Popular Article

PROSPECTS OF CHROMOSOME ENGINEERING IN CROP IMPROVEMENT

Sai Rekha, K.
Department of Rice, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu– 641003, INDIA
Corresponding author's E-mail: ssaishine@gmail.com

KEY WORDS:
Chromosome, Apomixis, Recombination

ARTICLE INFO
Received on:
05.04.2017
Revised on:
17.05.2017
Accepted on:
19.05.2017

ABSTRACT
The term ''chromosome engineering'' describes technologies in which chromosomes are manipulated to change their mode of genetic inheritance. The concept of "chromosome engineering" was introduced by the American researcher E. Sears in 1972, based on summarizing the results of his studies on the transfer of chromosome segment of *Aegilops umbellulata in the* genome of common wheat, carried out in 1956. Chromosome engineering in improving crop traits can achieve through: manipulation of whole chromosome sets, manipulation of individual chromosomes and manipulation of chromosome segments. The major tasks of chromosome engineering involves production of haploid plants through CENH3 modification, conversion of meiosis to mitosis through mutating spo11 rec8 osd1 (MiMe) and manipulating homologous recombination through spo11. The recent strategies of CE are recombineering and enhancer trapping but these are widely using in animal genetics and there is need to enhance these techniques especially in plant genetics for their improvement.

INTRODUCTION

Chromosomes are the rod-shaped, filamentous bodies present in the nucleus, which become visible during cell division. They are the carriers of the gene or unit of heredity. Chromosomes are not visible in active nucleus due to their high water content, but are clearly seen during cell division. Chromosome engineering is the controlled generation of chromosomal deletions, inversions, or translocations with defined endpoints. The term ''chromosome engineering'' describes technologies in which chromosomes are manipulated to change their mode of genetic inheritance. The concept of "chromosome engineering" was introduced by the American researcher E. Sears in 1972, based on summarizing the results of his studies on the transfer of chromosome segment of *Aegilops umbellulata* in the genome of common wheat, carried out in 1956. According to the works of E. Sears, the concept of chromosome engineering is more specialized and it involves (a) The targeted transfer of alien chromosomes and (b) The induced transfer of chromosome segments into the genome of cultivated plants from other species in order to improve crop traits.

Chromosome engineering in improving crop traits

Engineering centromeres to produce haploid plants

A fundamental difficulty in plant breeding is the need to produce functionally homozygous lines with consistent phenotypes. Molecular markers reduce the number of progeny that must be screened to recover useful trait combinations. However, several generations of selfing or backcrossing are required to create a new inbred. Haploid production has revolutionized breeding in crops where it can be efficiently performed. Haploids can accelerate genetic mapping and are beneficial for genomics because they remove heterozygosity. Microspores (pollen precursors) are the most common starting material because of their higher number per flower, but ovules have also been cultured. In some species, phenotypic variation arising from tissue culture (termed ''somaclonal variation'') can be deleterious.

Furthermore, regeneration is frequently too inefficient for production breeding and protocols are usually limited to a few genotypes. A more biologically interesting haploid production method involves crossing a crop to a distant relative in an interspecific or intergeneric cross. In a fraction of progeny, the genome from one parent is selectively eliminated after fertilization, yielding a haploid with chromosomes from the desired parent only. A classic example is the cross between cultivated barley (*Hordeum vulgare*) and *Hordeum bulbosum*, in which the *H. bulbosum*

chromosomes are missegregated and lost during embryogenesis. In many wide crosses, the seed is inviable and embryo rescue is needed to regenerate an adult plant. Maize haploid inducers, many derived from the classic ‘‘Stock6’’line, are rare examples of an intraspecies cross that produces genome elimination.

A recent discovery suggests a completely new strategy for creating haploid plants through centromeres. Centromeres are loci that nucleate kinetochores, the protein complexes that bind to spindle microtubules and mediate chromosome segregation during cell division. In the novel method, centromeres are subtly disabled by mutating a kinetochore protein. Crossing this centromere mutant to wild-type mixes two sets of chromosomes in the fertilized zygote. Chromosomes from the mutant parent (the ‘‘haploid inducer’’) have defective kinetochores and can be lost by missegregation during zygotic mitosis. Resulting adult plants are haploids with only chromosomes from their wild-type parent. This method mimics the genome elimination seen in wide crosses and potentially allows the process to be engineered into any plant.

A haploid inducer was created by altering the essential kinetochore protein CENH3, a variant of histone H3 that replaces conventional H3 in centromeric nucleosomes. Similar to conventional histone H3s, CENH3 has a C-terminal histone fold domain that complexes with other histones to form the nucleosome core and an N-terminal tail domain that protrudes from the nucleosome. Unlike conventional histones, CENH3s evolve rapidly, particularly in their N-terminal tail.

