Molecules and Cells

Minireview

Advances towards Controlling Meiotic Recombination for Plant Breeding

Kyuha Choi*

Department of Life Sciences, Pohang University of Science and Technology, Pohang 37673, Korea
*Correspondence: kyuha@postech.ac.kr
http://dx.doi.org/10.14348/molcells.2017.0171
www.molcells.org

Meiotic homologous recombination generates new combinations of preexisting genetic variation and is a crucial process in plant breeding. Within the last decade, our understanding of plant meiotic recombination and genome diversity has advanced considerably. Innovation in DNA sequencing technology has led to the exploration of high-resolution genetic and epigenetic information in plant genomes, which has helped to accelerate plant breeding practices via high-throughput genotyping, and linkage and association mapping. In addition, great advances toward understanding the genetic and epigenetic control mechanisms of meiotic recombination have enabled the expansion of breeding programs and the unlocking of genetic diversity that can be used for crop improvement. This review highlights the recent literature on plant meiotic recombination and discusses the translation of this knowledge to the manipulation of meiotic recombination frequency and location with regards to crop plant breeding.

Keywords: breeding, crossover, epigenetics, meiotic DSBs, meiotic recombination

INTRODUCTION

Meiosis is a specialized cell division process that occurs in sexually-reproducing organisms in which a single round of DNA replication is followed by two successive rounds of chromosome segregation, resulting in gametes that have half the chromosome number (n) of their parental cells (2n). During fertilization, two gametes fuse together to restore the number of chromosomes in the resulting zygote to the same number that the parent cells had prior to meiosis (2n). During meiosis, programmed double-stranded breaks (DSBs) are formed by an evolutionally conserved SPO11 topoisomerase-like complex and are then repaired resulting in either reciprocal crossover, or non-crossovers by using a homologous template. By these processes, gametes are produced with recombined chromosomes (Hunter, 2015; Mercier et al., 2015; Villeneuve et al., 2001). Therefore, meiosis profoundly affects the genetic diversity of populations, and consequently adaptation potential, through meiotic recombination, independent chromosome segregation, and random fertilization (Barton and Charlesworth, 1998). This population genetic diversity also represents an important resource for selection of desirable traits in crop breeding (Bevan et al., 2017; Chaney et al., 2016).

Extensive genomic diversity has been revealed in plants by next-generation sequencing (NGS) technologies and computational analyses. Innovations in DNA sequencing and bioinformatics allow for the detection of genetic variations within plant populations that are used to recombine or map genomic locations of favorable traits by linkage and association mapping (Bevan et al., 2017; Chaney et al., 2016; Soyk et al., 2017). Genomics and computational approaches such as genotyping-by-sequencing and LDhat that is a package of estimating historical recombination by analyzing patterns of linkage disequilibrium (LD) in population have also contributed to the map of fine-scale meiotic crossovers in plant genomes (Choi et al., 2013; Hellsten et al., 2013; Wijnker et al., 2013). Like other eukaryotes, plant meiotic crossover

Received 22 August, 2017; revised 10 November, 2017; accepted 17 November, 2017; published online 23 November, 2017

eISSN: 0219-1032

frequency is not uniform along a chromosome, but instead occurs frequently within narrow regions of approximately 1-2 kb called recombination hotspots (Baudat et al., 2013; Choi and Henderson, 2015; Lichten and Goldman, 1995; Mercier et al., 2015). Fine-scale maps show that plant meiotic crossover hotspots occur near gene promoters and terminators in euchromatin while crossovers are suppressed in heterochromatic regions (Choi and Henderson, 2015; Lambing et al., 2017). Importantly, the suppression of crossover formation within heterochromatin is one of main bottlenecks in attempts to recombine favorable traits in the place of unfavorable traits in crop plants such as wheat and barley (Bevan et al., 2017; Choulet et al., 2014; Mayer et al., 2012; Tomato Genome Consortium, 2012). Notably, the heterochromatin of crop genomes is large, covering hundreds of mega base-pairs, and also contains functional genes (Bevan et al., 2017; Choulet et al., 2014; Mayer et al., 2012).

Despite the excessive DSBs that are made during the initiation of meiotic recombination, only one to three DSBs result in crossovers along chromosome after repair. The rest of the DSBs result in either repair by a sister chromatid, or non-crossovers through repair by a different repair pathway, such as synthesis-dependent strand annealing (SDSA) (Gray and Cohen, 2016; Hunter, 2015; Mercier et al., 2015). The limited number of crossovers per chromosome is relatively conserved among species with the exception of a few fungal species that generate more crossovers, although at least one crossover is required to ensure proper chromosomal segregation (Mercier et al., 2015). Enhancing the crossover frequency in both euchromatin and heterochromatin should help breeders acquire desirable traits or remove undesirable traits along chromosomes in crop plant genomes, as both the resolution of genetic mapping and the recombination of desirable trait variations are dependent on meiotic crossover rates and locations (Bevan et al., 2017).

This review describes the recent advances in understanding and control of plant meiotic recombination in Arabidopsis, including meiotic DSBs as well as the genetic and epigenetic factors that contribute to crossovers. This is followed by a discussion of the application of these advances to crop breeding using the CRISPR (clustered regularly interspace palindromic repeats) system.

