Field Tests on Transgenic Potatoes in Mexico

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Abstract

In 1990, Monsanto scientists reported the development of transgenic Russet Burbank potatoes resistant to mixed virus infections. This was the first attempt to obtain coated protein-mediated protection against PVX and PVY with a single transformation event. Since this achievement, several field tests have been conducted at different locations and the effectiveness of these tests showed that resistance to PVX and PVY was confirmed and could prevent yield losses due to dual infections by these viruses.

In January 1991, a collaborative program between Monsanto and CINVESTAV was initiated. The main purpose was to apply the double coat protein gene strategy to Mexican potato varieties. This was the first project supported by ISAAA for technology transfer between a private company and a research center in a developing country. Funds for the project were provided by The Rockefeller Foundation.

As a result of the project, resistance to virus infection was successfully achieved and transgenic-Alpha potatoes are becoming available. Field tests were conducted at Prosser, Washington during the summer of 1992 and at CINVESTAV Irapuato in the following summer. This particular experience exposed CINVESTAV and INIFAP scientists to several important regulatory issues, risk assessment and biosafety regulations.

After five years of research development, some of the expected impacts of this project have been evaluated. The most relevant results of this program are related to the field release of transgenic potatoes in Mexico for the benefit of small farmers. Scientific results of this development were published elsewhere (Rivera, 1995) and a summary of these findings is presented in the present document.

Status of potato cultivation in Mexico

Potato is ranked fifth among the priority crops in Mexico after maize, beans, wheat, and cotton. In 1992, potato occupied approximately 72,000 ha with an average production of 16.8 tons per ha. Total annual production in the same year was in the range of 1.21 million tons and valued at US$240 million. Approximately 80% of the production is used for consumption as table potatoes (fresh market), 7% for industry (potato chips, flour, starch, and alcohol production) and 13% for seed.

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From the total surface for potato cultivation, almost 57% is under irrigation where yields average 20.5 tons per ha; the remaining 43% is rainfed with average yields of 11.7 tons per ha.

The Dutch variety Alpha occupies approximately 60% or 45,000 ha of the total 80,000 ha of potato fields in the country. The second most important variety is Lopez (15,000 ha), and the remaining acreage is occupied by other Mexican varieties and varieties such as Atlantic (chipper), White, Rose, and Bintje.

The Alpha variety is very well adapted to Mexican fields and is very popular because of its versatile qualities for cooking, as a chipper or for industrial use to produce flour, starch, and alcohol. Despite its extreme susceptibility to late blight (*Phytophthora infestans*) and virus diseases, Alpha has retained its position as the major variety for many years, and there is no indication that it will be replaced in the short term.

Two types of potato farmers can be easily distinguished in Mexico. Industrialized farmers with modern, irrigated and well-organized farms utilize certified seed produced by themselves or through their own farmer organizations. Phytopathological problems are usually under control with heavy applications of pesticides and integrated pest management. On the other hand, there are small farmers that rarely use certified seed, produce potatoes under non-irrigated conditions and can not afford a large number of chemical applications. Their potato seed is usually the remainder of the previous year's harvest or that which could not be sold in the market because of small size or low quality.

Genetic engineering of potatoes for virus resistance

Improvement of potato by conventional breeding and selection is laborious, time-consuming and inefficient because it is a tetraploid species. New approaches for plant improvement like genetic engineering have been shown to be an excellent tool to improve existing varieties by selectively adding specific traits such as virus resistance. Genetically engineered resistance to virus diseases was one of the first practical demonstrations of this powerful technology (Beachy *et al.*, 1990). Several new strategies to obtain virus-resistant plants are now available and have been applied to almost all types of plant viruses (Wilson, 1993).

