Plant Mating Mystery Solved

UNIVERSITY PARK (Centre Co.) — A gene long suspected of controlling the self-incompatibility mating system in plants has finally been caught in the act by a team of Penn State biologists.

Led by Teh-hui Kao, associate professor of biochemistry and molecular biology, the team is the first to show directly that this gene determines whether a plant is able to fertilize itself.

For more than 130 years, since Darwin observed that some plants can fertilize themselves with their own pollen while others cannot, scientists have been trying to understand exactly what controls this aspect of plant mating. Now, in a paper published in the Feb. 10 issue of the journal Nature, the Penn State biologists have provided the first direct evidence confirming a theory of genetic self-incompatibility that is the foundation for years of research in plant genetics.

The cornerstone of this theory is the self-incompatibility gene, or "S gene." According to the theory, a plant that cannot fertilize itself has an S gene that is "turned on," enabling it to produce in its pistil a protein that recognizes and rejects its own pollen. If a plant's S gene is "turned off," it does not produce the S protein, so it is able to fertilize itself.

"A protein identified in the early 1980s seemed to be the predicted S protein," said Kao, "but our strongest clues until now were only from indirect evidence."

By harnessing standard genetic-engineering techniques, Kao's team was able to neutralize the gene in a group of petunia plants, reversing their inherited inability to fertilize themselves and enabling them to produce seeds. The biologists also inserted the gene into another group of plants, giving them the ability to reject pollen with a specific genetic identity.

The biologists performed two experiments to show that a plant's ability to produce seeds when self pollinated depends on the presence or absence of an active S gene. In the first experiment, they disabled an S gene in a line of self-incompatible plants, then attempted to fertilize them with their own pollen.

"We reasoned that if an S protein is required for self-incompatibility interactions between pistil and pollen then inhibition of its synthesis should lead to the breakdown of self incompatibility," Kao said.

Each plant has two varieties of the S gene, called S alleles, which it inherits from the parent plants. Kao used petunia plants that had alleles called S2 and S3. He used a genetic engineering technique to produce an "antisense" S3 allele whose DNA sequence order is the reverse of a normal S3 allele's. Normal alleles produce RNA in a normal sequence order that makes genetic "sense."

"Antisense RNA is able to block the synthesis of protein from sense RNA in a mysterious way that we do not yet understand," Kao said.

Next, the team, including post-doctoral fellow Hyun-Sook Lee and graduate student Shihshieh Huang, incorporated the antisense S3 allele into a bacterium that they then used to infect the petunia leaves. From these leaves, they grew transgenic plants containing the three alleles, S2, S3 and antisense S3.

"Although this is a standard procedure, it turned out to be the most critical step in this experiment," Kao says. "We struggled for about a year before we were able to successfully grow transgenic petunias."

The team tested these transgenic plants, found they were not producing any S3 protein and attempted to fertilize them with S3 pollen.

"A normal plant with S2 and S3 alleles, when pollinated with S3 pollen, will reject the pollen because the S allele types match. The flower's pistil recognizes the pollen as 'self pollen,' fertilization fails, and the plant does not produce seeds," said the Penn State biologists. "But our transgenic petunias produced the same large number of seeds as you would get from compatible pollination, showing that they had lost the ability to reject self pollen."

Kao says this is the first successful attempt to use the antisense approach in any self-incompatible plant species.

Growers of self-incompatible crops such as apples could benefit from this part of Kao's research, according to George Greene, associate professor of pomology at Penn State.

Because apples are self-incompatible, commercial apple growers typically mix, in a single orchard block, three varieties that they carefully select to provide sources of compatible pollen. Cultivation of a single self-compatible variety would increase efficiency. Greene said by reducing several cultural and harvesting problems.

In their second experiment, Kao's team put an S3 gene into petunias that contained S1 and S2 alleles. A normal plant with S1 and S2 alleles will accept S3 pollen because the S3 allele carried by the pollen is different from the S1 and S2 alleles carried by the flower's pistil. However, Kao's team found that some of the transgenic plants produced no seeds at all when pollinated with S3 pollen.

