Biosafety
ISSN: 2167-0331

Ilardi, Biosafety 2014, 3:2
DOI: 10.4172/2167-0331.1000e151

Editorial Open Access

Could Transgenic Plants Expressing Virus-Derived Sequences Create New Routes for Virus Evolution?

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Rec date: Jul 13, 2014, **Acc date:** Jul 14, 2014, **Pub date:** Jul 18, 2014

Editorial

Virus resistance is a major objective in the development of genetically engineered crops. The biotechnological strategies developed to control plant viruses can be classified into three major categories based on the type of nucleic acid sequence used [1]. The first category makes use of sequences derived from the viral genomes; a concept known as pathogen-derived resistance (PDR). The second involves plant-derived genes; and the third is non-viral, non-plant derived sequences, such as antibodies directed against the specific virus. So far, the only solutions implemented in commercial agriculture have exploited the first strategy, PDR, and consist of fourteen events regarding seven plant species and eight viruses [2]. Shortly after the publication of the first scientific reports on transgenic plant resistant to virus, there were concerns regarding the potential impacts that these plants could have on the environment and in particular regarding the risk that, under particular conditions, they could lead to the generation of new viruses and thus new diseases. In this context, hetero-encapsidation, synergism and recombination, all well-known phenomena naturally occurring in nature when plants are simultaneously infected with more than one virus, were reanalyzed under a new perspective.

Hetero-encapsidation occurs when the genome of one virus is encapsidated by the coat protein (CP) of another virus. In transgenic plants this hetero-encapsidation could result from the CP expressed by the transgene. As the CP can carry determinants for pathogenicity and vector specificity, if hetero-encapsidation occurs, then some properties of the incoming virus could, in principle, change. For instance, a vector-non-transmissible virus could become transmissible after hetero-encapsidation with a transgenic CP derived from a vector-transmissible virus. This event, for instance, could enable a hetero-encapsidated virus to be transmitted to a different host plant. However, it is worth noting that changes in vector specificity and host range are a single-generation event; in the next viral generation the original CP will be synthetized and the parental virion restored. It follows that in the case of hetero-encapsidation, new viruses are not stably produced. In any case, the potential risk associated with hetero-encapsidation can be easily eliminated through modification of the CP gene, so that the transgenic protein is either unable to assemble viral particles, or no longer interacts with the vector, while still conferring resistance to the target virus.

In nature, synergism can occur in plants infected by two unrelated viruses. This results in an increase in symptoms and/or virus titer that neither virus is able to create independently. In the majority of cases analyzed, the phenomenon was mostly driven by the expression of viral RNA silencing suppressor proteins [3], which have never been used as a tool to confer transgenic virus resistance. However, since other plant virus derived proteins could be involved in this phenomenon, transgenic virus derived proteins might, under particular combinations, increase the susceptibility of heterologous viruses. It is therefore necessary to determine whether the viral proteins expressed from the transgene could induce synergistic responses. However, synergism in itself does not modify existing viruses nor does it create novel viruses.

A different scenario could arise if a gene flow occurred from a transgenic plant to an infecting virus by recombination. Recombination between viruses is one of the driving forces of virus evolution [4], and, in principle, it could also occur between virus-derived transgene transcripts and the genome of an infecting RNA virus. Because recombination alters the genome of the incoming virus, these changes are then potentially transmittable to the virus progeny. This raises the question of whether recombination in virus-infected transgenic plants could lead to the creation of novel viruses and thus to the emergence of new diseases. Many studies have focused on recombination in transgenic plants expressing viral genes. A crucial factor was found to be the selective pressure applied to the infecting virus. In fact, high selective pressure in favor of recombinant viruses enhances their creation, whereas, an extremely limited number of recombinant viruses have been found without selective pressure [5].

The emergence of a recombinant virus requires not only the creation of a new viable virus, but also that selection pressure gives it an advantage over the parental virus. So, are the recombinants occurring in infected transgenic plants different from those in non-transgenic plants? Could they have a selective advantage over the parental and "naturally recombinant" viruses? Experiments were therefore carried out showing that, at least in those conditions, the recombinants detected were very similar to those observed in nature [6,7].

Beside the initial PDR approaches that made use of the expression of viral proteins to confer resistance, the majority of the transgenic virus-resistant plants are now obtained through RNA silencing technology. In this case any viral genome sharing a sequence homology with the virus-derived transgene will be the target of the degradative RNA silencing mechanism activated by the expression of double-stranded virus-derived transgenic RNA molecules [8,9]. RNA silencing does not require the expression of transgenic proteins, thus circumventing the possible risk of complementation, hetero-encapsidation and/or synergy on behalf of incoming viruses. In addition, the very low RNA steady-state level of transgenic transcripts as well as their molecular structure (dsRNAs) reduces the likelihood, if any, of a hypothetical recombination between the transgenic RNA and the genome of an incoming virus. In this context, a minor event to be considered is if the breakdown of transgenic RNA silencing, by infection with a non-target virus, results in an increase in the

transgenic dsRNA steady-state level. The ability of a heterologous virus to overcome transgenic RNA silencing has been shown in some cases but not all [10,11]. If the increase in the transgenic RNA level occurs it could, in principle, enhances the very low chance that transgenic dsRNA could be used as a substrate for recombination. However, as mentioned above, RNA silencing does not require the expression of proteins. Therefore to prevent a recombinant event incorporating a functional gene sequence, it is only necessary to introduce a frame-shift or stop-codon in the transgene sequence used.

Based on current scientific knowledge it seems that, if well-conceived, virus-derived transgenic plants, especially those based on the RNA silencing technology do not raise important biosafety concerns regarding the potential creation of new routes for virus evolution.

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