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# Crop improvement through centromere mediated engineering and its application

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The creation of homozygous lines for all significant traits is necessary for the stable reproduction of crop varieties through seeds. These true-breeding lines are created using a conventional method that involves repeated backcrossing or self-pollination for seven to eight generations. However, complete homozygosity can be attained in a single generation using doubled haploid technology. Either in vivo or in vitro techniques are used to produce haploids. Methods for in vitro haploid induction, such as anther culture and microspore embryogenesis, are costly and time-consuming. Additionally, a lot of species are resistant to other cultures. Specific crop species are the only ones that can use in vivo technologies like interspecific crosses, pollen that has been severely irradiated, and parthenogenesis. Although many still require in vivo haploid induction techniques, seed-based methods are less expensive and labour-intensive.

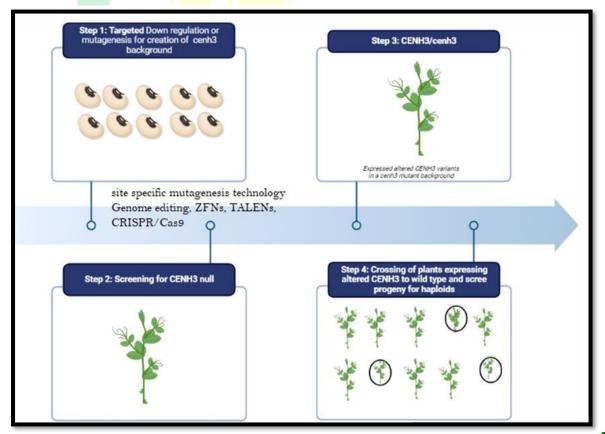
Plant breeding can be greatly speed-up by the creation of haploid plants, which having chromosomes from only one parent. Haploids can be produced from a heterozygous individual and transformed into diploids can leads to rapid production of homozygous lines. Haplozymes are typically created using two techniques. First, it is possible to create haploid plants from cultured gametophyte cells, but many species and genotypes are resistant to this technique. Second, in extremely rare interspecific or intergeneric crosses, where chromosomal elimination occurs following fertilization however, chromosome elimination in plants as a result of some interspecific crosses has been described, but it happens on rare or natural occasions. The zygote, created by the fusion of pollen sperm and eggs, inherits both parental chromosome sets but loses one of them during genome elimination.



Kinetochores are built on DNA structures known as centromeres. During mitotic and meiotic cell divisions, they act as handles to which spindle fibers connect so as to pull chromosomes through the mother cell and split them between the daughters. The existence of CENH3, a histone H3 variation that takes the place of conventional H3 in centromeric, epigenetically identifies them. CENH3, which also contributes to centromeric identity, is hypothesized to enhance spindle fiber attachment to the chromosome through the development of kinetochores. All eukaryotes, including significant agricultural crops, contain a universal protein called CENH3.

#### Major protein for centromere engineering: CENH3

Similar to regular histone H3s, CENH3 has an N-terminal tail domain that extends from the nucleosome and a C-terminal histone fold domain that forms a complex with other histones to form the nucleosome core. The CENH3 homologs are very different from each other, especially in their N-terminal tail, in contrast to the nearly invariant histone H3.



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## Steps for creation of Centromere mediated haploid production



### **Applications for Plant breeding**

#### 1. Clonal seed production:

It is possible to induce apomixis de novo in plants by modifying sexual processes. The first step is to change meiosis by turning it into a mitotic-like division, producing clonal gametes with the somatic level of ploidy Arabidopsis. The second step is to produce seeds from these unreduced gametes, without any genetic contribution from another gamete.

#### 2. Construction of mapping population:

Mapping population can be constructed by simply crossing an  $F_1$  with a parent who induces haploidy to produce haploid seeds and population's parental allele frequencies and recombination rate were comparable to those of earlier RIL groups. This population can be utilized to successfully map QTL.

#### 3. Speedy assemblage of chromosome substitution lines (CSLs):

In conventional breeding method, the capacity of two homozygous lines to create the best hybrid when crossed is selected for in order to take advantage of heterosis. The plants that are heterotic and the ones that exhibit the desired phenotype are so distinct. Reverse breeding is a cutting-edge plant breeding technique that advocates a top-down method. The parental lines are then replicated after the best hybrid has been chosen. By eliminating crossover formation in the heterozygote and creating doubling haploid plants from gametes free of crossovers can creates homozygous parental lines from any heterozygous plant. Due to the loss of most, if not all crossovers, chromosomes segregate randomly, but balanced gametes are produced at a rate close to the expected rate. These gametes have been turned into haploid plants using centromere-mediated genome elimination and then self-pollination resulting in doublehaploid diploid plants that are homozygous at all loci in the genome. From these, both the original hybrid and a set of substitution lines have been obtained.

Thus, this new technique of centromere mediated haploid production introduced in Arabidopsis holds lots of potential for Plant Breeding community in future for development of variety for meeting the requirement of teeming population of the world.