Unleashing Meiotic Crossovers in Crops

Raghavendra V. C.*, Yashaswini R, Channabasava and Premsagar S. P.

Phd Scholar, Dept. of Genetics and Plant Breeding, College of Agriculture, UAS Raichur - 584104

Corresponding Author
Raghavendra V. C.
Email: raghuvcagri@gmail.com

OPEN ACCESS

Keywords

Crossing over, Recombination, Meiotic

How to cite this article:

Raghavendra, V. C., Yashaswini, R., Channabasava and Premsagar, S. P. 2023. Unleashing Meiotic Crossovers in Crops. *Vigyan Varta* 4(5): 4-7.

ABSTRACT

Meiotic crossovers shuffle chromosomes to produce unique combinations of alleles that are transmitted to offspring, generating genetic diversity on which natural or human selection can act. However, meiotic crossovers are relatively rare, typically one to three per chromosome, limiting the efficiency of the breeding process and related activities such as genetic mapping. Several genes that limit meiotic recombination were identified which has several mechanisms that actively antagonize CO formation in parallel i.e., FANCM, RECQ4 and FIGL1. Disrupting these anti-CO factors provokes a large increase in CO frequency without affecting meiotic progression. Plant breeding relies on meiotic crossovers to combine favourable alleles into elite varieties which occurs during meiosis, a specialized cell division.

INTRODUCTION

Crossing Over (Recombination)

Crossing over refers to the reciprocal physical exchange of chromosomal DNA between non-sister chromatids of homologous chromosomes. The concept of recombination emerged during the early 20th century, following the post-Mendel era of heredity research when Thomas Hunt Morgan proposed theory of gene linkage and crossing-over in 1913. The first cytological proof of the crossover theory came from Harriet Creighton and Barbara McClintock in 1931, who were able to correlate cytological and genetic exchanges in Drosophila and maize respectively

Process of Meiotic Recombination: (Fernandes et al., 2018)

I. Double strand break formation

In most organisms, meiotic recombination is initiated by the programmed formation of DNA double-strand breaks (DSBs), catalyzed by a highly conserved topoisomerase-like protein, Sporulant Deficient 11 (SPO11), and several other associated proteins. Spo11 appears to be

universally conserved among eukaryotes that undergo meiosis.

II. 3' end resection

Biochemical analysis in yeast shows that after DSB formation, Spo11 remains covalently attached to the 5' ends of the break via a conserved tyrosine residue that is required for SPO11 activity. Endonucleolytic cleavage and removal of SPO11 are carried out by the MRX–N complex [Mre11–Rad50–Xrs2 (Nbs1)] and the 5' ends are then further resected by Exo1 to yield 3' single-stranded DNA (ssDNA) tails.

III. Single end invasion and D loop formation

Replication Protein A (RPA), a conserved ssDNA binding protein, binds 3' ends to protect them from degradation, remove secondary structures, and facilitate the loading of recombinase. Following resection and protection of the ssDNA ends, RPAs are replaced by RecA-related recombinases, such as RAD51 and Disrupted meiotic cDNA1 (DMC1) assist the 3′ single-stranded DNA ends of DSBs to invade either the intact sister chromatid or a homologous chromosome as a repair template. This invasion forms a D-loop intermediate.

IV. DNA synthesis

Following single-end invasion, the 3' end primes for DNA synthesis using the complementary strand of the invaded chromatid as the template.

V. Second end capturing

The template strand that is displaced by synthesis anneals to the other resected end of the broken chromatid at the opposite side. This is called second end capturing. At this stage the predominant repair mechanism in plants is for the extended invading strand to disassociate and reanneal to the other end of the original DSB, termed synthesis-dependent strand annealing (SDSA), which results in non-crossover (NCO) products. Alternatively, DNA synthesis proceeds, and then second-end capture and double Holliday junction (dHJ) intermediates facilitate reciprocal exchange of DNA between homologous chromosomes, forming a crossover (CO) products.

VI. Pathways of meiotic recombination

The repair of meiotic DSBs yields COs or NCOs. Two different classes of crossovers have been identified, namely class I and class II. 70% (range from 60–90%) of crossovers are class I crossovers, which are dependent on the meiosis-specific ZMM proteins (Zip1, Zip2, Zip3, Msh4, Msh5, Mer3, Spo16, and Spo22/Zip4). Zip1 is a structural component of the synaptonemal complex that holds pairs of homologous chromosomes together. Zip2 is a XPF-like Helix-hairpin- helix containing protein and Zip3 is a SUMO E3 ligase, whereas Msh4 and Msh5 are homologs of the E. coli mismatch repair protein MutS implicated in stabilizing dHJ intermediates. The number of class I crossovers (~60) matches the number of Zip2, Zip3, and Msh4- Msh5 foci observed in yeast pachytene nuclei. Mer3 is a DNA helicase required for Holliday junction branch migration. Finally, Spo16 and Spo22/Zip4 are involved in synaptonemal complex assembly, and they are unique because, in contrast to the other ZMM proteins, they are not essential for crossover interference. Synthesis-dependent strand annealing (SDSA) pathway results in non-crossover (NCO) products. In addition to the Types I and II CO pathways, an alternative CO pathway may exist. Double mutants in both Type I and Type II factors, such as msh4 mus81, have a residual 5–10% of CO's. However, the

mechanism that generates these COs is unclear (Wang and Copenhaver, 2018).