HIGH RESOLUTION MAPPING OF PLANT MEIOTIC RECOMBINATION INITIATION SITES

In Arabidopsis, SPO11-1 and SPO11-2 are homologs of the archaeal DNA topoisomerase TOPOVI subunit A (TOPOVIA) that interact with MEIOTIC TOPOISOMERASE VIB-LIKE (MTOPVIB) to form a DNA topoisomerase VI-like hetero-tetrameric complex that catalyzes DSBs to initiate meiotic recombination (Fig. 1A) (Grelon et al., 2001; Hartung et al., 2007; Vrielynck et al., 2016). During DSB formation, the catalytically active tyrosine residue of SPO11 is covalently attached to 5' end of the DNA by a phosphodiester bond. Subsequent endo- and exonuclease activities of the MRNS (MRE11, RAD50, NBS1/XRS2, SAE2/COM1) complex with EXOI process the 3' region near the SPO11 attachment site, which releases SPO11 protein-oligonucleotide as a complex from the DNA (Fig. 1A) (Cannavo and Cejka, 2014; Choi et al., 2017; Garcia et al., 2011; Lam and Keeney, 2014; Neale et al., 2005; Pan et al., 2011). Accordingly, purified SPO11-associated oligonucleotides (~20-40 nucleotide) have been sequenced and used to generate high resolution genome-

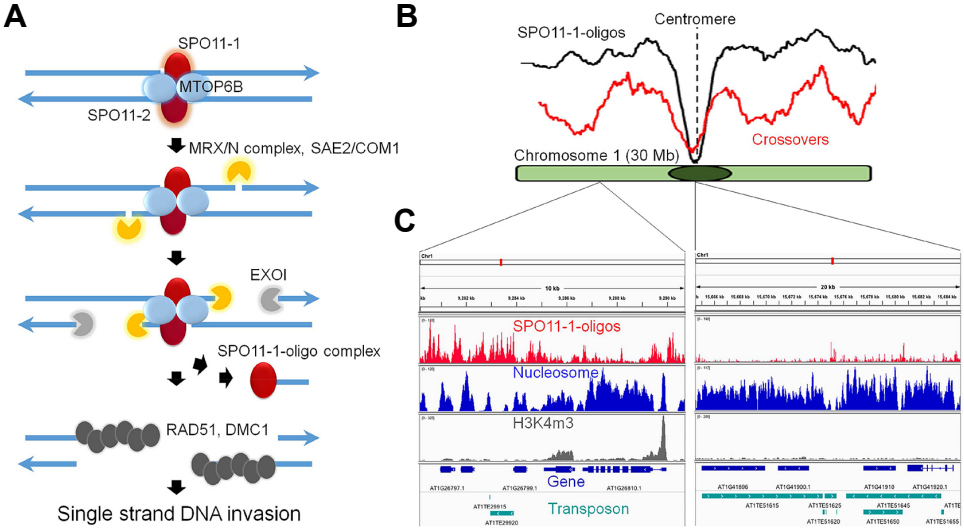

Fig. 1. Genome-wide meiotic DSB map in a plant. (A) Production of the SPO11-1 protein-DNA oligonucleotide complex during meiotic DSB formation. (B) Landscapes of SPO11-1-oligonucletides (oligos) and crossovers along Arabidopsis chromosome 1. (C) Representative snap shots for meiotic DSBs, nucleosome occupancy, and H3K4m3 levels, in the chromosomal arm and centromeric region of Arabidopsis chromosome 1. A DNA transposon, Helitron (AT1TE29920) located at a gene promoter displays high DSB levels and low nucleosome occupancy (left image), while centromeric transposons (AT1TE51615, AT1TE51645, Gypsy retrotransposon) display low levels of DSBs (right image).

wide maps of meiotic recombination initiation sites in fungi and mice (Fowler et al., 2014; Lam and Keeney, 2014, 2015; Lange et al., 2016; Pan et al., 2011). The first plant meiotic DSB map was developed by sequencing Arabidopsis SPO11-1 oligonucleotides (30-50 nt) and revealed both conserved and plant-specific features compared with fungal and mammalian maps (Choi et al., 2017).

As in yeasts, meiotic DSB location and frequency in plants are mainly determined by nucleosome occupancy (Fig. 1B-1C) (Choi et al., 2017; Fowler et al., 2014; Pan et al., 2011). In mammals, the PRDM9 protein directs meiotic DSBs to specific DNA sequences (Baudat et al., 2013; Clément and de Massy, 2017; Lange et al., 2016). Since plants and yeasts do not have PRDM9, the nucleosome-depleted gene promoters are the highest DSB hotspots (Choi et al., 2017). Notably, plant gene terminators and introns are also DSB hotspots with low nucleosome occupancy (Fig. 1C), yet gene terminators are not DSB hotspots in yeast, even though they also have lower nucleosome occupancy (Choi et al., 2017; Pan et al., 2011). The DSB hotspots that were identified in Arabidopsis gene terminators are consistent with the occurrence of crossover hotspots in Arabidopsis and monkey flower plants (Choi et al., 2013; 2016; Hellsten et al., 2013; Wijnker et al., 2013). Bird genomes also display crossover hotspots in both gene promoters and terminators, implying that a similar pattern of recombination hotspots is present in plants and birds (Singhal et al., 2015).

Strikingly, Arabidopsis DSB hotspots occur within specific DNA transposon families (such as Helitron, Tc1/mariner, and pogo) that are nucleosome-depleted, while retrotransposons and nucleosome-occupied DNA transposons are DSB coldspots (Fig. 1C) (Choi et al., 2017). The specific DSB hotspot transposons are enriched in pericentromeres and gene regulatory regions of proximal promoters and introns, whereas DSB coldspot transposons are often found within centromeres. Each transposon family also displays a distinct distribution pattern across chromosomes (Choi et al., 2017; Underwood et al., 2017a). The DSB hotspot DNA transposons are significantly associated with plant immunity genes such as defensin and the NBS-LRR gene family. This association suggests that the DNA transposons may play a role in enhancing the recombination frequency of these genes during adaptation. Hence, the detection of meiotic DSB hotspot inside transposons upon sequencing Arabidopsis SPO11-1 oligonucleotides expands the concept of McClintock's Controlling Elements from modifying transcription to meiotic recombination that contributes to genome diversity and evolution (Chuong et al., 2016; McClintock, 1956; Slotkin and Martienssen, 2007). Compared with current meiotic DSB maps in other species, the SPO11-1-oligonucleotide maps in Arabidopsis show the strongest quantitative correlations between levels of meiotic DSBs, nucleosome occupancy, and AT sequence richness that excludes nucleosomes in both gene regulatory regions and DNA transposon hotspots (Choi et al., 2017). In addition, the meiotic DSB coldspots in transposons, pericentromeres, and centromeres are associated with heterochromatin marks of DNA methylation and H3K9 dimethylation (me2) (Choi et al., 2017; Underwood et al., 2017b). The inhibition of DSB formation in centromeres is required to limit meiotic, non-allelic, homologous recombination-induced genome instability (Sasaki et al., 2010). Decreased levels of DNA cytosine methylation or H3K9me2 lead to an increase in the number of meiotic DSBs in the heterochromatic regions including centromeres with reduced nucleosome occupancy. This finding demonstrates a crucial role for these epigenetic marks in suppressing meiotic DSB formation on a genomic scale (Choi et al., 2017; Underwood et al., 2017b).

