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The Potato Story

Wojciech K. Kaniewski
Thomas Jefferson University, Philadelphia, PA

Peter E. Thomas
United States Department of Agriculture Agricultural Research Service, Prosser, WA

The need for genetic improvements of American potato was recognized as a primary target for plant genetic engineering. As immediate needs, virus and insect resistance were recognized as important and attainable goals. Russet Burbank was selected as the recipient variety, because it is highly vulnerable to virus and insect production losses, and it is the predominant American variety. The development of resistance to the Colorado potato beetle and to potato leafroll virus were selected as priority goals, because these are the most economically important pests of potato in the United States and around the world. This article describes potato research and the struggles to develop commercial products, as well as the safety, initial acceptance, and final commercial failure of developed products. Opportunities for developing countries and subsistence farmers are emphasized.

Key words: Colorado potato beetle, potato, potato leafroll virus, potato virus Y, Russet Burbank, transgenic resistance.

In the summer of 1986, Dr. Peter Thomas was unexpectedly contacted by Monsanto scientists. They posed a question: "What is the most economically important crop/virus combination in the United States?" The answer was simple: Russet Burbank potato and potato leafroll virus. Monsanto next wished to know if Thomas would be interested in testing the feasibility of developing virus-derived coat protein (CP) mediated transgenic resistance in potato, the objective being to solve potato virus disease problems in an approach that would markedly reduce or eliminate the need for pesticides. As he was not interested in moving to St. Louis for a short-term position, he suggested that I might be willing to take on the assignment, after my United States Department of Agriculture (USDA) contract as a visiting scientist in his laboratory was completed. I accepted Monsanto's offer and began preparations.

I became pessimistic about the possibility of achieving commercially credible resistance by the virus coat protein transgenic approach after reading the first coat protein paper (Powell-Abel et al., 1986). Would any farmer be interested in only a slight delay of symptom development? It was Roger Beachy who convinced me that improvements in the expression of transgenic resistance might be possible and was certainly worth a try.

Monsanto assigned a group of molecular biologists to clone CP genes from different viruses and to genetically transform tobacco and tomato plants with these transgenes, so that the first transgenic plants would be available upon my arrival in St. Louis. In the meantime, in Prosser, Washington, we were working in a number of areas designed to select and evaluate virus resistance in potato, including selection of aggressive field isolates of potato viruses and the development of sensitive virus assay methods (Kaniewski & Thomas, 1988) capable of quantifying the presence and levels of coat protein in transgenic plants.

In early 1987, I joined the Monsanto team in St. Louis. Large numbers of transgenic tobacco lines were already waiting for resistance evaluation. To my great surprise, I found transgenic lines that resisted all infections by the virus sources homologous to the transgenes under laboratory conditions. Because we found that level of resistance did not always correlate with level of coat protein expression, we began immediately to test all lines directly for virus resistance. As a result, we soon found transgenic tobacco lines that were extremely resistant to tobacco mosaic virus, alfalfa mosaic virus, and cucumber mosaic virus. This convinced us that transgenically imparted resistance was a reality and that we should begin to generate products for tomato and potato.

We started by showing that potato X potexvirus (PVX) and potato Y potyvirus (PVY) CP transgenes produced high levels of resistance in tobacco. Next, we transformed Russet Burbank with a construct containing the CP genes of both PVX and PVY (the first double gene construct) and potato leafroll virus (PLRV) as soon as potato become transformable in 1988 (Newell et al., 1991). Among 16 PVX/PVY transgenic potato lines produced, four were moderately resistant; one, line 303, was extremely resistant to challenge with both viruses

(Lawson et al., 1990). Field testing between 1989 and 1992 confirmed the very high levels of resistance in line 303 under field conditions (Kaniewski et al., 1990; Kaniewski & Thomas, 1993). Research into the nature of this resistance revealed that line 303 was not infectible by either PVX or PVY by mechanical inoculation, nor was it infectible by PVY by aphid or graft transmission, but it was very susceptible to PVX by grafting. When plants were grafted with double infected scions, only PVX moved freely in this potato line, convincing us that there is more than one mechanism of CP-mediated resistance.

We worked extensively on mechanisms of transgenic resistance. We are certain that any one mechanism proposed so far cannot accommodate all our experimental data; therefore, more than one mechanism must be responsible for transgenic virus resistance. We applied various experiments to understand why transgenic lines with equal expression of transgene proteins could be extremely resistant or completely susceptible to viral infection, but we still cannot answer this question.

