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VIRUS RESISTANCE BY GENETIC ENGINEERING: PROGRESS WITH WHITE CLOVER MOSAIC VIRUS

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ABSTRACT

Recent advances in gene transfer in plants made it possible to produce new varieties of crops with novel characteristics using genetic engineering. One of the most significant findings has been the recent report that introducing one gene from a virus into the plant is able to make the plant resistant to that virus. *Abel et al.* (Science, 1986) showed that the coat protein gene of tobacco mosaic virus (TMV) introduced into tobacco confers a good level of resistance to subsequent challenges by TMV. These results are reviewed, and progress is reported from our project aimed at engineering resistance to while clover mosaic virus

Genetic Engineering of Plants

The first successful genetic engineering of plants was reported in 1983, when three groups announced that they made plants cells resistant to an antibiotic called kanamycin (reviewed by Fraley *et al.*, 1986). Stable inheritance of these introduced genes by progeny plants was announced the following year. Progress since the initial reports has been astonishingly rapid. We are now at the stage where the capacity to genetically engineer certain species of plants is possible in any laboratory with tissue culture facilities, and can be routinely performed as part of undergratuate teaching courses. Although certain species, most notably the cereals, are proving rather difficult, it seems probable that the research effort currently being expended in their direction will overcome these problems.

In essence, genetic engineering provides the capacity to add single genes to a plant without otherwise altering its genetic characteristics. Because the genetic code is the same in all organisms, the single genes to be added can come from any source: other plants, animals, bacteria, or fungi. It will usually be necessary to modify the gene so that it is suitably expressed in its new host plant. For example, imagine that we wanted to engineer a sweeter-tasting kiwifruit. The sweet-tasting gene would need to be expressed in the kiwifruit berries when they are ripe; nothing would be gained if the sweetness gene was turned on in leaves or roots of the kiwifruit vines.

What traits can be engineered into crops?

One problem with the rapid progress in our ability to introduce genes into plants is that this capacity has temporarily outrun our knowledge to apply it. In particular, there are not many single genes around that will do something useful for crop plants. Making kiwifruit kanamycin-resistant won't sell many extra fruit in Japan! In consequence there is currently a large number of research programmes aimed at isolating useful genes that can subsequently be introduced into plants.

Genes that confer herbicide resistance were the first such useful genes to be reported. The earliest work (Comai *el al.*, 1985) utilised a gene from bacteria that had been mutated to give resistance to the herbicide glyphosate, marketed under the trade name Roundup. Since that time a number of different herbicide-resistance genes have been developed by different groups. The selectivity provided by these genes will allow farmers much greater flexibility in the choice of which herbicide application patterns in favour of post-emergent applications. Perhaps more importantly, they will result in the widespread use of herbicides that are much more effective and much safer environmentally than those currently in use.

New insect resistance genes for plants have also been reported. These genes have been isolated from bacteria which have evolved proteins that are toxic to various species of insect. Producing the proteins in the leaves of tobacco plants causes insects that feed on the leaves to die (Vaeck *el al.*, 1987). In addition to these toxin genes, various feeding deterrents are known to be present in many plants seeds. One of these, a trypsin inhibitor from cowpeas (which apparently blocks the insects digestive system), has been reported to reduce insect feeding on plants engineered to contain the inhibitor protein (Hilder *et al.*, 1987). Clearly insect resistance is another promising area where genetic engineering can reduce crop losses and reduce the incidence of hazardous chemicals in the environment.

In this review, we will concentrate on a third type of gene that has been reported to produce a useful phenotype in plants. Last year, researchers at Monsanto, in collaboration with a group at Washington University in St Louis, published a paper which is already proving to be a landmark in the area of plant genetic engineering (Abel *el al.*, 1986). They reported that they could get resistance to a virus in plants that had been engineered to contain one of the proteins from that virus. This result contains an elegant twist to it; the researchers used the pathogen itself as a source of a resistance gene.

Virus coat proteins are resistance genes

Tobacco mosaic virus (TMV) is the best characterised plant virus. It contains three genes on its RNA (see Fig 1a): one for the enzyme with which it replicates, one for a protein required for its movement through a plant, and a third for the so-called coat protein. The coat protein provides the packaging in which the viral RNA encapsidates itself, so that it is protected when it is outside the cell moving between plants. The coat protein may also have other roles in the viral life cycle (for example, it appears to help in cell-to-cell movement).