Regulation of crossover formation

Crossover formation is essential for proper chromosome segregation at meiosis I, therefore their number and position should be exclusively maintained by various means (Wang and Copenhaver, 2018). Despite the stochastic nature of crossing-over, which to a first approximation can occur at any site along the chromosomes, crossovers are subject to tight regulation. Following are the some of the various mechanisms that regulate crossover formation:

i. **Crossover assurance**
ii. **Crossover interference**
iii. **Crossover homeostasis**
iv. **Crossover invariance**
v. **Crossover patterning**

Manipulation of crossover frequency in plants

In addition to the genetic diversity resulting from the random segregation of paternal and maternal homologues during meiosis, CO formation results in new allelic combinations that may carry advantageous functional innovations. However, the low number of COs often limits the genetic variation that can be captured in plant breeding programs. In the vast majority of species, the mean number of COs per chromosome rarely exceeds three per bivalent. This holds true irrespective of the physical size of the chromosome and despite an excess in CO precursors. The lower the CO frequency, the larger the region that is swept; i.e., where genetic diversity is erased. Thus, by increasing CO frequencies new favorable alleles could be introduced not only through increased allele reshuffling but also through reduced genetic variance loss in regions subjected to selection. Following are the some of the approaches of manipulation of crossover frequency (Blary and Jenczewski, 2019).

i. Altering crossover distribution
ii. Increasing crossover rate between divergent chromosomes
iii. Chromosome engineering
iv. Use of polyploids
v. Using environment
vi. Knocking-out anti-crossover factors

Meiotic recombination frequency in autotetraploids

To determine MRFs in autotetraploids, they identified plants with a stable and complete tetraploid chromosome set of 4X = 20 among the progeny of a colchicine-treated diploid, homozygous line 3A. Seeds from these plants (n = 3652, progeny from five individual plants, three generations after polyploidization) exclusively showed yellow fluorescence, indicating stable expression of both markers after polyploidization. The tetraploid derivatives, with four copies of the marker chromosome, were backcrossed to non-transgenic autotetraploid Arabidopsis thaliana. This results in plants carrying two copies of the transgenic chromosome 3.

To exclude a dependence of MRF on the copy number and to strictly compare with diploids having a single marker chromosome, they backcrossed the double-copy lines once more to wild- type plants. The intensity of red and green fluorescence depends on the dosage of the marker genes. With the double-copy F1 plants as reference, plants with one gene copy each were selected and used for the final test crosses (Pecinka et al., 2011). They have taken into account that markers could have been separated by recombination already during the backcross,

but nevertheless be transmitted through the same gamete. However, in this case, they would segregate like uncoupled markers in the selfed progeny, and they have retrospectively eliminated such plants from the analysis. The single copy plants were backcrossed once more to the tetraploid wild type, again in reciprocal orientation, or allowed to self-pollinate. Again, female and male MRFs were significantly different from each other ($P < 0.0000001$), but more importantly, single-copy autotetraploid MRFs were significantly higher in comparison to diploid MRFs (selfing MRF = 20.5%, $P < 0.0001$; female MRF = 15.0%, $P < 0.001$; male MRF = 28.0%, $P < 0.001$).

Meiotic recombination frequency in allotetraploid

Arabidopsis arenosa is a natural autotetraploid and close relative of Arabidopsis thaliana. Crosses between tetraploid Arabidopsis thaliana and Arabidopsis arenosa give rise to fertile allopolyploid hybrids that resemble Arabidopsis suecica, a species originating from an ancient hybridization event between these species. They crossed the homozygous tetraploid line 3A with non-transgenic tetraploid Arabidopsis arenosa (4 × = 32) and identified plants with stable and full parental chromosome sets of 4 × = 26. Chromosomes derived from the two parental genomes in Arabidopsis suecica and in newly established hybrids were shown to pair in a diploid-like manner. To verify the strict exclusion of multivalent formation or pairing between non-homologous chromosomes, they performed a cytological analysis of the resulting hybrids during the transition from meiotic metaphase I to anaphase I. Chromosomes from different parental origin were distinguished by fluorescence in situ hybridization with differentially labeled species-specific centromeric repeat probes.

Furthermore, enhanced recombination could help stabilize the chromosome complement and segregation during complex meiosis, or enhance the formation of bivalents in allotetraploids. Polyploidy-enhanced MRF might thus have a dual impact, enhancing the generation of new diversity, while at the same time, promoting genome stability through proper chromosome segregation.

REFERENCES

Blary, A. and Jenczewski, E., 2019, Manipulation of crossover frequency and distribution for plant breeding. Theoretical and Applied Genetics, 132 (3): 575-592.

Fernandes, J. B., Séguéla-Arnaud, M., Larchevêque, C., Lloyd, A. H. and Mercier, R., 2018. Unleashing meiotic crossovers in hybrid plants. The Proceedings of the National Academy of Sciences, 115 (10): 2431-2436.

Pecinka, A., Fang, W., Rehmsmeier, M., Levy, A. A. and Mittelsten Scheid, O., 2011. Polyploidization increases meiotic recombination frequency in Arabidopsis. BioMed Central, 9, :1-7.

Wang, Y. and Copenhaver, G. P., 2018. Meiotic recombination: mixing it up in plants. Annl. Rev. Plant Biolology, 69: 577-609.