RESISTANCE TO PLRV INFECTION IN TRANSGENIC 'RUSSET BURBANK' AND 'RANGER RUSSET' REGENERANTS TRANSFORMED WITH THE CCAT PROTEIN GENE OF PLRV.

by
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Introduction

The breeding of new potato varieties has some new techniques available from biotechnology. One of these is the insertion of foreign genes into potatoes to obtain greater resistance to pathogens. We can insert genes from the actual causal agents disease and obtain potatoes that express a gene involved in the disease process. This gene interferes with the pathogen by expressing itself in the wrong way or at the wrong time. In this report, I will describe work in which the gene that encodes the coat protein of potato leafroll virus (PLRV) was isolated and engineered so that it would be expressed in plants. The gene was vectored into potato by use of the Agrobacterium infection system. This process results in the insertion of a piece of bacterial DNA, also containing the coat protein gene, into a potato chromosome (see Figure 1). The virus gene is expressed as if it were one of the potato's genes. The functioning of the gene is a stable process and it will be passed along to progeny potato plants whether multiplied clonally or sexually. It is possible to obtain new varieties that have the inserted gene and no other observable changes in morphology or performance. Thus it is a way of making small changes in varieties that the industry is already accustomed to, and, in fact, would prefer to continue using if certain problems associated with that variety could be lessened.

The expression of a viral gene like the coat protein gene of PLRV should not change the culinary constitution of the potato in any way. This technique is a logical extension of traditional breeding. Traditional breeding has made use of crosses between familiar varieties and breeding clones throughout history. In modern times, this process has embraced the wild relatives of potatoes as sources of valuable resistance factors. The end product is always a potato that yields, processes, and tastes like an acceptable potato variety even though it has "exotic" blood in it. Gene engineering increases the options we have to improve potato with "new blood," so to speak. Gene transfer also makes possible the preservation of the entire variety genotype while adding a trait, something that is not possible with traditional breeding procedures.

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Gene Insertion and Screening:

Transformation of potato was initiated by dipping pieces of stem into a suspension of Agrobacterium harboring the vector plasmid. The stems were cultured on media that contained kanamycin, an antibiotic that is used to select transformed plants in tissue culture. The first noticeable change was the formation of a disorganized tissue called callus. After some weeks, differentiated leaves and stem developed on the callus. These shoots were removed and cultured sterilely in glass tubes. The stems developed roots, and from then on behaved very much like normal potato plants that can be grown in soil, will reach a normal size and will produce tubers which are true to the type of the original variety.

The shoots were assayed to see if the inserted gene was being read (transcribed) properly. Transcription was found to be occurring satisfactorily in a number of plants, and twelve transformants were selected for more detailed studies.

The clones that were selected included six regenerants from 'Russet Burbank' and six from 'Ranger Russet.' We multiplied these using micropropagation techniques and transplanted cuttings into small pots. We placed green peach aphids carrying PLRV on the plants, let them feed for a week, and then killed the aphids with insecticide.

The concentration of virus in the plants was determined by use of an immunologically based test called ELISA. This test is normally used to detect virus. This presence/absence type of assay is just one of the uses of ELISA; it can also be used to measure the amount of virus in a plant if different concentrations of purified virus are placed on the plate with test samples. This is how we employed this test for our purposes.

Results and Discussion:

Twelve regenerants, six from Ranger Russet and six from Russet Burbank were selected on the basis of positive Northerns displaying strong signals. These twelve clones and the untransformed clones were subjected to aphid inoculation.

Although all plants were exposed to the same inoculum challenge not all plants became infected. The results in Table 1 are presented as means of all plants that were considered infected. Clones RR612.3.6 and RB612.55.2 had four infected plants out of eight inoculated each. The untransformed controls became completely infected. Three measurements (primary) of titer were made on the plants that were taken out of tissue culture. These are termed measurements of primary titer because they are from infection the primary plantlets. These plants produced tubers, and the tubers were replanted after sprouting. One measurement was taken on these tuber-derived plants. This is referred to as the measurement of secondary titer (See Table 1). The untransformed controls were the highest in titer if the average of the three measurements was taken into consideration.

Four transformants of Russet Burbank and two of Ranger Russet were significantly lower than controls in the primary infection titer. The same clones were significantly less than control Russet Burbank evaulated for secondary infection titer, whereas two additional Ranger Russet clones had lower secondary titers than the controls.

The correlation between primary and secondary infection titers was significant for Russet Burbank, but not significant for Ranger Russet. Therefore low titer in the primary infection is not always followed by low infection in the plants derived from tubers in clones derived from Ranger Russet. There is a significant relationship between primary and secondary titer in the case of regenerants from Russet Burbank. (See Figure 2).

Protein is the end-product of a two step process. First the DNA of the gene is transcribed into a fragment of RNA which is called "messenger" RNA (mRNA). The mRNA is then "translated" into a protein. Our plants did not produce coat protein at a level that we could detect. One of the goals of this work was to induce the plant to produce the actual coat protein of the virus. We did not succeed in this goal, but it appears that protein synthesis is not necessary. We were able to have an impact on the virus with transcription of mRNA alone.

The PLRV virus has a single strand of RNA which is released into the plant cell. This is called the positive strand. In order to produce more copies of the RNA for progeny virus particles, a complementary copy of the positive strand must be replicated. This is called the negative sense strand, and is the template for future positive-sense strands. The explanation for titer reduction may lie in the interference of the plant produced mRNA which is exactly complementary the coat protein codon of the negative sense strand of the RNA that PLRV.

Table 1. Titer of PLRV in potato foliage expressed as nanograms virus per gram fresh weight of foliage (ng/g FW). Primary titers were taken at five (first), seven (second) and nine (third) weeks from initiation of inoculation. Secondary titer was measured in plants derived from tubers harvested from original regenerants at eight weeks.

