ELIMINATION OF POTATO LEAFROLL DISEASE AND TUBER NET NECROSIS IN TRANSGENIC RUSSET BURBANK POTATOES

by
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ABSTRACT

Genes from the potato leafroll virus were incorporated into the genome of Russet Burbank potato using a transformation process. Among many transgenic lines created, we have identified a few that have completely effective resistance against the potato leafroll disease and leafroll-induced tuber net necrosis. The resistance is effective in the field against the strains of the virus that occur in the Columbia Basin. Both foliage and tubers appear identical on the resistant transgenic and nontransgenic Russet Burbank.

INTRODUCTION

The Problem: Potato virus disease pressure is intense in the Columbia Basin of the Northwest. A thriving potato industry has evolved here because potent pesticides were available to control the nematode and insect vectors that spread virus diseases. At this point in time, control of potato leafroll virus is still dependent entirely on pesticides. Without pesticides, we routinely have 100% leafroll disease in the fall even when we start with the best of certified seed. The leafroll problem in the Columbia Basin is compounded by the fact that the major variety, Russet Burbank, and other cultivars often develop net necrosis when infected by leafroll. Net necrosis is a dark discoloration of vascular tissues in the tuber. It renders tubers unfit for processing into french fries and other products and reduces their fresh market value.

Despite their central importance in potato culture in the Columbia Basin, key pesticides used to control potato virus diseases are being driven off the market by public concern about their human health and environmental impacts. Concurrently the incidence of leafroll virus disease is increasing, and tuber quality, affected by leafroll-induced net necrosis, is decreasing rapidly.

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This Presentation is part of the Proceedings of the 1994 Washington State Potato Conference & Trade Fair.

The Columbia Basin is certainly losing competitive advantage to other areas of the nation and world where leafroll disease pressure is less intense and potatoes can be grown with less disease loss. If alternative disease control measures are not developed, the industry may move to other areas of the nation and the world.

The goal of the research reported here is to develop the alternative, pesticide-free methods needed to control potato leafroll disease in the Columbia Basin. We are pursuing several approaches to this goal, and we will report here that we have achieved complete control of leafroll disease by development of transgenic resistance. We believe we can now grow transgenic Russet Burbank free of the pesticides previously needed to control the disease.

Virus Disease Resistance: Development of disease resistance is one of several classical approaches to virus disease control. The others include 1. cure of disease (chemotherapy coupled with meristem culture, for example, is routinely used to eliminate virus from infected clones), 2. maintenance and use of pathogen-free seed or propagules (potato seed certification programs do this), 3. restriction of long distance movement of pathogens into new areas (quarantines may do this), 4. restriction of local dissemination into and among crop plants (use of pesticides to prevent spread of viruses by insects and nematodes does this), and 5. restriction of the deleterious effects of disease (processing leafroll-infected potatoes before net necrosis develops in storage does this). The use of a combination of these approaches is usually most effective in controlling disease with minimum use of pesticides, and this general approach has been termed integrated pest management.

There are two types of resistance, genetic and induced. Genetic resistance is controlled by genes and is passed from one generation to the next by inheritance of the genes. Among the approaches to disease control, genetic resistance has long been considered the ideal means of controlling disease. It is often difficult to achieve, but once achieved, disease resistance has the potential of replacing all other approaches, is easy and inexpensive to apply, and has no undesirable environmental or health impacts.

In the past, genetic resistance genes were always derived from other plants by breeding. A number of distinct disadvantages are often associated with the use of genetic resistance derived from other plants. Resistance genes often are not available in plants that will intercross with the susceptible domestic cultivar. Even when resistance genes are available in genetically compatible plants, many years are usually required for their incorporation into cultivars that contain all of the other characteristics needed for a commercially successful cultivar. Transfer of gene from different but related species is sometimes possible using new technologies including ploidy manipulation, embryo rescue, and protoplast fusion, but such genes are frequently inseparably linked with undesirable characteristics of the other species. Furthermore, many plant virus resistance genes are not fully effective or universally applicable against all isolates of the virus.

Induced resistance has the same effect as genetic resistance but is conferred on by a prior event. It is not heritable, but ultimately has a genetic basis. Several types of induced resistance are known. Immunization of people or animals against specific diseases by injections of attenuated pathogens is a common practice in medicine and is an example of a type of induced resistance with which most people are familiar. Plants infected with one strain of virus are often resistant to other strains of the same virus. This is the most effective type of induced resistance known in plants and is called cross protection. This type of resistance has been utilized effectively when mild strains of the virus (strains that cause only marginal losses) were available to protect against severe strains that occur in the environment.

