalities in \textit{T\textsubscript{1}} and \textit{T\textsubscript{2}} generation (Figure 3a-f). The most pronounced abnormality was in the stamen morphology (Figure 3g-h). The plants in later generations too showed abnormalities in flowers, mostly in the stamens. The progeny plants of silenced lines either failed to set fruits or set fruits with very few seeds (Figure 3i-j). These seeds on germination showed a variable degree of viability. The viable seeds albeit in reduced number were propagated to the next generation.

The examination of pollens revealed that strongly silenced lines produced pollens with the deformed shape. Unlike WT pollens that were round with a vegetative and generative cell (Figure 3k-l), the pollens of silenced lines showed the deformed
shape and were either empty or contained the remnant of chromosome fragments (Figure 3m-n). It is likely that pollen grains collapsed either due to lack of nourishment in deformed anthers or MSH2-silencing affected some other gene.

The viability of WT and MSH2-RNAi pollens was examined by Alexander staining. In WT, all pollens exhibited a red-purple fluorescence, strongly reflecting the high male gametophytic fertility of these plants (Figure 3o). In contrast, MSH2-RNAi lines such as 1T21-11 displayed mostly inviable pollens (Figure 3p-r). As a consequence, MSH2-RNAi lines set fruits bearing ca. 5% seeds. The tomato line 1T21-11 with the highest reduction in MSH2 expression showed a maximum loss in the fertility with > 90% blue stained pollen grains (Figure 3p). The fruits of 1T21-11 line bore very few viable seeds along with underdeveloped seeds (Figure 3j).

**MSH2-RNAi lines display tetraploid meiosis progression**

The deformities in the anther morphology are reported to be associated with defects in male meiosis in tomato (Rick, 1948; Jeong *et al*., 2014) and Arabidopsis (Panoli *et al*., 2006). Considering that observed stamen abnormalities could be associated with meiosis, we examined meiosis progression in the MSH2 silenced lines. The pollen mother cells (PMCs) were isolated at different stages of floral bud development as outlined by Brukhin *et al*. (2003) from MSH2 silenced lines and WT. A complete meiotic progression was examined for WT which progressed normally with characteristic landmarks for different stages of the meiosis, such as zygotene (Figure 4a), pachytene (Figure 4b), diplotene and diakinesis (Figure 4c-d) followed by metaphase (Figure 4e), early and late anaphase (Figure 4f-g), early and late telophase (Figure 4h-i).

In strongly silenced MSH2-RNAi lines, irrespective of the stage of floral bud development, predominant short condensed chromosomes were observed in the majority of the meiocytes. These meiocytes were either arrested at the prophase I (Figure 4j-k) or completed later stages of meiosis (Figure 4l-q). The abnormal increase in the number of chromosomes in meiocytes was suggestive of either premature loss of sister chromatid cohesion or increase in ploidy leading to the formation of diploid tetrads (Figure 4q). In these lines, very few meiocytes were observed with WT complement of chromosomes.
To quantify the chromosome number in meiotic cells of MSH2-RNAi lines, we used Centromere Protein C (CENP-C) antibody that specifically labels the centromere. Immunolabelling of WT prophase I meiocytes with CENP-C antibody showed 12 centromeres at pachytene indicating the likely presence of 12 bivalent chromosomes (Figure 5a-c). In contrast, the meiocytes of MSH2 silenced line at a stage equivalent to prophase I showed 48 centromeres (Figure 5d-f). There are two possibilities to explain the presence of 48 centromeres. First, a loss of sister chromatid cohesion in the prophase I may lead to the appearance of 48 sister chromatids. Second, an increase in ploidy to tetraploid in the pollen mother cell followed by an absence or delay in sister chromatid cohesion may result in 48 chromosomes. Analysis of meiotic stages favors the second scenario where we can observe the occurrence of diploid tetrads where the sister chromatids separate during anaphase II. Consistent with above, among the meiocytes examined, most contained 48 chromosomes as signified by CENP-C, however, in few meiocytes, the meiosis proceeded to anaphase I with segregation of 24 chromosomes to each pole (Figure 5g-i). Beyond this, other stages of meiosis I and meiosis II was rarely observed indicating a block in the meiosis of the silenced lines. However, a few cells displayed 12 centromeres indicating that silenced lines generate haploid spores albeit at highly reduced level.

We observed that only a few tetraploid cells completed meiosis II with the formation of diploid tetrads at the end of meiosis II (Figure 4k-o). The analysis of relative proportions of meiocytes revealed that in 1T21-11 line, 25% meiocytes displayed abnormal tetraploid chromosomes, 67% tetraploid meiocytes and 8% displayed diploid meiosis (Table 2). Similarly, the 2T27-5 line had 25% meiocytes with abnormal tetraploid and 75% tetraploid nuclei giving rise to diploid microspores. Consistent with this, the anthers of MSH2 silenced lines contained largely empty shrunken pollen at the end of meiosis. Taken together these results confirm that MSH2-RNAi meiocytes display abnormal meiotic progression resulting in tetraploid meiocytes. The tetraploid meiocytes were observed only in 1T21-11 and 2T27-5 lines with high silencing of MSH2 transcript. The 3T21-4 line with moderate MSH2 silencing and 5T23-3 line with no MSH2 silencing displayed diploid meiocytes similar to WT (Figure S6).

**Analysis of T-DNA integration sites in MSH2-RNAi lines using FPNI-PCR**
We also examined whether the tetraploid meiocytes or defect in cytokinesis was caused by inactivation of some important gene due to insertion of the transgene. To locate the site of transgene insertion, FPNI-PCR was performed on genomic DNA of \(1T_2\text{-}11\) and \(2T_2\text{-}7\text{-}5\) tetraploid \(MSH2\text{-RNAi}\) lines. The amplified PCR product flanking the right border of inserted T-DNA was cloned and sequenced. The flanking sequence of line \(1T_2\text{-}11\) showed 98% homology to \(Tic22\) gene in chromosome No. 9 (Table S1, Figure S7a,b) and that of \(2T_2\text{-}7\text{-}5\) showed hit in \(TRM32\) gene, a member of the TON1 Recruiting Motif superfamily (Table S1, Figure S7c,d). While \(TRM1\) reportedly associates with cortical microtubules, not all TRM proteins associate with microtubules. Moreover, the sequence diversity among the TRM family members with varied transcriptional expression patterns indicates that different members may have different roles (Struk and Dhonukshe, 2014). Considering that both \(1T_2\text{-}11\) and \(2T_2\text{-}7\text{-}5\) \(MSH2\text{-RNAi}\) lines show tetraploidy, it is unlikely that insertional inactivation of these genes leads to tetraploidy in the silenced lines.

