12. Heteroencapsidation in Plant Virus Infection

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Introduction

One of the most promising recent advances in crop protection is the use of pathogen-derived sequences to protect plants against virus infection. In 1986, Powell Abel *et al.* first showed that tobacco plants transformed with the coat protein (CP) gene of tobacco mosaic virus (TMV) subsequently showed resistance to infection with the virus. Since this time, coat protein-mediated protection against many other plant viruses has been shown as has resistance conferred by other viral transgenes.

While the mechanism of pathogen-derived resistance is still not fully understood, plants expressing viral coat proteins are already being deployed as a cost-effective method of control. It is essential that any potential environmental risk from the release of CP transgenic plants be addressed now, before any potential damage may be caused. The main areas of concern are heteroencapsidation of superinfecting virus in the transgenically expressed coat protein and recombination between the genome of the incoming virus and the transgenic sequences.

Heteroencapsidation is widespread in the natural environment when mixed infections occur and has been documented in economically important plant viruses *eg* barley yellow dwarf virus in *Avena sativa* (Creamer *et al.*,1990 ; Wen and Lister, 1991). By such interactions coat protein properties of one virus can be conferred on a different virus, thereby potentially altering vector relationships and possibly the ability for a virus to move in a non-host plant. Every infection of a CP transgenic plant is essentially a double infection and hence may result in the production of progeny virus with novel phenotypes. Heteroencapsidation in CP transgenic plants has been shown for related (Lecoq *et al.*, 1990) and unrelated viruses (Candelier-Harvey and Hull, 1993).

The aim of our work is to study basic factors which could lead to, and affect potential levels of, heteroencapsidation in CP transgenic plants. Thus, viruses which have different structures and different capsid stabilization have been chosen as donors for transgenic CPs and superinfecting viruses. This poster reports initial observations with Alfalfa mosaic virus (AIMV) as donor of CP and superinfection by a range of viruses.

Results

- transgenically expressed AIMV CP heteroencapsidates CMV but not TMV, TRV or PEBV (Table 1).
- CMV of subgroups I and II heteroencapsidates in AIMV CP. IC-RT/PCR shows that for strain Q (subgroup II) 60% of plants sampled showed heteroencapsidation (Fig. 1), while for strain lx (subgroup I) 92% of AIMV CP transgenic plants heteroencap-

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sidate.

- quantification of heteroencapsidation of Q strain CMV (Fig. 2) indicates that 1 % of virus particles are heteroencapsidants *ie* CMV RNA contained within AIMV particles.
- the amount of heteroencapsidation varies within transgenic plants with leaf position, this is shown in Fig. 3.
- the amount of heteroencapsidation in each leaf changes with time (Fig. 3).

Conclusions

- transgenically expressed coat protein is capable of heteroencapsidating superinfecting viral genomes.
- it appears that the relationship between superinfecting and coat protein donor virus determines whether heteroencapsidation will occur. AIMV is closely related to CMV (Family Bromoviridae) but not to TMV (Genus Tobamovirus) or TRV and PEBV (Genus Tobravirus).
- heteroencapsidation may be related to particle stabilization factors. AIMV and CMV stabilize their isometric and quasi-isometric particles by protein/RNA interactions while TMV, TRV and PEBV use protein/protein interaction to stabilize their rod-shaped particles.
- heteroencapsidation is transient, reaching a peak at 14dpi over the whole plant. The time of the peak differs according to leaf position and appears to be related to virus concentration.

Future work

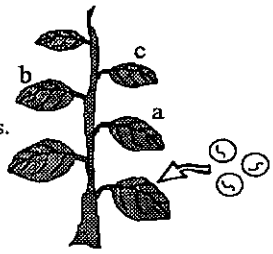
We will continue our studies of the factors affecting heteroencapsidation in transgenically expressed coat protein by superinfecting with viruses from other groups, particularly those closely related to AIMV with similar particle shapes and stabilization strategies. We will also employ different donor transgenes *eg* nepovirus (isometric particles stabilized by protein/protein interaction) and tobamovirus (rod-shaped particles stabilized by protein/protein interaction) to investigate whether similar factors influence all transgene/superinfecting virus associations.

