Researchers Develop First Hypoallergenic Soybeans

Soybeans are used in everything from printing ink and biofuels to baby formulas and salad dressing. But this so-called miracle crop isn't for everyone. Worldwide, 6 to 8 percent of children and 1 to 2 percent of adults suffer food allergies. Those allergic to soybeans must avoid eating foods containing its proteins.

Research suggests a single protein called Gly m Bd 30K/P34 is to blame. "Soybeans are in many processed foods, and here in the Western Hemisphere it's hard to avoid them unless you're very proactive," notes Eliot M. Herman, who discovered P34 in the early 1990s. Now, using a biotech approach, he and colleagues have shut off the gene that makes the allergenic protein in the crop's seed.

"This is probably the first time a dominant human allergen has been knocked out of a major food crop using biotechnology," says Herman, an ARS plant physiologist. He'll publish the advance along with co-investigators Rick M. Helm, an associate professor at the University of Arkansas for Medical Sciences in Little Rock; Tony J. Kinney, a crop-genetics research leader at the DuPont Experiment Station, Wilmington, Delaware; and Rudolf Jung at Pioneer Hi-Bred International, a DuPont affiliate.

The team resorted to biotechnology after analysis of the soybean genome revealed the P34 gene in domestic cultivars and in their wild cousins. "The simplest approach would be to find a soybean without the protein, but we found that all the domestic varieties and wild soybeans had it," says Herman, who recently transferred from Beltsville to the Donald Danforth Plant Science Center in St. Louis, Missouri, but is assigned to ARS' Plant Genetics Research Unit, Columbia, Missouri.

More Than Just a Sneeze

Soybeans are considered one of the "big eight" food allergen sources. The others are eggs, milk, peanuts, wheat, fish, shellfish, and tree nuts. And while P34's exact function still isn't known, the researchers do know it belongs to a class of proteins called cysteine proteases. It causes 65 percent or more of allergic reactions in soy-sensitive individuals and does so by binding with IgE antibodies circulating in their bloodstreams.

A severe but rare reaction to soybeans is anaphylactic shock, in which a sensitized person's airways rapidly constrict. Usually, though, the legume causes nonfatal reactions, including hives, itching, and diarrhea.

"The only cure is avoidance of the food product. The difficulty with this is the number of products derived from peanuts, soybeans, and other legumes," notes Helm, an immunologist specializing in food allergens at the Arkansas Children's Hospital Research Institute.

Follow the Biotech Road

Although scientists appear to have shut off the gene for making P34, farmers aren't likely to see the knockout soybeans commercialized just yet. More tests are needed to prove they're indeed hypoallergenic, or less allergy causing. So far, though, the researchers are encouraged by early results from human blood serum tests in which antibodies that normally bind to P34 couldn't detect the allergen in knockout beans.

The hypoallergenic beans will also have to pass muster on an agronomic checklist that includes seed production, yield, pest resistance, oil and protein composition, and other criteria important to soy farmers and processors.

To expedite research that could eventually yield a new commercial cultivar, Pioneer Hi-Bred International is propagating the researchers' most promising knockout strain on field plots in Hawaii. The tropical climate there allows for two crops per season versus one on the U.S. mainland.

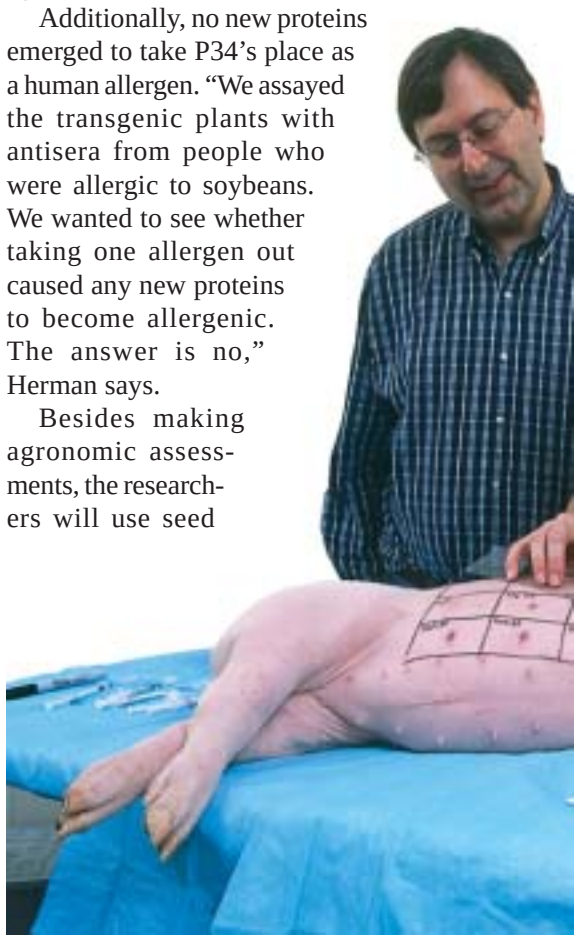
The knockout strain, derived from the soybean cultivar Jack, got its start from a clump of embryonic plant cells called a callus. The scientists cultured the material in the lab specifically for the purpose of knocking out the P34 gene. To do this, they used a biotech method called gene-silencing.