The most efficient haploid inducer adds an N terminal GFP tag to the protein and replaces the hypervariable tail of CENH3 with the tail of conventional H3 (termed ‘‘GFP-tailswap’’). When cenh3 GFP-tailswap plants were crossed to wild-type, up to 50% of F1 progeny were haploid. All wide crosses described above produce a mixture of haploid progeny and diploid hybrids, in which chromosomes from both mutant and wild-type parents are kept. Microspore culture produces haploids with paternal chromosomes and paternal cytoplasm. Crossing a CENH3-based haploid inducer (as the female) with a wildtype male shifts paternal chromosomes into the maternal cytoplasm. Cytoplasmic male sterility is useful for producing hybrid seed and facile cytoplasm exchange is likely to be one of the major applications of haploid inducers based on CENH3 alterations.

How can CENH3 engineering create a haploid inducer in crops?

TILLING or insertional mutagenesis could create a cenh3 mutation. Without a cenh3 mutant, gene silencing methods, such as RNAi, should inactivate the endogene in any plant. The promoter for the RNAi transgene might need to be expressed in gametophytes, to ensure that endogenous CENH3 is absent from pollen or egg cell chromosomes. The commonly used 35S promoter is often poorly expressed in gametophytes. Mutant CENH3 transgenes could be synthesized with altered codon usage to evade RNAi and should probably be expressed from the native CENH3 promoter. CENH3 is a small protein, thus a single transgene can contain an RNAi transgene as well as a transgene expressing a mutant variant. Thus, a haploid inducer could conceivably be made in a single transformation. Haploids in A. thaliana were produced through seeds; as such, CENH3 engineering might avoid the need for tissue culture and, in some crops, potential somaclonal variation. Ideally, the method could offer haploid technology to breeders without access to highly standardized tissue culture facilities.

Can other centromere alterations create a haploid inducer?

GFP-tailswap is not the only CENH3 variant that induces genome elimination. GFP-tagged full-length CENH3 also induces haploids, at a lower frequency, and many other alterations to CENH3 might cause missegregation in a cross. It has been suggested that alterations to the CENPC protein could also cause genome elimination. The potential for engineering other kinetochore proteins to produce haploids will depend on their behavior during DNA replication. After fertilization, both mutant and wild-type chromosomes are replicated during S phase, prior to the first zygotic mitosis. If a kinetochore protein is removed during DNA replication and reloaded onto both chromosome sets from a common pool, there will be no difference between chromosomes from the two parents and therefore no genome elimination. Pre-existing CENH3 at kinetochores is probably retained during DNA replication and partitioned equally between the two replicated sisters. This explains why chromosomes from the mutant and wild-type retain their different behaviors, even if additional CENH3, presumably a mixture of mutant and wild-type protein, is loaded after S phase. CENP-C binds to centromere DNA directly,

which might increase the chance that it remains associated with replicated chromosomes.

Manipulating meiotic recombination frequency through chromosome engineering

A high meiotic recombination rate is useful for introgressing traits controlled by a small number of genes into another genetic background. Regions of the genome with suppressed recombination, often correlating with a high percentage of heterochromatin, pose particular difficulties. Meiotic recombination is initiated by double-stranded breaks catalyzed by the nuclease Spo11, which is broadly conserved in eukaryotes. Processing of the double-stranded break can yield a crossover outcome (resulting in recombination) or a non-crossover repair event.

Molecular understanding of this process is deepening, suggesting opportunities for engineering elevated recombination rates. Two types of engineering could, in principle, elevate meiotic recombination. First, chromosome structure might be altered to allow easier access by recombination factors. Although this is a promising approach, radical changes in chromatin structure might affect gene expression in unwanted ways. In the future, it might be possible to produce local changes in chromatin structure, perhaps with engineered sequence-specific DNA binding proteins fused to enzymes that modify epigenetic marks.

A second strategy for increasing meiotic recombination is to focus on recombination proteins themselves. In addition to Spo11, several other proteins that help to initiate recombination have been discovered through forward genetic screens and reverse genetic approaches using gene expression profiling to identify candidates. Furthermore, DNA helicases are key controllers of recombination rate in yeast and manipulating such proteins in plants might increase meiotic recombination.

A related problem for plant breeders is introgressing traits from wild relatives that are so distantly related that chromosome pairing in meiosis I is difficult. Such homeologous pairing can be genetically controlled, as shown by the wheat Ph1 locus, which prevents recombination between homeologs. The recent discovery that Ph1 down regulates cyclin-dependent kinases offers hope that the meiotic cell cycle machinery can be manipulated to allow homeologous recombination.

Chromosome engineering for apomixis

Hybrid seeds have greatly increased agricultural productivity, but their genotype cannot be propagated through sexual reproduction. It is thought that apomixis alternates with sexual reproduction, allowing such plants to multiply favorable genotypes yet still create variation when necessary. Apomixis is often described as a potentially revolutionary technology for agriculture, because it could perpetuate vigorous hybrids indefinitely; however, attempts to introgress the trait into crops have not succeeded. Although there are many ways for apomixis to occur in nature, a common route for scientists seeking to engineer it is to divide the process into three steps. First, meiosis must be bypassed or altered so that the plant produces diploid gametes without recombination. Second, embryogenesis should begin without fertilization. Third, endosperm development must also be triggered without fertilization. Chromosome engineering has had notable recent success in achieving the first step.