Clone	Nanograms virus per gram fresh weight of foliage			
	Primary			Secondary
	35 days	49 days	63 days	56 days
cv. Russet Burbank				
RBUT	715 a[1/]	901 a	856 ab	1332 ab
RB612.1.4	462 abc	768 a	590 abcd	1485 a
RB612.3.1	149 c	211 c	273 cd	259 c
RB612.5.3	731 a	783 a	1043 a	1055 abc
RB612.46.1	284 bc	371 bc	398 d	204 c
RB612.55.2	216 c	214 c	198 d	560 bc
RB612.77.1	164 c	212 c	321 cd	609 bc
cv. Ranger Russet				
RRUT	749 a	901 a	1086 a	885 abc
RR612.2.3	392 abc	694 ab	665 abcd	540 bc
RR612.3.6	235 bc	263 c	379 bcd	273 c
RR612.12.1	345 bc	566 abc	772 abc	730 abc
RR612.21.1	290 bc	291 c	389 bcd	405 c
RR612.27.1	574 ab	792 a	737 abc	521 bc
RR612.28.2	337 bc	713 ab	831 ab	1529 a

[1/]Means not sharing the same letter are significantly different at the $P < 0.05$ level, using Duncan's Multiple Range Test.

Figure 1. Diagram of the Agrobacterium infection gene transfer method. The gene of economic interest is placed in the Transfer DNA of the vector plasmid which is then inserted into the potato chromosome.

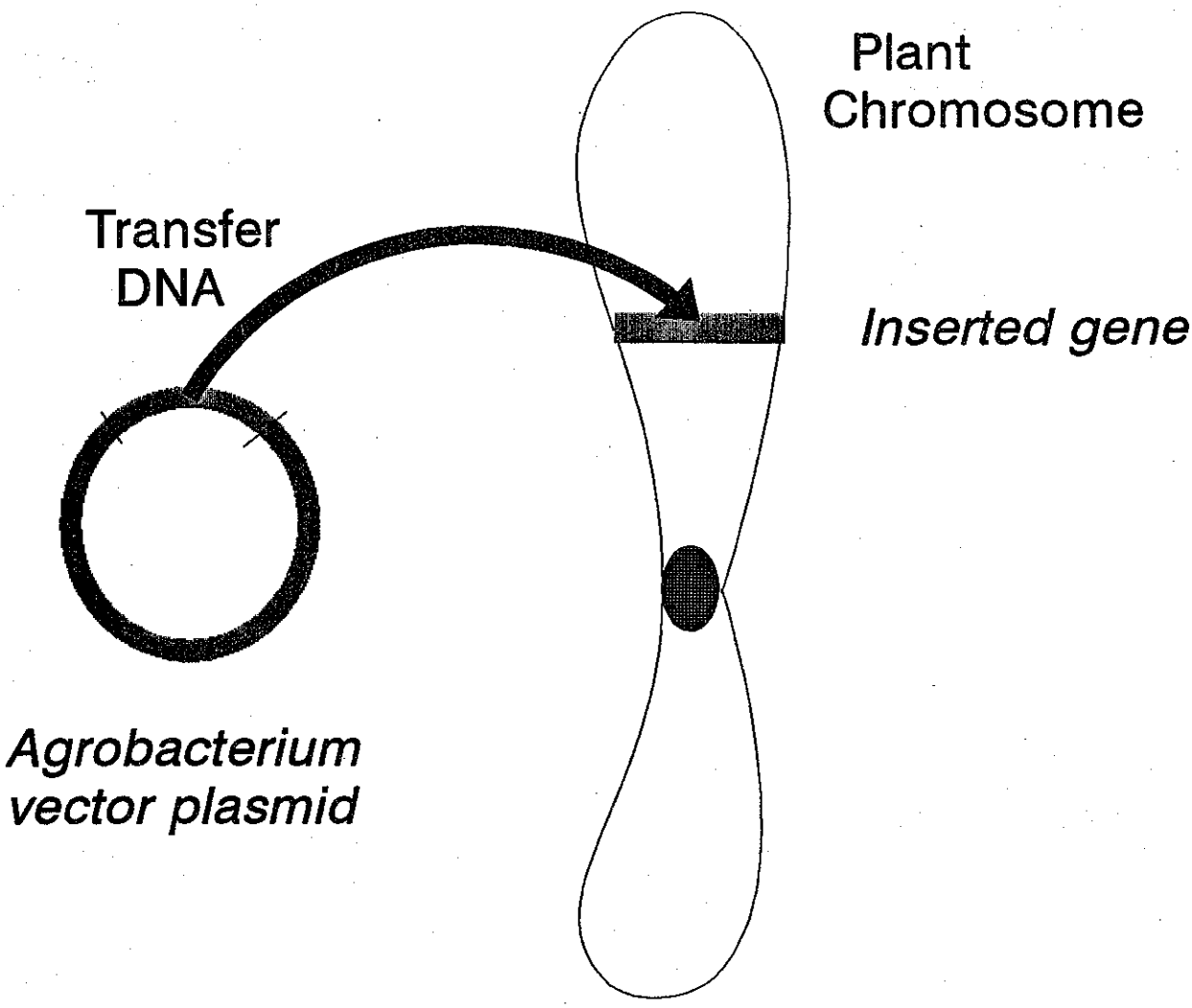

Figure 2. Plot of primary titer versus secondary titer for 'Ranger Russet' (open square) and 'Russet Burbank' (closed square). The correlation for 'Russet Burbank' (regression line is also presented) clones is significant, while that of 'Ranger Russet' is not.