As genetic engineering and plant transformation techniques advanced in recent years, scientist began to test whether a cross protection type of resistance could be developed by the transfer of virus genes to the plant. This genetically-engineered protection against virus infection was first demonstrated in plants developed to express the tobacco mosaic virus coat protein gene. The general applicability of this approach to achieve resistance against plant viruses has been demonstrated with several virus in several plant species. The expression of viral genes within the plant appears to interfere with expression of the same genes of the invading virus, and this prevents the invading virus from fulfilling functions that are essential for its establishment in the plant and its survival. In effect, the process of introducing the viral genes into a plant converts an induced resistance into a genetic resistance. The viral genes in the plant are passed from one generation to the next by breeding, and they confer resistance on the new generation.

Major advantages to this approach over traditional breeding for resistance are that it may be achieved more rapidly, and resistance can be added as a single characteristic to an established variety with little or no influence on other characteristics of the variety.

MATERIALS AND METHODS

This work is an example of cooperation between private industry (Monsanto Co.), U.S. Department of Agriculture, and state university scientists. The Russet Burbank cultivar was transformed with genes from potato leafroll virus, individual transformed plants were propagated as lines, and the transgenic lines were evaluated for resistance at Prosser, Washington, Parma, Idaho, and Hermiston, Oregon.

Initial Screening: In initial screening for resistance, transgenic potato lines were propagated in tissue culture, transplanted into small soil pots and then transplanted into replicated field plots 2-3 weeks later. The field plants were inoculated about 2 weeks after transplanting by placing aphids that had been reared on potato leafroll-infected plants on each plant in the field. The plants were evaluated for symptom development throughout the growing season, and they were tested for virus infection using the highly sensitive virus assay called ELISA.

Selected plants were also assayed for virus infection by aphid and by graft transmission to index hosts in the greenhouse. Tuber yields were determined at the end of the season, and tubers were evaluated for net necrosis at harvest and after a period of cold storage. Fifty tubers of each line were eye-indexed in the winter. This means that an eye from each tuber was grown in the greenhouse, and the young plants were evaluated for visual symptoms and assayed for infection using ELISA.

Natural Exposure: The most resistant lines selected in initial screening, were exposed to natural infection without pesticide treatment in replicated four-row plots placed in a solid stand of Belrus potatoes at Hermiston, Oregon. Each plot was bordered by four rows of Belrus on each side and five Belrus plants in the rows at each end. The two center Belrus rows were pesticide treated to prevent movement of crawling aphids from plot to plot. The plots were planted with tissue culture-produced plantlets in late May. They were inspected for aphid infestation and symptom development throughout the season. Tuber yields were determined at the end of the season, and tubers were evaluated for net necrosis at harvest time and in the spring after cold storage. Fifty tubers of each line were eye-indexed, evaluated for visual symptoms, and assayed for infection using ELISA.

RESULTS AND DISCUSSION

Initial Screening: In the first year of evaluation, 1990, little or no resistance was observed among transgenic lines in initial screening trials conducted at Prosser.

Many new transformed lines were evaluated in initial screening trials the following year. Plants in all the lines developed leafroll symptoms following inoculation, but a few withstood the infection a little better than other lines or nontransgenic Russet Burbank. While nontransgenic Russet Burbank and most other lines declined and tended to die prematurely following infection, these lines continued to grow despite severe foliage symptoms. The virus content of infected plants was lower in these lines, and both incidence and severity of net necrosis was markedly reduced. In subsequent testing, these lines proved to be highly resistant to movement of virus from plant to plant in the field. Tuber yields in the lines with reduced symptoms were much higher than in the nontransgenic Russet Burbank lines used as controls.

A wide range of resistance was identified among many new transgenic lines evaluated in initial screening tests in 1992, and 16 lines were selected for further testing. Plants in the most resistant lines appeared to be unaffected by leafroll disease. They did not have foliar symptoms at the end of the season, and few had any tuber discoloration that could be interpreted as leafroll-induced net necrosis. Virus could not be detected in plants of the most resistant plants using the ELISA assay nor could virus be transmitted from them to susceptible host species by aphid or graft transmission methods. Tubers harvested from the 16 resistant lines and from control Russet Burbank were eye indexed in the winter.