ENHANCING CROSSOVER RATES BY DISRUPTING ANTI-CROSSOVER GENES

Following the dissociation of SPO11-oligonucletide complexes and 5' to 3' single-strand resection, DMC1 recombinase and its cofactor RAD51 lead the invasion of the homologous duplex with the 3' end of single-stranded DNA (ssDNA), producing a recombination intermediate molecule (Fig. 1A) (Cloud et al., 2012; Da Ines et al., 2013; Gray and Cohen, 2016; Mercier et al., 2015). Two main DNA repair pathways - class I and class II - process these recombination intermediates to generate the limited number of crossovers (Fig. 2A). In the class I interfering pathway, ZMM (ZIP1, MSH4, MSH5 and MER3) proteins stabilize the recombination intermediate molecules and contribute to most crossovers (85-90%) via MLH1/MLH3. In the class II non-interfering pathway, 10-15% of crossovers rely on MUS81 (Fig. 2A) (Lambing et al., 2017; Mercier et al., 2015). In Arabidopsis, *zmm* mutants, including *zip4*, *hei10, msh4, msh5* and *shoc/zip2,* display significantly reduced fertility due to limited crossovers and produce only approximately 5 seeds per silique. Suppressors of *zmm* were identified using forward genetic screens of *zmm* mutant seeds. These *zmm* suppressors restore *zmm* fertility to wild-type levels (~50 seeds/silique) and also display 2-6 fold higher crossover rates than wild-type plants (Crismani et al., 2012; Girard et al., 2015; Séguéla-Arnaud et al., 2015, 2017). The genetic mapping and analyses of *zmm* suppressor mutants revealed three independent pathways of anti-crossover factors that limit crossover frequency. Any combination of mutations among these three pathways additively increases the number of MUS81-dependent crossovers (Table 1).

Firstly, FANCONI ANEMIA COMPLEMENTATION GROUP M (FANCM) helicase and its cofactors limit crossover formation (Crismani et al., 2012; Girard et al., 2014). The *fancm* mutation leads to a 3-fold increase in crossover frequency in a homozygous background, although no somatic phenotypic changes were detected. However, the *fancm* mutants display wild-type crossover rates in hybrid plants, indicating that the strong crossover suppression may be due to the DNA heteroduplex state in recombination intermediate molecules (Borts and Haber, 1987; Fernandes et al., 2017b; Girard et al., 2015; Ziolkowski et al., 2015).

Secondly, the RECQ4A and RECQ4B proteins in Arabidopsis are two redundant orthologs of BLM/Sgs1 helicase that form a complex with TOP3α and RMI1 that has the strongest anti-crossover activity observed so far (Séguéla-Arnaud et al., 2015; 2017). Arabidopsis *recq4a recq4b* double mutants show a 6.2-fold increase in crossover frequency in homozygous

Table 1. Arabidopsis genes involved in meiotic crossover frequency and location

Complex/Pathway	Gene	AT number	*zmm sup*	Increased fold CO rate in mutant or transgene		Reference
				Inbred context	Hybrid context	
FANCM helicase	*FANCM*	AT1G35530	yes	3	1	Crismani et al., 2012; Fernandes et al., 2017b
	MHF1	AT5G50930	yes	1.5-2	n.d	Girard et al., 2014
	MHF2	AT1G78790	yes	1.5-2	n.d	Girard et al., 2014
RTR complex	*RECQ4A*	AT1G10930	yes	6	5	Fernandes et al., 2017b
	RECQ4B	AT1G60930				
	TOP3a	AT5G63920	yes	3	n.d	Séguéla-Arnaud et al., 2015
	RMI1	AT5G63540	yes	3	n.d	Séguéla-Arnaud et al., 2017
FIGL1 helicase	*FIGL1*	AT3G27120	yes	2	2	Girard et al., 2015
	FLIP	AT1G04650	yes	1.2	n.d	Fernandes et al., 2017a
Class I CO pathway	*HEI10*	AT1G53490	no	2	2	Serra et al., 2017; Ziolkowski et al., 2017
non-CG methylation	*CMT3*	AT1G69770	no	*	*	Underwood et al., 2017b
H3K9me2	*SUVH4*	AT5G13960	no	*	n.d.	Underwood et al., 2017b
	SUVH5	AT2G35160	no			
	SUVH6	AT2G22740	no			
Anti-crossover	*MSH2*	AT3G18524	no	n.d	1.4	Emmanuel et al., 2006

n.d. indicates that crossover rate is not determined.
Star (*) indicates that crossover frequency is increased in pericentromeric regions.