Commercial realities meant that the PVX/PVY double gene product concept was abandoned, as improvements in seed certification programs were found to effectively control PVX. This double gene construct was donated in 1991 to CINVESTAV (Centro de Investigación y Estudios Avanzados, Mexico) to be used in local potato varieties by subsistence farmers.

Our major effort was directed toward PLRV resistance in Russet Burbank. CP-expressing plants were first generated in 1988. Although a few of these lines were statistically more resistant than controls, none were sufficiently resistant under field conditions, which required that new generations of plants be produced with improved CP gene constructs. Hundreds of transgenic lines were generated from a total of 22 various PLRV CP constructs and assessed for resistance in both the growth chamber and field trials. Many transgenic plant lines were found to be significantly more resistant than controls, but only one of these was seen to be completely resistant to the homologous virus strain in growth chamber experiments. Resistant lines were field tested and their practical value confirmed, especially by virus spread tests (Thomas, Kaniewski, & Lawson, 1997). To our surprise, the only line that was not infectible in growth chamber assays became completely infected under natural exposure in field tests. We isolated virus from field-infected plants and retested the plants with this isolate in the growth chamber. Although it was still immune to the homologous virus strain, this most resistant transgenic line was shown to be completely susceptible to the field isolate, despite the fact that the CP gene sequences of both viruses were identical. Commercialization of the most resistant PLRV CP lines was considered but ultimately rejected, because the lines were not good enough according to strict Monsanto product standards.

When the CP approach failed to produce potatoes highly resistant to PLRV, the Monsanto administration decided in early 1991 that research would be discontinued and the virus team disbanded if highly PLRV resistant potatoes were not demonstrated by the end of that year. In a frantic race, five new constructs containing the PLRV replicase region were prepared and efficacy tested. This was more desperation than science. Two of the constructs (full-length nonmodified replicase and a truncated replicase) delivered plants immune to the homologous PLRV isolate in growth chamber experiments (Lawson, Thomas, & Kaniewski, 2001). Still, concern remained, because it was already known that TMV resistance in tobacco, as mediated by the replicase gene, was effective only against the homologous strain of the virus. Despite this skepticism, the new replicase potato lines were exposed in field plots at Prosser to the range of PLRV variants that occur naturally in the Columbia Basin. Although most lines were susceptible in varying degrees, some remained virus free throughout the season (Thomas, Lawson, Zalewski, Reed, & Kaniewski, 2000). The nonspecificity of resistance in these lines was confirmed later by field exposure of each line to each of 64 different PLRV isolates collected from throughout the United States.

We announced the development of highly PLRV-resistant potato plants in the fall of 1991 and recommended commercialization of selected lines expressing the full-length nonmodified replicase gene. This PLRV replicase gene was later donated to Mexico for use in a triple virus-derived gene construct (PVX/PVY/PLRV) that would be used for triple virus protection in local potato cultivars.

As the Virus Team described its success in transgenic resistance, the Insect Control Group concurrently announced season-long control of Colorado potato beetle (CPB) in potato (Perlak et al., 1993) using a synthetic *Bt* gene. Monsanto decided to commercialize *Bt* insect-resistant potato plants first, to later be replaced with lines that combined both insect and virus resistance in the same germplasm. Four local commercially important varieties were transformed with the *Bt* gene (Russet Burbank, Atlantic, Snowden, and Superior). Resistant lines were selected for commercialization, extensively characterized for regulatory approvals, and concurrently

Table 1. Russet Burbank potato line selection for resistance to PLRV and CPB.

Explants transformed	14,989
Plants obtained	1,730
Plants resistant to CPB (feeding assay)	1,021
Plants with no spec-strep (PCR)	701
Lines planted in the field	616
PLRV resistant lines	323
Lines with more than 10 ppm of Bt protein	122
Lines with high yield and without net necrosis	47
Lines with no PLRV in sprouts	31
Lines selected for initial seed increase	19
Lines commercialized	3

increased for seed stock. These lines were commercially available in 1995 and sold to farmers under the brand name NewLeaf by NatureMark, a newly created Monsanto subsidiary.

