Technology To Improve the Quality of Sweet Potato 'Seed'

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Sweet potatoes are grown commercially by bedding whole potatoes in the field and transplanting cuttings from the sprouts produced in the beds to the production field. Thus, it is one of many crops, including potatoes, sugarcane and strawberries, grown by vegetative propagation.

Commonly, varieties of these vegetatively propagated crops decline because disease-causing pathogens, including viruses and bacteria, gradually accumulate in the planting stock over years of production. An added problem in sweet potato production is the high frequency of mutations, which can cause defects such as changing the flesh color from the normal orange to yellow or white (Figure 1). The frequency of mutation in sweet potatoes is thought to be greater than in other crops because the part of the plant used for propagation, the storage root, does not have "eyes" as does a potato tuber, but must instead produce buds from vegetative cells. This process is thought to favor mutation.

In modern production, the rate of decline of varieties is slowed by programs that carefully produce foundation seed from stock free of viruses and mutations. In Louisiana, foundation seed is produced at the Sweet Potato Research Station at Chase and sold to growers to use in crop production.

Virus-tested Plants

Virus-tested sweet potato plants can be produced by a plant tissue culture process known as meristem-tip culture, in which the smallest possible piece (less than 0.5 mm) is removed from the growing tip of a selected plant. This meristem tip is regenerated in tissue culture into a new plant (Figure 2), which then must be thoroughly tested to be certain that all viruses have been eliminated.

This involves a time-consuming, laborious method of grafting the tissue-culture derived plant at least three times to a seedling of an indicator plant, the Brazilian morning glory. This plant develops obvious symptoms when infected with known sweet potato viruses. In addition, because mutations can occur during meristem-tip culture, each virus-tested plant must be grown in the field to be certain that it remains true to the original characteristics of the particular variety.

Once the mericlone (the plant derived from a single meristem) has passed each of these tests, it is maintained in tissue culture by cutting the stem into segments containing at least one node on which is a preformed bud. A new plant can grow from this bud with minimal risk of mutation. Since this only minimizes without entirely eliminating the risk of mutation, this tissue culture stock must be periodically retested to be certain it is true to type.

The tools of molecular biology offer promise for improving the process of producing sweet potato foundation seed in several ways. The most important is in improving the efficiency, accuracy and reliability of testing for the presence of viruses, other pathogens and mutations, not only during the initial process of generating the stock tissue cultures, but also during the subsequent process of producing foundation seed and even farmers' seed in the field.

Photo by Don R. LaBonte

Figure 1. A sweet potato root with a section that has mutated from the normal orange flesh to light yellow flesh.

Virus Detection

Two groups of viruses found in Louisiana sweet potatoes are a current focus of research: the aphid-transmitted potyviruses and the whitefly-transmitted geminiviruses. It is possible to isolate each of these viruses in other host plants more suitable for virus purification and characterization.

Each of the most important potyviruses that infect Louisiana sweet potatoes has been purified and used to

Photo by Christopher A. Clark

Figure 2. Tissue culture plantlets of Beauregard sweet potato.

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Photo by John Chaney

These are newly harvested sweet potatoes from the farm of Carl Ducote of Bunkie, La. They are the Beauregard variety, which was developed at the LSU AgCenter and is the country's most popular variety.

prepare antisera in cooperation with colleagues at the International Potato Center in Lima, Peru. These antisera have proved useful in serological assays to identify the viruses transmitted into the Brazilian morningglory indicator plants.

Unfortunately, sweet potato plants contain unusually large concentrations of a number of substances that interfere with serological assays, including latex, polyphenols and polysaccharides. Thus, this type of test has not proved reliable in detecting these viruses directly from sweet potato. In the case of geminiviruses, detection using serological approaches has not been successful in most crops.

An alternative to using serology, which detects primarily the protein coat associated with viruses, is to detect the nucleic acid component of the viruses. A variety of methods can be used for this purpose.

PCR-based Assay

One method developed at the LSU AgCenter to detect the sweet potato geminiviruses is a polymerase chain reaction (PCR)-based assay. First, the sequence of nucleotide bases that make up a portion of the DNA of this virus was determined. This sequence was then used to design short segments of DNA that could be used as primers in the synthesis of a DNA product using the virus DNA as a template. Thus, if virus DNA is present in an extract from a plant, when the PCR test is run using these primers, a DNA product is produced that can be identified using gel electrophoresis.

This method has the advantage of both speed and sensitivity. But, although it is useful for confirming the presence of geminiviruses in a few samples, it is too expensive for use in cases requiring large numbers of samples be tested.

Hybridization Assay

Research is underway to determine if molecular hybridization assays can be developed that could screen large numbers of samples less expensively for sweet potato geminiviruses. Hybridization assays involve extracting DNA from plant samples, placing the extracts on spots on sheets of nitrocellulose membrane and then adding a specific probe consisting of virus-specific DNA that is labeled with chemiluminescent marker. If virus DNA is present in the spot on the membrane, the probe will bind to it, and then the marker will produce a chemical reaction that can be observed (Figure 3).

