

Potential Ecological Risks of Transgenic Plants: the Particular Case of Plants Expressing Viral Genes

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Abstract

Tepfer, Mark. 1995. Potential Ecological Risks of Transgenic Plants: the Particular Case of Plants Expressing Viral Genes. Arab J. Pl. Prot. 13(1):27-20

Many laboratories, including ours, have introduced viral sequences into the genome of host plants as a means of creating artificial virus resistance genes. These genes are of particular interest in the numerous cases where natural resistance genes are not available. It can be argued these new viral genes will have a positive impact on the environment, particularly if their use allows reduction in use of pesticides for control of the biological vectors of plant viruses (insects, fungi, nematodes...). However, there is concern that plants expressing viral genes could have a potential for negative environmental impact as well. These concerns are based on two classes of modifications of plant/virus interactions. One class of interactions is based on the ability of viral proteins synthesized by the plant to complement certain functions of the infecting virus. For instance, in plants synthesizing a virus coat protein, the infecting virus can be encapsidated in particles composed, at least in part, of the protein synthesized by the plant,

which can lead to changes in vector transmission. Complementation by viral proteins can thus lead to changes in virus phenotype. There is also evidence that plants expressing viral sequences can also be a source of modifications in the genotypes of infecting viruses. This would be the case if there is recombination between the viral sequences expressed by the plant and the infecting virus. Though potential mechanisms for changes in plant/virus interactions in transgenic plants have been identified, we know very little about how this might be translated into potential changes in the epidemiology of virus diseases. In some cases, for instance with coat protein genes, there may be ways of eliminating certain factors of risk. Further studies of potential mechanisms should allow us to devise other means of risk reduction or elimination.

Key words: Virus disease control, transgenic plants, plant transformation, risk assessment.

Introduction

Among the very first transgenic plants expressing a gene of potential agronomic interest were ones in which expression of viral sequences conferred virus resistance (33). Since then, development of virus resistance genes has been the most striking application of the concept of pathogen-derived resistance (38), and has led to a real flowering of resistance strategies based on expression of genes encoding viral coat protein, replicase, movement protein, as well as several types of non-coding viral sequences (for review, see 8, 48). It is clear that these new resistance genes have an enormous potential for positive impact on agriculture, since natural resistance genes are often not available for introduction into crop varieties by classical plant breeding. For instance, more effective virus resistance should allow reduction of the use of the pesticides that are currently necessary for controlling the biological vectors of virus transmission (insects, fungi, nematodes). Another positive impact will come from the importance of virus resistance in ensuring

crop yields. In Europe and North America, where over-production is often perceived as a greater agronomic problem, yield security would seem a less important priority, but from a world-wide perspective, increasing yield will certainly be necessary for insuring adequate nutrition. It is clearly of particular importance to assure yield security of local crops that play a key role in nutrition, which can be threatened by viral diseases (e.g. in Thailand, problems of papaya ringspot virus on papaya).

Nonetheless, in recent years, as transgenic plants have approached the stage of commercial release, several questions have been raised concerning biosafety issues specific to plants expressing viral genes (4, 16, 32, 44).

1. In cases where transgenic plants are sexually compatible with wild relatives, will transfer of the virus resistance gene to the wild relative enhance its fitness (i.e. weediness)?
2. It is generally accepted that recombination has

played an important role in plant virus evolution. Will recombination between the transcripts of the viral gene expressed by the plant and the genome of an infecting virus lead to new viral genomes?

3. In the specific case of plants expressing a cucumber mosaic virus (CMV) satellite RNA gene, will deleterious forms of the satellite RNA arise by mutation?
4. In plants expressing coat protein genes, will heterologous encapsidation of an infecting virus enhance its transmission by vector organisms? There are other less well studied cases of dependence of a virus on the presence of a second (helper) virus. Will expression in transgenic plants of sequences of the helper virus increase the gravity of infection by the dependent virus or enlarge its host range?

Attempting to evaluate the gravity of the potential risks associated with each of these questions is extremely complex, and often leads us to areas where current knowledge is insufficient. Without being exhaustive, each of these questions will be discussed here in turn, in an effort to present the complexity of the debate concerning the potential impacts of these risk factors. In some cases, means of reducing potential risk will be described.

Transmission of virus resistance genes to wild relatives

Particularly in the area where a crop plant originated, there are often wild relatives that can hybridize with the cultivated varieties. This fact is of course well known to plant breeders, since such wild relatives are one of the most important sources of genes of interest to be introduced into modern crop varieties by classical breeding. In Europe, which is the area of origin of relatively few crops, compatible wild forms exist for beet, kale crops (*Brassica* species), carrot, endive, etc. Clearly, in these cases, there is good reason to expect that there will be gene flow from transgenic crop plants to their wild relatives.

