Researchers solve plant sex cell mystery

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Graduate student Tim Kelliher and Professor Virginia Walbot examine corn tassels in the field on the Stanford campus. (Photo: L.A. Cicero)

(Phys.org) -- Although farmers have been manipulating plant germlines since the Neolithic, plant sex cells have stubbornly guarded the secret of their origin. The surprisingly simple answer – low oxygen levels – could change the way we breed plants.

The sex life of corn has gotten a lot of prurient attention over the years. By 5,000 B.C., agriculturalists in the Americas were already producing the first hybrid corn varieties by cross-pollinating plants to generate larger plants or colored kernels.

Today, hybrid seed production in corn is a multibillion-dollar industry, and crossbreeding is fundamental to the production of most other species as well. But despite plant reproduction's central role in agribusiness, researchers have never answered a basic question: Where do plant sex cells come from?

The answer, according to Stanford biology Professor Virginia Walbot and graduate student Timothy Kelliher, is surprisingly simple. In a set of elegant experiments – Walbot prides herself on "thinking of experiments you can do with basically no money" – the researchers demonstrated that low oxygen levels deep inside the developing flowers are all that is needed to trigger the formation of sex cells.

The discovery isn't only of academic interest.

"Controlling plant reproduction is fairly fundamental to modern agriculture," Walbot said.

In a corn industry that still detassels seed corn by hand as a way of controlling who fertilizes whom, a technique that switches sex cell production on or off could allow for dramatically increased control over plant crossbreeding.

The research paper appeared recently in the journal *Science*.

When two flowers love each other very much

All flowering plants produce pollen within structures called anthers, which in corn grow from the distinctive cluster of male flowers we know as the tassel. But before these anthers mature, they are arranged in a clover shape deep within the plant. The central cells within each of these clover-like lobes will turn into sex cells and, eventually, pollen.

The mechanism behind this development was unknown in plants. In animals, surrounding cells signal the germ line to begin forming from a single "founder cell." Walbot and Kelliher were leaning toward this view, having identified two promising signaling molecules, MAC1 and MSCA1. Plants that lacked the protein MAC1 developed too many germ cells. Plants that lacked MSCA1 had none at all.

Clearly, MAC1 was important for organizing the non-sexual cells around germ cells, while MSCA1 was necessary for cells to develop into sex cells. But the connection between the two, and what initially led to their expression, remained unclear.

A role for redox

Although most researchers assumed that, as in animals, sex cells were developing from a special set of cells with a predetermined predilection for the role, Walbot and Kelliher saw two clues that implied otherwise.

First, the physical arrangement of the sex cells didn't point to the existence of a single "founder." In fact, it suggested a scenario where "your position as a cell mattered more than who your parents were," Kelliher said.

Second, the way the MSCA1 enzyme operated suggested that oxygen levels might play a role in the signaling process.

The environment inside a plant can be either "oxidizing" – where oxygen is plentiful, and oxidation is favored – or "reducing" – where oxidation is prevented, usually by a lack of reactive oxygen, and the opposite process of reduction is favored. MSCA1 happened to send its signal through reduction – meaning that different oxygen levels might have different developmental effects.

To test the theory, the researchers inserted a probe deep into the immature anther tissue of corn. What they found was telling: unusually low oxygen levels – likely a side effect of the rapidly growing anthers' metabolic activity – at the precise time that cells were beginning to turn into sex cells.

Corn hose

To see if low oxygen alone was responsible for sex cell development, the researchers threaded a plastic hose into the developing anther and piped in mixtures of gases.

High concentrations of oxygen drastically decreased the number of sex cells. High concentrations of nitrogen gas, which is inert and provides a reducing environment, increased sex cell formation.

"It was a remarkably easy experiment," said Walbot. "We had the initial results in two days."

The researchers showed that low oxygen levels could even cause cells outside the anther lobes – which would never normally produce pollen – to develop into sex cells.

All together, Walbot explained, the evidence suggests that naturally occurring variations in oxygen levels inside the growing anther causes the central cells to become hypoxic first: "The cells that are most hypoxic then get to throw the switch."

Once oxygen levels drop below a certain threshold, MSCA1 is finally able to go to work and reduce its target, causing central cells to become sex cells. These cells then release MAC1, which in turn ensures that the outside cells don't become germline.

It's an inside-out differentiation pattern, utterly unlike what animal germlines do – which may explain why it took so long to be discovered.

"The plant takes advantage of its own structure to create this developmental signal," said Kelliher. "And then any cell can create the next generation as long as it's in the right place – you don't have to be specially designated. It's kind of a romantic idea."

Children of the corn research

Keeping a close watch over this entire plant fertility process is crucial for the hybrid seed industry. Fields are usually planted with two varieties of seed corn that are going to be crossbred. In order to keep plants from fertilizing themselves – which results in inferior-quality plants – all of the tassels of one species need to be removed.

This is an enormous task that requires specialized detasseling machines, followed up by people who check for plants that were missed.

"Currently they remove the tassels on 1 million acres of corn each year, at 20,000 plants per acre," said Walbot. "That's billions of hand-detasseled plants."

Sterile varieties of corn have been developed that don't require detasseling, but self-perpetuating versions have proven difficult to perfect. A low-oxygen sterilization method could make automated hybridization much simpler, allowing it to be applied to a large number of varieties.

"We leave those applications to industry," said Walbot. But the effects of the research could be wide-ranging. Assuming that the findings hold true for all flowering plants, as a number of research groups are now seeking to confirm, the discovery could open up a new level of fertility control for a huge array of crops.

Stanford University is currently seeking a patent on some of the paper's findings.

More information:

www.sciencemag.org/content/337/6092/345.abstract

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