Research in Plain English

Development of a Magnetic Capture Hybridization Real-Time PCR Assay for Detection of Tumorigenic *Agrobacterium vitis* in Grapevines

Research in Plain English provides brief, non-technical summaries of journal articles by Cornell faculty, students, and staff.

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Crown gall formation. Photo by Tom Burr.

Background:
Agrobacterium vitis (*A. vitis*) is the bacterial agent that causes grape crown gall. An infected vineyard can have severe economic consequences for grape production. Current management strategies for preventing crown gall include planting resistant rootstock, planting in field sites without a history of grown gall, and using planting material free of pathogens. Yet, *A. vitis* persists systemically in grape vines. This presents grape growers an additional challenge: Although nursery stock may appear non-infected, cuttings may still carry the pathogen. Once *A. vitis* is present in a grape field, it can persist for years in both living and dead grape tissue. Thus, the best solution for preventing crown gall is to use clean plant material.

Experimental Design:
Current methods for testing *A. vitis* lack sensitivity and are time consuming. For example, callusing or flushing cuttings with buffer may take up to six weeks to confirm *A. vitis* absence or presence. The goal of this study was to develop a

diagnostic test that improves accuracy for detecting A. *vitis* that would yield results in a time-efficient manner.

Polymerase chain reaction (PCR) assays are becoming increasingly popular for detecting A. *vitis* with improved sensitivity and specificity. However, primers with PCR must distinguish between tumorigenic and nontumorigenic strains, which inhibit the detection of a wide range of A. *vitis* strains. Consequently, researchers in this study used real-time PCR to increase detection sensitivity.

Real-time PCR is more sensitive and less time consuming than traditional PCR because it does not require further analysis such as gel electrophoresis. One limitation, though, is that tests require a threshold level of target nucleic acid. Researchers first employed the Powerfood kit (Mo Bio Laboratories Inc., CA) to extract all bacteria DNA. Target DNA can then be diluted from non-target bacteria; however, this can result in false negatives. Considering these issues, researchers tested target nucleic acid enrichment via magnetic capture hybridization (MCH) and immunomagnetic separation (IMS) followed by real-time PCR. MCH uses tiny magnetic beads to which a "biotinylated oligonucleotide probe" (i.e. the capture probe) is attached. This capture probe-bead complex binds with the targeted nucleic acids from A. *vitis*. A magnetic force is then used to separate these target nucleic acids, bound together in beads, from non-target DNA, which can be washed away. IMS uses specific antibodies targeted for bacteria cells to concentrate target A. *vitis* bacteria. Like IMS, a magnetic force is subsequently used to separate target cells from non-target cells and PCR inhibitors. Finally, template DNA, released from bacterial cells, is now ready for real-time PCR. View the Burr Lab's visual depiction of the MCH process.

Both MCH and IMS have the potential to significantly improve efficiency and accuracy of grapevine indexing for A. *vitis*, and as such, the objective of this study was to compare these technologies.

Results:

- MCH and IMS real-time PCR were 1,000-fold more sensitive than Powerfood DNA extraction and 10,000-fold more sensitive than direct real-time PCR.
- MCH real-time PCR assays detected only tumorigenic strains, whereas non-tumorigenic strains remained negative after real-time PCR.
- IMS was effective for detecting A. *vitis* on grapevines but also detects non-tumorigenic strains that are often present.
- MCH, IMS, and Powerfood DNA extraction allowed detection of A. *vitis* in naturally infected grapevines, though MCH was by far the most accurate.

Conclusions:
Because of the adverse economic affects of crown gall on grape production,

efficient and accurate indexing methods must be available to test for its casual bacteria A. *vitis*. Researchers in this study found real-time PCR tests to be time efficient, reliable, and accurate detection methods for A. *vitis*. Researchers demonstrated that MCH (a nucleic acid probe binds target sequences) and IMS (antibodies target the pathogen) are simple and rapid methods. Both MCH and IMS were 10,000-fold and 1,000-fold more sensitive than direct real-time PCR and Powerfood DNA extraction followed by real-time PCR. Researchers found MCH to be the most sensitive method for A. *vitis* detection. An established protocol using methods such as MCH real-time PCR has the potential to better understand the distribution of bacteria in grapevines and promote healthy vineyard management.

The Bottom Line:
Real-time PCR is an effective diagnostic test for detecting A. *vitis*, the causal bacterial agent for crown gall. Tests developed by researchers at Cornell improved the sensitivity and accuracy by "orders of magnitude" for being able to detect A. *vitis* in grape vines.

For more information about crown gall in vineyards please see these related Appellation Cornell articles: Grapes 101 (May 2015) and Research Focus (March 2012).

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