Once they knew for sure they had silenced P34 in the callus, they regenerated the cells into whole plants and propagated them further for their seed. After obtaining a uniform plant population by selecting for three generations, they checked the knockout beans' growth, development, and P34 activity against those of an unaltered control group.

"The yield looks perfectly normal. The plants develop and grow at a normal rate. The seeds set at a normal rate, and they all seem to have the same kinds of protein, oil, and other good stuff in them," Herman reports. "It looks like silencing P34 doesn't hurt the plant's agronomic characteristics."

Additionally, no new proteins emerged to take P34's place as a human allergen. "We assayed the transgenic plants with antisera from people who were allergic to soybeans. We wanted to see whether taking one allergen out caused any new proteins to become allergenic. The answer is no," Herman says.

Besides making agronomic assessments, the researchers will use seed



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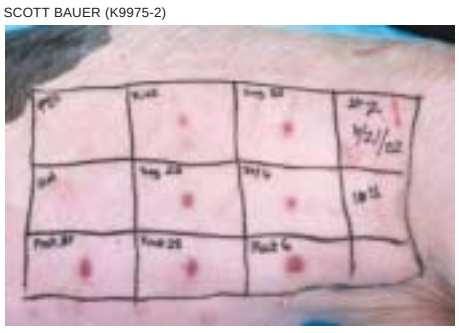

Above: Red areas on the pig's skin indicate allergic reactions to food substances. Below: Professor Rick Helm (right) and technician Gael Cockrell (center), both with the University of Arkansas, perform an allergy test on the skin of an anesthetized, soybean-sensitive pig. ARS plant molecular biologist Eliot Herman observes. The test is similar to that used with humans.