**Root tip cells show diploid nuclei with sporadic defect in cell plate formation**

In the present study, \(MSH2\text{-RNAi}\) tomato lines generated tetraploid meiocytes. However, no predominant enlarged organ size, a characteristic of increased ploidy was observed during the vegetative growth of plants. Even though the silenced lines displayed abnormal floral organs, these too showed no significant enlargement of floral structures. To ascertain whether \(MSH2\) silencing leads to tetraploidy in the progeny of the plants, we examined somatic chromosomal count in root tips of silenced and control seedlings. The nuclei in WT root tips displayed diploid chromosome number. Interestingly the \(MSH2\text{-RNAi}\) lines \(1T_2\text{-}11\) and \(2T_2\text{-}7\text{-}5\) with tetraploid meiocytes also displayed diploid chromosomal nuclei in root tip tissues indicating that these plants were diploid. At the same time, few cells showed no intervening cell wall between the nuclei suggesting that \(MSH2\) silencing affected the cell plate formation (Figure 6). The loss of intervening cell wall resulted in the formation of several syncytial nuclei within a cell (Figure 6e,f). In contrast, the weakly silenced \(MSH2\text{-RNAi}\) lines with diploid meiocytes exhibited root tip with uniformly sized diploid nuclei with distinct cell plate formation (Figure 6c) indicating that loss of cell plate was a feature specific to lines with a higher degree of \(MSH2\) silencing.
To confirm whether loss of cell plate formation was related to the tetraploidy, we examined root tips of tomato tetraploid line, LA3255. The root cells of the LA3255 line showed normal tetraploid nuclei with no defect in the cell plate formation (Figure 6d). Consistent with tetraploid nature of the LA3255 line, the staining of root tip nuclei with DAPI showed larger nuclei in LA3255 line compared to diploid wild-type and MSH2-RNAi lines nuclei (Figure S8).
DISCUSSION

The DNA mismatch repair is the highly conserved biological pathway that maintains the fidelity of DNA replication and genetic stability. In the mammalian system, the defects in MMR lead to abnormalities in meiosis and increases predisposition to certain types of cancer (Li, 2008). Consistent with the vital role of MMR, the MSH2 in tomato appeared recalcitrant to mutagenesis, as evident by the absence of nonsynonymous mutations in M2 lines screened by TILLING. In the mammalian system, the polymorphism in MSH2 significantly increases the risk of breast cancer (Hsieh et al., 2016). The paucity of polymorphism in MSH2 as revealed by EcoTILLING of tomato accessions is in conformity with the indispensable nature of the gene. Though a missense and few silent mutations were identified, these had no influence on the protein function as predicted by SIFT.

In plants, the role of MSH2 gene in MMR has been mainly inferred based on its homology with yeast and mammalian genes. In Arabidopsis, a MSH2 T-DNA insertional mutation accumulates mutations in microsatellites and other loci indicating a role of MSH2 in MMR (Leonard et al., 2003; Hoffman et al., 2004). MSH2 reportedly plays a role in repair of the UV-B induced DNA damage in the mammalian system, (Peters et al., 2003; Seifert et al., 2008). In plants as well, MSH2 likely plays a role in the repair of UV-B induced Cyclobutane Pyrimidine Dimer (CPD). The Arabidopsis MSH2 mutant on UV-B exposure shows enhanced formation of CPD suggesting a role of MSH2 in the repair of UV-B damage (Lario et al., 2011). Similarly, in strongly silenced tomato MSH2-RNAi lines, thymine dimer formation is exacerbated by exposure to UV-B. Also, MSH2-deficient plants show inefficient repair of thymine dimers even on exposure to visible light that stimulates photoreactivation repair. From the preceding, it is apparent that strongly silenced MSH2-RNAi lines have low in vivo MMR activity as evident by enhanced UV-B damage.

The propagation of Arabidopsis MSH2 T-DNA insertional mutant line revealed the accumulation of a wide variety of mutations such as morphological abnormalities, reduction in seed/silique development and loss of germination efficiency (Hoffman et al., 2004). Analogously, strongly silenced tomato MSH2-RNAi lines also show multiple abnormalities throughout the plant development, mainly during floral development with
abnormal anthers. The lines with abnormal stamens showed reduced fruit set and highly reduced seed numbers in the fruits. In contrast to Arabidopsis MSH2 mutant lines where seed set on average was reduced to only 50% (Hoffman et al., 2004), in tomato silenced lines, it was less than 10%.

The reduction in seed set can result from the failure in the embryo development. Considering that the MSH2 silenced lines developed abnormal anthers, reduced seed set may have resulted from the failure to make viable pollens in the deformed anthers. Consistent with this, Alexander staining showed nonviable pollens with blue staining in highly silenced lines. Since WT and moderately silenced lines showed viable pollens with red staining, it can be assumed that MSH2 silencing leads to loss of pollen viability in tomato. Thus the reduced seed set in the silenced lines is in conformity with the loss of pollen viability.

The successful pollen development in plants requires operation of proper meiosis in the developing anther (Ma, 2005). In several plants defects in the anther formation are associated with defective meiosis or meiotic arrest (Walbot and Egger, 2016). In tomato, heat-induced abnormal anther development in a conditional male sterile mutant is associated with reduced pollen set and loss of pollen viability (Rick and Boynton, 1967). It is, therefore, likely that silencing of MSH2 expression affected anther development that in turn may have affected the progression of meiosis.