References

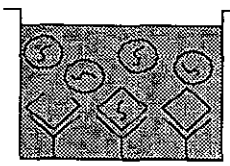
1) Candelier-Harvey, P. and Hull, R. (1993) : Transgenic Research **2**:227-235.
2) Creamer, R. and Falk, B.S. (1990) : Journal of General Virology **71**:211-217.
3) Lecoq, H., Ravelonandro, M., Wipf-Scheibel, C., Monison, M., Raccah, B. and Dunez, J. (1990) : Molecular Plant-Microbe Interactions **6**:409-406.
4) Powell-Abel, P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T. and Beachy, R.N. (1986) : Science **232**:738-743.
5) Wen, F. and Lister, R.M. (1991) : Journal of General Virology **72**:2217-2229.

Experimental Approach

Tobacco plants expressing AlMV coat protein infected with a range of plant viruses.

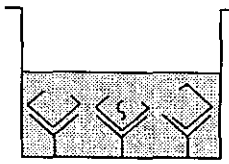


Samples from systemically infected leaves a, b, and c collected at 14 and 21 days post inoculation.



Sap extract from virus infected transgenic plants added to anti-AlMV antibody coated microtitre plate

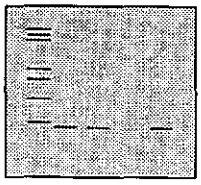
Unbound, native virus washed from the microtitre plate.



Reverse transcription of heteroencapsidated virus carried out in the microtitre plate.

Synthesised cDNA recovered from the microtitre plate and subjected to PCR.

PCR products are run on agarose gels, stained with ethidium bromide and samples positive for heteroencapsidation are identified.



The amount of heteroencapsidation in positive samples is assessed by competitive, quantitative RT/PCR, carried out using a non-homologous competitor fragment.
The point of equivalence between the target and competitor can be easily read from the gel without further treatment.

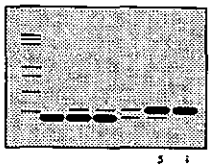
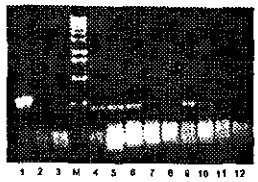


Figure 1.

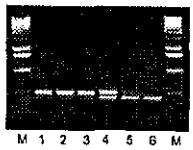
Agarose gel of RT/PCR products following immune capture



Lane 1 - non-transgenic plant infected with AlMV (positive control for IC-RT/PCR)
Lane 2 - non-transgenic plant infected with Q-CMV (negative control)
Lane 3 - uninfected AlMV CP transgenic plant
Lanes 4 to 12 - Q-CMV infected AlMV CP transgenic plants
Heteroencapsidation is indicated by the presence of a 436bp PCR product. The control sample (lane 1) shows a 514bp product from AlMV. M is the 1Kb marker.

Figure 2.

Agarose gel of quantitative RT/PCR of a heteroencapsidated sample



Lanes 1 to 5 - qRT/PCR of a constant amount of immune captured heteroencapsidatedCMV with decreasing amounts of the competitor fragment. (200, 100, 50 10 and 1pg).
Lane 6 - immune captured CMV alone (control)
The CMV target gives a PCR product of 436bp while the competitor fragment gives a 519bp product.
M is the 1Kb marker.

Figure 3.

Heteroencapsidation of Q-CMV in AlMV CP

Levels of heteroencapsidation change with leaf position and time post inoculation

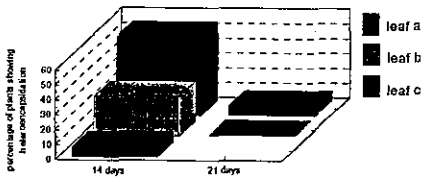


Table 1.

Can transgenic AlMV CP heteroencapsidate viruses from different groups ?

Superinfecting Virus	Heteroencapsidation in AlMV CP transgenic plant
Cucumber mosaic virus - strain Ix (CMV) subgroup I Cucumovirus (Bromoviridae)	Yes
Cucumber mosaic virus - strain Q (CMV) subgroup II Cucumovirus (Bromoviridae)	Yes
Tobacco mosaic virus (TMV) Tobamovirus	No
Tobacco rattle virus (TRV) Tobravirus	No
Pea early browning virus (PEBV) Tobravirus	No