Synthetic Clonal Reproduction Through Seeds

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Engineering cloning through seeds (apomixis) in food crops would revolutionize agriculture by fixing hybrid vigor and allowing the perpetuation of any elite heterozygous genotype (*1*). The first component of apomixis, the formation of diploid clonal gametes, can be introduced in a sexual plant, with *Arabidopsis* mutants (*MiMe* and *dyad*) (*2*, *3*). However these clonal gametes participate in normal fertilization, giving rise to progeny with more chromosomes than their parent. Clonal gametes would be turned into clonal seeds if fertilized by a strain whose chromosomes are eliminated from the resultant progeny (Fig. 1A). Genome elimination can be induced by manipulating the centromere-specific histone CENH3 (*4*). By co-expressing green fluorescent protein (GFP)–CENH3 and GFP-tailswap variants in the *cenh3-1* mutant, we made a line with increased fertility that causes genome elimination when crossed to a parent with diploid gametes (*5*) [supporting online material (SOM) text]. We named this line *GEM* for Genome Elimination induced by a Mix of CENH3 variants. When *GEM* was crossed to a tetraploid wild type (which has diploid gametes) or to *osd1*, a diploid mutant that produces a very high proportion of diploid male and female gametes (*2*), it induced conversion of diploid gametes into uniparental diploid progeny in addition to giving triploid and aneuploid progeny (table S1). However, uniparental diploid plants from *osd1* crosses are not clones, because the diploid gametes produced by *osd1* are recombined (*2*, *5*) (SOM text and fig. S2).

We then crossed *dyad* and *MiMe* plants as female to the *GEM* line and screened for clones in the F1 generation, which comprised diploids, triploids, and aneuploids (table S2). Because the genetic background of the parents differed, we could trace the origin of the chromosomes. *MiMe* × *GEM* gave an average of 14 viable (i.e., able to germinate) seeds per fruit; 34% (53/155) were diploid (table S2). Among these diploid plants, 98% (52/53) had only maternal chromosomes, lacking any paternal contribution for the eight diagnostic loci tested (Fig. 1B). Furthermore, these maternal diploids (named diploid eliminants) retained the heterozygosity of the mother plant at all tested loci (Fig. 1B). Likewise, *dyad* × *GEM* resulted in 0.9 viable seeds per fruit, 13% (29/220) of which were diploid eliminants retaining full maternal heterozygosity (table S2 and Fig. 1C). Although not fully penetrant, this demonstrates clonal propagation through seed in a manner akin to the outcome of apomixis.

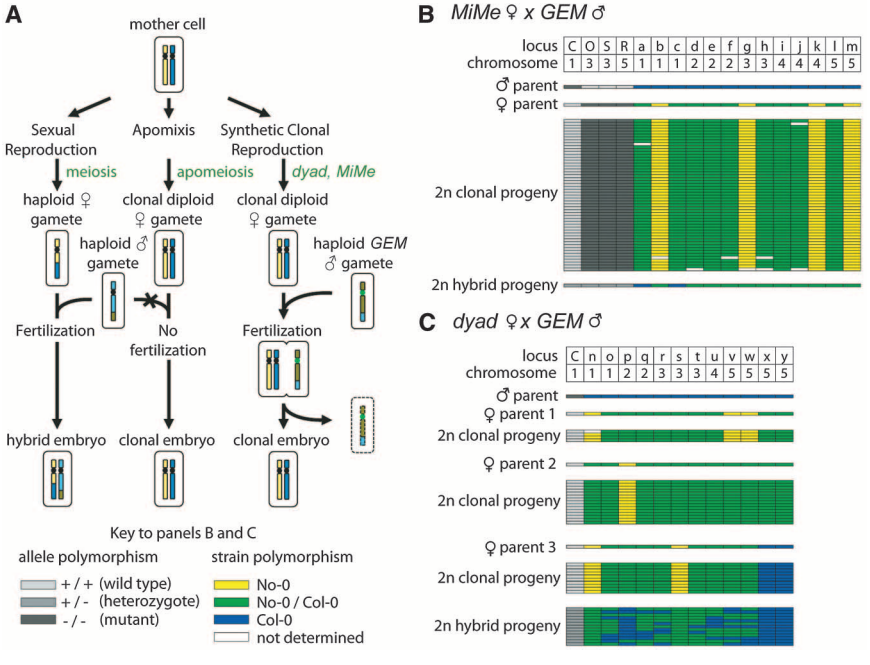

Fig. 1. (**A**) In natural apomixis, clonal seeds develop without fertilization. To induce clonal seed from sexual plants, we fertilized clonal gametes with a parent whose chromosomes are modified to be eliminated after fertilization. (**B** and **C**) Parents and diploid progeny were genotyped for polymorphic loci (table S3). Each row represents one plant, and each column is a locus.

Because *MiMe* also produces male diploid clonal gametes (*2*), we tested for clonal male inheritance by crossing *GEM* as the female to a *MiMe* male. Although seed viability was lower in this cross, likely because the Col-0 strain is sensitive to paternal genome excess (*6*), 42% of progeny were diploids that lacked maternal contribution and had the heterozygosity of the male parent (fig. S3A and table S2). Thus, these plants are clones of their *MiMe* father, mimicking male apomixis (*7*). We crossed a maternal *MiMe* clone to *GEM* for a second generation and obtained 14 seeds per fruit. In this population, 24% (19/79) of progeny were diploid and genetically identical to their mother and grandmother (fig. S3B and table S2), demonstrating clonal propagation through seed for more than one generation.

Our experiments show that clonal reproduction can be engineered in a sexual plant by manipulating two to four conserved genes controlling meiosis and chromosome segregation. The system we have described still relies on crossing, whereas to achieve the full benefits of apomixis the plant needs to be self-propagating. However, this proof of principle suggests a new strategy for development of apomixis in food crops.

References and Notes

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5. Materials and methods are available as supporting material on *Science* Online.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6019/876/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 to S3
References

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