Consistent with this view, the MSH2 silenced lines showed a meiotic arrest in the anthers. The majority of pollen mother cells (PMC) of MSH2-RNAi lines fail to complete the meiotic cycle. These PMCs initiate meiosis as evident by the onset of zygotene. However, the meiosis gets stalled at the final stage of prophase I – diakinesis, as the chromosomes are at their most condensed form. The stalling at diakinesis is seemingly related to the increase in the ploidy as CENP-C stained cells showed the presence of 48 univalent chromosomes. The stalling of the meiosis is not total, as few CENP-C stained cells also showed 24 bivalent chromosomes, which completed meiosis presumably generating diploid pollens. However, most pollens were shrunken, and only a few showed Alexander staining. It is plausible that loss of pollen viability was largely for the diploid pollens. From the preceding, it is likely that incomplete meiosis and the formation of nonviable pollens contribute to the reduced seed set in the strongly-silenced MSH2-RNAi lines.
The process that generates tetraploid nuclei in the PMC remains to be investigated. In several plants including tomato during microsporogenesis, the nuclei can transfer to the neighboring cell by cytomixis (Weiling, 1965; Mursalimov et al., 2013). Such intercellular transfer of nuclei can influence the ploidy level of the produced pollens. However, such cytomixic transfer of nuclei most probably does not contribute to tetraploid nuclei, as in tomato cytomixis is observed only ca. 15% of PMC (Weiling, 1965). The occurrence of a large number of polyploid pollen mother cells does not favor cytomixis to be the cause of tetraploid nuclei. The probability that tetraploid meiocytes arise due to $MSH2$-silencing-induced endomitosis also seems remote. In Chrysanthemum, the fusion of two PMC forms tetraploids, albeit this process occurs at very low frequency (Kim et al., 2009), and cannot account for high numbers of tetraploid meiocytes in $MSH2$ silenced lines.

The root cells of $MSH2$-RNAi-silenced lines show defective cytokinesis with multiple nuclei in syncytial cells. The similar syncytial cells may also form due to defective cytokinesis during meiocytes archesporial cells development. It is likely that the fusion of nuclei in syncytial cells gives rise to the tetraploid nuclei in PMC of $MSH2$-RNAi-silenced lines. The fusion of nuclei resulting in the stochastic formation of tetraploid PMC initials has been documented in mutants defective in cell plate formation. In Arabidopsis, the mutations in sterol methyl transferase and callose synthase lead to defect in cell plate formation predominantly in the floral tissues. Both mutants also form tetraploid meiocytes and diploid gametes in both male and female sporogenesis (de Storme et al., 2013). The formation of tetraploid meiocytes in $MSH2$ silenced lines is analogous to tomato $pmcd1$ mutant, where cytokinesis failure in meiocytes archesporial cells generates tetraploid nuclei (de Storme and Geelan, 2013a).

The $MSH2$ silenced lines show the formation of the very few viable pollens and the progeny of these plants were exclusively diploid. The strongly reduced seed set in silenced lines seems to indicate that only diploid embryos survived post-fertilization. Another reason could be defective endosperm development due to unbalanced 1:1 ratio rather than normal 1:2 ratio of the paternal and maternal genome (Scott et al., 1998). The absence of triploid progeny may also result from the existence of strong triploid block in members of Solanaceae including tomato (Ehlenfeldt and Ortiz 1995; Nilsson, 1950). The absence of triploid or tetraploid progeny in $MSH2$-RNAi lines is also
consistent with the reported absence of triploid/tetraploid plants in progeny of tomato *pmcd1* mutant that shows a cytokinesis defect and generates diploid pollens (de Storme and Geelan, 2013a).

The pre-meiotic doubling of chromosome occurs in unisexual and parthenogenetically reproducing animal species (Itono et al., 2006). It is believed that in higher plants such chromosomal doubling rarely occurs (de Storme and Geelan, 2013b). In higher plants, the doubling of chromosomes reportedly occurs after the onset of meiosis via meiotic restitution (de Storme and Geelan, 2013b). Considering that doubling of chromosomes in *MSH2* silenced line occurs before the onset of meiosis, it is of a novel nature. The very high frequency of tetraploid meiocytes also hints for a likely function of *MSH2* in the regulation of chromosomal doubling via a mechanism that remains to be deciphered. Since the formation of tetraploid meiocytes in *MSH2* silenced line is quite similar to *pmcd1* mutant, the failure of cytokinesis appears to be the main contributor to this phenomenon.

In plants, MSH2 heterodimerizes with MSH3, MSH6, and MSH7. However, none of these heterodimers reportedly have any function in the regulation of meiosis. Conversely, the *MSH4* and *MSH5* mutants in Arabidopsis and rice though exhibit normal vegetative growth, show severe fertility reduction likely related to meiotic defects (Higgins et al., 2004; Lu et al., 2008; Luo et al., 2013; Wang et al., 2016). The *MSH2*-silenced lines also affected *MSH5* and *MSH7* transcript levels which may have affected the process of meiosis. The manifested tetraploid meiocytes formation and later failure in meiosis might be due to the reduction of these three transcripts. Alternately, the increased microsatellite instability caused due to low activity of the MSH2 protein may contribute to defective cytokinesis and tetraploid meiocytes formation.

Similar to *MSH2* silencing, loss of function lines in 40S ribosomal protein S6 kinases in Arabidopsis generate tetraploid PMCs and show a high degree of pollen abortion (Henriques et al., 2010). Likewise, mutations in glucan synthase-like 8 and sterol methyl transferase 2 lead to defects in somatic cell plate formation and the occasional fusion of syncytial nuclei (Chen et al., 2009; Guseman et al., 2010). It is, therefore, likely that T-DNA insertion in a meiosis/cytokinesis related gene may have caused the tetraploidy. The examination of two silenced lines indicated insertion in *TIC22* and *TRM32* genes; both of these genes have no reported role in meiosis or cell
plate formation. Despite having an insertion in widely unrelated genes, both the silenced lines showed identical phenotype suggesting a close correlation between MSH2 silencing and tetraploidy.

The tetraploid formation in MSH2 silenced lines is more akin to the proposal that DNA replication or repair defects might produce signals that block cytokinesis and yield tetraploids in cancer cells (Ganem et al., 2007). While it is not reported in plants, in mice embryo fibroblasts, deficiency of MSH2 in conjunction with P53 revealed that MSH2 plays a role in preventing polyploidization of cells (Strathdee et al., 2001). In mammalian cells, MSH2 has been identified as a target of the E2F transcription factors (Ren et al., 2002) wherein the loss of MSH2 inhibits the interaction of E2F with MSH2, thus affecting the E2F signaling. It is believed that disruption of the E2F signaling affects the progression of cell cycle and promotes failure of cytokinesis. Since MSH2 have a probable role in mammalian cytokinesis, it is plausible that MSH2 may affect cytokinesis in plants in a similar fashion. Nonetheless, it remains to be determined whether MSH2 alone or with its interacting partners plays a direct or an indirect role in regulation ploidy and cytokinesis in plants. It is also likely that MSH2 silencing leads to wide ramification in plant cells and observed change in the ploidy level reflect its downstream effect.