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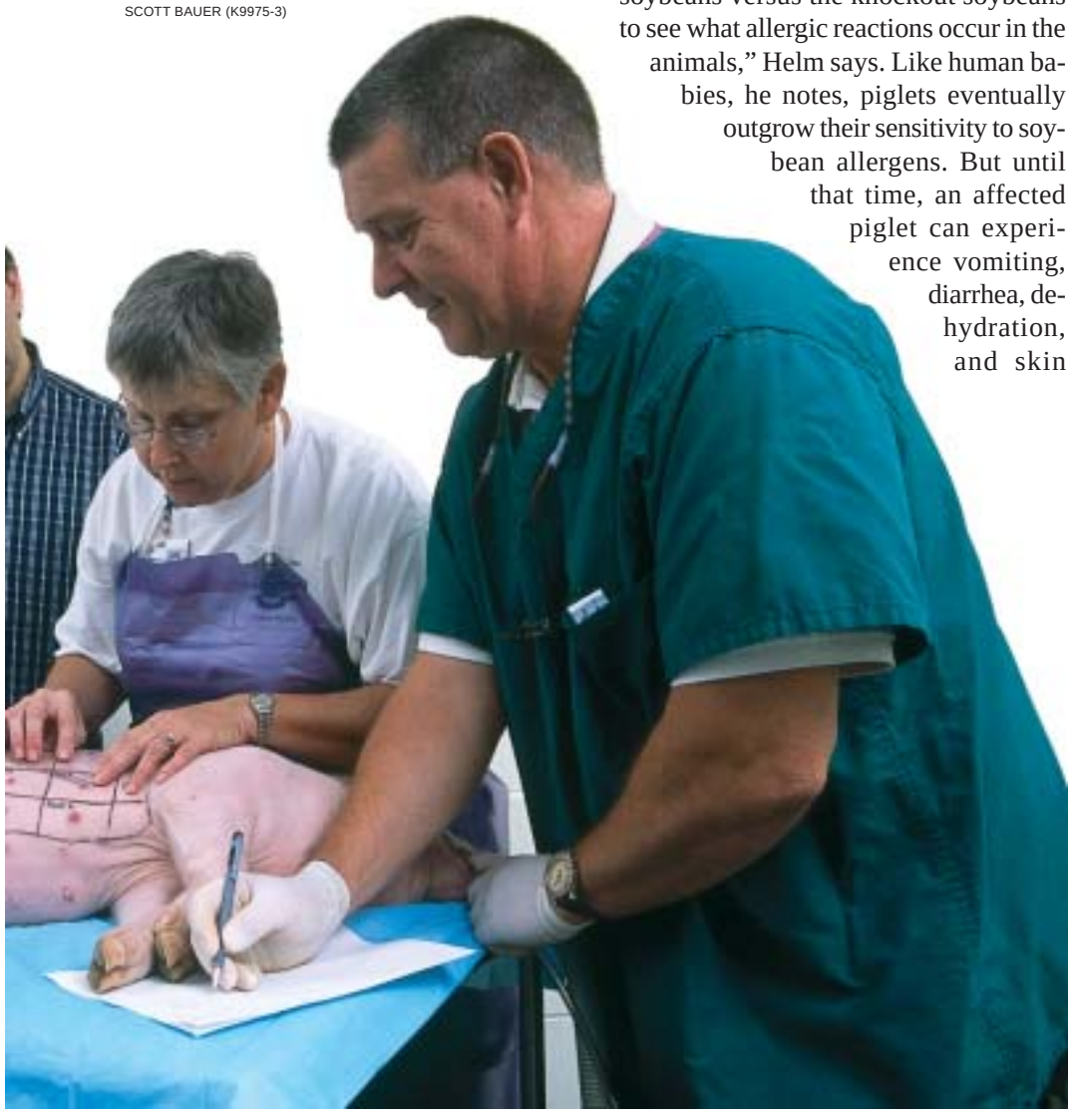

harvested from the Hawaiian bean plots to begin trials with piglets, which require lots of feed as they grow and develop. Helm will lead the studies at Little Rock using part of a $784,000 grant that he, Herman, Glenn Furuta, and Susan Hefle were awarded by USDA's Initiative for Future Agriculture and Food Systems. They were recognized for their development of an animal model to predict the allergenicity of genetically modified food. Furuta is at Children's Hospital/Harvard Medical School. Hefle is with the University of Nebraska.

Their study will include skin-prick allergenicity tests and feeding trials using newborn piglets. "We'll use unaltered soybeans versus the knockout soybeans to see what allergic reactions occur in the animals," Helm says. Like human babies, he notes, piglets eventually outgrow their sensitivity to soybean allergens. But until that time, an affected piglet can experience vomiting, diarrhea, dehydration, and skin rashes. "Because this seems to be a food allergy problem, it should be manageable by getting rid of whatever the pigs are allergic to," adds Herman. Information from the pig studies and others could serve as the springboard for clinical trials with humans.

"We're doing human testing only in the in vitro sense," says Herman. "We use human blood serum to test for P34 in the laboratory." But doing clinical tests with human subjects is a responsibility that will ultimately rest on the shoulders of whoever decides to commercialize the knockout beans as a hypoallergenic variety, he adds.

Miracle Crop Market

The potential effect such beans could have on the industry and on consumers may be far reaching, not so much because of the number of soy-sensitive people there are, but because of the multitude of products derived from the crop, which generated $12 billion in 2000 U.S. farm sales. Some products that could potentially benefit from hypoallergenic soybeans include baby formulas, soy milk, flour, cereals, grits, and pet food.

"You're never going to make a completely allergy-free soybean plant because you're not going to be able to eliminate all the proteins in it," says Helm. Rather, "we're looking to make a safer product." Many soy-sensitive consumers are also allergic to other foods, particularly those among the big eight. Singling out soy's major allergen could shorten the list of products they need to avoid.—By **Jan Suszkiw,** ARS.

This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at http://www.nps.ars.usda.gov.

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