One would expect that gene flow to wild relatives would be relatively neutral in most cases, i.e. no selective advantage would be conferred by the gene transferred. However, in the case of resistance genes, whether they confer protection against a herbicide or a pathogen, one could well imagine that such genes could increase the fitness of the wild relative and thus enhance its weediness. In the case of virus resistance genes, how could one evaluate this potential impact? The first question is whether the wild relative is naturally sensitive or resistant to the virus. Obviously, in the latter case, one would not expect that transmission of a novel gene conferring resistance to the virus would confer any particular advantage. In cases where the wild relative is

sensitive to the virus, it would be wise to attempt to determine if the resistance gene could confer an advantage, even though it may be difficult to extrapolate from experimental conditions to the field. Nonetheless, it would be extremely pertinent to make crosses between the wild relative and the transgenic crop variety, and then evaluate the fitness of the hybrid, both when infected or not by the virus. To the best of my knowledge, this type of experiment has never been carried out. Though there has been considerable effort applied to seeking resistance genes in wild relatives to cultivated plants, more generally, there is a lack of information on the impact of viruses on the fitness of weedy plants.

To give an example, recent years have seen the spread in Europe of a new weedy type of beet, which apparently derives from spontaneous crosses between sugar beet and a wild relative (Boudry, unpublished data). It would be of great interest to know if the weedy beets are sensitive or resistant to the major viruses that affect sugar beet, and if they are sensitive, whether transfer of a virus resistance gene could enhance their weediness. This question has also been extensively discussed in the United States, concerning the potential impact of transfer of resistance genes from a transgenic squash rendered resistant to zucchini yellow mosaic virus and watermelon mosaic virus 2 to wild varieties of the same species.

A last point worth mentioning is that under certain circumstances there could be a distinct benefit if the wild relatives were rendered resistant, as well as the crop plant. This would be the case if the wild relatives were the only source of the virus infecting the crop plant. There may be a few situations where this would pertain, but the vast majority of known plant viruses have broad host ranges, often being able to infect hundreds of plant species.

Recombination between viral sequences expressed by the plant and an infecting viral genome

In the previous section I have discussed an area of potential risk that only concerns a small proportion of transgenic plants expressing viral genes, those that can cross with a wild relative. In contrast, questions concerning recombination in plants expressing viral sequences are relevant to all such plants.

It has long been recognized that recombination has played a key role in plant virus evolution (1, 9, 47). For instance, it has recently been proposed that the potyvirus genus arose from a fungus-transmitted ancestor virus infecting graminaceous monocots via capture by recombination of genes conferring transmission by aphids. Through a burst of speciation, this would have allowed potyviruses to infect nearly all dicotyledonous plants, becoming one of the most agronomically important virus groups (40). The rapid increase in recent

years in information concerning the sequence of viral genomes has led to a significant increase in the number of cases where recombination between virus genomes has almost certainly occurred, including luteoviruses (24, 34), tobnaviruses (13), potyviruses (2) and nepoviruses (Le Gall et al., unpublished data). It has also become clear that recombination between sequences transcribed from the plant genome and a viral genome has occurred in at least one strain of a luteovirus (28), a potyvirus (39) and a cucumovirus (26). Thus, since recombination is well documented in plant viruses, it came as no surprise to people in the field when it was shown that recombination could occur in transgenic plants expressing a truncated coat protein gene when infected with a viral genome carrying a deletion in the coat protein gene (11).

Since co-infection of plants by more than one virus is not infrequent, and, as we have seen above, recombination between viral genomes is a regular occurrence, the important question is whether recombination in transgenic plants can be the source of qualitative or quantitative changes in the generation of novel viral genomes, relative to what occurs already in multiply infected plants. One situation where a qualitative increase could be expected is when viral genes have been transferred to the genome of a non-host plant as a means of conferring resistance to a virus that normally infects the plant (see for instance 6, 42). When such plants are infected with a virus, we clearly have co-existence in the same cells of viral sequences that are not normally present in the same plant, due to differences in host range, which could allow recombination to lead to truly novel viral genomes.