In summary, our study indicates that MSH2 silencing leads to tetraploid meiocytes formation in tomato. Additionally, MSH2 silencing also affects cytokinesis with multiple nuclei in syncytial cells. Though the precise mechanism of MSH2 action remains to be deciphered, these phenotypes indicate an additional role for MSH gene family. Our results also suggest that similar to cytomixis, the formation of syncytial cells may contribute to the tetraploid formation in higher plants. Uncovering the role of MSH2 in the regulation of cytokinesis and nuclear fusion will be of great interest in future studies and may contribute to an alternate route to the polyploid formation.
EXPERIMENTAL PROCEDURES

Screening for SNPs/mutations in MSH2 gene

The population used for the EcoTILLING was same as described earlier in Mohan et al. (2016) and Upadhyaya et al. (2017). Genomic DNA was isolated from tomato accessions or EMS-mutagenized M₂ lines following the procedure of Sreelakshmi et al. (2010). MSH2 gene sequence was obtained from SGN (solgenomics.net, Solyc06g069230). CODDLE software (blocks.fhcrc.org/proweb/coddle) was used to predict the most deleterious region of the gene. The primers for SNP/mutation screening were designed using Primer3web version 4.0.0 (bioinfo.ut.ee/primer3). (Table S2). The SNP/mutation detection and haplotype assignment were carried out as described by Mohan et al. (2016). The sequence variations (Substitutions and Indels) were detected by using ‘Multalin’ software (http://multalin.toulouse.inra.fr/multalin/, Corpet (1988). The SNP frequency was calculated using the formula: (Total number of SNPs detected / total length of the screened fragment) X 1000 (Frerichmann et al., 2013). PARSESNP (Taylor and Greene, 2003) was used to analyze and display the variation in gene sequences, and SIFT (Sorting intolerant from tolerant; Sim et al., 2012) was used to predict the deleterious variations for protein functions.

Cloning of a fragment of MSH2 gene in pHELLSGATE 12 RNAi vector

For RNAi silencing, a 301 bp region in 4th exon of MSH2 showing no significant homology with any other tomato gene was selected. The 301 bp region from MSH2 cDNA was PCR amplified using primers set with the attB sequence at the 5’ end (FP- AGATTCTCCAGTGATTGTTGCT; RP- CAGATCCTGTACCAAATCCCTA). The PCR product was cloned into a pDONR201 vector with attP1 and attP2 sites using BP Clonase enzyme (Invitrogen). The gene fragment from the intermediate clone was transferred to the pHELLSGATE12 vector (CSIRO, Australia) by recombination between attL1/attL2 and attR1/attR2, mediated by LR Clonase enzyme (Invitrogen). The confirmed clone was transformed into Agrobacterium tumefaciens C58C1 strain.

Generation of MSH2-RNAi lines

Tomato (cv. Arka Vikas) transformation was performed as described earlier
(Sharma et al., 2009) yielding ten independent transgenic lines. The phenotyping of the transgenic plants was continually carried out in successive generations. The lines with 80-90% MSH2 transcript silencing showed anther abnormalities. Therefore these lines were manually pollinated to propagate to next generation. Nonetheless, few T2 lines with very strong anther-defects did not survive as they produced insufficient viable pollens and failed to set fruits. The plants were carried forward till T4 generation. The following nomenclature was adopted for progressive generations; Line No. 2T0 indicates T0 line No.2, 2T15 indicates T1 progeny No. 5 of Line No. 2, 2T25-7 indicates its T2 progeny No. 7 of T1 plant No.5 of T0 Line No. 2.

**Southern blot analysis**

Genomic DNA (~15 µg) of T0 transgenic lines was digested with BamHI and SalI to release a fragment size of 2.2Kb (Figure S3), while genomic DNA (~15 µg) of T2 transgenic lines was digested EcoRI or NotI or Pacl (Figure S4) at 37°C overnight, separated on a 0.8% (w/v) agarose gel, and transferred to Hybond N+ membranes. αP32-dCTP labeled 2.2 Kb BamHI, and SalI digested (Figure S3) and NPTII (Figure S4) fragment was synthesized by PCR and used as the probe. The primers for amplification of NPTII probe amplify a 554 bp fragment in the coding region of NPTII. Prehybridization, hybridization, washing and developing of the blot was performed according to Sambrook and Russell (2001).

**RNA Isolation and Quantitative Real-Time PCR**

RNA was isolated from young leaves with three independent replicates using the method as described earlier (Gupta et al., 2014). Complementary DNA (cDNA) was prepared from 2 µg of RNA using high-capacity cDNA Archive kit (Applied Biosystems, USA). Quantitative real-time PCR was also performed as described earlier by (Gupta et al., 2014). Gene-specific primers were designed from the sequences obtained from the SGN database using Primer 3 software (Table S3) while real-time data analysis were same as described by (Gupta et al., 2014).

**Analysis of in-vivo MSH2 activity**
The *in-vivo* MSH2 activity was determined by measuring the amount of thymine dimer formation on exposure to UV-B light as described by Lario *et al.* (2011, 2015). Three samples of juvenile 6th to 7th node leaves were exposed for 6 h to UV-B radiation (2 Wm⁻², TL 20 W/01-RS, Philips narrowband) in dark room at 22°C. Wild-type leaves were treated similarly albeit without UV-B radiation. One sample was frozen at the end of 6 h treatment. The second and third sample were transferred to the darkness and white light (100 µmol m⁻² sec⁻¹) respectively for 24 h. Samples were snap frozen and stored at -80°C till DNA extraction and thymine dimer determination.

The UV-B induced thymine dimers were quantified as described by Stapleton *et al.* (1993). Six µg of genomic DNA isolated from UV-B treated and control leaves was denatured, dot blotted onto Hybond N+ membrane (Perkin Elmer life Sciences, Inc.) and baked at 80°C. The membrane was blocked, washed and incubated with thymine dimer-specific monoclonal antibodies (Sigma-Aldrich, 1:2000 in TBS) for 3 h at room temperature with gentle agitation. The dimers were visualized by incubating with alkaline phosphatase conjugated secondary antibody (Sigma, 1:3000) and detection by nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate reaction. The bands were quantified by densitometry using Image J software. The thymine dimers were quantified using three independent biological replicates. However, in the figures, a single representative blot and the values derived from it using ImageJ software is shown.

