**Title:**

Self-cloning crops, or How a scientific egg hunt led us to virgin births in plants

**Introduction:**

The use of hybrid seeds transformed agriculture in the 20th century. However, the progeny of hybrids have highly variable yields due to genetic segregation (Mendel’s laws). Therefore, farmers have to purchase new hybrid seeds every year. But hybrid seeds are expensive to produce, especially for crops that are naturally self-pollinating. As a result, hybrids are under-utilized for many major crops, especially in developing countries. If hybrids could make seeds genetically identical to their parents, farmers need not buy new seeds each year. In nature, many plant species have evolved to reproduce asexually and make progeny that are clones, a process called “apomixis”. But apomixis is not found in any of the major crops. So for over a quarter century scientists have dreamed of “synthetic apomixis”, i.e., to make hybrid crop plants that reproduce as clones, so that hybrid seeds can be accessible to farmers in the developing world.

When we started this project, our original goal was something else altogether. My lab had been studying a fundamental problem in plant biology: How does an egg cell when fertilized become a zygote? The zygote is a totipotent stem cell, programmed to make an embryo, and eventually a whole new plant. The egg cell is terminally differentiated. Some genes must be switched on after fertilization that lead to reprogramming of the egg cell. We had isolated zygotes and identified many newly transcribed genes. Which of these genes might be a master switch for the transition of the egg cell to a zygote? One gene caught our attention, because it was related to a known gene called BABY BOOM (BBM) in Arabidopsis that could induce embryo growth on leaves. This gene, which we called BBM1, was silenced in the maternal genome, and expressed only from the paternal genome. If BBM1 was a master switch to convert an egg cell into a zygote, it made sense that the gene would be maternally silenced. If so, forcing the egg cell to express BBM1 might convert the egg to a zygote, and produce an embryo without fertilization. This was the hypothesis we set out to test.

**Methods:**

We made rice plants with a transgene in which the BBM1 gene was driven by an egg-cell specific promoter, so that BBM1 would be ectopically expressed in the egg cell (BBM1-ee). To eliminate meiosis, we used CRISPR-Cas9 with guide RNAs targeting three meiotic genes, REC8, PAIR1 and OSD1. The ploidy of plants was determined by flow cytometry.

**Results:**

Our hypothesis that BBM1 is a master regulator that can switch an egg cell into a zygote was validated: The BBM1-ee rice plants produced progeny without fertilization (“parthenogenesis”)! Asexual progeny are haploid, because they have not received the chromosomes of the sperm cell, and therefore they are sterile. But what if we could eliminate meiosis? At a conference in France, I saw a poster from the labs of Raphael Mercier and Emmanuel Guiderdoni, describing rice plants called MiMe (substitution of Mitosis for Meiosis). These rice plants were mutants for three meiotic genes, and produced unrecombined diploid gametes (eggs and sperm). We realized that combining MiMe with BBM1-ee would produce diploid progeny, due to induction of parthenogenesis in the diploid egg cell. Moreover, the progeny would be genetically identical to the mother plant, i.e. clones, meaning that synthetic apomixis could be achieved. A few emails and Skype calls, and a collaboration was established. We then proceeded to make MiMe mutants in our BBM1-ee plants, using CRISPR-Cas9 for genome editing. Several months later, we had transgenic BBM1-ee plants with MiMe mutations. These plants were predicted to produce both tetraploid progeny by sexual reproduction, and diploid progeny asexually through parthenogenesis. To our delight, we did obtain diploid progeny at different frequencies. The diploid progeny were predicted to be clones, asexually derived from diploid egg cells that had evaded meiosis. We propagated them to get progeny “grand clones” and “great grand clones”. To prove beyond doubt that the clones were genetically identical to the parent plants, we sequenced the genomes of the clones and grand clones. The parental plant was heterozygous at multiple nucleotide positions throughout the genome. As predicted, the two generations of clones were also heterozygous at all these genomic positions. For a sexual progeny to inherit this heterozygosity by random chance was negligible. We had made rice plants that cloned themselves!

**Conclusions:**

So it was that a search for genes that turn egg cells into zygotes unexpectedly provided a key to the long-sought goal of synthetic apomixis. Of course, we have only shown feasibility, and implementation in farmer’s fields will require improved efficiencies, field trials, etc. But a stubborn barrier has been breached, and that is promising for the future of agriculture in a world where increasing demand for food runs up against limited land and resources.

**References**

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