Based on Monsanto plans to combine virus and CPB resistance in future products, the Virus Team began to combine CPB and PLRV resistance, as major potato cultivation constraints in the US, in lines that would later be named NewLeaf Plus (Figure 1) and to combine CPB and PVY resistance in lines named NewLeaf Y. New vectors were constructed with appropriate insect/virus gene combinations, and large-scale transformations were begun. To expedite the selection process, we decided to skip all expression assays and growth chamber assays of virus resistance. All lines produced in 1993 (Table 1) were first subjected to CPB larva feeding test; those that survived this test were subjected to polymerase chain reaction (PCR) screening for the presence of unnecessary backbone coding sequences. Plant lines that passed both these tests were micropropagated and transplanted directly to field plots. They were screened for virus resistance and agronomic performance directly at two field sites in the spring of 1994 (Kaniewski & Thomas, 1998). Based on field virus resistance, *Bt* protein content, and agronomic performance (Thomas & Kaniewski, 1998), 19 lines were selected for seed production and regulatory approval (Table 1). After several years of intensive evaluation, three of these Russet Burbank lines were commercialized in 1998. During this period of field selection and evaluation, no pesticide was ever required to control infection by any virus in the resistant Russet Burbank lines. A similar strategy was applied to generate NewLeaf Y lines in both Russet Burbank and Shepody cultivars. These were also commercialized in 1998 (Duncan et al., 2002).

Figure 1. Major constraints of potato production. NewLeaf Plus, unaffected (top); Russet Burbank defoliated by CPB (middle); Russet Burbank infected with PLRV (bottom).

Figure 2. NewLeaf Plus field (left) is protected from PLRV—.no sprays, no infection. The conventional potato field on the right is 100% PLRV infected, despite sprays.

During the process of registration, the food and environmental safety of these new products were discussed extensively. The USDA raised, as the major problem, the issue that in transgenic potatoes the PLRV transgene is present in all cells, whereas in potatoes infected with PLRV, the virus is present only in phloem cells. To address this issue we designed an experiment where we could detect PLRV RNA in individual cells with a sensitivity about 100 times higher than that commonly achieved in immunological tests. This investigation revealed that viral RNA is not confined to the phloem (as previously believed) but is present in almost all cells of infected potatoes, with highest concentrations detectable in phloem cells. Moreover, we detected minus-strand RNA in nearly all cells of infected plants, thereby demonstrating that PLRV not only moves to cells away from the phloem but also multiplies there (Holt, Kaniewska, Lavrik, & Kaniewski, 1997).

The USDA also asked for information concerning the probability of transencapsidation of unrelated virus RNA with coat protein produced by the PVY transgene. We therefore designed an experiment to compare the frequency of transencapsidation of RNA of the closely related virus potato A potyvirus (PVA) with PVY CP in mixed infections, to that of transencapsidation of PVA RNA by PVY coat protein in NewLeaf Y potato. The results showed that the frequency of transencapsidation in transgenic potato is at least 100 times lower than is common in natural mixed infections. Although insect transmission of transencapsidated virions can be eliminated by utilizing a CP gene with a single mutation, the USDA recommended use of the native CP gene, due to the proven low probability of transencapsidation and the lack of the HC Pro gene, which would be responsible for synergistic effects, in our transgenic potatoes.

Table 2. Biotechnology provides tool to manage PLRV problem in commercial potatoes.

	Idaho	Basin
Grower benefits (average savings per acre)		
Insecticide	$39	$78
Net necrosis	$102	$86
Total	$141	$164
Environmental benefit		
Insecticide replacement potential (lbs.)[a, b]	1,030,000	815,000

Note. Data from Thomas and Kaniewski (in press) and Thomas et al. (1994).
[a] 80% of Burbank acres planted to NewLeaf Plus.
[b] Total equivalent to 30,000 spray plane sorties.

NewLeaf Plus was commercially grown mainly in the Pacific Northwest (Figure 2). It produced healthy potato crops, free of net necrosis with a markedly reduced or zero requirement for insecticide application (Table 2). Farmers and processors enjoyed most of the benefits of NewLeaf Plus, although consumers were receiving potatoes of superior quality. The production of NewLeaf Y was localized mainly in the central and eastern United States and Canada. It eliminated seed reinfection by PVY in these regions—a great benefit to seed growers. Farmers benefited not only from higher yields of higher quality tubers in potato crops free of CPB damage and PVY infection, but also a markedly reduced need for pesticide. Shortly after introduction, it was impossible to produce enough seed potatoes to meet the demand. Consumer questionnaires in the United States

and Canada showed high preference for transgenic potatoes, mainly because of their superior quality, no need for pesticide use, and competitive pricing.