**Meiotic Chromosome Preparation**

Floral buds at the appropriate stages of the meiosis were picked according to Brukhin *et al.* (2003). The buds were fixed and stored in Carnoy’s solution [ethanol: chloroform: glacial acetic acid, (6:3:1)] at 4°C or -20°C. Fixed inflorescences were rinsed with Ethanol: glacial acetic acid (3:1) at defined intervals. Procedure for meiotic chromosome preparation was same as described by (Ross *et al.*, 1997). Chromosomes were counterstained with AT-specific fluorescent dye, 4′-6-diamidino-2-phenylindole (DAPI, 1 µg/ml) (Sigma Laboratories). All images were captured using a 63X (1.4 N.A.) oil-immersion objective on a Leica SP5 confocal microscope. Images were further processed with Adobe Photoshop CS6.
Mitotic Chromosome Preparation

Roots tips (1.5-2 cm) from 5-day-old seedlings were fixed in Carnoy’s solution for 24 h followed by washing with 70% (v/v) ethanol (15 min. 3X) and storage at 4°C. Procedure for mitotic chromosome preparation was same as described by (Babich et al., 1997). Then the tips were observed at 100X under Nikon compound microscope (Nikon Eclipse 80i) and photographed (Nikon NIS elements Basic version 3.1). Root tip nuclei were also assessed for nuclear size in somatic tissue by fixing in ethanol:acetic acid (3:1) fixative for at least 1 h, cleared in 70% ethanol and stained with 1µg/mL 4',6-diamidino-2-phenylindole (DAPI) solution.

Immunofluorescence

For immunofluorescence, synaptonemal complexes were prepared as described by Ross et al. (1997) and Chelysheva et al. (2010). 60% (v/v) acetic acid treatment was done at 45°C for 5 min with continuous stirring with a hook to remove maximum cytoplasm debris. Chromosomes were counterstained with DAPI and observed under the microscope to ensure proper slide preparation. The slides were microwaved in 10 mM citrate buffer pH 6 for 45s at 850 W for proper fixing of the chromosomes to slides. Slides were first immersed in 0.1M phosphate buffered saline (PBST): pH 7.4 containing 0.01% (v/v) TritonX-100 for 2×5 minutes. To block non-specific antibody binding, 100 µl of blocking buffer [1% (w/v) BSA in PBS] was applied directly to the slides. 100 µl of primary CENPC (Centromere protein C) antibody (1:100) in blocking buffer [PBS + 0.1% (v/v) Triton + 1% (w/v) BSA] was applied directly to the slides, covered with parafilm and incubated overnight at 4°C in a moist chamber. The slides were washed (2×5 minutes) with PBST before adding 100 µl of the secondary antibody. Secondary antibody, goat anti-rabbit 488 (Molecular Probes; diluted 1:500) was incubated for 90 minutes at room temperature. Finally, the slides were washed for 2×5 minutes and mounted in DAPI (10 µg/ml) in Vectashield antifade mounting medium. Slides were examined using a 63X (1.4 N.A.) oil-immersion objective on a Leica SP5 confocal microscope. Images were further processed with Adobe Photoshop CS6.

Fusion primer and nested integrated PCR (FPNI-PCR)
To locate the unknown sequences flanking the insertion sites in the transgenic lines, FPNI-PCR described by Wang et al. (2011) with few modifications was used. The primers used for the FPNI-PCR are listed in Table S4. The cycling conditions were followed according to Wang et al. (2011). All PCR products were electrophoresed on a 2.0% (w/v) agarose gel and FPNI-PCR products showing sizes more than 500 bp were chosen for sequencing. The purified PCR product was sequenced using Sanger sequencing (Macrogen, South Korea). The sequences were aligned to the tomato genome sequence (Sol Genomics Network, https://solgenomics.net/) using BLAST program.

ACKNOWLEDGEMENTS
The authors wish to thank Lorinda Anderson (Colorado State University, USA) for providing CENPC antibodies. This work was supported by International Atomic Energy Agency (IAEA) (grant no. 15632/R0-4 to RS), Department of Biotechnology, India (grant no. BT/PR/5275/AGR/16/465/2004; BT/PR/7002/PBD/16/1009/2012) to RS and YS, and University Grants Commission (research fellowship to SS).

Authors Contributions
Conceived and designed the experiments, SS, AKP, RS, YS, MR. Performed the experiments, SS, AKP. Analyzed the data SS, AKP, RS, YS, and MR. Wrote the paper, SS, YS, RS and MR.

SUPPORTING INFORMATION
Additional SUPPORTING INFORMATION may be found in the online version of this article.

Figure S1. In silico prediction of most deleterious MSH2 gene region by CODDLE and distribution of SNPs detected in MSH2 gene by EcoTILLING.

Figure S2. Multiple sequence alignment of mRNA sequences from tomato MSH family. The black box indicates the sequence of MSH2 used for making MSH2-RNAi construct.
**Figure S3.** Generation of MSH2-RNAi transgenic tomato lines and Southern blot of T₀ transgenic lines.

**Figure S4.** Southern blot of T₂ MSH2-RNAi lines 1T₂1-11, 2T₂7-5 and 11T₂4-1.

**Figure S5.** Quantification of thymine dimer levels in genomic DNA of WT and UV-B-treated MSH2-RNAi lines, 3T₂1-4 and 1T₂1-11.

**Figure S6.** Male meiosis in MSH2-RNAi tomato line No. 3T₂1-4 (a–f) and 5T₂3-3(g–l).

**Figure S7.** Sequence analysis of FPNI-PCR product of Line No. 1T₂1-11 and 2T₂5-5.

**Figure S8.** DAPI staining of root tip nuclei of WT and different MSH2-RNAi lines.

**Table S1.** The gene sequences flanking right border of T-DNA obtained using FPNI-PCR.

**Table S2.** List of primers used for screening for mutations and SNPs in MSH2 gene.

**Table S3.** List of genes and the primers used for transcript analysis of MMR pathway.

**Table S4.** List of genes and the primers used for FNPI-PCR.
REFERENCES


**Table 1.** List of SNPs detected in *MSH2* gene in different tomato accessions along with amino acid changes. The scores predicted by SIFT suggests that none of the amino acid changes would affect MSH2 protein activity. **Note:** A SIFT score of less than 0.05 predicts the base substitution to be intolerant.