Virus symptoms were not observed in plants grown from the tubers of the most resistant selections, and virus could not be detected in these plants using ELISA. Although the field plots were not designed for accurate yield determinations, it was clear that yield of nontransformed Russet Burbank was markedly reduced by the leafroll disease, while there was little or no effect of the disease on the resistant transgenic plants. Except for their resistance to leafroll, the transgenic plants were indistinguishable from standard Russet Burbank potato.

Natural Exposure: Two transgenic lines selected for resistance in initial screening trials in 1992 (02-05 & 03-04), six transgenic lines selected for resistance in 1993 trials (08-47, 09-04, 11-04, 11-13, 11-16, and 11-18), and one transgenic line that was susceptible in 1993 screening trials (07-33) were subjected to natural exposure trials in 1993. Tubers produced were eye indexed in a greenhouse during the following winter and were evaluated for net necrosis at harvest and again in the spring after cold storage.

Among the five most resistant transgenic lines, virus was detected by ELISA in only three plants produced by eye indexing tubers in a greenhouse in the winter (Fig. 1). These plants had mild symptoms. In contrast, 67% of nontransgenic Russet Burbank and 70% of the susceptible transgenic line, 07-33, were infected according to eye indexing. Furthermore, the infected, susceptible plants had the typical, severe leafroll symptoms. Incidence of infection in the remaining three transgenic lines (02-05, 03-04, and 08-47) was markedly reduced compared with the nontransgenic Russet Burbank controls.

Three of the five most resistant transgenic lines (11-4, 11-13, and 11-18) were devoid of net necrosis at harvest time, and incidence of net necrosis in two resistant lines (09-04 and 11-18) was very low (Fig. 2). In contrast, more than 40% of tubers in the nontransgenic Russet Burbank and in the susceptible transgenic, 07-33, had net necrosis. Net necrosis incidence in the remaining three transgenic lines (02-5, 03-04, and 08-47) was markedly reduced compared with the nontransgenic Russet Burbank controls. One of the five most resistant lines (11-13) remained free of net necrosis even after storage, but a few tubers in the other four most resistant lines contained some necrosis (Fig. 2) following storage. However, it is doubtful that the necrosis observed in these lines after storage was caused by leafroll since only three plants among the five most resistant lines were infected with leafroll according to the winter eye index results. Net necrosis observed in the resistant transgenic lines was less severe than in nontransgenic Russet Burbank and was more restricted in the stem end.

These results indicate that five lines of transgenic Russet Burbank potato have been identified that can be grown essentially free of potato leafroll disease in the Columbia Basin without pesticides. These lines remain essentially free of leafroll disease and leafroll-induced net necrosis under natural exposure and even when they are artifically inoculated as young plants in the field with potato leafroll virus. These lines appear to be identical to standard Russet Burbank in all characteristics except their resistance to leafroll. Additional lines with somewhat less resistance to initial infection were highly resistant to spread of virus from plant to plant in the field.

Figure 1. Transgenic Resistance to Potato Leafroll Virus - Percent leafroll infection in transgenic and nontransgenic Russet Burbank potato tubers (grown without pesticide), Hermiston, Or. - 1993.