A complex but efficient solution for creating clonal diploid gametes is to combine three mutations that affect meiotic chromosomes and meiotic cell cycle progression. Removing the SPO11 nuclease prevents meiotic recombination. Chromosomes in spo11mutants segregate randomly in meiosis I, because they cannot pair with their homolog. In meiosis I, sister chromatids normally segregate to the same side of the spindle, because their centromeres are held together by the meiosis specific cohesin protein REC8. Whenspo11and rec8mutations are combined, sister chromatids segregate to opposite sides of the spindle in meiosis I, effectively turning this division into mitosis.

The final mutation, osd1, prevents the onset of meiosis II, leaving two diploid gametes with the same genotype as the parent plant. spo11 rec8 osd1 mutants are termed ‘‘MiMe’’, because they convert meiosis into mitosis. In MiMe plants, an astonishing 85% of female gametophytes and 100% of the pollen have the diploid genotype of the parent plant. The challenge of engineering apomixis now shifts to coaxing the diploid embryo sac to form a seed without fertilization.

Homologous recombination for chromosome engineering

Balancer chromosomes are special, modified chromosomes used for genetically screening a population of organisms to select for heterozygotes. Balancer chromosomes can be used as a genetic tool to prevent crossing over (genetic recombination) between homologous chromosomes during meiosis. To suppress crossing over, balancer chromosomes are the products of

multiple, nested chromosomal inversions so that synapsis between homologous chromosomes is disrupted. This construct is called a crossover suppressor. If crossing over between a balancer chromosome and the balancer's homolog does occur during meiosis each chromatid ends up lacking some genes and carrying two copies of other genes. Recombination in inverted regions leads to dicentric or acentric chromosomes. Progeny carrying chromosomes that are the products of recombination between balancer and normal chromosomes are not viable (they die).

Balancer chromosomes always contain a lethal recessive allele. This means that if an organism receives two copies of the balancer chromosome, one from the mother and one from the father, then the organism will not live. So any organism that is homozygous for that chromosome will not live to pass on its genes. However, offspring that only get one copy of one balancer chromosome and one copy of a wild type or mutant chromosome will live to pass on its genes. After only a few generations the population will be entirely heterozygous so that you can be guaranteed of its genotype on at least those two chromosomes. Balancer chromosomes also come with some sort of physical marker. This marker can be actually associated with the DNA in the chromosome such as the Green Fluorescent Protein that fluoresces in ultraviolet light, or it can be an easily distinguishable physical characteristic.

Future strategies of chromosome engineering

Recombineering

Recombineering is a genetic and molecular biology technique based on homologous recombination systems, as opposed to the older/more common method of using restriction enzymes and ligases to combine DNA sequences in a specified order. Recombineering is widely used for bacterial genetics, in the generation of target vectors for making a conditional mouse knockout, and for modifying DNA of any source often contained on a bacterial artificial chromosome (BAC), among other applications.

The biggest advantage of recombineering is that it obviates the need for conveniently positioned restriction sites, whereas in conventional genetic engineering, DNA modification is often compromised by the availability of unique restriction sites. In engineering large constructs of >100 kb, such as the Bacterial Artificial Chromosomes (BACs), or chromosomes, recombineering has become a necessity. Recombineering can generate the desired modifications without leaving any 'footprints' behind.

Enhancer trapping

Transposable elements based mutagenesis or transposon tagging is a powerful technology with one limitation: it can identify only genes that have a recognizable mutant phenotype following element intregation. Many of the genes that one mutates either do not result in visible phenotypes or cause the death of the organism. Such genes will never be recovered from a screen based on transposan tagging. An enhancer trap is a method in molecular biology that allows hijacking of an enhancer from another gene, and so, identification of enhancers. The enhancer trap construct contains a transposable element and a reporter gene.

REFERENCES

Aakash Goyal, Pankaj Kumar Bhowmik and Saikat Kumar Basu. 2009. Minichromosomes: The second generation genetic engineering tool. *Plant Omics Journal*, **2**(1):1-8 (1836-3644).

Pershina, L.A. 2014. Plant Chromosome Engineering Is an Area of Biotechnology. *Russian Journal of Genetics: Applied Research*, **4**(4): 311–317.

Simon W.L. Chan. 2010. Chromosome engineering: power tools for plant genetics. *Trends in Biotechnology*, **28**(12): 75-78.

How to cite this article?
Sai Rekha, K. 2017. Prospects of chromosome engineering in crop improvement. *Innovative Farming*, **2**(2): 122-125.