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Table 2: Number of male meiocytes in different *MSH2*-RNAi lines with reference to WT and the number of seeds per fruit in the respective lines.

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Figures Legend

**Figure 1.** Relative transcript levels of members of *MSH* gene family in leaves of five different *MSH2*-RNAi lines. (a) *MSH1*, (b) *MSH2*, (c) *MSH3*, (d) *MSH4*, (e) *MSH5*, (f) *MSH6*, (g) *MSH7*. The transcripts levels were expressed after normalization with two internal controls, *β*-actin and ubiquitin. Note: line No. 1T21-11 shows the reduction in *MSH2*, *MSH5*, and *MSH7* transcript. The statistical significance was determined by Student’s t-test. * P<0.05.

**Figure 2.** Determination of the *in-vivo* activity of MSH2 by quantification of thymine dimer levels using a thymine dimer-specific antibody in UV-B irradiated WT and *MSH2*-RNAi lines by dot-blot assay. (a, b) Quantification of thymine dimers in WT and *MSH2*-RNAi plants after 6 h with or without UV-B exposure (untreated). (a) Representative dot-blot. The label on top of each spot indicates the line number of *MSH2* silenced line. (b) Quantification of thymine dimers in WT and *MSH2*-RNAi lines by Image J analysis. (c, d) Quantification of thymine dimers in WT and *MSH2*-RNAi plants. Thymine dimers were quantified either immediately after 6 h exposure with UV-B, or after 24 h of white light, or dark incubation. (c) Representative dot-blot. The labels on the right of the blot indicate the line number of *MSH2* silenced line. (d) Quantification of thymine dimers in *MSH2*-RNAi lines by Image J analysis. Note: The thymine dimers in (b) and (d) were quantified using three independent biological replicates. The statistical significance was determined by Student’s t-test. * P<0.05.

**Figure 3.** Abnormal flower, anther, fruit and pollen morphology manifested by *MSH2*-RNAi T2 Plants. (a) WT flower (b-d) Abnormal floral morphology, particularly in stamens, in strongly *MSH2* silenced lines. (e-f) Flowers of partially *MSH2* silenced lines, 5T23-3 and 3T21-4 are nearly similar to the WT. (g) Anther cones of line WT. (h) Abnormal anther cones of line 1T21-11. Note the absence of characteristic yellow coloration in 1T21-11. (i-j) Vertical cut section of red ripe fruit showing highly reduced seed set in line 1T21-11 (i) compared to WT (j). (k-l) WT pollen grains are bicellular with vegetative and generative cells compared to empty (m) and shriveled (n) pollen...
grain in line 1T2-11 (K-N, Scale bar, 20 µm). (o-r) Alexander staining showing viable pollen grains in WT (o), line 1T2-11 (p) 2T7-5 (q) and 2T5-5 (r). (l-o, Scale bar, 200 µm).

**Figure 4.** Male meiosis in wild-type and *MSH2-RNAi* tomato lines. Representative meiotic stages of wild-type (a–i) and *MSH2-RNAi* (j–q) lines from zygotene to telophase. In *MSH2-RNAi* meiocytes, few univalent chromosomes were observed that were assumed to be in early prophase I, (j–k). A proportion of male meiocytes of *MSH2-RNAi* lines underwent tetraploid meiosis that progressed from prophase to telophase (l–q). Note the formation of diploid tetrads (q). Scale bar, 10 µm.

**Figure 5.** Immunolocalization of centromere marker protein- CENPC in nuclei of WT and *MSH2-RNAi*, 1T2-11 line during prophase I. For each nuclei, the CENPC and DAPI staining along with merged signals is shown. (a–c) Zygotene nuclei with 12 centromere spots are likely indicating 12 bivalents in WT (a) with DAPI (b) and merged image (c). (d–f) Assumed zygotene nuclei with 48 centromere spots in 1T2-11 line (d) are likely indicating 48 univalents along with DAPI (e) and merged image (f). (g–i) Assumed zygotene nuclei with 24 centromere spots in line 1T2-11 (g) likely indicating the presence of 24 bivalents along with DAPI (h) and merged images (i). The nuclei were examined using a 63X (1.4 N.A.) oil-immersion objective on a Leica SP5 confocal microscope. Scale Bar, 10 µm.