Abel and coworkers cloned a DNA copy of the TMV coat protein gene, and inserted it between signals that would ensure

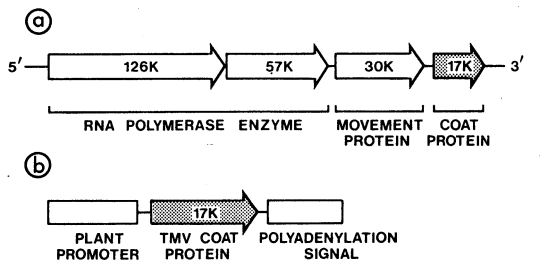

Figure 1. A TMV resistance gene. Part a shows the linear RNA genome of TMV, with the location of genes shown by arrows, and their probable identity indicated below. Part b shows the hybrid gene used by Abel *et al* (1986) to obtain resistance to TMV. A cloned DNA copy of the TMV coat protein was placed downstream of a plant promoter (the 35S promoter of cauliflower mosaic virus), and in front of a polyadenylation signal (from the napaline sythase gene). These signals drive expression of the TMV gene in most cells of engineered plants.

that the TMV protein was expressed after it was introduced into plants by genetic engineering. This step is illustrated in Fig 1b (ironically, part of these signals were obtained from yet another plant virus, cauliflower mosaic virus). They showed by using antibodies against the TMV coat protein that the gene was expressed in the leaves of the engineered plants. They then inoculated progeny of the engineered plant with TMV. Progeny that contained the TMV coat protein gene (¾ of the total, as expected) showed a good level of tolerance to TMV, while progeny that lacked the coat protein gene (¼) were normally susceptible. The level of tolerance varied with the strength of the viral inoculum. With low concentrations of TMV applied to the leaves, the engineered plants often did not develop any symptoms at all. At higher concentrations the resistance tended to break down, and a proportion of the engineered plants did develop infection, although (compared with the control plants) it was delayed.

The finding that viral resistance can be obtained by introducing a copy of the viral protein into plants has been extended to four unrelated plant viruses: alfalfa mosaic virus (Tumer *el al.*, 1987; Loesch-Fries *el al.*, 1987; J. Bol, personal communication), cucumber mosaic virus, potato virus X (R. Fraley, personal communications), and tobacco rattle virus (J. Bol, personal communication). The level of protection is directly related to the amount of coat protein produced by the engineered plants: higher expression results in higher tolerance to the virus. The mechanism of resistance has not been established as yet. The most commonly accepted idea is that there is a cellular "receptor" in susceptible plants that binds to the viral coat protein during uncoating of the virus in the cell. Expressing the coat protein in the engineered plant is hypothesised to block the receptor, prevent uncoating, and therefore make the plant resistant to infection.

The success of experiments with a number of different plant viruses suggests that this strategy may provide a general mechanism for obtaining viral resistance. The spectrum of protection provided by particular coat protein genes has not been clearly established, although it appears that closely related viruses will be inhibited (R. Fraley, personal communication). A crucial unanswered question is whether the resistance obtained is sufficiently high to be useful under field conditions. Plants containing viral coat protein genes are currently in field trials in the USA to determine this point.

Progress with white clover mosaic virus

Plant Disease Division has been working for several years on white clover mosaic virus (WClMV), which is extremely widespread in New Zealand pastures. It causes mosaic and mottling in white clover plants, and reduced dry matter production and nitrogen fixation in field trials with spaced plants. As with most viruses, the extent of the losses from the endemic infection is very difficult to quantify precisely, but it is probable that they are considerable. There is no known resistance gene available for breeders to incorporate into their new clover cultivars.

These features caused us to choose this virus for a genetic engineering approach. The project was initiated during 1985, before the results of Abel *el al* (1986) were known, since it was already apparent at that time that this approach stood a good chance of success. An additional consideration was that Derek White of Grasslands Division of the DSIR had developed techniques to modify white clover using genetic engineering, so that any gene could be introduced into new varieties.

Very little is known about the structure of any members of the potexvirus group (to which WClMV belongs). Before any engineering could be attempted, it was necessary to determine the nucleotide sequence of at least part of the virus in order to locate the coat protein gene. We have cloned and sequenced the complete WClMV genome, the first member of this important viral group to be so characterised.

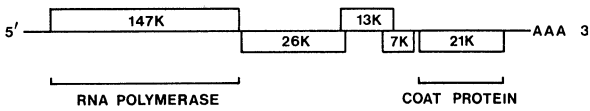

Figure 2. The genome of white clover mosaic virus. The organisation of open reading frames (probable genes) on the WClMV RNA is shown by boxes, with the identity (where this is known) indicated below.

The virus has at least five genes, as indicated in Fig 2. We have confirmed the identity of the coat protein gene by comparison to publish data, and by directly expressing a cloned copy of the gene *in vitro*. The protein expressed in this way is the same size as authentic coat protein, and reacts to antibody prepared against purified virus. Having established that we have a genuine clone of the coat protein, we are now in the process of placing the gene between appropriate signals so that it will be expressed in plants. The resulting gene will then be introduced into plants to see whether it confers resistance to white clover mosaic virus.

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