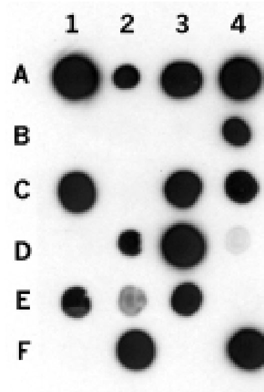

Figure 3. A hybridization assay involves extracting DNA from plant samples, placing the extracts on spots on sheets of nitrocellulose membrane and then adding a specific probe consisting of virus-specific DNA labeled with chemiluminescent marker. If virus DNA is present, the probe will bind to it, and the marker will produce a spot.

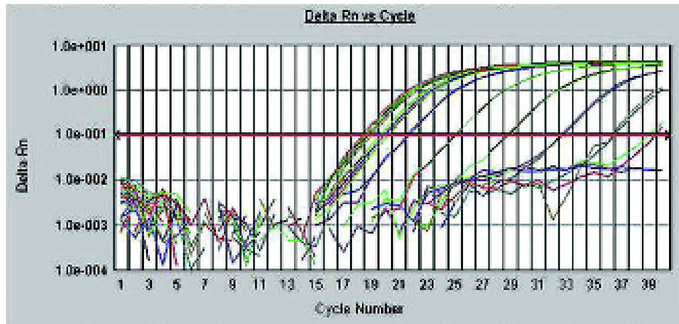

Figure 4. Each curve in this Real-time PCR represents a different unknown sample. From the geometric phase of each curve, a scientist is not only able to detect the presence of a virus, but also calculate the absolute or relative amount of virus present.

Real-time PCR

A different approach is being tested for the potyviruses. Real-time PCR is an adaptation of the basic PCR technique that increases the specificity and allows relative quantification of the target nucleic acid. In addition to using specific primers as in simple PCR, a fluorogenic probe complementary to the DNA between the primers is used. As the PCR reaction amplifies the target DNA, the fluorescent signal is released from the probe and the fluorescence generated can be measured as the reaction proceeds (Figure 4).

In preliminary experiments, promising results have been obtained for detection as well as quantification of one each of the potyviruses and geminiviruses directly from infected leaves. Future investigations will explore whether real-time PCR can be used to detect viruses in the tissue culture plants and obviate the need for graft indexing and also to quantify viruses in different breeding lines of sweet potatoes to determine if they have virus resistance.

Mutation Detection

Dramatic color changes in root “seed” stock are easy to spot and eliminate; however, during meristem-tip culture and routine tissue culture multiplication, it can be difficult to detect more subtle changes that can affect root shape and yield. This is of particular concern because of the small number of tissue culture plants used to generate the tens of thousands of plants required for planting acres of land for grower foundation seed. A mutation in one of these initial increase plants would be multiplied greatly and go virtually undetected until the crop is harvested.

Using DNA fingerprinting technology, we have been able to detect variation in some mutant versus true-to-type plant fingerprint profiles. Unfortunately, some plants with varying degrees of mutations differ little in fingerprint profiles. The problem is that just one probe, akin to one used to detect a specific virus, is not available. Mutations likely occur in many different genes, hence our interest in using a new technique called DNA microarrays to examine the entire plant genome.

Essentially, DNA microarrays contain tens of thousands of probes for detecting thousands of active plant genes simultaneously. The detection process is similar to the one described above for viruses. In this case, however, we extract plant RNA, which contains a class of RNA called messenger RNA or RNA transcripts. These RNA transcripts are used in the plant cell as a template to generate the actual protein for a given gene. Comparing DNA microarrays for true-to-type plants and mutant plants should show us genes inactivated (no messenger RNA) because of a mutation. This is an expensive and laborious technique but may ensure our ability to maintain and propagate true-to-type plants of a variety.

Resistance to Viruses and Other Pathogens

Disease resistance has been used to control many important sweet potato diseases caused by bacteria, fungi and nematodes. It is the most economical means of disease control from a farmer’s perspective because it can reduce reliance on chemicals for disease control. However, it can take many years to identify a suitable source of resistance for any particular disease. Using time-consuming screening methods, it can require many more years to incorporate resistance into a desirable variety.

Breeding for resistance appears even more daunting for sweet potato viruses. Symptoms often do not correlate with effects viruses have on their host plants. There is a complex of viruses potentially involved. And efforts to date have identified few sources of virus resistance in sweetpotato germplasm.

Biotechnology offers several tools that might be applied to these problems. As indicated, real-time PCR is being investigated as a means of quantifying viruses in plants that could be used to identify whether breeding lines are able to resist viruses by suppressing replication of the virus. Future research will be directed at determining if molecular markers can be used to identify resistant lines without the necessity of labor-intensive screening methods. ■

Photo by John Wozniak

Mary Hoy, research associate, is one of the scientists working to improve the quality of sweet potato seed.