**Figure 6.** Mitosis in WT and T3 *MSH2-RNAi* lines root tip cells of progenies of (a-d). WT (a), empty vector control lines (b), Progenies of *MSH2-RNAi*, 2T2-1 line bearing diploid meiocytes (c), and tetraploid line LA3255 (d) showing clear separation of the diploid nucleus in mitotic cells of root tip by intervening cell plate. (e-f). Progenies of strongly silenced *MSH2-RNAi* line 2T5-1 (e) and 1T2-11 (f) with tetraploid meiocytes show the absence of cell plate between dividing mitotic nuclei. Scale Bar, 100 µm.
Figure S1. *In silico* prediction of most deleterious *MSH2* gene region by CODDLE (a) and distribution of SNPs detected in *MSH2* gene by EcoTILLING (b). (a) *In silico* prediction of most deleterious *MSH2* gene region by CODDLE. The tomato *MSH2* gene consists of 7322 bp with 13 exons and 12 introns. The probability curve traced in blue represents the region of the gene where mutations would most likely deleteriously affect the function of encoded protein. Based on above prediction, the segment encompassing exons 3 to 9 was chosen for EcoTILLING. (b) Distribution of SNPs detected in *MSH2* gene by EcoTILLING. Red boxes denote exons interconnected with introns (solid red line). The black upright triangle indicates missense or nonsynonymous changes in the DNA sequence. The purple upright triangle indicates silent or synonymous changes. The numbers on the left and right of pictorial diagram respectively represent the accession number and total length of genomic DNA.
Figure S2. Multiple sequence alignment of mRNA sequences of tomato MSH family. Black box indicate the sequence of MSH2 used for making MSH2-RNAi construct.
Figure S3. Generation of MSH2-RNAi transgenic tomato lines and Southern blot of T₀ transgenic lines. (a) Schematic representation of the construct used for MSH2 silencing. The construct contains one spliceable intron with the targeted MSH2 sequence forming a hairpin when the construct undergoes transformation. AttB1 and attB2 represent the two short stretches of sequences that participate in the recombination reaction of the Gateway system. T35S indicates 35S terminator. The restriction sites for BamHI and SalI are also indicated. (b-c) Southern blot of T₀ transgenic lines. Genomic DNA of T₀ plants was digested with BamHI and SalI to release the insert. The Southern blot was probed with radiolabelled NPTII–NOS probe of 2.2 Kb size. Numbers on the top of lanes 1T₀ to 6T₀ in panel (b) and lanes 7T₀ to 1T₀ 1 in panel (c) indicate the plant number of respective T₀ lines. L- 1 Kb DNA Ladder (Fermentas). WT- Wild type genomic DNA (negative control); Plasmid-, Plasmid DNA bearing MSH2-RNAi construct. Probe- Radiolabelled NPTII–NOS 2.2 Kb probe. Note: The NPTII–NOS probe was obtained by digesting MSH2-RNAi plasmid with BamHI and SalI. The asterisk (*) indicates the presence of NPTII–NOS sequence in the transgenic lines.
**Figure S4.** Southern blot of T<sub>2</sub> MSH2-RNAi lines 1T<sub>2</sub>1-11, 2T<sub>2</sub>7-5 and 11T<sub>2</sub>4-1. (a) Schematic representation of the construct used for MSH2 silencing. Black arrow represents the site of the restriction enzyme used for digesting Genomic DNA in Blot (b), (c) and (d). DNA was digested with EcoRI (b), with NotI (c), and PacI (d). L-1 Kb DNA Ladder (Fermentas). WT- Wild-type genomic DNA (negative control); Plasmid-, Plasmid DNA bearing MSH2-RNAi construct. The blots were probed with radiolabelled NPTII sequence. The asterisk (*) indicates the presence of NPTII sequence in the transgenic lines.
Figure S5. Quantification of thymine dimer levels in genomic DNA of WT and UV-B-treated $MSH2$-RNAi lines, 3T$_2$1-4 and 1T$_2$1-11. (a) Quantification of thymine dimer with different dilutions of genomic DNA. Numbers on top of the blot indicate the spotted amount of genomic DNA (µg) and on the left indicate plant number. (b) Quantification of thymine dimer formation in $MSH2$-RNAi lines in (a) by ImageJ analysis.
Figure S6. Male meiosis in MSH2-RNAi tomato lines. Representative meiotic stages of 3T₂1-4 (a–f) and 5T₂3-3 (j–q) lines from zygotene to telophase. The 3T₂1-4 line with moderate MSH2 silencing and 5T₂3-3 line with no MSH2 silencing displayed diploid meiotic chromosomes. Scale bar, 10 µm.
Figure S7. Sequence analysis of FPNI-PCR product of Line No. 1T_{11}-11 and 2T_{55}-5. The FPNI-PCR product from Line 1T_{11}-11 showed 98% homology to tomato chromosome 9 in NCBI gene database (a) to Tic22 gene of tomato in SGN database (b). The FPNI-PCR product from Line 2T_{55}-5 showed 100% homology in chromosome 10 in NCBI database (c) and to TRM32 gene of tomato in SGN database (d).
Figure S8. DAPI staining of root tip nuclei of WT and different MSH2-RNAi lines. (a) WT, (b) empty vector control, (c-e) progenies of T3 MSH2-RNAi lines- 1T21-1-11-1 (c), 2T27-5-1 (d), 3T2 -4-2 (e) and LA3255 (f). Tetraploid tomato line LA3255 shows distinct enlarged tetraploid root tip nuclei in comparison to normal diploid nuclei in WT, control and MSH2-RNAi lines. Scale Bar, 50 µm.
Supplementary Table S1: The gene sequences flanking right border of T-DNA obtained using FPNI-PCR. Bold lowercase letters indicate bacterial right border T-DNA sequence and uppercase letters indicate flanking genome sequence from transgenic *MSH2-RNAi* plants.

