

## **VIGS System Promises to Hasten Development of Varieties Resistant to Fusarium Head Blight**

By Don Lilleboe\*

VIGS is not the name of a new candy bar. But it does have all the makings of a sweet ingredient in the ongoing effort to control Fusarium Head Blight (scab) in wheat and barley.

VIGS — virus-induced gene silencing — is a system designed for use in the analysis of plant gene function. “This tool will allow us to assign functions to particular genes — particularly in the context of Fusarium resistance,” explains one of its primary developers, Steve Scofield of the USDA-ARS Crop Production and Pest Control Unit at West Lafayette, Ind. “By testing genes [with this system] and finding which ones are essential for Fusarium resistance, it allows us to understand the mechanism of resistance.”

The results of this gene identification process can be used by both traditional plant breeders and molecular biologists, Scofield adds. “They could tell the breeders which genes are most essential for Fusarium resistance; they also could be [tools] molecular biologists would use in making transgenic plants.”

While the concept of identifying genes that contribute to disease resistance is hardly new, the VIGS system is a faster and more-efficient approach. The prior methodology, Scofield explains, was a slower, more-laborious process that involved placing a new DNA construct — a “trans gene” — into plants, followed by regeneration of the entire wheat or barley plant.

However, the VIGS system — as the name implies — uses a virus. It’s based on a phenomenon known as RNA-mediated gene silencing, which Scofield describes as follows: “If a large amount of a double-stranded RNA molecule is produced in the cell, the cell recognizes that, and it activates a mechanism that targets for destruction whatever sequence is inside that double-stranded RNA.”

This phenomenon — which has been on the scientific radar screen for only about a decade (and occurs in both plants and animals) — may have evolved as a way for cells to

protect themselves against foreign RNA and DNA, Scofield theorizes. “Viruses certainly would fall in that category,” he says. “In fact, you can see this is a mechanism of viral resistance in plants.”

Scofield and his co-principal investigators — USDA-ARS researcher Joseph Anderson and Purdue scientist Ligrang Kong — use that mechanism to essentially “trick” the plant. “We have a plant gene we want to turn off,” he explains. “We put just a small fragment of that gene inside the virus to trigger this mechanism; and because all the viral RNA then becomes specifically targeted for destruction, the sequence of that plant gene is also part of the targeted sequence. So along with the viral RNA being attacked, the ‘messenger RNA’ of the gene we want to silence is destroyed” before it can serve its function.

Through this process, the investigators are able to see how genes work by observing plants where a particular gene is turned off. “If the plant starts off being resistant to *Fusarium* — and we switch off the gene and then infect the plant with *Fusarium* and see it is no longer resistant — we have very strong evidence that the gene we turned off was part of the resistance mechanism,” Scofield illustrates.

A breeder could use that information in analyzing specific wheat or barley lines where those particular genes were over-expressed or possessed some variation that made them more effective. “Then the breeder would incorporate those genes into the cultivated varieties they’re developing,” Scofield explains. Conversely, a molecular biologist could take the VIGS-generated information and design a plant “where that particular gene was over-expressed or put under some new control so that the resistance mechanism could be fortified.”

So where, along the continuum of progress, are Scofield and his colleagues at in terms of developing and refining the VIGS system?

“We’re really in the early stages,” he reports. They have the assay; but their initial work was done with foliar plant tissue, e.g., a leaf rust mechanism. For *Fusarium* head blight, the process must work in the heads of wheat and barley plants. “It’s been a major effort getting the parameters worked out as to when to infect the plant to get the virus to move up into the head and silence the genes at the correct time,” Scofield relates.

Presently, “we’re just beginning to test the ‘short list’ of genes that other scientists have identified as possibly being involved in the resistance mechanism,” he continues.

That work — which is partially funded by the U.S. Wheat and Barley Scab Initiative — involves approximately 30 genes. Scofield expects the screening to take about one year, during which time “we should learn how to streamline the process, make it more ‘high-throughput,’ and certainly disseminate the information so other people can do this themselves.”

At the core of the VIGS system development, Scofield emphasizes, is this inescapable fact: “The breeding efforts and genetic engineering efforts to improve resistance [to FHB] all need to know the answer to the question of what genes are essential for the resistance we have.” Wheat and barley plants with FHB resistance do exist, he points out; “but the problem is we don’t understand how that resistance operates.” The VIGS system “will greatly streamline our efforts to find out what genes are essential in that existing mechanism.”

It’s akin to building a house, Scofield says. A solid foundation must be laid before the structure can be framed, finished and utilized. He expects the VIGS system to be an important part of that foundation when it comes to constructing improved FHB resistance in wheat and barley. “The breeders are doing what they need to be doing right now: crossing the resistant lines into ones the farmers want to grow,” the USDA-ARS scientist concludes. “But the information we generate will allow them to do that more expeditiously, more cleanly.”

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