"The transgenic plants that failed to produce any seeds at all had normal levels of S3 protein for a plant containing an S3 gene, which enabled them to acquire the ability to completely reject S3 pollen," Kai said. "The transgenic plants that produced a few seeds when pollinated with S3 pollen had levels of S3 protein that were much lower than normal, and those transgenic plants producing the most seeds did not have any detectable amount of S3 protein."

He said that this experiment shows that S-protein levels alone control a plant's ability to reject its own pollen — or pollen whose S allele type is identical to one of those contained in the flower's pistil.

"The ability to prevent plants from fertilizing themselves could double the yield and reduce by one-third to two-thirds the labor costs involved in hybrid seed production," said Richard Craig, professor of plant breeding and the Styer Professor of Horticultural Botany at Penn State.

Virtually all commercially important vegetables and many important flowers are produced from F1 hybrid seeds, the result of crossing two purebred plant lines. In order to assure the uniformity of hybrid seeds, growers typically must remove by hand the pollen-producing organs from the seed-producing parent plants, then discard the seed produced by the pollen parents — sacrificing half the seed crop.

"If the plants were 100 percent self-incompatible, you could harvest seed from every plant while using much less costly and more efficient fertilization procedures," Craig said. "In addition, Dr. Kao's work could provide the key to producing hybrids in many crops where this technique previously has been either inefficient or impossible."

"Confirmation that the S gene encodes the key protein in self-recognition comes as a huge relief to scientists who have published analyses based on that assumption," said Andrew Clark, professor of biology at Penn State and an authority on the molecular evolution of S alleles.

Craig said, "Many generations of scientists have devoted their lives to understanding the beautiful system of self-incompatibility in plants. Dr. Kao has added something to this effort that we have been seeking for half a century. His impressively simple and elegant contribution to our understanding of this biological process brings it into the era of modern molecular biology."

Keo said his team's next research goals are to determine whether the S protein, a ribonuclease, digests the pollen's RNA or blocks its growth in some other way and to identify exactly which of the protein's amino acids recognize self-pollen.

"We have not yet captured the holy grail of this field, which is to determine the precise bio-chemical mechanism of self-incompatibility," Kao said, "but this goal is the focus of our work, which looks like it could turn out to be a lifetime project.'

This research was supported by grants from the National Science Foundation and the United States Department of Agriculture.

Farmers Win Top Awards In Contest

DEKALB, Ill. — Richard Schmaltz of Doylestown, Pa. won the state first-place award in the National Grain Sorghum Producers (NGSP) yield and management contest, non-irrigated division. This is his third consecutive win.

R. Gregory Manners of Ringoes, N.J. won the state first-place award in the National Grain Sorghum Producers (NGSP) yield and management contest, non-irrigated division. This is his third consecutive win.

Also, Schmaltz won the award with DeKalb DK37 sorghum which produced 90.52 bushels per acre. The yield ranked him among DeKalb's 14 national and state first-place winners.

He will be honored along with other state and national winners at the NGSP's annual convention in Nashville, Tenn. DeKalb Plant Genetics also will present him with an award during a special reception.

Manners won the award with DeKalb DK37 sorghum which produced 109.95 bushels per acre. The yield ranked him among DeKalb's 14 national and state first-place winners.

He also will be honored at the NGSP's annual convention in Nashville, Tenn. DeKalb Plant Genetics also will present him with an award during a special reception.

Schmaltz, who has been farming 40 years and has been a DeKalb dealer more than 30 years, grows corn, sorghum, soybeans and wheat on 575 acres. He plants 195 acres of that to sorghum.

Manners, who has been farming 21 years, plants 80 of his 700 acres to sorghum. On the other acreage he grows corn, soybeans, oats, rye, hay, sorghum/Sudangrass. He also raises about 50 head of cattle.

1 FARM EQUIPMENT

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1150 MF, field ready, $5500; AC-D19, gas, good condition, $2800; IH W/450, gas, w/pully, $1500. 717/872-8445.

12' JD roller harrow, $675; 18' JD grain drill, on rubber, $200; 16' Oliver grain drill, large rubber tires, $275, 3 axle 9-ton trailer, $1600, NI manure spreader $425 Other items, selling out 609-723-2574.

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