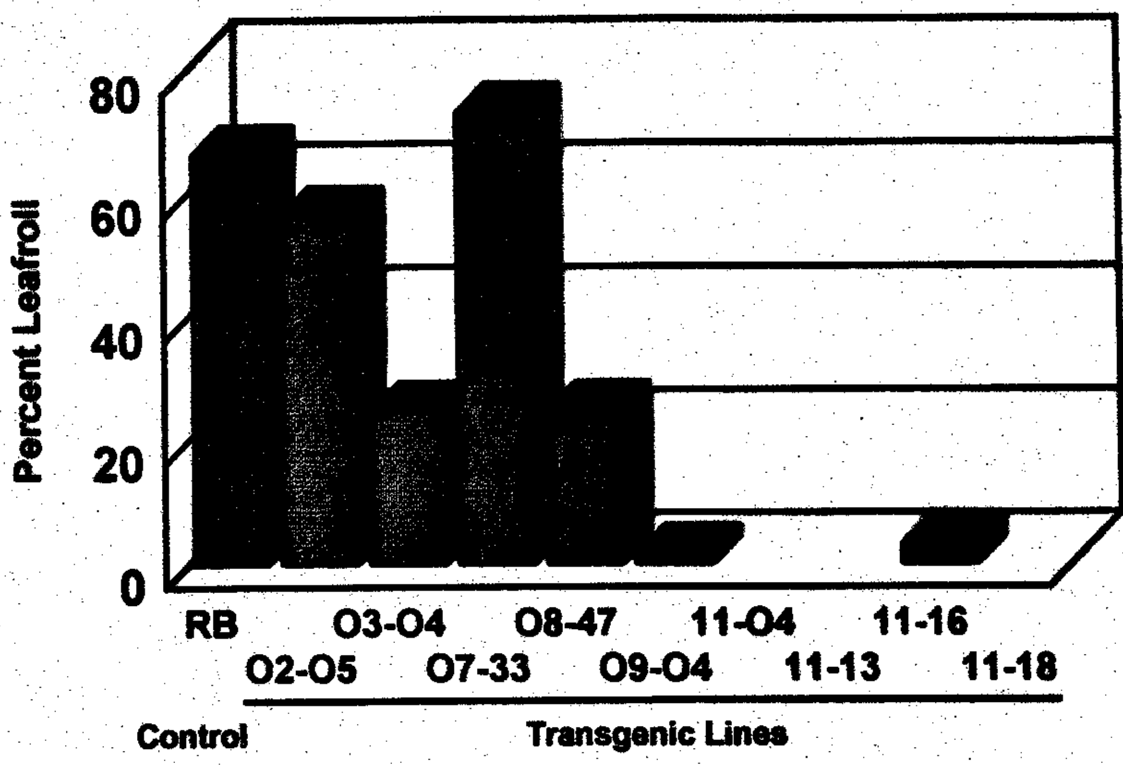
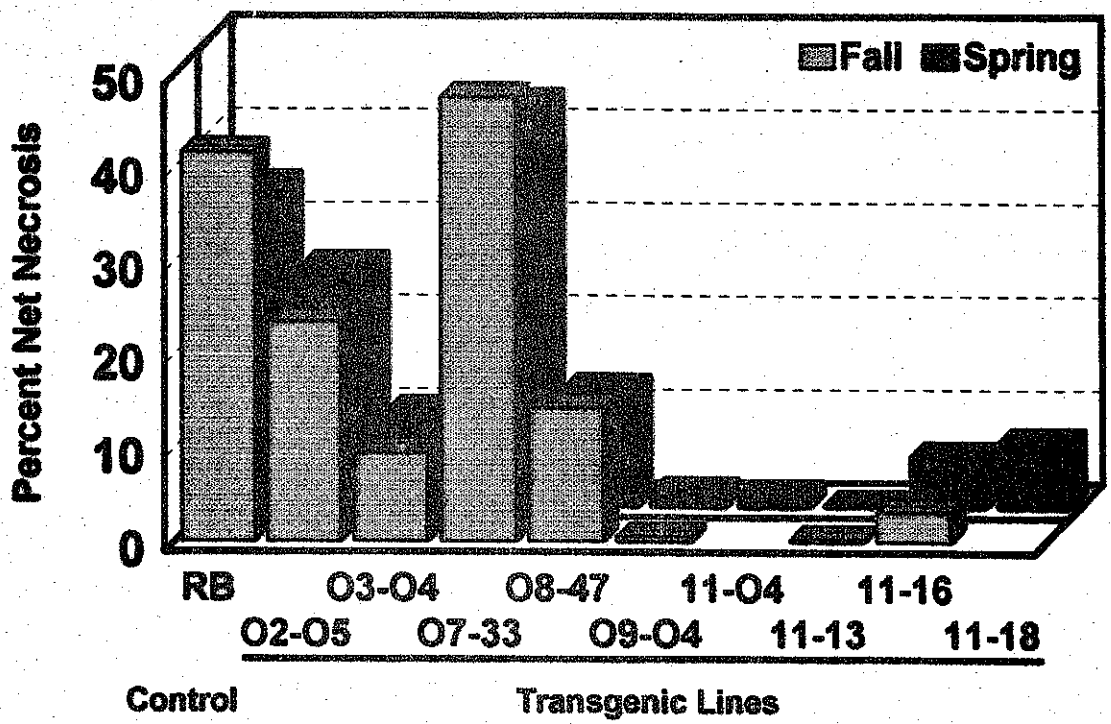


Figure 2. Transgenic Resistance to Net Necrosis - Percent net necrosis in transgenic and nontransgenic Russet Burbank potato tubers grown without insecticide, Hermiston, Or. - 1993. Percent net necrosis was determined at harvest time before storage and in the spring after storage.



The following papers were not available for publication in the 1994 Proceedings:

WSPC - Nutritional Advantage Marathon Program - Jim Knaub.

Progress on Commercialization of Transgenic Potatoes in the Northwest for Pest Resistance and Other Characteristics - Jim Zalewski, Hybritech.