In 1990, Monsanto scientists reported transgenic Russet Burbank potatoes resistant to mixed virus infections (Lawson *et al.*, 1990). This was the first attempt to obtain coated protein-mediated protection against two different viruses (PVX and PVY) with a single transformation event. The vector used, pMON9898, contained two chimeric genes, each harboring a coat-protein gene (*PVXCP* or *PVYCP)* under the control of the Ca MV 35S promoter and a termination signal. The selectable marker gene, *NPTII* that confers resistance to kanamycin, also was included. With this vector, Monsanto scientists were able to obtain several potato lines that expressed both coat-protein genes and were resistant to infection by PVX and PVY after mechanical inocu-

lation. Four selected lines were field-tested for virus resistance, plant growth and tuber yield (Kaniewski *et al.*, 1990). Since this first field experience, several tests at different locations have confirmed that resistance to PVX and PVY is effective in the field and can prevent yield losses due to dual infections by these viruses.

CINVESTAV-Monsanto collaboration project

A collaborative program, between CINVESTAV and Monsanto to introduce coat-protein genes into Mexican potato varieties and to confer genetically engineered resistance to potato virus PVX and PVY, was initiated in January 1991. This program was the first project supported by ISAAA and the main aim was to transfer the technology developed in a private company to a developing country. ISAAA identified the Mexican needs for potato culture, contacted Monsanto and obtained financial support for the project from The Rockefeller Foundation. This project was expected to have an impact in five areas:

* Productivity
* Environment
* Technology transfer
* Biosafety procedures
* Personnel training

The project was divided into four phases and the objectives of each phase are described in the following section.

PHASE I. Transformation and regeneration protocols for alpha variety

The main objectives of this phase were: 1. to train CINVESTAV scientists in the transformation and regeneration of potato variety Russet Burbank and, 2. to adapt these protocols to Alpha variety.

From the experiments performed with the vector PMON9898, 108 independent lines were selected for further analysis. After the ELISA test for PVX coat-protein, a total number of 50 lines were propagated (50 replicates per line) and sent to Prosser to be tested in the field. These lines were the top in the PVX expression test. After three months of training at Monsanto, CINVESTAV experts returned to Mexico to establish the transformation protocols under Mexican conditions.

PHASE II. Improvement of PVX and PVY vectors for transformation of potato with *Agrobacterium tumefaciens*

This phase has three objectives: 1. construction of an improved vector; 2. training in gene expression assays of transgenic plants and 3. training in viral protection assays of transgenic plants.

The first objective was focused on the construction of an improved version of the

PVX and *PVY CP* gene vector. The new vector constructed in this phase, plasmid pMON18770 contained the coat protein genes of PVX and PVY under the enhanced version of 35S promoter from cauliflower mosaic virus (35S CaMV). It also contained the *NPTII* gene for kanamycin resistance. As a selectable marker for bacteria, PMON18770 has also a gene that confers resistance to spectinomycin and streptomycin. This vector was previously used to obtain the potatoes to be released to the farmers. The transformation of Alpha potatoes with this vector was performed at CINVESTAV.

The training assays in this phase were conducted with some of the transgenic potato lines obtained in Phase I. More than 200 transgenic lines were analyzed for the expression of *NPTII* by ELISA. After the NPTII test, 50 lines showing the highest expression were selected. These lines were then evaluated for the expression of PVX and PVY coat proteins also by ELISA procedures. After PVX assays, the 30 highest expressors were selected to be field-tested. The viral protection assays included virus purification, infectivity assays, and inoculation of transgenic plants.

PHASE III. Production of transgenic potatoes at CINVESTAV

The original objective of Phase III, production of transgenic Alpha variety, was modified by the addition of new Mexican varieties Norteña and Rosita into the project.

The transformation of Alpha potato, at CINVESTAV with the improved vector was initiated during the period 1992-93. The study included transformation, selection of the best expressor lines and analysis and propagation of the selected clones. In December 1992, it was decided to include other varieties into the project to increase the impact of the technology among small farmers. The varieties chosen were Rosita and Norteña (developed by the National Potato Program). Finally, during 1993, the methodology for tissue culture of these varieties was developed and reported. In 1994, the conditions for an efficient transformation were determined. Minor adjustments were required over the previously reported protocol for Alpha.