As already mentioned, in plants expressing viral genes, any virus infection will be the equivalent of infection of non-transgenic plants by more than one virus. Considering the scale at which transgenic plants are likely to be cultivated, it is plausible that this will lead to an increase in the frequency of recombination, as compared to what occurs in multiply infected plants. However, in terms of the potential for creation of new virus genomes, one can argue that quantitative changes in recombination are not important, since over a long time scale, all potential recombinants would have been created, and the selective advantage of all potential recombinants would already have been tested in nature. Initially, this idea is intuitively attractive, and could be thought to be based on the idea of co-evolution of host and pathogen. However, on further reflection, it is clear that the hypothesis that all recombinants would have been tested in nature is based on a fallacious postulate. It assumes that all combinations of viruses would have co-infected a great enough number of host plants under a sufficient number of conditions for even extremely rare recombination events to have occurred, and for the fitness of all potential recombinants

to have undergone natural selection under all possible conditions. In fact, a situation approaching this would only be the case of viruses and their hosts had co-evolved under stable conditions over sufficient time for optimization of their interaction to have allowed them to approach equilibrium. It is clear that human activity has allowed viruses to interact with new hosts, either directly by introducing new plants, new viruses, and new vectors into all inhabited environments, or indirectly by the effects of human activity on ecosystems. The continuing appearance of new viral diseases in both animals and plants is clear indication that virus-host interactions are far from evolutionary equilibrium. In the plant field, it is often difficult to determine that a viral disease is in fact new, rather than simply being newly noticed. There are a few cases at least where the disease symptoms are striking enough that it is reasonable to propose that the disease is itself new. Just recently, it was shown that what could be considered a new virus disease, cucurbit aphid-borne yellows luteovirus (CABYV), is in fact a recombinant between two previously known viruses, beet western yellows luteovirus and pea enation mosaic virus (Gibbs and Cooper, unpublished data). This of course also clearly suggests that novel virus genomes can still appear by recombination in co-infected plants, and thus could also in transgenic plants expressing viral sequences.

Mutation of cucumber mosaic virus (CMV) satellite RNA in plants expressing satellite RNA genes

Certain CMV strains naturally include a small (330-400 nt), linear, supernumerary RNA, termed satellite RNA, with rather remarkable properties. CMV satRNA is not necessary for the helper virus; however, the satRNA is entirely dependent on the helper virus for all essential functions (replication, encapsidation, etc.); its sequence has essentially no homology to that of CMV, yet it can be replicated to extremely high levels by the helper virus; CMV satRNA does not encode proteins. For more detailed reviews concerning the general properties of satRNAs, see reviews by Colmer and Howell (3) and Roossinck et al. (36). A more complete discussion of the use of CMV satRNA in plant protection, as well as risk-related questions concerning CMV satRNAs will soon be published (Jacquemond and Tepfer, unpublished data).

CMV satRNAs would be of anecdotal interest, except that in many cases their presence leads to an important reduction in the amount of virus replicated, and hence to an almost complete attenuation of CMV symptoms on most host plants. Since CMV can have a major impact on many plant species, starting nearly ten years ago, several laboratories have introduced CMV satRNA genes into plants as a means of attenuating the symptoms of CMV infection (14, 17, 25, 29, 37, 45, 50). In all cases, the protection observed was quite

effective, yet so far plants expressing CMV satRNA genes have only been field tested in China (45, 49). The reason for this is that certain satRNAs, instead of attenuating symptoms, can lead to a spectacular increase of symptom severity. The best known case of this is the lethal necrosis induced on tomato by CMV strains including certain satRNAs (20), which can take on epidemic proportions, leading to nearly complete loss of tomato crops in certain regions of southern Italy (21) and Spain (19). This response is specific to tomato and to certain closely related *Lycopersicon* species, and is only induced by certain satRNA variants. More recently, satRNA variants that induce an increase in mosaic symptoms on tomato (10) and tobacco (15, 43) have been described, as well as ones responsible for a novel top stunting in tomato (12, 19).

Considerable effort has been focused on determining the molecular determinants of tomato lethal necrosis. Necrogenic satRNAs differ only very slightly from non-necrogenic ones; in fact, point mutations at any of four closely clustered positions of a non-necrogenic satRNA can render it necrogenic on tomato (5, 27, 41). Similarly, a small number of point mutations in a different part of the satRNA are responsible for the aggravated mosaic on tobacco and tomato (27, 41).

