

Rethinking the Risks of Viral Transgenes in plants

This commentary provides background for the new Bioscience Resource Project publication:

Latham J.R. and Wilson A.K. (2008). Transcomplementation and synergism in plants: implications for viral transgenes? *Molecular Plant Pathology* **9**(1) 85-103.

Part I: Transcomplementation and its implications

Today marks the online publication, in the journal *Molecular Plant Pathology*, of the Bioscience Resource Project's newest biosafety review: 'Transcomplementation and synergism: implications for virus-resistant transgenic plants?'

This review, which represents a conceptual reanalysis of the risks of viral proteins expressed in transgenic plants, is particularly timely because virus resistance and the biosafety of viral transgenes are currently under active discussion in more than one US regulatory agency.

The immediate causes of discussion are two recent events. The first is that, having convened an expert advisory panel, the EPA (US Environmental Protection Agency) is currently formulating a new policy for the regulation of transgenic viral coat proteins in plants (Federal register, Vol **72**, No. 74, April 18, 2007). The policy that the EPA is seeking to replace is the present case-by-case assessment of each new application. Instead, the EPA is now considering submitted comments on a new policy: a blanket approval for transgenic plants containing plant viral coat proteins (1).

The second event is the submission of the first application in ten years for USDA/EPA approval of a transgenic virus-resistant plant: a plum resistant to plum pox virus (designated the C5 or Honeysweet plum). This plum contains and expresses a full-length gene for plum pox virus coat protein (Petition 04-26401p).

The developments noted above are proximal causes, but the very existence of a regulatory discussion, and presumably the reason EPA felt the need to convene an expert panel at all, stems from the underlying fact that the use of intact viral transgenes to generate virus-resistant plants is controversial, and has been so almost ever since it was first proposed (de Zoeten 1991). Most often, objections and concerns have focussed on the now well-established fact that viruses recombine with viral transgenes (e.g. Greene and Allison 1994; Schoelz and Wintermantel 1993; Varrelmann *et al.* 2000). Recombination may allow novel viruses to be created and such viruses are known to be an important source of disease outbreaks (e.g. Dolja and Carrington 1992). This possibility prompted Mark Gibbs and colleagues to write in 1997: "only the foolhardy will have firm views on the likely outcome of the introduction of virus-resistant transgenic crops into agriculture." (Gibbs *et al.* 1997).

Recombination to create novel viruses has not been the only biosafety issue noted in the scientific literature however. A somewhat less well-known subject of discussion has concerned

the presence of transgenic viral proteins in resistant plants and specifically the possibility that infecting viruses (i.e. non-target viruses) may utilise these viral proteins to enhance their own infection, a process frequently referred to as transcomplementation. Despite various expressions of concern (e.g. de Zoeten 1991; Falk *et al.* 1995 and Power 2001), the potential biosafety implications of viral proteins in transgenic plants have never received detailed attention. Important questions such as: can transcomplementation occur in plants that are virus-resistant? What viruses can take advantage of transcomplementation, and by what proteins? What viral lifecycle traits can be enhanced by transcomplementation? And what hazards may result from transcomplementation? have never previously been comprehensively examined.

Our new analysis is intended to remedy that gap. In doing so it proposes that, in addition to conferring resistance to the target virus, inserting viral genes into transgenic plants (without specifically preventing protein expression from the transgene) is likely to confer upon these plants, either individually or at the population level, genetic vulnerability to viral infections. This conclusion is based principally on our comprehensive review of the large body of scientific literature which documents (a) plant viral synergisms, (b) gene exchanges between viruses and, (c) transcomplementation by transgenes. Approximately 150 such papers demonstrate that proteins from one virus very frequently facilitate infection by viruses of other species. This is true for a diverse array of viral proteins (including coat proteins) and for an equally diverse array of related and unrelated non-target viruses. Remarkably, a single transgenic viral protein in a single host species may confer enhanced vulnerability to well-established viral diseases and it even may confer susceptibility to viruses which do not normally infect plants (Cooper *et al.* 1995; Dasgupta *et al.* 2001).

Vulnerability to established and novel viruses represents perhaps the most well documented and simple consequence of transcomplementation. Transcomplementation however often takes more complex and subtle forms which nevertheless may have significant agronomic, epidemiological and phytosanitary implications.

To give an example, transcomplementation may enhance viral transmission through seeds (Kuhn and Dawson 1973). Seed transmission of plant viruses can be very significant in epidemiological terms and can also have important implications for the success of phytosanitary procedures in relation to international trade in seeds.

Part II: The need for new regulations

The conceptual basis of much transgenic regulation, including (unless it is changed) that by EPA over viral proteins, is the case-by-case assessment of each specific transgene. As with any a case-by-case approach, viral transgenes present a problem: how to accurately distinguish non-transcomplementing (i.e. no-risk) proteins from transcomplementing proteins? A substantial part of the review is devoted to an assessment of whether current knowledge of plant virology is adequate for this task. Thus the literature on transcomplementation demonstrates that for any single transgene in a single crop plant, there can be many distinct viruses whose infection can be facilitated by transcomplementation, and for each of these viruses there can be many potential

endpoints that may lead to plant pest risks (e.g. enhancement of insect acquisition, expansion of viral host range, more rapid infection, *etc.*). Added to this complexity are ecosystem variables such as the identity of neighbouring crops which are also important determinants of the results of transcomplementation. In other words a very large number of possible routes by which a plant pest risk may result from transcomplementation have been documented but there is little understanding of the specifics for each crop/virus/transgene/ecosystem combination.

In contrast to the solution agreed by the EPA's panel (blanket deregulation), we argue that the only solution compatible with current scientific understanding is to eliminate the possibility of transcomplementation entirely by preventing the expression in transgenic plants of plant viral proteins. Avoidance of expression would require insertion of multiple stop codons (or frameshift mutations) into transgenes of viral origin.

We are not the first to make this proposal (e.g. Miller *et al.* 1997; Hammond *et al.* 1999; Tepfer 2002) but our new analysis makes a compelling case that active regulatory protection is needed from transgenes which otherwise may worsen disease problems, invite crop failures and enhance the evolution and spread of new diseases. This case is made still more compelling since protein expression is not actually necessary to achieve transgenic viral resistance (e.g. Waterhouse *et al.* 1998; Masmoudi *et al.* 2002; Niu *et al.* 2006).

Thus the USDA and the EPA do indeed need a new policy for plant viral proteins. Our research suggests they need to abandon the old case-by-case approach and recognise that the most complete and rigorous analysis available shows that intact coat proteins (and indeed all functional plant viral proteins) require not blanket approval but blanket disapproval.

Footnotes

(1) The only exceptions are coat proteins from viruses not found in the US, which would still need case-by-case approval.

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