Developing 'silencing-based' GM resistance to viruses

H. Barker & K.D. McGeachy

Crops that are grown from vegetatively produced planting material (such as potato) can suffer with virus diseases that accumulate and increase each planting season. Because aphids transmit many of the important viruses that infect potato, substantial amounts of insecticides are applied to seed and ware potato crops to kill aphids, but frequently are only partially effective in controlling virus spread. Growing virus resistant cultivars could provide an economic and environmentally acceptable control method but incorporation of resistance genes by conventional breeding is slow and expensive and therefore few cultivars contain resistance genes to more than one or two viruses. Use of genetic manipulation to introduce transgenes, based on expression of plant virus sequences, that induce resistance is one means by which these problems can be overcome. More specifically the development of transgenes based on expressing short non-translatable virus RNA sequences simultaneously in sense and antisense orientation has been found to be a promising way to overcome some of the problems associated with the first generation of virus resistance transgenes. Expression of sense and antisense RNA from the transgene induces a strong form of virus resistance through an RNA-mediated defence mechanism that is based on post transcriptional gene-silencing. We are developing this technology in order to obtain resistance to a wide range of potato viruses and have already developed resistance to one of the most important potato viruses, *Potato virus Y* in tobacco, an excellent model species for virus resistance work.

Figure 1 A transgenic plant was grafted with a PVY^{O} infected shoot from a wild-type plant (left) and was allowed to remain on the plant as it grew. Three weeks after inoculation the shoot from the transgenic plant (right) was found to be virus-free and remained non-infected as the plant grew. Typical mottling symptoms of PVY^{O} can be readily seen in the wild-type shoot (left), but not in the resistant transgenic shoot (right).

A sequence encoding approx 800 nucleotides from the genome of an O strain of PVY (PVY^{O}) was cloned and incorporated into a plant transformation vector in sense and antisense orientations and used to produce transgenic tobacco lines. The sense and antisense transgenes were introduced into the same progeny plants by cross-fertilisation. Of 30 F_1 lines containing the both transgenes, 9 lines were resistant to mechanical inoculation with the homologous PVY^{O} isolate. Some resistant lines were also tested using the most severe form of challenge inoculation that can be used, namely graft inoculation (Figure 1). Despite the severity of the challenge inoculation, plants remained non-infected. Resistance was assessed in a number of ways; plants developed no symptoms, virus could not be detected by enzyme-linked immunosorbent assay, and attempts to recover infectious virus by inoculation of sap from resistant plants were unsuccessful.

This deliberate targeting of gene silencing-based virus resistance has been successful and has provided information on how to extend the method to other viruses. More advanced transgenes that are designed to induce silencing resistance are being constructed and tested at the moment. It is hoped that the technique will eventually produce a universal means by which resistance can be induced against a wide range of viruses and can be applied to many crops.