MOLECULAR CHARACTERIZATION OF THE POTATO LEAFROLL LUTEOVIRUS AND DEVELOPMENT OF GENETICALLY ENGINEERED RESISTANCE

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Introduction

Potato leafroll luteovirus (PLRV) is difficult to control and is responsible for significant worldwide economic losses in potato (*Solanum tuberosum L.*). In addition to reducing yields, PLRV reduces the quality of several potato varieties by causing net necrosis of the tubers. Luteoviruses are phloem-limited and transmitted by aphids in a circulative nonpropagative manner.

PLRV particles are composed of a single-stranded positive-sense RNA molecule and 180 identical coat protein subunits.

Materials And Methods

Complementary DNA clones representing the 5883 nucleotides of a Canadian PLRV isolate were generated, restriction-mapped, and sequenced (1, 2). Three PLRV coat protein gene constructs were used to transform tobacco and the potato varieties Desiree and Russet Burbank via an *Agrobacterium tumefaciens* mediated gene transfer (3, 4). One construct possessed 12 nucleotides of the untranslated leader sequence 5' to the coat protein AUG and the other construct, which was also inserted in the reverse orientation to produce negative sense RNA, had 192 nucleotides from this leader sequence. Introduced as a chimaeric gene under the control of a duplicated CaMV promoter, transcription levels of the PLRV coat protein gene varied considerably between transformants.

Results and Discussion

Within one of the cDNA clones an open reading frame (ORF) encoding the 23 kDa coat protein was identified and further characterized (1, 2). Comparison of the deduced amino acid sequence with the coat protein of other luteoviruses showed significant homology. Other similarities included a 17 kDa ORF within the ORF encoding the 23 kDa coat protein and termination of the latter with an amber codon which is immediately followed by a large ORF capable of encoding a 56 kDa coat protein extension.

Results show that significant levels of sustained resistance were obtained with each construct resulting in PLRV titres as low as 1 % of the level observed in controls as determined by enzyme-linked immunosorbent assays (3, 4). Virus transmission from PLRV inoculatec transgenic Russet Burbank was reduced substantially and was correlated with virus titre. Field trials have been used to demonstrate that the incidence and level of net necrosis caused by PLRV is reduced in the genetically modified Russet Burbank. A line of the genetically modified Russet Burbank with agronomic characteristics of the original variety has been selected for release.

Both the pattern and level of protection were the same for constructs producing positive and negative-sense RNA suggesting a similar mechanism of resistance. This resistance will have practical applications for the control of PLRV and may also help understand the mechanisms of virus infection. We are currently looking at other PLRV genes and preliminary results indicate that expression of the 17 kDA protein also confers resistance.

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2. Keese P *et al.* Journal of General Virology 1990;71: 719-724.
3. Kawchuk LM *et al.* Molecular Plant Microbe Interactions 1990;3: 301-307.
4. Kawchuk LM *et al.* Molecular Plant-Microbe Interactions 1991;4: 247-253.