At the end of 1994 there was a significantly large number of transgenic lines for the three varieties (Alpha, Rosita and Norteña). At that stage, initial tests in greenhouse were performed for virus resistance (ten plants for each line were mechanically inoculated with PVX and PVY).

Selected lines were propagated *in vitro* to produce a sufficient number of plants for field test. Micropropagated plants were then cultivated in the laboratories of a private company, Biotechnology 2000, at león Gto. Fifty plants of each line were tested and at least 50 minitubers were obtained for each line for the second field test scheduled for the same year.

PHASE IV. Field evaluation of transgenic potato plants

The main objectives of this phase were the field evaluation of transgenic potato plants and the implementation of regulatory issues related to the release of transgenic potatoes in Mexico. During 1992-93, only a few tests had been performed in Mexico

after the USA. To deal with this type of applications, a Biosafety Committee was organized by the Direction General of Plant Health from the Ministry of Agriculture and Rural Development (Dirección de Sanidad Vegetal de la Secretaria de Agricultura, Ganadería y Desarrollo Rural, SAGAR). This committee was composed of scientists and personnel from different Mexican institutions. It is fair to say, that this project somehow contributed to the establishment of this committee since field testing with transgenic potatoes was the first application submitted by a Mexican institution.

After the experience acquired during the USA testings, a test was performed in Irapuato, Mexico (CINVESTAV facilities). The permission granted to perform this test was the first to be awarded to a Mexican institution.

A second test was performed in Irapuato in 1995. This test included Alpha, Norteña and Rosita lines that were obtained at CINVESTAV, and propagated *in vitro* by Biotechnology 2000. The objective of this test was to evaluate virus resistance and all the plants were inoculated with PVX and PVY. At the same time, Biotechnology 2000 produced minitubers of the same lines for future field evaluations.

The application submitted to the Biosafety Committee clearly indicated that no virus inoculation was planned for this test. In addition, the flowers of the transgenic lines were to be removed to reduce the possibility of cross-breeding with commercial potatoes in the area. This precaution was suggested since little is known about potato pollen contamination. This test was performed near the city of Arandas, Jalisco, a new area which is being developed for potato seed production. This was again the first test with transgenic material in Jalisco. The main parameters observed in this test were: rate of emergence, early vigor, plant type, pest and disease susceptibility, tuber yield, quality and storability. Based on these parameters, the lines were graded in an arbitrary scale from 1 to 10 Lines above 5 were considered for further evaluations.

The tubers obtained in Arandas are the first transgenic tubers obtained directly from the field. These tubers will be propagated (again in Arandas) to produce enough seed for future multiplication trials and eventually for seed delivery to farmers. Additionally, it will be the first time that tubers obtained from the field are to be used as seeds. All previous tests were performed with plantlets grown *in vitro* or minitubers obtained under greenhouse conditions.

References

1) Beachy, R.N., S. Loesch-Fries and Tumer, N. (1990) : Coat protein mediated resistance against virus infection. Ann.Rev. Phytopathol. **28**: 451-474.
2) Kaniewski, W., Lawson, C. and Sammons, B. (1990) : Field resistance of trangenic Russet Burbank potato to effects of infection by potato virus X and potato virus Y. Bio/Technology **8** : 750-754.
3) Lawson, T.W.A. (1993) : Strategies to protect crop plants against viruses: Pathogen-derived resistance blossoms. Proc. Natl. Acad. Sci. **90** : 3134-3141.

4) Rivera-Bustamente, R. (1995) : An example of transfer of proprietary technology from the private sector to a developing country. *In* : Plant Biotechnology Transfer to Developing Countries. Ed. D.W. Altman and K.N. Watanabe. R.G. Landes Company, Wilson