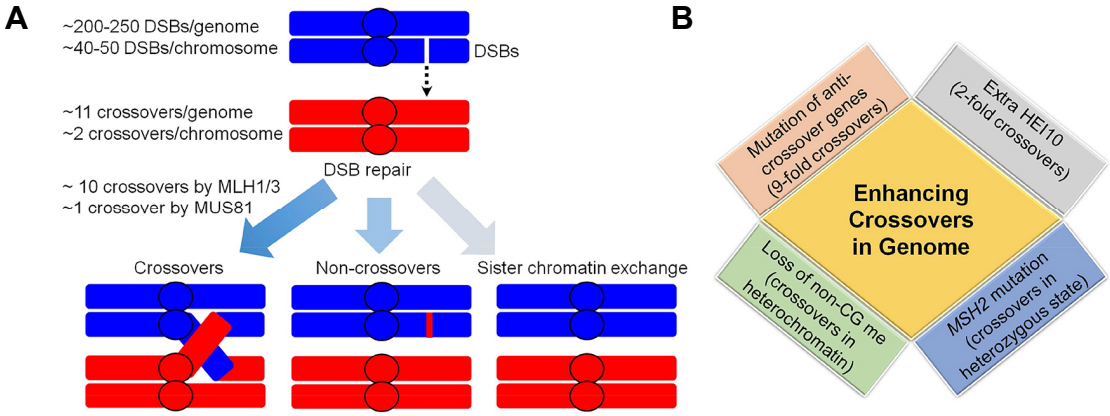

Fig. 2. Control of crossover frequency and location in plant genomes. (A) Limitation of crossover number in the Arabidopsis genome. During Arabidopsis male meiosis, an average of 250 meiotic DSBs and 11 crossovers occur throughout the genome. (B) A strategy for plant breeding programs to increase crossovers in both euchromatin and heterochromatin of hybrid lines by genetic and epigenetic disruptions. Enhancing the number of crossovers in the genome helps accelerate genetic mapping of desirable traits and generation of new varieties.

backgrounds and even an approximately 5-fold increase in heterozygous states. The recombination intermediates in *recq4a recq4b* mutants appear to be less sensitive to DNA heteroduplex-mediated crossover suppression than those in *fancm* mutants, and strongly favor being repaired to crossovers (Borts and Haber, 1987; Fernandes et al., 2017b). Finally, the AAA-ATPase FIDGETIN-LIKE 1 (FIGL1) and its interacting factor FLIP restrict the invasion by single-stranded DNA and thus crossover formation by controlling the dynamics of recombinase DMC1 and RAD51 (Fernandes et al., 2017a; Girard et al., 2015). Mutating *figl1* results in an approximately 2-fold increase in crossover frequency in both inbred and hybrid lines (Girard et al., 2015). Genetic analyses of these three pathways revealed that the highest increase in crossover rate in hybrid plants (9-fold) was obtained with the *recq4a recq4b figl1* triple mutants (Fernandes et al., 2017b). Like *recq4a recq4b figl1*, the *recq4a recq4b fancm* mutant also displays a 9-fold increase in crossover frequency in in-

bred lines. This crossover rate is slightly higher than that of a *recq4a recq4b* double mutant hybrid line because the *fancm* mutation is not present to additively increase crossovers in hybrid lines. Notably, the *recq4a recq4b figl1 fancm* mutant that is defective in all three pathways does not show a significantly higher crossover frequency than the *recq4a recq4b figl1* mutant when tested in hybrid plants (Fernandes et al., 2017b). Ultimately, the studies on *zmm suppressors* in Arabidopsis indicate that the mutations of *RECQ4A, RECQ4B* and *FIGL1* anti-crossover genes can be applied to crop plant breeding programs to increase crossover frequency (Fig. 2B).

DOSE-DEPENDENT INCREASE IN CROSSOVERS BY HEI10

Besides the genetic disruptions of anti-crossover genes for enhancing crossovers, the natural variations in meiotic crossover-promoting factor genes such as RNF212, PRDM9 and HEI10, also contribute to regulating crossover frequency and distribution (Kong et al., 2008; Sandor et al., 2012; Ziolkowski et al., 2017). In Arabidopsis, recombination quantitative trait loci (rQTL) mapping between Col-0 and Ler-0 accessions shows that one of two genetic variations of trans-acting modifiers maps to the *HEI10* locus, while the other remains to be elucidated (Ziolkowski et al., 2017). *HEI10* encodes a conserved meiotic E3 ubiquitin ligase required for crossover formation through the ZMM pathway (De Muyt et al., 2014; Gray and Cohen, 2016; Hunter, 2015). A single substitution is found in the HEI10 protein sequence (R264G) between Col-0 and Ler-0, Bur-0 and Cvi accessions. The lines that have the R264G substitution in HEI10 display a lower crossover rate than Col-0 and Ct-1, which express the R264 HEI10 variant (Ziolkowski et al., 2017). Like in mammals, Arabidopsis *hei10* mutants display haploinsufficiency in crossover frequency, and HEI10 is also able to promote crossover formation in a dosage-dependent manner (De Muyt et al., 2014; Qiao et al., 2014; Serra et al., 2017; Ziolkowski et al., 2017). Adding a single extra genomic copy of the R264 *HEI10* variant results in a 2-fold increase in the total crossover number and a 4-fold increase when combined with *recq4a recq4b* mutations in hybrid plants (Serra et al., 2017). It remains undetermined whether the extra *HEI10* transgenes can increase crossover frequency even further in a *recq4a recq4b figl1* mutant background. Since an extra HEI10 gene lead to a dosage-dependent increase in crossover frequency in hybrid plants, this might be a useful breeding approach for increasing crossovers in hybrid plants where the HEI10 elite cultivar can be crossed with diverse cultivars.

