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Globodera Alliance Newsletter

Using Molecular Markers to Identify Potato Varieties Resistant to Potato Cyst Nematodes

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Testing for Resistance

Testing whether a potato variety is resistant to potato cyst nematodes (PCN) isn't easy. To begin with, PCN are quarantine pests. There are only two facilities in the USA – one in Idaho, one in New York – where conducting experiments with PCN is even allowed. These facilities have to meet strict phytosanitary regulations to ensure that PCN never escapes.

Figure 1: Examining cysts found on the root of a potato greenhouse pot assay. (Photo: X. Wang)

Next, testing for resistance takes a lot of time. In a typical greenhouse pot assay, a tuber or tissue culture plantlet is placed in soil infested with PCN eggs. The plants have to be grown for 12—16 weeks or longer, until cysts have had time to develop on the roots. The next stage can be as simple as uprooting the plant, and looking to see if any cysts are present on the roots, or as difficult as meticulously sifting through the soil to find cysts – each about 1/2 mm in diameter – and then counting them. Looking directly at the roots works reasonably well with race Ro1 of the golden cyst nematode, as the cysts do not dislodge easily, but does not work well for the pale cyst nematode or race Ro2 of the golden nematode, as the cysts detach readily and remain in the soil.

Figure 2: Greenhouse bioassay for *G. rostochiensis* using potato varieties and breeding lines. (Photo: X. Wang)

The difficulty of testing is especially acute for potato breeding programs that seek to develop PCN-resistant varieties, because most quarantine facilities don't have the space to grow hundreds or thousands of candidate varieties each year, let alone the staff and resources necessary to process that many samples.

Molecular Markers

In principle, it should be possible to test for resistance by looking at a potato plant's DNA. Nematode resistance genes, like all genes, are encoded by short stretches of DNA. In an ideal world, where we knew what all nematode resistance genes looked like (i.e., their DNA sequence), we could predict whether a potato is resistant or susceptible just by sequencing its DNA. Unfortunately, we don't yet live in that ideal world – in part because it still costs too much to completely sequence the DNA of a potato – and in part because we don't yet know the sequence of the most commonly used nematode resistance genes.

It is nevertheless currently possible to test for the presence of molecular markers that are in close proximity to known resistance genes. Current technology allows molecular geneticists to determine the approximate location of a resistance gene, narrowing its location down to a region about one million base pairs long. [By way of context, a typical resistance gene is about 10,000 base pairs long, and the entire potato genome has about one billion base pairs of DNA]. Because of the way that DNA is passed down from generation to generation, regions of DNA that are physically close to each other tend to be inherited together.

Knowing that a resistance gene is somewhere within a million base pair region makes it possible to track it indirectly – by tracking any other segment of DNA in that region – as the segment and resistance gene will tend to both be present, or both be absent, in any given potato. For a tracked segment to be useful it should be unique, i.e., not found in susceptible potatoes. Since most nematode resistance genes originated in wild species, and were crossed into potato relatively recently, the DNA surrounding most resistance genes is also from wild species. Thus, in practice, many segments near resistance gene are unique, and easily differentiated from cultivated potato DNA.

The most widely deployed nematode resistance gene is the *H1* gene, which originated in *Solanum tuberosum* subspecies *andigena*, a close relative of modern potato (*S. tuberosum* subspecies *tuberosum*). *H1* confers high levels of resistance against race Ro1 of the golden nematode, but is ineffective against the pale cyst nematode or race Ro2. Two markers that track segments of DNA in close proximity to *H1* are widely used. One of these markers, TG689, was developed in the USA, while 57R was developed in Europe. Recent testing by the NY potato breeding program showed that 57R was, on average, slightly more accurate than TG689, as the presence of 57R correctly predicted resistance in 99.7% of individuals, while the presence of TG689 predicted resistance in only 98.3% of individuals tested. Neither marker proved good at predicting susceptibility (47% for 57R versus 41% for TG689), presumably because other resistance genes, not linked to 57R or TG689, were also present in some of the potatoes tested. Even so, because resistance is the trait that breeders are most interested in, either of these markers is easily "good enough" for routine resistance breeding. Either marker costs only a few dollars per sample, and only a few hours are needed to get an answer. A huge reduction in cost compared to inoculating with live nematodes, and far quicker, too.

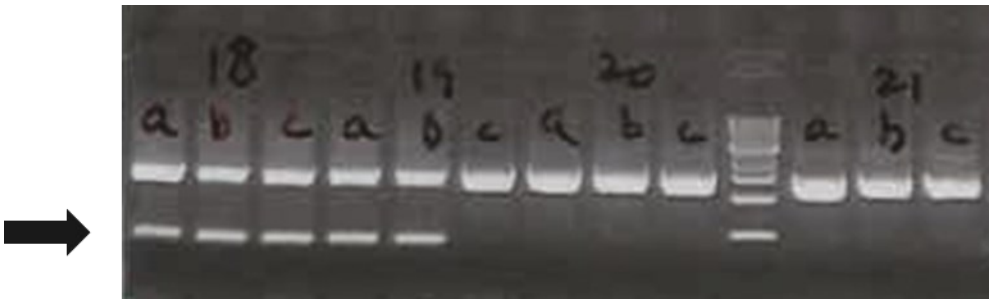

Figure 3: The 57R marker in action. The arrow on the left shows a segment of DNA present in most potatoes with the *H1* resistance gene. This image shows five DNA samples from varieties that have *H1*, and seven DNA samples from varieties that lack *H1*. A DNA size ladder, fourth lane from the right, is also shown. (Photo: W. DeJong)

