Asexual plant reproduction may seed new approach for agriculture

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An HHMI scientist has moved a step closer to turning sexually-reproducing plants into asexual reproducers, a finding that could have profound implications for agriculture.

Farmers throughout the world spend an estimated $36 billion a year to buy seeds for crops, especially those with sought after traits such as hardiness and pest-resistance. They can't grow these seeds themselves because the very act of sexual reproduction erases many of those carefully selected traits. So year after year, farmers must purchase new supplies of specially-produced seeds.

This problem is sidestepped by some plants—such as dandelions and poplar trees—that reproduce asexually by essentially cloning themselves. Jean-Philippe Vielle-Calzada, a Howard Hughes Medical Institute (HHMI) international research scholar, wondered whether he could learn enough about the genetics of asexual reproduction to apply it to plants that produce sexually. In an advance online publication in *Nature* on March 7, 2010, Vielle-Calzada and his colleagues report that they have moved a step closer to turning sexually-reproducing plants into asexual reproducers, a finding that could have profound implications for agriculture.

"Agricultural companies and farmers around the world have a tremendous interest in this method," says Vielle-Calzada, a plant researcher at the Center for Research and Advanced Studies of the National Polytechnic Institute in Irapuato, Mexico. "It would allow them to simplify the labor-intensive cross-hybridization methods they now use to produce hearty seeds with desirable traits."

As with animals, sexually-reproduction in plants involves the generation of male and female gametes that each carry half of the organism's genes. Flowering plants exhibit the most advanced form of sexual plant reproduction, producing pollen-derived sperm cells that join with egg cells to produce seeds. Each seed, then, is genetically unique. There are several types of asexual reproduction in plants, but all produce the same result: genetically identical daughter plants.

Vielle-Calzada's quest to develop an asexual seed began a decade ago, when he decided to investigate apomixis, a specific type of asexual reproduction. Many species of plants use apomixis to generate viable seeds without the fusion of sperm and egg. This method of asexual reproduction results in the formation of seeds that are essentially clones of the main plant and has great potential for crop improvement. In apomixis, reproductive cells retain the full complement of chromosomes, rather than losing half their genes via meiosis, as happens in sexual reproduction. About 350 families of flowering plants rely on apomixis to reproduce, but nearly all plants used for food reproduce sexually.

Vielle-Calzada studied apomixis in *Arabidopsis thaliana*, a small flowering mustard plant with a compact and well understood genome. *Arabidopsis* was also selected because it does not reproduce asexually. "We've been trying to induce apomixis in a species that doesn't practice it," he says.

In the research reported in Nature, Vielle-Calzada and scientists from Mexico, France, and the United States homed in on a reproductive structure of *Arabidopsis* called the ovule. Each tiny ovule produces a single female gamete, which, when fertilized, grows into a seed. The team used a genetic screen to identify genes that are active in the ovule - reasoning that measuring gene activity would lead to important insights into which proteins are essential for guiding asexual reproduction.

The researchers netted a number of interesting genes in their screen, but one in particular, Argonaute 9, caught their attention immediately. The large family of Argonaute proteins has gained widespread attention among researchers because the proteins control which gene products—either RNA or proteins—a cell makes. Argonautes do this by slicing up messenger RNA before it can be translated into proteins. The identification of Argonaute activity in the ovule was all the more interesting, says Vielle-Calzada, because Argonaute proteins had never been seen in *Arabidopsis* reproductive cells before.

Next, Vielle-Calzada and his colleagues mutated the Argonaute 9 gene and watched what happened next. The results were swift and provocative. Instead of producing a single gamete, most of the ovules with the disrupted Argonaute gene produced several gametes, which were abnormal. Instead of carrying half of the species' chromosomes, they carried the full complement of genetic material— implying that they had not undergone meiosis.

"By cutting off the function of Argonaute, we caused a 'schizophrenic' reaction of the cells in the ovule, which were not supposed to become gametes," Vielle-Calzada says. "It looks like Argonaute normally prevents those cells from being transformed into gamete precursors." That suggested that Argonaute 9 prevents the initiation of apomixis in *Arabidopsis*.

The finding raises the possibility that many—or maybe even all—plants have the ability to reproduce through apomixis, but that potential is suppressed by Argonaute 9. "It's possible that plants have a very old memory that allows them to reproduce asexually," Vielle-Calzada says.

The team then searched inside the ovule to look for the pieces of RNA that Argonaute 9 degraded. They found that Argonaute chewed up 2,600 snippets of RNA. The experiment "was a complete tour de force for the lab," Vielle-Calzada says. "It required a lot of ovules and a lot of fiddling."

After mapping those RNA sequences back to the *Arabidopsis* genome, the team discovered that more than half were produced by transposons. Transposons, also called "jumping genes," are mobile genetic elements that copy and insert themselves throughout the genome. Their function remains somewhat mysterious, although some evidence suggest they are important in controlling gene expression.

"It seems that Argonaute 9 silences transposons in the ovule of Arabidopsis," Vielle-Calzada says. "The open question now is, 'Why?'" His working hypothesis is that squelching the transposons prevents apomixis, but his lab is working to prove the connection. "These results are exciting because they suggest for the first time that transposons could be controlling early development in plants," he says.

Though he has made great progress, Vielle-Calzada is still working toward creating a fully asexual *Arabidopsis* plant. His current mutants do not develop completely asexual seeds. But by highlighting the role of Argonaute 9 in plant reproduction, Vielle-Calzada has moved a step closer to a slew of agricultural possibilities. "Now we just need to discover how to trigger the second and final step of making sexual plants asexual," he says.

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