EPIGENETIC ACTIVATION OF CROSSOVERS IN HETEROCHROMATIN

Although genetic disruption of the anti-crossover genes and adding extra copies of the HEI10 gene result in the formation of more crossovers in gene-rich chromosome arms, crossovers in the pericentromeres and centromeres remain suppressed in Arabidopsis (Fig. 1B) (Fernandes et al., 2017b; Serra et al., 2017). The heterochromatic features DNA cytosine methylation, H3K9me2, H2A.W, and transposons are highly enriched in the pericentromeres and centromeres (Lippman et al., 2004; Lister et al., 2008; Stroud et al., 2013; Yelagandula et al., 2014). DNA demethylation was expected to increase crossovers in the heterochromatic regions; however, the loss of DNA CG methylation maintenance by mutation of *MET1* or *DDM1* surprisingly led to a reduction in the number of crossovers around the centromeres (Melamed-Bessudo and Levy, 2012; Yelina et al., 2012). Furthermore, an increase in crossovers within subtelomeric regions was also observed, ultimately reshaping the crossover distribution along chromosomes in *met1* and *ddm1* mutants (Melamed-Bessudo and Levy, 2012; Yelina et al., 2012; 2015). The cytosine methylation in CHG contexts is introduced into plant genomes by CHROMOMETHYLASE3 (CMT3) and CMT2 cooperatively, while the methylation in CHH contexts is introduced by CMT2 (Du et al., 2012; Lindroth et al., 2001; Stroud et al., 2013; 2014; Zemach et al., 2013). During CHG methylation by CMT3, the chromo and BAH (Bromo-Adjacent Homology) domains of CMT3 simultaneously bind to H3K9me2 on the nucleosome, while histone methylation is catalyzed by the histone methyltranferases KRYPTONITE/SUVH4, SUVH5 and SUVH6 (Du et al., 2012). The SRA domain of KRYPTONITE directly recognizes the DNA methyl cytosines that are maintained by CMT2 and CMT3 (Johnson et al., 2007). Therefore, CMT2/3 and SUVH4/5/6 proteins reinforce recruitment of each other and catalysis of non-CG methylation and H3K9 methylation, respectively, on the same nucleosome (Du et al., 2012; Stroud et al., 2014). Non-CG methylation also can be introduced by the *de novo* DNA methyltransferases DOMAINS REARRANGED METHYTRANSFREASE1 (DRM1) and DRM2 (Cao and Jacobsen, 2002; Cao et al., 2003). Notably, the loss of non-CG methylation and/or H3K9me2 enhance crossover frequency around pericentromeres but not centromeres, even though the number of meiotic DSBs substantially increases in centromeres (Underwood et al., 2017b). The increased crossovers in pericentromeric regions occur in both inbred and hybrid *suvh4 suvh5 suvh6* or *cmt3* mutant lines (Underwood et al., 2017b). Therefore, the epigenetic disruption of non-CG methylation or H3K9me2 can be translated to crop breeding programs that introduce new combinations of genetic alleles into the large pericentromeric regions (Tables 1 and 2; Fig. 2B).

MANUPULATION OF MEIOTIC RECOMBINATION FOR CROP IMPROVEMENT

The dependence of plant crop breeding on meiotic crossover frequency and crossover distribution can delay breeding time and restrict both combination and genetic mapping of desirable traits. To accelerate crop breeding and unlock genetic diversity, we can use the CRISPR system as a precise genome editing tool to manipulate the genomes of higher eukaryotes and control of meiotic recombination (Doudna and Charpentier, 2014; Hsu et al., 2014; Kim, 2016; Puchta, 2017). CRISPR approaches represent DNA-free gene editing tools that can be applied directly to the genomes of elite crop cultivars to implement genetic variations that are

Table 2. Crop plant orthologous genes to *Arabidopsis FIGL1, RECQ4* and *CMT3* genes

Arabidopsis gene	Plant species	Locus
FIGL1	Rice	LOC9271031
	Wheat	AK331006
	Maize	LOC100193153
	Tomato	LOC101262887
	Soybean	LOC100789161, LOC100776024
RECQ4A	Rice	LOC_Os11g48090(A), LOC_Os04g35420(B)
RACQ4B	Wheat	AK334643
	Maize	LOC100274706
	Tomato	LOC101260976
	Soybean	LOC100800006, LOC100817867
CMT3	Rice	OsCMT3a(LOC_Os10g01570), OsCMT3b(LOC_Os03g12570)
	Wheat	AK332918
	Maize	Zmet2(GQ923937)
	Tomato	LOC101265056, LOC101267211
	Soybean	LOC100799480

known to contribute to desirable traits, such as productivity and herbicide resistance (Kim, 2016; Puchta, 2017; Soyk et al., 2017; Wolter and Puchta, 2017; Yin et al., 2017). In addition, CRISPR or RNAi systems can be used to mutate or knock-down the genes encoding anti-crossover and epigenetic factors simultaneously, which may lead to an increase in crossover frequency in both euchromatin and heterochromatin (Tables 1 and 2; Fig. 2B). The DNA mismatch repair protein MSH2 represents an additional target for disruption using these systems, as mutation of the *MSH2* gene attenuates heterozygosity-mediated crossover suppression and results in a 40% increase in crossover rate (Emmanuel et al., 2006). Finally, CRISPR based tools can be further modified with either meiotic specific expression, or fusion with a meiotic protein as an effector, to induce meiotic recombination at a specific target site and potentially increase genetic and epigenetic variations of favorable traits that involve linked alleles such as clustered plant immunity genes (Deng et al., 2017).

CONCLUSION

Meiotic recombination has a profound effect on genetic diversity, which is crucial for both the adaption of plants to their environment and the improvement of crop traits of agricultural value. CRISPR tools can now be used to precisely edit the genomes of elite cultivars. However, the natural variation in the wild relatives of crop plants, landraces, and diverse cultivars also provide a valuable resource for crop improvement, as does genetic variation by chemical and radiation-driven mutagenesis. The discovery of anti-crossover and epigenetic factor genes that affect meiotic crossover in Arabidopsis will add new branches to crop breeding programs that aim to create new combinations of favorable traits and unlock unexplored genetic diversity. To further understand the mechanism of crossover formation in plants, the meiotic factors that control DSB formation and act in the ZMM pathway that limits crossovers must be elucidated. In addition to the first plant SPO11-1 oligonucleotide maps, genomic profiling of meiotic proteins will provide insights into how meiotic recombination is controlled, and how it can be induced at specific sites in plant genomes.

ACKNOWLEDGMENTS

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) and was funded by the Ministry of Education (2017R1D1A1B03028374), Next-Generation BioGreen Program (SSAC grant PJ01137901 RDA Korea) and POSTECH Basic Science Research Institute (4.0014843.01).

REFERENCES

Barton, N.H., and Charlesworth, B. (1998). Why sex and recombination? Science *281*, 1986-1990.

Baudat, F., Imai, Y., and de Massy, B. (2013). Meiotic recombination in mammals: localization and regulation. Nat. Rev. Genet. *14*, 794-806.

Bevan, M.W., Uauy, C., Wulff, B.B.H., Zhou, J., Krasileva, K., and Clark, M.D. (2017). Genomic innovation for crop improvement. Nature *543*, 346-354.

