Seedling Quality Testing at the Gene Level

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Nursery managers are all too familiar with the critical importance of determining the proper lifting window for nursery stock. Plants that are harvested too early are damaged during the lift-and-pack process and also store poorly. Currently, the best physiological test for determining the lifting window is to measure cold hardiness by the whole plant freezing or electrolyte leakage tests. For example, conifer nurseries in British Columbia use 0 ^{o}F (-18 ^{o}C) as the hardiness level when it is safe to begin harvesting. While these cold hardiness tests are useful, they typically take one to several weeks to produce results and a series of tests must be done during the fall to track cold hardiness development. Wouldn't it be great if there were a quick and accurate test to determine exactly when the cold hardiness process started?

Genomic Testing— Genomics, or gene-expression analysis, is a relatively new discipline that allows us to look inside plant tissues at the chemical signals that trigger specific physiological events such as the development of cold hardiness (Figure 1). In living organisms, each developmental step and every interaction with the environment is orchestrated by DNA encoded genes. Therefore, the physiological condition of a plant can be determined by analyzing the activity profile of its genes.

Sounds great, but the trick is to identify which gene or genes are involved in the cold hardiness process. Gene expression analysis uses microarrays or biochips to simultaneously examine thousands of genes from a sample of plant tissue and determine their level of activity. In this way, plant response to environmental cues can be closely examined and this information used to identify the genes that are involved in hardening. Once these indicator genes have been identified, then a chemical assay can be developed to measure their activity. Changes in the expression of specific genes are thus an accurate and early indicator for the development of cold tolerance. And, because it can identify the start of the hardening process, genomic testing is much more useful that traditional cold hardiness tests that only provide information several weeks after hardiness has already developed (Figure 1).

The Research— I know that this sounds like Star Wars technology but researchers in Europe have already identified the genes involved in the cold hardiness of Scots pine (*Pinus sylvestris*) and European beech (*Fagus sylvatica*) seedlings. The study was performed in 4 countries (Denmark, the Netherlands, Scotland, and Sweden) and involved both research institutes and

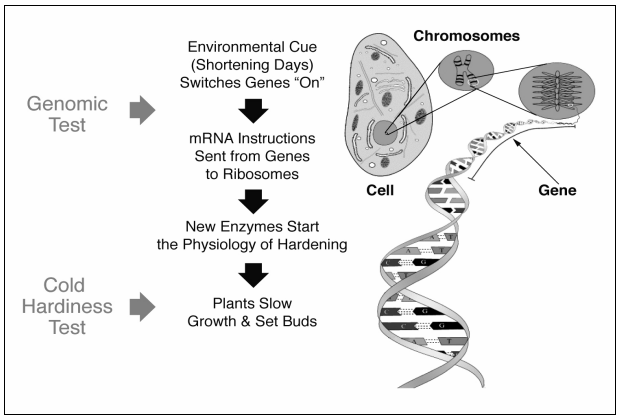

Figure 1 – Genomics tests of physiological and morphological processes such as cold hardening will give nursery managers an early warning, compared to traditional seedling quality testing.

operational forest nurseries. The main objective was to monitor shoot cold tolerance and bud dormancy of pine and beech seedlings before, during, and after refrigerated storage with the shoot electrolyte leakage (SEL) test. Because pine and beech represent broadleaved and gymnosperm trees, they differ in the morphological and perhaps physiological development of cold hardiness. These cold hardiness test results were correlated with gene expression using genomics technology, which led to the development of a rapid, predictive molecular diagnostic test.

Seedlings were grown in climate-controlled environments for the initial identification of the relevant hardiness genes, followed by outdoor nursery trials to monitor the actual development of cold hardiness. A standard provenance of each species was tested at each research location along with seedlings from a local seed source. This testing procedure allowed comparisons of most parameters that are known to influence dormancy and cold hardiness such as geographic origin, genetic background, and nursery cultural history

Dehydrins are one of several proteins that were already known to be specifically associated with the onset of cold hardiness in red-osier dogwood, rhododendron, and blueberry. The European research trials identified the specific dehydrins and other proteins that are linked to cold hardiness in Scots pine and European beech seedlings. Once the specific genes were identified, the researchers used genomics technology to identify when they were activated. These genetic response data were analyzed with sophisticated statistical techniques, which revealed 3 different gene groups that were correlated to the cold hardening process. In samples from different provenances, genes from each group displayed a characteristic gene expression profile during the acquisition of frost hardiness.