Going from the Greenhouse to the Field

Current practice in the NY breeding program is to evaluate all clones that have survived two years of evaluation for agronomic performance in the field with 57R, to identify those most likely

to be resistant. The marker-based resistance status is taken into account when making decisions about which clones to save after the third field season. Several years later, for any clone nearing release as a cultivar, resistance/susceptibility is confirmed by conducting inoculations with live nematodes. In NY, where resistant varieties serve a crucial role in controlling the golden nematode, it is important to be 100% (and not 98.3% or 99.7%) sure that when a new variety is said to be resistant, it truly is. For this level of certainty, only a potato bioassay will do.

There is, unfortunately, no single resistance gene comparable to *H1* that confers complete resistance against the strains of pale cyst nematode present in Idaho. The locations of several genes of smaller effect are known, however, and a marker (HC) against one of them has been developed in Europe. Markers are especially useful in situations where resistance requires many genes, in part because inoculations can't always detect the presence of small effect genes, and in part because when a low level of resistance is detected, it doesn't identify which small effect gene(s) are present. Breeders need tools to track each gene independently, so they can work to assemble them all in a single potato. The HC marker is a good start, but additional markers are needed before breeders can effectively breed for high levels of resistance. Along this vein, researchers in NY have recently developed a marker linked to a gene from *S. vernei* that gives moderate levels of resistance to both the golden and pale cyst nematodes. Researchers in Idaho are pursuing similar efforts to develop markers for other PCN resistance genes.

About the GLOBAL Project

GLOBAL stands for "Globodera Alliance", an international group of research, extension, and education professionals working to eradicate *Globodera spp.* in U.S. potato production.

GLOBAL Project members include scientists from the University of Idaho, Oregon State University, Cornell University, U.S. Department of Agriculture (USDA), Agriculture and Agri-Food Canada, The James Hutton Institute, and the French National Institute of Agricultural Research.

For periodic updates on this ongoing work visit:

www.globodera.org
twitter.com/globodera.org
facebook.com/GloboderaAlliance

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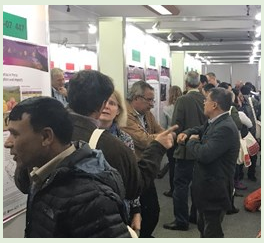

GLOBAL Scientists Present at World Potato Congress

The 10th World Potato Congress was held in Cusco, Peru, the center of diversity for potato. Approximately 900 participants from around the world gathered to share research on all aspects of potato. The congress opened with addresses from the President of Peru, Martín Vizcarra; Vice-President, Mercedes Aráoz, the Governor of the Cusco region, Mayor of Cusco, and the Minister of Agriculture and Irrigation. All joined in singing the national anthem. This level of dignitaries reflected the profound influence that potato has had on Peru for centuries. Romain Cools, President of the World Potato Congress and Barbara Wells, Director General of the International Potato Center then welcomed participants. In addition to scientific talks and exchanges, a field day was held at the INIA (National Institute for Agrarian Innovation) Experiment Station and Potato Park. The experiment station specializes in native varieties, showcasing the wide genetic diversity of thousands of potato varieties. The Potato Park is an Indigenous Biocultural Heritage Area that protects and improves the crop and biodiversity of the Andean region.

GLOBAL project scientists Drs. Jonathan Whitworth and Rich Novy from USDA -ARS and Dr. Joe Kuhl from the University of Idaho presented at the conference. Dr. Kuhl gave an overview of GLOBAL project research, focused on "Risk assessment and eradication of *Globodera* spp. in the U.S. Production of Potato." Dr. Whitworth presented, "Seeking host resistance in potato to three *Globodera* species. Dr. Novy presented, "Breeding and development of *Globodera*-resistant potato varieties with long tuber shape and russet skin for production in the western United States".

Posters presented at the conference by GLOBAL Project Investigators can be found at **https://www.globodera.org/science-posters**

Upcoming Events:

Potato Association of America Conference

July 22-26, 2018 — Boise, Idaho

At this year's PAA conference, GLOBAL Project will present a symposium on the **Impact of Quarantined Pests on the Potato Industry**. For more information go to: **www.uidaho.edu/paa2018**

Organization of Nematologists of Tropical American Annual Meeting

August 19-23, 2018 — Arequipa, Peru

At ONTA, GLOBAL Project will present a symposium on the **Potato Cyst Nematode** featuring a global perspective on the spread of PCN. For more information go to: **www.ontaweb.org/**

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GLOBAL Project scientists, advisory board, and support staff tour the Plant Breeding Facility at Cornell University, a GLOBAL partner agency

GLOBAL Advisory Committee consists of potato industry, state and federal regulatory and academic individuals who have volunteered their time and efforts. We thank them!

GLOBAL Advisory Committee

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