Borts, R.H., and Haber, J.E. (1987). Meiotic recombination in yeast: alteration by multiple heterozygosities. Science *237*, 1459-1465.

Cannavo, E., and Cejka, P. (2014). Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. Nature *514*, 122-125.

Cao, X., and Jacobsen, S.E. (2002). Role of the arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing. Curr. Biol. *12*, 1138-1144.

Cao, X., Aufsatz, W., Zilberman, D., Mette, M.F., Huang, M.S., Matzke, M., and Jacobsen, S.E. (2003). Role of the DRM and CMT3 Methyltransferases in RNA-Directed DNA Methylation. Curr. Biol. *13*,

2212-2217.

Chaney, L., Sharp, A.R., Evans, C.R., and Udall, J.A. (2016). Genome Mapping in Plant Comparative Genomics. Trends Plant Sci. *21*, 770-780.

Choi, K., and Henderson, I.R. (2015). Meiotic recombination hotspots - a comparative view. Plant J. *83*, 52-61.

Choi, K., Zhao, X., Kelly, K.A., Venn, O., Higgins, J.D., Yelina, N.E., Hardcastle, T.J., Ziolkowski, P.A., Copenhaver, G.P., Franklin, F.C.H., et al. (2013). Arabidopsis meiotic crossover hot spots overlap with H2A.Z nucleosomes at gene promoters. Nat. Genet. *45*, 1327-1336.

Choi, K., Reinhard, C., Serra, H., Ziolkowski, P.A., Underwood, C.J., Zhao, X., Hardcastle, T.J., Yelina, N.E., Griffin, C., Jackson, M., et al. (2016). Recombination Rate Heterogeneity within Arabidopsis Disease Resistance Genes. PLOS Genet. *12*, e1006179.

Choi, K., Zhao, X., Lambing, C., Underwood, C.J., Hardcastle, T.J., Serra, H., Tock, A.J., Ziolkowski, P.A., Yelina, N.E., Martienssen, R.A., et al. (2017). Nucleosomes and DNA methylation shape meiotic DSB frequency in Arabidopsis transposons and gene regulatory regions. bioRxiv *160911*, https://doi.org/10.1101/160911.

Choulet, F., Alberti, A., Theil, S., Glover, N., Barbe, V., Daron, J., Pingault, L., Sourdille, P., Couloux, A., Paux, E., et al. (2014). Structural and functional partitioning of bread wheat chromosome 3B. Science *345*, 1249721.

Chuong, E.B., Elde, N.C., and Feschotte, C. (2016). Regulatory activities of transposable elements: from conflicts to benefits. Nat. Rev. Genet. *18*, 71-86.

Clément, J., and de Massy, B. (2017). Birth and death of a protein. Elife *6*, e29502.

Cloud, V., Chan, Y.-L., Grubb, J., Budke, B., and Bishop, D.K. (2012). Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. Science *337*, 1222-1225.

Crismani, W., Girard, C., Froger, N., Pradillo, M., Santos, J.L., Chelysheva, L., Copenhaver, G.P., Horlow, C., and Mercier, R. (2012). FANCM limits meiotic cxrossovers. Science *336*, 1588-1590.

Da Ines, O., Degroote, F., Goubely, C., Amiard, S., Gallego, M.E., and White, C.I. (2013). Meiotic recombination in Arabidopsis is catalysed by DMC1, with RAD51 playing a supporting role. PLoS Genet. *9*, e1003787.

De Muyt, A., Zhang, L., Piolot, T., Kleckner, N., Espagne, E., and Zickler, D. (2014). E3 ligase Hei10: a multifaceted structure-based signaling molecule with roles within and beyond meiosis. Genes Dev. *28*, 1111-1123.

Deng, Y., Zhai, K., Xie, Z., Yang, D., Zhu, X., Liu, J., Wang, X., Qin, P., Yang, Y., Zhang, G., et al. (2017). Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. Science *355*, 962-965.

Doudna, J.A., and Charpentier, E. (2014). Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science *346*, 1258096.

Du, J., Zhong, X., Bernatavichute, Y. V, Stroud, H., Feng, S., Caro, E., Vashisht, A.A., Terragni, J., Chin, H.G., Tu, A., et al. (2012). Dual binding of chromomethylase domains to H3K9me2-containing nucleosomes directs DNA methylation in plants. Cell *151*, 167-180.

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

Fernandes, J., Duhamel, M., Seguela-Arnaud, M., Froger, N., Girard, C., Choinard, S., De Winne, N., De Jaeger, G., Gevaert, K., Guerois, R., et al. (2017a). FIGL1 and its novel partner FLIP form a conserved complex that regulates homologous recombination. bioRxiv *159657*, https://doi.org/10.1101/159657.

Fernandes, J.B., Seguela-Arnaud, M., Larcheveque, C., Lloyd, A.H., and Mercier, R. (2017b). Unleashing meiotic crossovers in hybrid plants. bioRxiv *159640*, https://doi.org/10.1101/159640.

Fowler, K.R., Sasaki, M., Milman, N., Keeney, S., and Smith, G.R. (2014). Evolutionarily diverse determinants of meiotic DNA break and recombination landscapes across the genome. Genome Res. *24*, 1650-1664.

Garcia, V., Phelps, S.E.L., Gray, S., and Neale, M.J. (2011). Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. Nature *479*, 241-244.

Girard, C., Crismani, W., Froger, N., Mazel, J., Lemhemdi, A., Horlow, C., and Mercier, R. (2014). FANCM-associated proteins MHF1 and MHF2, but not the other Fanconi anemia factors, limit meiotic crossovers. Nucleic Acids Res. *42*, 9087-9095.

Girard, C., Chelysheva, L., Choinard, S., Froger, N., Macaisne, N., Lehmemdi, A., Mazel, J., Crismani, W., and Mercier, R. (2015). AAA-ATPase FIDGETIN-LIKE 1 and Helicase FANCM Antagonize Meiotic Crossovers by Distinct Mechanisms. PLoS Genet. *11*, e1005369.

Gray, S., and Cohen, P.E. (2016). Control of meiotic crossovers: from double-strand break formation to designation. Annu. Rev. Genet. *50*, 175-210.

