Resistance of genetically modified potatoes to *Potato virus Y* under field conditions

André Nepomuceno Dusi[1], Cristiane Lopes de Oliveira[2], Paulo Eduardo de Melo[1] and Antonio Carlos Torres[1]

[1]Embrapa Hortaliças, Caixa Postal 218, CEP 70359-970 Brasília, DF, Brazil. E-mail: dusi@cnph.embrapa.br, paulo@cnph.embrapa.br, torres@cnph.embrapa.br [2]Universidade Católica de Brasília, QS 07 Lote 1 EPCT, CEP 71966-700 Brasília, DF, Brazil. E-mail: cristiane@cnph.embrapa.br

Abstract – The objective of this work was to evaluate the resistance of genetically modified clones of potato to *Potato virus Y* (PVY) under field conditions. Genetically modified plants were compared with nontransformed plants of the same cultivar. The plots were flanked with potato plants infected with both PVY^O and PVY^N strains (spread lines), in order to provide the experimental area with the source of virus, which was naturally spread by the native aphid population. The experiment was weekly monitored by visual inspections and by DAS-Elisa in the plants produced from the harvested tubers, in order to evaluate the resistance of transgenic plants throughout the plant growth cycle. By the end of the third year, no infection symptoms were observed in the 1P clone; clone 63P showed 1% of infection, in contrast to about 90% of nontransformed plants infected. The stable expression of resistance to PVY provided by the coat protein gene was obtained in genetically modified clones of potato plants cultivar Achat under field conditions, during three consecutive years.

Index terms: *Solanum tuberosum*, coat protein gene, genetically modified organism, transgenic potato, viral resistance.

Resistência de plantas de batata geneticamente modificadas ao *Potato virus Y* em condições de campo

Resumo – O objetivo deste trabalho foi avaliar a resistência de clones geneticamente modificados de batata ao *Potato virus Y* (PVY) em condições de campo. As plantas geneticamente modificadas foram comparadas com plantas não modificadas da mesma cultivar. As parcelas foram delimitadas com plantas infectadas com as estirpes PVY^O e PVY^N (linhas disseminadoras), para tornar disponível, na área experimental, a fonte de inóculo de vírus, que foi naturalmente disseminada pela população nativa de afídeos. O experimento foi monitorado semanalmente por inspeção visual e por DAS-Elisa nas plantas produzidas a partir dos tubérculos colhidos, para avaliar a resistência de plantas transgênicas ao longo do ciclo de crescimento. Ao final do terceiro ano, nenhum sintoma de infecção foi observado no clone 1P; o clone 63P apresentou 1% de infecção, em contraste com cerca de 90% de plantas-controle infectadas. A expressão estável da resistência ao PVY, conferida pelo gene da capa proteica, foi obtida em clones de batata geneticamente modificados da cultivar Achat, em condições de campo, durante três anos consecutivos.

Termos para indexação: *Solanum tuberosum*, gene da capa proteica, organismo geneticamente modificado, batata transgênica, resistência viral.

Introduction

Potato (*Solanum tuberosum* L.) is originated from the highlands of Bolivia and Peru in South America. It is an important crop, placed among the four most consumed foods in the world, together with maize, wheat, and rice (Food and Agriculture Organization of the United Nations, 2005). Even though potato has been one of the most extensively investigated crops, its culture in tropical regions is still facing major problems. Viruses, for example, are still a yield-limiting factor for the crop. Although rarely lethal, in many situations they affect the development of the plant, and consequently reduce the yield (Hooker, 1990).

Coat protein (CP) gene-mediated resistance in transgenic plants has been reported as an effective protection in several plant-virus systems (Hull & Davies, 1992). This approach is commercially used for some crops, such as potato and papaya (Lawson et al., 1990; Fitch et al., 1992). Currently, only virus-resistant papaya is still widely grown in the US and in China (James, 2008). However, the mechanism

eliciting this type of resistance is still unclear. In addition, Lindbo & Dougherty (1992) disclosed that CP-mediated resistance has been reported to induce different degrees of protection. They postulated that protection sometimes results from coat protein mRNA accumulation and is independent of a requirement for coat protein expression, thus implying that the mechanisms inducing resistance could be operating at more than one level. CP-mediated resistance is usually associated to RNA silencing, a mechanism in which the production of a double-stranded RNA, as an intermediate of the viral replication cycle, breaks the viral mRNA or blocks its translation (Zerbini et al., 2005).

