

Durability of transgene-mediated virus resistance: High-frequency occurrence of recombinant viruses in transgenic virus-resistant plants

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Strategies to protect plants against viral infections

Plant viral diseases cause significant economic losses to agriculture. Genetic engineering has permitted the development of a new strategy for the protection of plants against viral diseases. This strategy involves the introduction and expression of viral genes in the plant genome (1). Two different types of viral resistance following the expression of a viral sequence can be observed in transgenic plants. In one case, the synthesis of a functional viral protein or alternatively a mutated functional viral protein is necessary. Coat protein-mediated resistance to Tobacco Mosaic Virus (TMV) (2) is a good example of such resistance. The second type of resistance can be obtained with untranslatable as well as translatable transgenes and occurs in plants accumulating only low levels of transgenic mRNA and protein. This kind of resistance, referred to as homology-dependent resistance (3), is exclusively RNA-dependent and is due to post-transcriptional gene silencing (PTGS), which specifically prevents accumulation of transgenic mRNA and of genomic RNA from the invading virus. The response seems to be triggered by the presence in the host's cells of double stranded RNA or aberrant nucleic acids. Plant factors able to recognize and degrade viral RNA in a sequence-specific manner have been postulated by Lindbo *et al.* (4). PTGS is a form of stable but potentially reversible epigenetic modification (5), which is triggered by highly expressed single-copy loci, poorly transcribed complex loci or transcribed inverted repeats.

During the last decade, numerous virus-resistant transgenic plants expressing PTGS against different viral genes have been produced. Coat protein (CP) gene-mediated resistance became the most frequently used strategy and several such plants have recently been approved for commercial release in the USA.

The occurrence of new recombination types

This new strategy, transgressing the natural genetic barriers between taxons, raises questions on biosafety (6). For instance:

Does the use of transgenic virus-resistant plants in our agro-ecosystem increase the frequency of appearance of new viruses with an altered host range or virulence?

This could happen by recombination between the transgenic mRNA and the genomic RNA of a virus able to overcome the resistance. This hazard involves a direct interaction between the transgene RNA and the invading virus genome (7).

Recombination is thought to be a key mechanism in RNA virus evolution and has been suggested to occur by template switching of the viral RNA-dependent RNA polymerase during viral RNA replication (8). Sequence data show that the exchange of genetic material has happened not only between related viruses but also between genomes from quite different taxa. The frequency of such

events in plants grown in the field in the absence of selection pressure has not been determined (9). However, during a short time frame and in a given ecosystem the consensus sequence of a particular viral strain is remarkably stable (even though micro-heterogeneity is always present in a natural population) (10).

Investigations on recombination events in potato and conclusions

By studying recombination between related potato virus Y (PVY) strains during double infection of transgenic tobacco plants resistant to PVY-N605 alone, we have found that, in up to 25% of the double-infected plants, the N strain had recombined with the O strains to eliminate the viral sequence determining resistance.

?? The selection in transgenic plants eliminating the parental strain allows the propagation of recombinant viruses to a detectable level. This was not the case in wild type plants. This way, new recombinant viruses with altered host range or virulence, may be spread in the environment when homology-dependent virus-resistant plants are used in agriculture.

?? Furthermore, we have shown that a compounding risk, neglected until now, is due to the fact that a transgenic plant expressing homology-dependent resistance provides a new environment with an increased selective pressure favoring the mutated virus produced by recombination between co-infecting viruses.

?? These kind of data should be considered whenever transgenic virus resistant plants are used in an agro-ecosystem and new agronomic practices will have to be developed in order to avoid the loss of resistance of the transgenic plants as well as the appearance of new viral strains.

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This topic was also the subject of the issue Nr 5 of the series „focus Bioécrité CH” published in French and German by the agency BICS. This publication is displayed at the Conference or can be sent by the Centre BATS.