Grelon, M., Vezon, D., Gendrot, G., and Pelletier, G. (2001). AtSPO11-1 is necessary for efficient meiotic recombination in plants. EMBO J. *20*, 589-600.

Hartung, F., Wurz-Wildersinn, R., Fuchs, J., Schubert, I., Suer, S., and Puchta, H. (2007). The catalytically active tyrosine residues of both SPO11-1 and SPO11-2 are required for meiotic double-strand break induction in Arabidopsis. Plant Cell *19*, 3090-3099.

Hellsten, U., Wright, K.M., Jenkins, J., Shu, S., Yuan, Y., Wessler, S.R., Schmutz, J., Willis, J.H., and Rokhsar, D.S. (2013). Fine-scale variation in meiotic recombination in Mimulus inferred from population shotgun sequencing. Proc. Natl. Acad. Sci. USA *110*, 19478-19482.

Hsu, P.D., Lander, E.S., and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell *157*, 1262-1278.

Hunter, N. (2015). Meiotic Recombination: The essence of heredity. Cold Spring Harb. Perspect. Biol. *7*, a016618.

Johnson, L.M., Bostick, M., Zhang, X., Kraft, E., Henderson, I., Callis, J., and Jacobsen, S.E. (2007). The SRA methyl-cytosine-binding domain links DNA and histone methylation. Curr. Biol. *17*, 379-384.

Kim, J.-S. (2016). Genome editing comes of age. Nat. Protoc. *11*, 1573-1578.

Kong, A., Thorleifsson, G., Stefansson, H., Masson, G., Helgason, A., Gudbjartsson, D.F., Jonsdottir, G.M., Gudjonsson, S.A., Sverrisson, S., Thorlacius, T., et al. (2008). Sequence variants in the RNF212 gene associate with genome-wide recombination rate. Science *319*, 1398-1401.

Lam, I., and Keeney, S. (2014). Mechanism and regulation of meiotic recombination initiation. Cold Spring Harb. Perspect. Biol. *7*, a016634.

Lam, I., and Keeney, S. (2015). Nonparadoxical evolutionary stability of the recombination initiation landscape in yeast. Science *350*, 932-937.

Lambing, C., Franklin, F.C.H., and Wang, C.-J.R. (2017). Understanding and manipulating meiotic recombination in plants. Plant Physiol. *173*, 1530-1542.

Lange, J., Yamada, S., Tischfield, S.E., Pan, J., Kim, S., Zhu, X., Socci, N.D., Jasin, M., and Keeney, S. (2016). The landscape of mouse meiotic double-strand break formation, processing, and repair. Cell *167*, 695-708.

Lichten, M., and Goldman, a S. (1995). Meiotic recombination hotspots. Annu. Rev. Genet. *29*, 423-444.

Lindroth, A.M., Cao, X., Jackson, J.P., Zilberman, D., McCallum, C.M., Henikoff, S., and Jacobsen, S.E. (2001). Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. Science *292*, 2077-2080.

Lippman, Z., Gendrel, A.-V., Black, M., Vaughn, M.W., Dedhia, N., McCombie, W.R., Lavine, K., Mittal, V., May, B., Kasschau, K.D., et al. (2004). Role of transposable elements in heterochromatin and epigenetic control. Nature *430*, 471-476.

Lister, R., O'Malley, R.C., Tonti-Filippini, J., Gregory, B.D., Berry, C.C., Millar, a H., and Ecker, J.R. (2008). Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell *133*, 523-536.

Mayer, K.F.X., Waugh, R., Brown, J.W.S., Schulman, A., Langridge, P., Platzer, M., Fincher, G.B., Muehlbauer, G.J., Sato, K., Close, T.J., et al. (2012). A physical, genetic and functional sequence assembly of the barley genome. Nature *491*, 711-716.

McClintock, B. (1956). Controlling elements and the gene. Cold Spring Harb. Symp. Quant. Biol. *21*, 197-216.

Melamed-Bessudo, C., and Levy, A.A. (2012). Deficiency in DNA methylation increases meiotic crossover rates in euchromatic but not in heterochromatic regions in *Arabidopsis*. Proc. Natl. Acad. Sci. USA. *109*, E981-988.

Mercier, R., Mézard, C., Jenczewski, E., Macaisne, N., and Grelon, M. (2015). The molecular biology of meiosis in plants. Annu. Rev. Plant Biol. *66*, 297-327.

Neale, M.J., Pan, J., and Keeney, S. (2005). Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. Nature *436*, 1053-1057.

Pan, J., Sasaki, M., Kniewel, R., Murakami, H., Blitzblau, H.G., Tischfield, S.E., Zhu, X., Neale, M.J., Jasin, M., Socci, N.D., et al. (2011). A hierarchical combination of factors shapes the genome-wide topography of yeast meiotic recombination initiation. Cell *144*, 719-731.

Puchta, H. (2017). Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. Curr. Opin. Plant Biol. *36*, 1-8.

Qiao, H., Prasada Rao, H.B.D., Yang, Y., Fong, J.H., Cloutier, J.M., Deacon, D.C., Nagel, K.E., Swartz, R.K., Strong, E., Holloway, J.K., et al. (2014). Antagonistic roles of ubiquitin ligase HEI10 and SUMO ligase RNF212 regulate meiotic recombination. Nat. Genet. *46*, 194-199.

Sandor, C., Li, W., Coppieters, W., Druet, T., Charlier, C., and Georges, M. (2012). Genetic variants in REC8, RNF212, and PRDM9 influence male recombination in cattle. PLoS Genet. *8*, e1002854.

Sasaki, M., Lange, J., and Keeney, S. (2010). Genome destabilization by homologous recombination in the germline. Nat. Rev. Mol. Cell Biol. *11*, 182-195.

Séguéla-Arnaud, M., Crismani, W., Larchevêque, C., Mazel, J., Froger, N., Choinard, S., Lemhemdi, A., Macaisne, N., Van Leene, J., Gevaert, K., et al. (2015). Multiple mechanisms limit meiotic crossovers: TOP3α and two BLM homologs antagonize crossovers in parallel to FANCM. Proc. Natl. Acad. Sci. USA *112*, 4713-4718.

