Frogs help researchers find genetic mechanism for mildew susceptibility in grapevine

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This shows powdery mildew on a cabernet sauvignon grapevine leaf. Credit: USDA grape genetics publications and research.

A princess kisses a frog and it turns into a prince, but when a scientist uses a frog to find out more information about a grapevine disease, it turns into the perfect tool narrowing in on the cause of crop loss of Vitis vinifera, the world's favorite connoisseur wine-producing varietal.

MU researchers recently published a study that uncovered a specific gene in the Vitis vinifera varietal Cabernet Sauvingon, that contributes to its susceptibility to a widespread plant disease, powdery mildew. They studied the biological role of the gene by "incubating" it in unfertilized frog eggs.

The study, funded by USDA National Institute of Food and Agriculture grants, was lead by Walter Gassmann, an investigator at the Bond Life Sciences Center and University of Missouri professor in the division of plant sciences.

The findings show one way that Vitis vinifera is genetically unable to combat the pathogen that causes powdery mildew.

Gassmann said isolating the genes that determine susceptibility could lead to developing immunities for different varietals and other crop plants and contribute to general scientific knowledge of grapevine, which has not been studied on the molecular level to the extent of many other plants.

The grapevine genome is largely unknown.

"Not much is known about the way grapevine supports the growth of the powdery mildew disease, but what we've provided is a reasonable hypothesis for what's going on here and why Cabernet Sauvingon could be susceptible to this pathogen," Gassmann said.

The research opens the door for discussion on genetically modifying

grapevine varietals.

Theoretically, Gassmann said, the grapevine could be modified to prevent susceptibility and would keep the character of the wine intact—a benefit of genetic modification over crossbreeding, which increases immunity over a lengthy process but can diminish character and affect taste of the wine.

Grapevine under attack

Gassmann's recent research found a link between nitrate transporters and susceptibility through a genetic process going on in grapevine infected with the powdery mildew disease.

Infected grapevine expressed an upregulation of a gene that encodes a nitrate transporter, a protein that regulates the makes it possible for the protein to enter the plant cell.

Once the pathogen is attracted to this varietal of grapevine, it tricks grapevine into providing nutrients, allowing the mildew to grow and devastate the plant.

As leaves mature, they go through a transition where they're no longer taking a lot of nutrients for themselves. Instead, they become "sources" and send nutrients to new "sink" leaves and tissues. The exchange enables plants to grow.

The powdery mildew pathogen, which requires a living host, tricks the grapevine into using its nutrient transfer against itself. Leaves turn into a "sink" for the pathogens, and nutrients that would have gone to new leaves, go instead, to the pathogen, Gassmann said.

"We think that what this fungus has to do is make this leaf a sink for

nitrate so that nitrate goes to the pathogen instead of going to the rest of the plant," Gassmann said.

According to a report by the USDA, powdery mildew can cause "major yield losses if infection occurs early in the crop cycle and conditions remain favorable for development."

Powdery mildew appears as white to pale gray "fuzzy" blotches on the upper surfaces of leaves and thrives in "cool, humid and semiarid areas," according to the report.

Gassmann said powdery mildew affects grapevine leaves, stems and berries and contributes to significant crop loss of the Vitas vinifera, which is cultivated for most commercial wine varietals.

"The leaves that are attacked lose their chlorophyll and they can't produce much sugar," Gassmann said. "Plus the grape berries get infected directly, so quality and yield are reduced in multiple ways."

Pinpointing a cause

Solutions to problems start with finding the reason why something is happening, so Gassmann and his team looked at a list of genes activated by the pathogen to find transporters that allowed compounds like peptides, amino acids, and nitrate to pass.

Genes for nitrate transporters, Gassmann said, pointed to a cause for vulnerability to the mildew pathogen.

Over-fertilization of nitrate increases the severity of mildew in many crop plants, according to previous studies sited in Gassmann's article in the journal of *Plant Cell Physiology*.

The testing system for isolating and analyzing the genes began with female frogs.

Gassmann used frog oocytes (unfertilized eggs), to verify the similar functions of nitrate transporters in Arabidopsis thaliana, a plant used as a baseline for comparison.

A nitrate transporter, he hypothesized, would increase the grapevine's susceptibility to mildew.

"The genes that were upregulated in grapevine showed similarity to genes in Arabidopsis that are known to transport nitrate," Gassmann said. "We felt the first thing we had to do was verify that what we have in grapevine actually does that."

The eggs are very large relative to other testing systems and act as "an incubating system" for developing a protein. Gassmann and his team of researchers injected the oocyte with RNA, a messenger molecule that contains the information from a gene to produce a protein. The egg thinks it's being fertilized and protein reproduces and is studied.

"The oocyte is like a machine to crank out protein," Gassmann said. "We use that technique to establish what we have is actually a nitrate transporter."

The system confirmed that the gene isolated from grapevine encodes a nitrate transporter.

"We contributed to the general knowledge of the nitrate transporter family," Gassmann said. "It turned out to be the first member of one branch of nitrate transporters that, even in Arabidopsis haven't been characterized before."

The mounting knowledge of Vitis vinifera genes could make genetically modifying the strain to prevent the susceptibility easier.

"Resistance is determined sometimes by a single gene," Gassmann said. "Until people are willing to have the conversation of genetic modification, the only way to save your grapevines is to be spraying a lot."

Sharon Pike, Gassmann, other investigators from the MU Christopher S. Bond Life Sciences Center and post-doctoral student, Min Jung Kim from Daniel Schachtman's lab at the Donald Danforth Plant Science Center in Saint Louis, Mo. contributed to the report.

The article was accepted November 2013 into the *Plant Cell Physiology* journal.

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