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<td>gtttattagaataatgcataatttttaaaaaaggttaaatgtaataaatctgtgatatattttatatfaca gtcagagctatattctaaaga6GCCAAACAGAGATTCAATCGCCCTGTATTTCTTCAGG AAGGTAAGCTTAATGTATTGAGTTAGTATCCACCTATCCACTAGTGATAC AATGCTATATCTCCTCTTTGAAATTTTGTCAACCTCTGACTGACACACCA CCGGTGCCTCCTATAGTAGAAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC</td>
</tr>
<tr>
<td>2T2-5-5</td>
<td>RB</td>
<td>gtttacccgcaaatataatgtgcaataactcgtgataatttttaaaaggttaaatgtaataaatctgtgatatattttatatfaca gtcagagctatattctaaaga6GCCAAACAGAGATTCAATCGCCCTGTATTTCTTCAGG AAGGTAAGCTTAATGTATTGAGTTAGTATCCACCTATCCACTAGTGATAC AATGCTATATCTCCTCTTTGAAATTTTGTCAACCTCTGACTGACACACCA CCGGTGCCTCCTATAGTAGAAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC ACGTGCCCTATAGTAGAAGTCTATTACATTATTTTGATTTGATACACCC</td>
</tr>
</tbody>
</table>
**Supplementary Table S2** List of genes and the primers used for screening for mutations and SNPs for the regions predicted by CODDLE based on genome sequence of Solanaceae Genome version 2.5. The *MSH2* gene information and location of the primers on genome sequence is also indicated.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5’-3’)</th>
<th>Start Position</th>
<th>End Position</th>
<th>GC%</th>
<th>Tm</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>M13</td>
<td>Fp TGTAAACGACGCGCCAGT</td>
<td></td>
<td></td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rp AGGAAACAGCTATGACCAT</td>
<td></td>
<td></td>
<td>42</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td><em>MSH2</em></td>
<td>Fp TGTACCAATGTGCATTTCCTTCTT</td>
<td>42994468</td>
<td>42994445</td>
<td>33</td>
<td>58</td>
<td>1243 bp</td>
</tr>
<tr>
<td>Solyg06g069230 (Set I)</td>
<td>Rp TAGCTAAGAAAGAGGGGATTCAA</td>
<td>42993248</td>
<td>42986063</td>
<td>38</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><em>MSH2</em></td>
<td>Fp CTGACACAACATCTTGAAGGA</td>
<td>42993225</td>
<td>42993378</td>
<td>45</td>
<td>60</td>
<td>914 bp</td>
</tr>
<tr>
<td>Solyg06g069230 (Set II)</td>
<td>Rp CAGTAGCACATCCAATGAGAA</td>
<td>42992510</td>
<td>42992489</td>
<td>45</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><em>MSH2</em></td>
<td>Fp TTTTTTTTCCCGTCATTCATC</td>
<td>42992585</td>
<td>42992563</td>
<td>45</td>
<td>60</td>
<td>972 bp</td>
</tr>
<tr>
<td>Solyg06g069230 (Set III)</td>
<td>Rp GGGGTGGAGCTTTTAAGACATCA</td>
<td>42991635</td>
<td>42984452</td>
<td>45</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>
**Supplementary Table S3:** List of genes and the primers used for transcript analysis of MMR pathway.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene sequence id.</th>
<th>Primer Sequence (5’-3’)</th>
<th>Start position</th>
<th>End position</th>
<th>GC%</th>
<th>Tm</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH1</td>
<td>Solyc09g090870.2</td>
<td>Fp: GAGGAACCTAAAGGGGAGATTTTG</td>
<td>70267311</td>
<td>70267288</td>
<td>42</td>
<td>62</td>
<td>136 bp</td>
</tr>
<tr>
<td></td>
<td>(SGN)</td>
<td>Rp: TGAATCTAGAGCAGGTCTGAGTTG</td>
<td>70267199</td>
<td>70267175</td>
<td>46</td>
<td>63.6</td>
<td></td>
</tr>
<tr>
<td>MSH2</td>
<td>Solyc06g069230</td>
<td>Fp: TTAGCTGGTTCACTTTGAGTTG</td>
<td>42992524</td>
<td>42992501</td>
<td>42</td>
<td>62</td>
<td>102 bp</td>
</tr>
<tr>
<td></td>
<td>(SGN)</td>
<td>Rp: ATCTGGTGACTGATTTTGCTCT</td>
<td>42992446</td>
<td>42992425</td>
<td>42</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>MSH3</td>
<td>XM_010319297</td>
<td>Fp: TTCATAGAAAAGCTACTGCTGCTG</td>
<td>1814</td>
<td>1837</td>
<td>42</td>
<td>62</td>
<td>100 bp</td>
</tr>
<tr>
<td></td>
<td>(NCBI)</td>
<td>Rp: ACTTCTGTCCCTCCGTAATGAAAAG</td>
<td>1891</td>
<td>1914</td>
<td>42</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>MSH4</td>
<td>XM_010326283</td>
<td>Fp: AGAGATGAAGGAGACGGCTTTAT</td>
<td>1886</td>
<td>1909</td>
<td>42</td>
<td>62</td>
<td>139 bp</td>
</tr>
<tr>
<td></td>
<td>(NCBI)</td>
<td>Rp: TAAGTGCCAATAGATGCTCAACAC</td>
<td>2002</td>
<td>2025</td>
<td>42</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>MSH5</td>
<td>XM_010329008</td>
<td>Fp: GTAGACGTCTCTTGGAGGAACCTTGT</td>
<td>1088</td>
<td>1111</td>
<td>50</td>
<td>65.2</td>
<td>119 bp</td>
</tr>
<tr>
<td></td>
<td>(NCBI)</td>
<td>Rp: CACGTAAAGAGACCGAAACTTCTT</td>
<td>1184</td>
<td>1207</td>
<td>42</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td>Solyc01g079520.2</td>
<td>Fp: AAGGTATTAACGTGGTGGTCAGAC</td>
<td>78582874</td>
<td>78582893</td>
<td>46</td>
<td>65.3</td>
<td>108 bp</td>
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<tr>
<td></td>
<td>(SGN)</td>
<td>Rp: GCATCCATCTCATAAGCTCATAG</td>
<td>78583173</td>
<td>78583196</td>
<td>42</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>MSH7</td>
<td>XM_004242880</td>
<td>Fp: GGATTTGCTTTTGTCGATTG</td>
<td>1499</td>
<td>1520</td>
<td>41</td>
<td>58.4</td>
<td>116 bp</td>
</tr>
<tr>
<td></td>
<td>(NCBI)</td>
<td>Rp: TATAACTTCCCTCGGTGACACTTG</td>
<td>1592</td>
<td>1615</td>
<td>42</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>
**Supplementary Table S4:** List of genes and the primers used for FNPI-PCR. The common region is highlighted in bold.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5’-3’)</th>
<th>Primer use</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP1</td>
<td>GTAATACGACTCATAATAGGCACCAGCTGGT NTCGASTWTSGWGTT</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP2</td>
<td>GTAATACGACTCATAATAGGGCACACGCCTGGT NGTCAAWGANAWGAA</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP3</td>
<td>GTAATACGACTCATAATAGGGCAACGCCTGGT WGTGNAWANCAAGA</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP4</td>
<td>GTAATACGACTCATAATAGGGCACCACCTGGT AGWGNAGWANCAWAGG</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP5</td>
<td>GTAATACGACTCATAATAGGGCAACGCCTGGT NGTAWAASGTNTSCAA</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP6</td>
<td>GTAATACGACTCATAATAGGGCAACGCCTGGT NGACGASWGANWAGC</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP7</td>
<td>GTAATACGACTCATAATAGGGCAACGCCTGGT NGACGASWGANWAGA</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FP8</td>
<td>GTAATACGACTCATAATAGGGCAACGCCTGGT GNCACTWSCNTNTCTT</td>
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</tr>
<tr>
<td>FP9</td>
<td>GTAATACGACTCATAATAGGGCAACGCCTGGT NCAGCTWSCNTNTCTT</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>FSP1</td>
<td>GTAATACGACTCATAATAGGGC</td>
<td>2nd PCR primer</td>
</tr>
<tr>
<td>FSP2</td>
<td>ACTATAGGGCACCAGCTGGT</td>
<td>3rd PCR primer</td>
</tr>
<tr>
<td>PRB1</td>
<td>TTGACAGGATATATGCGGTTT</td>
<td>1st PCR primer</td>
</tr>
<tr>
<td>PRB2</td>
<td>AAGGGCGTGAAAAGGTTTATC</td>
<td>2nd PCR primer</td>
</tr>
<tr>
<td>PRB3</td>
<td>CCATTTGTATGTGCATGCCAA</td>
<td>3rd PCR primer</td>
</tr>
</tbody>
</table>