Séguéla-Arnaud, M., Choinard, S., Larchevêque, C., Girard, C., Froger, N., Crismani, W., and Mercier, R. (2017). RMI1 and TOP3α limit meiotic CO formation through their C-terminal domains. Nucleic Acids Res. *45*, 1860-1871.

Serra, H., Lambing, C., Griffin, C.H., Topp, S.D., Seguela-Arnaud, M., Fernandes, J., Mercier, R., and Henderson, I.R. (2017). Massive crossover elevation via combination of HEI10 and recq4a recq4b during Arabidopsis meiosis. bioRxiv *159764*, https://doi.org/10.1101/159764.

Singhal, S., Leffler, E.M., Sannareddy, K., Turner, I., Venn, O., Hooper, D.M., Strand, A.I., Li, Q., Raney, B., Balakrishnan, C.N., et al. (2015). Stable recombination hotspots in birds. Science *350*, 928-932.

Slotkin, R.K., and Martienssen, R. (2007). Transposable elements and the epigenetic regulation of the genome. Nat. Rev. Genet. *8*, 272-285.

Soyk, S., Lemmon, Z.H., Oved, M., Fisher, J., Liberatore, K.L., Park, S.J., Goren, A., Jiang, K., Ramos, A., van der Knaap, E., et al. (2017). Bypassing negative epistasis on yield in tomato imposed by a domestication gene. Cell *169*, 1142-1155.

Stroud, H., Greenberg, M.V.C., Feng, S., Bernatavichute, Y. V, and Jacobsen, S.E. (2013). Comprehensive analysis of silencing mutants reveals complex regulation of the Arabidopsis methylome. Cell *152*, 352-364.

Stroud, H., Do, T., Du, J., Zhong, X., Feng, S., Johnson, L., Patel, D.J., and Jacobsen, S.E. (2014). Non-CG methylation patterns shape the epigenetic landscape in Arabidopsis. Nat. Struct. Mol. Biol. *21*, 64-72.

Tomato Genome Consortium (2012). The tomato genome sequence provides insights into fleshy fruit evolution. Nature *485*, 635-641.

Underwood, C.J., Henderson, I.R., and Martienssen, R.A. (2017a). Genetic and epigenetic variation of transposable elements in Arabidopsis. Curr. Opin. Plant Biol. *36*, 135-141.

Underwood, C.J., Choi, K., Lambing, C., Zhao, X., Serra, H., Borges, F., Simorowski, J., Ernst, E., Jacob, Y., Henderson, I.R., et al. (2017b). Epigenetic activation of meiotic recombination in Arabidopsis centromeres via loss of H3K9me2 and non-CG DNA methylation. bioRxiv *160929*, https://doi.org/10.1101/160929.

Villeneuve, A.M., Hillers, K.J., Duffy, J.B., Kemphues, K.J., Villeneuve, A.M., Khodosh, R., and Hawley, R.S. (2001). Whence meiosis? Cell *106*, 647-650.

Vrielynck, N., Chambon, A., Vezon, D., Pereira, L., Chelysheva, L., De Muyt, A., Mézard, C., Mayer, C., and Grelon, M. (2016). A DNA topoisomerase VI-like complex initiates meiotic recombination. Science *351*, 939-943.

Wijnker, E., Velikkakam James, G., Ding, J., Becker, F., Klasen, J.R., Rawat, V., Rowan, B.A., de Jong, D.F., de Snoo, C.B., Zapata, L., et al. (2013). The genomic landscape of meiotic crossovers and gene conversions in Arabidopsis thaliana. Elife *2*, e01426.

Wolter, F., and Puchta, H. (2017). Knocking out consumer concerns and regulator's rules: efficient use of CRISPR/Cas ribonucleoprotein complexes for genome editing in cereals. Genome Biol. *18*, 43.

Yelagandula, R., Stroud, H., Holec, S., Zhou, K., Feng, S., Zhong, X., Muthurajan, U.M., Nie, X., Kawashima, T., Groth, M., et al. (2014). The histone variant H2A.W defines heterochromatin and promotes chromatin condensation in Arabidopsis. Cell *158*, 98-109.

Yelina, N.E., Choi, K., Chelysheva, L., Macaulay, M., de Snoo, B., Wijnker, E., Miller, N., Drouaud, J., Grelon, M., Copenhaver, G.P., et al. (2012). Epigenetic remodeling of meiotic crossover frequency in Arabidopsis thaliana DNA methyltransferase mutants. PLoS Genet. *8*, e1002844.

Yelina, N.E., Lambing, C., Hardcastle, T.J., Zhao, X., Santos, B., and Henderson, I.R. (2015). DNA methylation epigenetically silences crossover hot spots and controls chromosomal domains of meiotic recombination in Arabidopsis. Genes Dev. *29*, 2183-2202.

Yin, K., Gao, C., and Qiu, J.-L. (2017). Progress and prospects in plant genome editing. Nat. Plants *3*, 17107.

Zemach, A., Kim, M.Y., Hsieh, P.-H., Coleman-Derr, D., Eshed-Williams, L., Thao, K., Harmer, S.L., and Zilberman, D. (2013). The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell *153*, 193-205.

Ziolkowski, P.A., Berchowitz, L.E., Lambing, C., Yelina, N.E., Zhao, X., Kelly, K.A., Choi, K., Ziolkowska, L., June, V., Sanchez-Moran, E., et al. (2015). Juxtaposition of heterozygous and homozygous regions causes reciprocal crossover remodelling via interference during Arabidopsis meiosis. Elife *4*, e03708.

Ziolkowski, P.A., Underwood, C.J., Lambing, C., Martinez-Garcia, M., Lawrence, E.J., Ziolkowska, L., Griffin, C., Choi, K., Franklin, F.C.H., Martienssen, R.A., et al. (2017). Natural variation and dosage of the HEI10 meiotic E3 ligase control Arabidopsis crossover recombination. Genes Dev. *31*, 306-317.