Our experience in developing transgenic virus disease and insect pest resistance in potato, in securing regulatory approvals, and in bringing the product to commercialization, has provided guidelines for others attempting to develop similar products in other crops. To facilitate the efforts of others, we have summarized our experience in developing virus resistance (Kaniewski & Lawson, 1998), in conducting field evaluation (Kaniewski & Thomas, 1999), in virus isolation and purification (Thomas & Kaniewski, 2001), in perceived risks associated with transgenic crops (Thomas, Hassan, Kaniewski, Lawson, & Zalewski, 1998; Kaniewski, Rogan, & Cline, 2000), in applications in developing countries (Kaniewski & Beachy, 2003), and in product development history (Rogan et al., in press). Our work on securing regulatory approvals—especially in foreign countries—is a valuable source of various food and environment safety information.

Three NewLeaf Y and three NewLeaf Plus clones were approved by Canadian and United States regulatory agencies in 1998 and 1999 and allowed for commercialization in North America. Food and feed safety approvals of the tubers derived from NewLeaf Plus and NewLeaf Y potato clones were obtained after voluntary consultations with the United States Food and Drug Administration and mandatory reviews by the Canadian Food Inspection Agency, Health Canada, the Animal Plant Health Inspection Service (APHIS) of the USDA and the US Environmental Protection Agency. NewLeaf Plus and Y varieties have also been approved for food export to Japan, Mexico, and Australia. NewLeaf Superior is approved for cultivation in Bulgaria, Romania, and Russia.

Activists began their successful antibiotechnology campaign against our transgenic potatoes in 1999. McDonald's decision to ban genetically modified crops from its food chain had a major impact within the North American potato industry. Potato processors under pressure, not only from the McDonald decision, but also from export markets especially in Europe, were forced to suspend transgenic contracts. Monsanto was forced to withdraw from the potato business after the highly successful 2001 season. NatureMark was dissolved, and of course, all research toward further potato improvement—including areas of great promise, such as development of high solids, anti-bruising, herbicide resistance, late blight and other fungal resistances—came to a halt.

It is ironic that those activists who list reduction in use of pesticides as a major goal are those that have effectively blocked the most successful scientific approach to that end. Meanwhile, Monsanto continues to receive inquiries from unhappy American and Canadian farmers concerning future availability of these proven and effective products.

Acceptance of transgenic potatoes in many foreign countries where cost precludes large-scale application of effective pesticides to control virus diseases and insect pests is a highly encouraging development. Requests for assistance continue to come from many countries around the world. Real progress is underway to develop similar products to address regionally important virus and insect pests.

Using Monsanto constructs, the potato virus project in Mexico has now developed new transgenic lines of three varieties of Mexican potatoes resistant to PVX and PVY that are ready for planting by farmers. Mexican potato lines transformed with a triple gene construct to provide resistance to PVX, PVY, and PLRV (including the Alpha, Rosita, and Nortena varieties) are now at the line selection stage. Furthermore, Monsanto has licensed the use of the synthetic *Bt* gene for genetic transformation of six Russian (Nevsky, Lugovskoy, Elizaveta, Volzhanin, Golubizna, and Charodei), three Bulgarian (Kalina, Koral, and Bor), and three Romanian (Redsec, Coval, and Belint) leading potato varieties for CPB resistance. In Russia and Bulgaria, two years of field tests for line selection have been completed, and tests required for varietal registration are now in progress. In Romania, transgenic lines have been transformed, and the first line selection field tests are now under way. NewLeaf Plus and NewLeaf Y were recently tested for virus resistance on Mauritius, and the virus resistance of these lines has proven to be highly effective in this remote region, which means that existing products could be cultivated there, or the genes could be used for transformation of local varieties.

It is our experience that many countries around the world are eagerly seeking virus-, insect-, disease-, and herbicide-resistant potatoes and other crops. It is especially the small and subsistence farmers, who cannot afford the gamut of pesticides required for disease and pest control, that will benefit most from such products. New and potentially catastrophic strains of PVY that cause tuber necrosis have recently invaded American potato culture; these strains could probably be eliminated by using our PVY-resistant lines.

We believe that genetically improved potatoes (the existing products, as well as additional products devel-

oped in the future) will be grown as the standard crop in many countries around the world and will once again be grown in American soil in the near future.

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