EVALUATING PVY RESISTANT TRANSGENIC POTATOES *

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For the past two years we have been evaluating transgenic potatoes for resistance to potato virus Y (PVY). By using a procedure called transformation, a single gene from the virus was inserted into plant cells that were then regenerated into whole plants. The inserted viral gene is then passed from generation to generation during propogation of the plants. Although no one is really sure why, many researchers have observed that plants with viral gene insertions are in some cases resistant or even immune to infection by the virus from which the gene was taken. While this may seem like the ultimate solution to virus diseases, there are a number problems that have to be considered before these materials can be used.

Many variations occur during insertion of the gene and regeneration of plants to test for resistance. For example, the number of copies of the viral gene may be different in individual plants, the viral genes can be inserted into the plant genome in different places and the amount of product made from the individual inserted viral genes may not the same in all of the plants. So far it has not been possible to control these factors. Since any of them could effect the range of resistance observed, tuber quality, yield plant type or many other characteristics, it is essential to evaluate the plants as if they were advanced breeding lines.

In 1992 we obtained a single line of Russet Burbank from Hybritech Seed International, Inc. that had been transformed with the coat protein gene of PVY. These plants were grown, along with non-transgenic controls, in the greenhouse at the University of Wisconsin's Hancock Research Station. Some of both types of plants were inoculated with PVY by rubbing them with a high dose of virus. The control plants all developed severe symptoms of PVY whereas the transgenic plants remained free of symptoms. ELISA analysis confirmed the observation that the control plants were severely infected whereas the transgenic plants were free of virus. When the tubers were harvested from each type of plant the data showed about a 90% reduction in total tuber weight in the inoculated control plants compared to their uninfected replicate but there was no significant difference in total tuber weight between the inoculated and uninoculated transgenic plants.

In 1993, tubers from the lines tested in the greenhouse were used to establish plots to determine whether or not the progeny of the transgenic plants would express the observed resistance and be free of virus. We planted four replicates of four treatments. The replicates were tubers from either non-transgenic controls or from transgenic plants which had come from plants that had and had not been inoculated with virus in 1992. As expected, the plants that grew from tubers taken from plants not previously exposed to virus established a normal looking virus free-stand and the plots established with tubers from inoculated non-transgenic plants were devastated due to the severe dose of virus that had been given to the plants used to generate them in 1992. However, the plots arising from the transgenic tubers taken from plants inoculated in 1992 were indistinguishable from either uninoculated controls or the uninoculated non-transgenic plants. These observations were confirmed by ELISA readings and by the inspectors from the Seed Potato Certification Program. There were no differences in yield of plants grown from tubers of inoculated transgenic plants, uninoculated transgenic plants or uninoculated control plants. By comparision, there was a very significant reduction in yield from the plots established with tubers from inoculated

non-transgenic plants. Since the dose of virus used in these experiments was significantly higher than would likely occur in a real agricultural situation, we are very impressed by the degree of resistance the transgenic plants have shown so far. We will continue to monitor the tubers produced by these plants for several more years to be certain that they do not harbor a low level of virus that would require several generations to be detectable.

Since the above data present convincing evidence that high levels of resistance to PVY can be achieved by transforming potatoes with viral genes, we have undertaken a second experiment to address the issue of selecting exactly the right material for release. This year we are evaluating 57 lines of transgenic plants for resistance and horticultural characteristics. The varieties being screened are Russet Burbank, Superior, Shepody, and Snowden. To increase the "reality" factor with respect to virus resistance we are testing the plants for resistance to infection by aphid transmission. Each of the test lines were interplanted with plants infected with PVY to ensure a source of PVY inoculum for the aphids to spread. Aphid pressure in the plots was artificially elevated by introducing laboratory-reared green peach aphids onto the PVY source plants. The area surrounding the field was planted in oats to encourage the establishment of other vector aphid species that do not normally colonize potatoes. Field observations indicated that high populations of aphids were present on all plants in the plots and would provide good pressure for measuring resistance to virus spread by vectors.

The plants were evaluated for agronomic type, disease and insect incidence by Kevin Bula and Rick Hafner of the Seed Potato Certification Program and foliar samples were collected to measure virus infection by ELISA. Preliminary data indicate that several of the lines from each variety were off type or had other undesirable growth characteristics such as poor vigor and leaf shape. All of the Shepody and some of the other lines were susceptible to infection by aphid transmission of virus. On the other hand, 19 out of 31 lines of the Russet Burbanks, 1 of the 10 Superior and 6 of the 13 Snowden lines were free of virus as judged by ELISA testing. Many of these were true to type and had the appropriate growth characteristics.

This winter we have regenerated plants of Russet Norkotah that express the coat protein of PVY. These plants will be added to the testing protocol for the plants described above and evaluations of agronomic traits and virus resistance will continue. Next year another round of testing and evaluation will be done with those materials that make it through the initial screening. This is a complicated and tedious processes requiring the collaboration of entomologists, virologists, molecular biologists, certification personnel and support from industry. Eventually, we hope to combine our skills to select varieties that have retained their familiar traits but have the added value of virus resistance.

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