A Simple Explanation of How the Test Works—Enzymes are proteins that trigger all of the many physiological processes in organisms, and they are created out of amino acids in the cell nucleus. If you ever wondered why your nursery crops require so much nitrogen, each amino acid contains nitrogen and the proteins they constitute make up about half of the dry weight of a cell. Each type of protein has its own unique structure and function. An *E. coli* bacterium, one of the simplest organisms, contains over a 1000 different proteins that switch on and off at genetically-controlled times to perform the chemical reactions that sustain life.

To create an enzyme, the cell must first transcribe the genetic information stored in the DNA into messenger RNA (mRNA). The strand of mRNA then moves over to a ribosome which is an enzyme that can stitch the proper sequence of amino acids together using the mRNA blueprint. The long chain of amino acids is an enzyme that folds into its characteristic shape, floats free, and begins performing a specific reaction (Figure 1).

The N-Sure Cold Hardiness Test – The European research identified three indicator genes that together provide enough information to give an accurate estimate of the cold hardiness stage of the seedling. The corresponding genes dominate the hardening process in all of the Scots pine provenances that were studied and strongly correlated with SEL values. Activity of indicator genes, two differentially-regulated dehydrins and one control gene, is measured in a cold hardiness test developed by the company N-Sure. The dehydrin genes have different biological roles – one is involved in general drought and cold resistance and is active during growth and initial stages of hardening. The other one is highly specific for development of fully hardened buds, and the activity of the corresponding gene peaks when maximum cold hardiness has been attained. The assay is based on the relative activity of these 3 genes. The N-Sure test has been validated with many seed sources of Scots pine grown at different geographical locations in Europe with different nursery regimes and has proven to be highly consistent. The reason for this is that a biological process of crucial importance for hardiness is monitored. Recently, the test has been adapted for use with Norway spruce (*Picea abies)* as well.

The assay will be sold as a sampling kit that contains all necessities for taking and stabilizing a representative sample from a batch of seedlings. For example, a composite sample of bud tissue could be collected from seedlings receiving cultural treatments to stimulate dormancy. The stabilized sample can be sent by regular mail, and upon arrival in the test lab the result will be available within 24 to 48 hours (Figure 2). The company that will commercialize the tests is a spin-off from Wageningen University and Research Centre, the Netherlands, and is called N-Sure. The seedling assay will be part of the first market introduction series of N-Sure, planned for 2007. If there is interest from the US and Canadian nurseries, N-Sure will be looking for a business partner in Northern America for reselling the tests.
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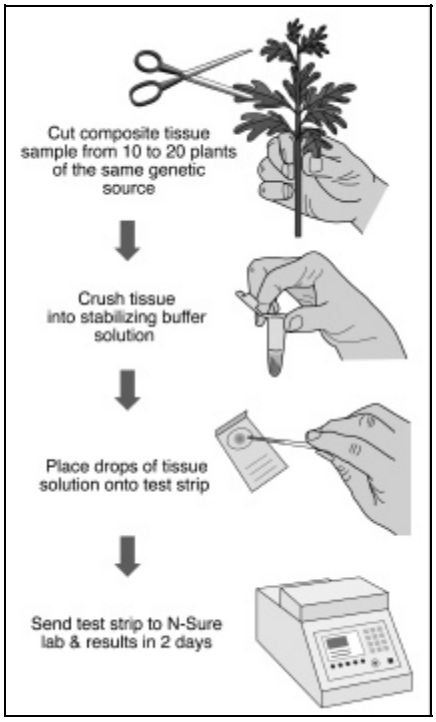

Figure 2 – The N-Sure test provides a quick and accurate way to monitor cold hardiness and dormancy of nursery stock.

Summary - Gene expression analysis is a promising new way to determine when the cold hardiness and dormancy process starts in plants. One of the most attractive features of genomic testing is that it provides a much earlier indication than traditional cold hardiness testing. While it has proven its usefulness in European Forest Nurseries, with pine, beech, and spruce, further operational testing needs to be done with North American species and nursery cultural practices.