Of course, for potential applications, it would be essential to avoid using deleterious satRNA genes. However, even using a gene based on a satRNA that has no known deleterious effects, there are potential risks. When such plants are infected with CMV, the satRNA will be multiplied to high levels, and the risk is that during these numerous replication cycles that a deleterious variant could arise by mutation and then be transmitted to a sensitive host plant where it could have deleterious effects. This is the reason that the most prevalent opinion, at least in Europe and North America, is that wild-type satellite RNAs have potential risks that are too great to envisage using them for plant protection at an agronomic scale. Based on current knowledge, this cautious approach may well be the most appropriate at this time, nonetheless, the situation is in fact extraordinarily complex. For instance, CMV satRNAs are only rarely found in naturally infected plants in the field. This suggests that there is strong counter-selection against the satRNA. Most likely this is due to the fact that the satRNA has a strongly negative effect on CMV titer. One result of this is that plants infected with CMV+satRNA are less good source plants for virus transmission than ones infected with CMV alone. However, if this were always the case, one would expect that counter-selection would have led to the complete disappearance of CMV satRNAs. Also, how then do you explain the epidemics of CMV satRNA-induced tomato necrosis that have caused such wide-spread damage in Italy and Spain? Of

course, the only clear conclusion is that we are lacking critical information on the regulation of the epidemiology of the CMV satRNAs.

Are there ways that the satRNA could be modified to avoid its potential deleterious effects? It has often been proposed to delete the segment that seems most directly involved in inducing lethal necrosis, but it is far from clear that this could be done without destroying the protective properties of the satRNA. Another problem with this approach is that, since new deleterious effects are still being associated with satRNAs (increased mosaic, top stunting, etc., see above), it hardly seems plausible to be able to modify all the domains that are involved in symptom aggravation. Another possibility for risk reduction that has often been proposed is to modify the satRNA to prevent its encapsidation in CMV particles, and thus render the satRNA non-transmissible. This also would seem perhaps difficult to achieve, but a recent report (30) that a certain satRNA variant is not encapsidated, at least by certain helper strains, gives some hope that biological containment of satRNAs could be achieved.

Heterologous encapsidation of an infecting genome in plants expressing a coat protein genes

Based on previous knowledge, it had been predicted that a viral genome infecting a plant expressing coat protein could be encapsidated by the protein expressed by the plant, either alone or in combination with the coat protein encoded by the viral genome (4, 16, 32, 44). This has now been shown to indeed occur, first by Farinelli et al. (7), who showed that when plants expressing the coat protein of the N strain of potato virus Y (PVY) are infected with a different strain, PVY-O, the viral particles synthesized contain both types of coat proteins. More recently, it was shown that when plants expressing the coat protein of an aphid-transmissible strain of plum pox potyvirus (PPV) are infected with a non-transmissible strain of zucchini yellow mosaic potyvirus (ZYMV), particles composed of the ZYMV genome encapsidated in coat protein of both ZYMV and PPV were formed, and that the presence of the PPV subunits sufficed to render these hybrid virus particles aphid transmissible (23). Similar results with PPV have also been obtained by Maiss (unpublished data).

The important question is whether this potential for modifying vector specificity could have an impact on virus epidemiology. A first important point is that the genome of the virus is not affected; thus, the virus does not retain any modification in vector transmission beyond the initial infection. A second critical point is that heterologous encapsidation is a natural phenomenon that can be observed when plants are infected with more than one virus of the same group. Thus, the question can be

restated to ask whether heterologous encapsidation in transgenic plants could have an impact greater than what is due to the same phenomenon occurring during multiple infections. In cases of heterologous encapsidation of closely related viruses, as in the experimental systems described above, it is reasonable to suggest that the potential for serious impact is slight. There are some specific cases where greater caution is clearly warranted. The members of a little-known virus group, the umbraviruses (31), are entirely dependent on a helper virus, usually a luteovirus, for encapsidation and vector transmission. The association between an umbravirus and its helper luteovirus can cause quite serious agronomic losses, as in the case of groundnut rosette disease in Africa (35). The obvious danger is that plants expressing the coat protein of a luteovirus could be sufficient to supply encapsidation and vector transmission to an associated umbravirus, thus freeing the umbravirus from the strict constraints of co-infection. In contrast to the umbraviruses, the luteovirus group is quite well studied. Potato plants expressing the potato leafcurl luteovirus (PLRV) coat protein have been described, and are resistant to the virus (22). Fortunately, these plants do not express the readthrough domain of the PLRV coat protein, which is necessary for recognition by insect vectors (18), and thus these plants should not be able to assist umbraviruses. It should also be noted that the domain of potyvirus coat proteins that is the site of recognition by vectors can be deleted without impairing the coat protein's protective functions.