Since 1994, Embrapa has been working on the development of genetically modified (GM) potatoes transformed with the *Potato virus Y* (PVY) coat protein gene, and two potato Achat clones (named 1P and 63P) were selected for expressing resistance to PVY under greenhouse conditions (Romano et al., 2001). Both clones are being submitted to environmental and food safety risk assessment analyses as part of a biosafety project of Embrapa.

Resistance evaluations for those clones, under greenhouse conditions, indicated resistance to all PVY strains reported in Brazil. The 1P clone showed extreme resistance and the 63P clone showed intermediate resistance when challenged by mechanical inoculation (Dusi et al., 2001).

The objective of this work was to evaluate the resistance of genetically modified clones of potato to PVY under field conditions.

Materials and Methods

The GM clones were previously selected from the cv. Achat for their resistance to PVY (Romano et al., 2001). Plants of both the 1P and 63P clones were released into the environment at Embrapa Hortaliças, DF, Brazil (15°56'S, 48°08'W, 99.8 m above sea level). The first field trial occurred in 2004, and the assay was repeated in 2005 and 2006. The nontransformed parental cv. Achat was used as control in all experiments.

Each trial was performed in an experimental area of 120 m^2 located 30 m away from the conventional potato field. The assay was conducted in a randomized block design with three treatments (1P, 63P and the nontransformed parental) randomly distributed. There were 20 replicates of five plants in the first year, with a distance of 0.80 m between the lines and of 0.30 m between plants (1.2 m^2). The plots were flanked with PVY-infected plants of the Achat cultivar to ensure the presence of inoculum in the experimental area (spread lines) (Figure 1). Seed tubers of the spread lines were obtained from plants contaminated by aphids with both PVY^O and PVY^N strains, to ensure the aphid transmission of the virus under field conditions. The virus-free seed tubers of both 1P and 63P GM clones and of nontransformed 'Achat' were produced at Embrapa Hortaliças.

The planting dates were May 21, 2004; April 20, 2005; and May 3, 2006. Preliminary studies of the aphid population fluctuation indicated that this period of the year had the highest number of aphid flights, thus ensuring better epidemiological conditions for the virus spread. The cultural practices were standard for the region, including irrigation every two or three days. Also, insecticide and fungicide sprays were applied when needed, focusing mainly on *Diabrotica* sp. control.

No artificial inoculation of the GM and the nontransformed control plants were made, and only the natural spread by the virus vector (aphids) was recorded. The experiment was monitored weekly by visual inspections. On the second and third years, ten plots (replicates no. 1, 2, 7, 8, 10, 12, 15, 16, 17 and 18) were sown with tubers harvested in the previous year to determine both the decay of the seed in a three-year period and the stability of the resistance in the long run. On the other ten plots, new virus-free

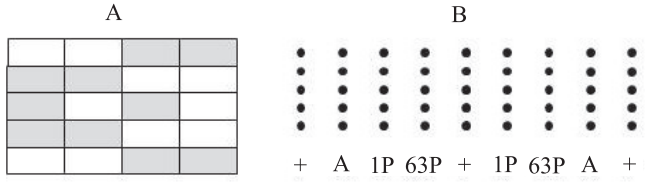

Figure 1. Croquis of the experimental field. A, replicates in gray represent the plots in which virus-free potato seeds of the nontransformed cv. Achat control were used each year; B, detail of the distribution of the clones, including the infected lines between plots (example): the 1P, 63P and nontransformed Achat (A) clones were flanked by PVY infected plants (+).

nontransformed Achat seed-tubers were sown each year to determine the infection pressure of the specific year.

