

transgene could activate retrotransposon RNA, which becomes heteroencapsidated and transmitted horizontally to other species.

1.5. Risk Reduction and Control

The main question to be addressed is whether the risk on field release of the transgenic plant is significantly more than the risk from the nontransgenic plant. To answer this, a wide range of controlled release experiments have been performed.

It is likely that it will take some time for a full risk assessment on the viral transgenic plants to be performed and commercial and other pressures will be very strong for field release. There are two approaches to risk reduction and control that can be put into effect relatively soon. One is to use biological containment (7). In this approach, the region(s) of the transgene giving the undesirable properties are deleted, while retaining those that give the desirable protection property. A good example of this approach is found in the potyviral CP, which has an amino acid triplet (asp, ala, gly; DAG), which is involved in the interactions with the aphid vector (38–40). Mutations of this motif, or its removal (which does not affect the protection ability of the CP; ref. 41), would render heteroencapsidation with the transgene unable to confer aphid transmissibility on the superinfecting virus. Much more difficult is to avoid recombination, but targeted research on this may reveal methods.

The second approach is to design methods for monitoring the effects of field release. For small-scale releases, it is relatively easy to design monitoring procedures for analyzing pollen flow into related weeds and for detecting heteroencapsidants or recombinants. This will be much more difficult, if not impossible, for large-scale releases, in which the approach should be to educate farmers and extension service personnel to identify any unusual event that might be associated with the transgenic plants. This will be the challenge for the future.

1.6. Protocol for Detecting Heteroencapsidation

The basis of this protocol, modified from that of Candelier-Harvey and Hull (29), is to immunecapture the heteroencapsidant, using an antiserum to the transgenically expressed CP, and to detect any encapsidated superinfecting viral (or retrotransposon) RNA by PCR.

2. Materials (All chemical Analar grade from BDH, Poole, UK, or Sigma-Aldrich, Poole, UK)

2.1. Solutions

1. Coating buffer: 1.59 g Na_2CO_3 , 2.93 g NaHCO_3 , 1 L H_2O , pH 9.6.
2. Phosphate-buffered saline (PBS): 4.6 g Na_2HPO_4 , 3.45 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 8.5 g NaCl , 1 L H_2O , pH 7.2.