In a sense, the ability of coat protein synthesized in transgenic plants to confer vector transmission can be seen in the larger context of complementation and synergy between viruses. There are quite a few cases where infection by two viruses leads to a dramatic increase in replication in one of the partners, and a worsening of symptoms. However, very little is known about the molecular basis for any of these synergistic

interactions. One such interaction, in which co-infection of tobacco with a potyvirus and potato virus X (PVX) leads to greatly increased titers of PVX, has recently been studied in more detail by Vance et al. (46). They showed that expression in transgenic plants of the 5'-proximal part of a potyvirus genome was sufficient to mimic the synergy obtained when PVX and the potyvirus were co-inoculated. Thus, in all cases where functional viral proteins are synthesized in transgenic plants, it is worth considering whether this could confer the potential to mimic a synergistic interaction, or in other ways provide functional complementation to an infecting virus.

Conclusions

At some final stage, all the foregoing considerations must be put into a larger context, to go beyond strictly technical considerations of potential risk, to arrive at a risk/benefit analysis taking into account the various needs of society. There will be a few cases in which the decision to release plants expressing virus-derived genes will be easy to make, for instance when the benefits to society (or the losses if the resistance gene is not used) are so great that the potential risks described here would seem minor in comparison. There may also be cases where the potential benefits of a virus-derived resistance gene are slight, for instance when equivalent resistance can be created by classical breeding. There are of course many cases where the appropriate judgment will be much harder to make. One last point is that what is perceived as acceptable risk is certainly quite different in various parts of the world. For instance, it is clear that the perception of the debate on the importance of the potential risks described above is quite different in Europe, as compared to the United States. It is to be expected that the social and political perspectives of developing countries will also give them unique points of view on the risk/benefit analysis of the agronomic use of transgenic plants, including those expressing viral genes.

المخلص

تبرفر، مارك. 1995. المخاطر البيئية الممكنة للنباتات المحورة وراثياً: الحالة الخاصة بالنباتات المحورة وراثياً بواسطة جينات من أصل فيروسي. مجلة وقاية النبات العربية. 13(1): 20-27

التي يركبها النبات بدور مكمل لنشاط هذه الفيروسات. ففي النباتات المحورة وراثياً، التي تصنع بروتينات الغطاء الفيروسي، يمكن للفيروس المعدي أن يكون جزيئات فيروسية ذات غطاء بروتيني - على الأقل جزئياً - يركبها النبات العائل، ويمكن أن تحدث تغيرات في إمكانية نقل الفيروس بواسطة النواقل. وبالتالي فإن استعمال هذه الطريقة قد يؤدي إلى تغيرات في الأنماط الشكلية للفيروسات. كما أن هناك براهين بأن استعمال النباتات المحورة وراثياً، والتي تنتج بروتينات فيروسية، قد يحدث تغيرات في الأنماط الوراثية للفيروسات المُعدية، وذلك عن طريق إعادة التآلف بين تتالي النوكليوتيدات النباتية ونوكليوتيدات الفيروس. وبالرغم من معرفتنا بإمكانية حدوث هذا النوع من التغيرات في التفاعل ما بين الفيروس والنبات العائل، إلا أن معرفتنا قليلة جداً

أدخلت العديد من المختبرات، بما فيها مختبرنا، أجزاء من المادة الوراثية للفيروسات النباتية إلى المادة الوراثية النباتية بهدف خلق مورثات مقاومة لهذه الفيروسات. ولهذه المورثات أهمية خاصة في الحالات التي لم يتمكن العلماء فيها من إيجاد هذه المورثات في الطبيعة. ويمكن القول بأن ادخال مثل هذه المورثات إلى النبات ذو آثار إيجابية في البيئة وبخاصة إذا أدى استعمالها إلى الإقلال من استخدام المبيدات المستعملة في مكافحة النواقل الطبيعية للأمراض الفيروسية على النباتات (حشرات، نيماتودا، فطور). إلا أن هناك مخاوف من أن يكون لهذه النباتات المحورة تأثيرات سلبية في البيئة. وتتجم هذه المخاوف من احتمال حدوث نوعين من التحولات في التفاعل ما بين الفيروسات والنبات العائل. يرتكز إحداها على إمكانية قيام بعض البروتينات الفيروسية

من بعض عوامل المخاطرة. كما أن متابعة دراسة احتمالات الخطر يسمح لنا بإيجاد الطرق التي يمكنها تقليل هذه المخاطر أو إزالتها.
كلمات مفتاحية: مقاومة أمراض فيروسية، نباتات محورة، تقويم المخاطر.

بالنسبة للتغيرات في وبائية الأمراض الفيروسية، التي قد تحدث من جراء ذلك. يمكن في بعض الحالات، مثل استعمال جينات الغلاف البروتيني، اتباع بعض الطرق التي تقلل

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