A green water-pan trap was placed in the middle of the experimental plot each year and weekly sampled to verify the aphid incidence in the area (Webb et al., 1994). Each year, at the end of the crop cycle (on August 30, 2004; August 10, 2005; and August 9, 2006), all the plants were individually harvested and their tubers were kept in a cold chamber. After natural dormancy breaking, one tuber from each plant was planted in a pot, visually inspected and individually tested for PVY by DAS-Elisa (Dusi et al., 2001) and Western-blot testing. Data of yield in 2006 was tested for both normality and equal variance prior to ANOVA.

Results and Discussion

The translation of the CP gene in the 1P and 63P clones was detected neither by DAS-Elisa nor by Western-blot testing.

None of the 1P clone plants and only one plant of the 63P clone became naturally infected with PVY by the virus vector (aphids). In contrast, almost all of the nontransgenic parental plants showed symptoms of virus infection at the end of the experiment. The percentage of virus dissemination among control plants (susceptible) varied from 40 to 90% (Table 1).

Based on both the infection of the control plots and the presence of aphids during the experimental period (Figure 2), the resistance was confirmed in the field. Similar results were observed previously under greenhouse conditions (Dusi et al., 2001).

In 2006, the yield of tubers from the plots that were exposed for three years under field conditions were recorded. Data passed both normality and variance homogeneity tests. There was no effect for block. The coefficient of variation (CV) was 19.7%. The yield of the GM clones (4.2±0.21 and 4.3±0.31 kg per plot for 1P and 63P respectively) was significantly higher $p<0.01$ than that of the nontransgenic Achat control (3.1±0.19 kg per plot) due to the lack of PVY infection on those clones. The viral infection affects the development of the plant, among other symptoms that negatively affect the yield (Hooker, 1990).

The potato growers that adopt the current recommended technology can use the same seed for three to four crop cycles maximum (Brune et al., 1999). The cost of the seed potato represents up to 35% of the total production cost. Due to the relatively high cost of the seed, small growers tend to reuse the same seed for several crop cycles, or even use tubers of unknown origin and unknown health status as seeds to reduce production costs. However, this practice results in the continuous reduction of the yield. For these growers, the adoption of the GM potato with resistance to PVY will allow the repeated use of the same seed for more cycles without loss of its health status, as reinfection with PVY will not occur.

Further analyses to determine the environmental impact and the food safety of the GM potato clones are underway to comply with the Brazilian law, for their application for commercial release.

Table 1. Plants of potato clones infected with *Potato virus Y* in 2004, 2005 and 2006.

Clones[(1)]	Infected plants (%±SE)		
	2004	2005	2006
1P	0	0	0
63P	0	0	1±1.0
Aa	45±4.8	40±10.7	48±9.2
Ab	45±4.8	80±6.7	90±4.5

[(1)]Aa, nontransformed control in the first year in the field; Ab, nontransformed parental plants control, cv. Achat, with one to three years in the field.

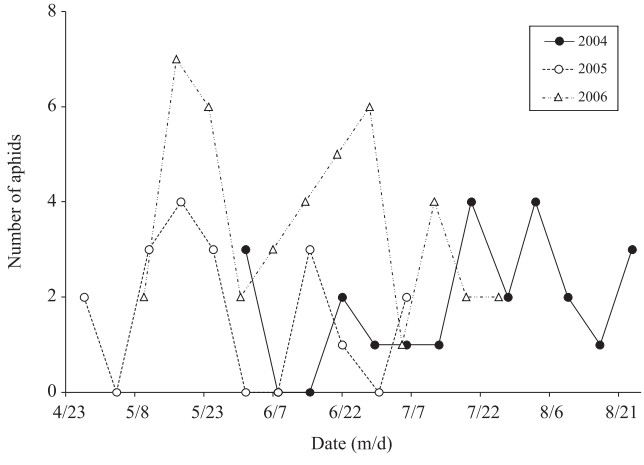

Figure 2. Number of aphids caught on a weekly basis by the green water trap in 2004, 2005 and 2006.

Conclusion

The genetically modified potato clones 1P and 63P containing the coat protein gene of *Potato virus Y* (PVY) are resistant to PVY, as confirmed by a three-year assay under field conditions.

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