**Update on Agrobacterium-Mediated Transformation of Plants**

**Agrobacterium tumefaciens and the Plant: The David and Goliath of Modern Genetics**

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THE FUTURE OF GENETICALLY MODIFIED (GM) CROPS

Since the first reports on GM crops in the 1980s (Bevan, 1984), the amount of debate surrounding the generation of transgenic plants and the political and economical ramifications of their development have seldom been far from the public eye. It could be said that attention on the use of transgenically modified crop plants is substantially greater than on any other area of plant science. To date, the majority of commercially produced GM crops have been created by biolistically delivering transgenes into embryonic tissue (Padgette et al., 1995; James, 2002). However, as our understanding of the mechanisms of T-DNA integration grows and further information is reported on the proteins involved, the biolistic approach may well be superseded by a more sophisticated and “natural” solution to the problem.

*Agrobacterium tumefaciens* is a soil-borne bacterium that, in nature, is capable of inserting a defined fragment of its DNA into the genome of dicotyledonous plants (for review, see Tzfira and Citovsky, 2002; Gelvin, 2003). It has been the focus of research for a wide spectrum of biologists, from bacteriologists to molecular biologists to botanists, for a number of years. Moreover, the unique process by which it delivers trans-kingdom DNA into host plant cells has become a staple in almost all undergraduate plant science courses. However, it is important to remember that *A. tumefaciens* remains at the cutting edge of plant science. Our understanding of the molecular mechanisms of DNA integration has grown immeasurably in recent years, and in a climate of intense political debate surrounding the future of transgenic crops, the future role of *A. tumefaciens* in the controlled creation of transgenic crops or simply as the model type IV secretion system and as a research tool would appear to hang in the balance.

The aim of this review is to discuss recent advances and future prospects in the field of *A. tumefaciens*-mediated plant transformation (for more detailed review, see Tzfira and Citovsky, 2002; Gelvin, 2003). To achieve this end, an overview is provided of the molecular mechanisms involved in T-DNA generation, transfer, and integration in the host genome, together with an outline of the most recent developments in the identification of plant proteins interacting with T-DNA. These data provided insights into the value of *Agrobacterium* both as a model system and of its potential future importance in the area of transgenic crop production. This leads, in the second part of the review, to discussion of the controversy surrounding the release of GM crop plants into the environment.

*Agrobacterium: IS IT A TYPICAL PATHOGENIC BACTERIUM?*

*Agrobacterium* is a soil-borne bacterium that, in the presence of a wounded plant, moves toward it, attaches itself to the wound site, and proceeds to transform the cell. The sugars and phenolic compounds exuded by the wounded plant not only signal the pathogenic opportunity to the bacterium but also induce transcription of the virulence genes. These virulence genes are located on a specific plasmid known as the tumor-inducing (Ti) plasmid, which also contains the transferred DNA (T-DNA). Virulence proteins have roles ranging from transcriptional activation to T-DNA processing and export, with certain proteins also having a function in the host (Fig. 1). *Agrobacterium* has evolved to transfer the T-DNA, which codes for: (a) plant hormone producing enzymes that stimulate growth of a tumor, and (b) metabolic enzymes responsible for producing opines, metabolizable only by *Agrobacterium*. The resultant crown gall is a microcosm where the bacteria can thrive.

Virulence proteins VirD1 and VirD2 act together in the processing of the single-stranded T-DNA from the Ti-plasmid, during which the VirD2 protein becomes covalently bound to the 5′ terminus (Fig. 2). This covalent association is thought to remain until the last stages of T-DNA integration into the host genome because localization experiments have shown the accumulation of VirD2 in the nucleus. One of the other vital virulence proteins is VirE2. This virulence protein was originally shown to be a single-stranded DNA-binding protein that binds in a sequence-nonspecific manner. Many more roles have been put forward for this versatile protein from involvement in cytoplasmic trafficking and nuclear im-
port to formation of a transmembrane pore (Dumas et al., 2001).

Other virulence proteins play a role in constituting the membrane channel, representative of the bacterial type IV secretion system, through which the T-DNA and certain virulence proteins are secreted. The type IV secretion system is thought to be related to the ancient conjugation system, which allows the secretion of DNA-protein complexes (for review, see Christie, 2001). Bacterial type IV secretion systems have been implicated in the pathogenicity of many human illnesses including whooping cough (*Bordetella pertussis*), stomach ulcers (*Helicobacter pylori*), and Legionnaires’ disease (*Legionella pneumophila*) by translocation of effector or virulence proteins into the host. The type IV secretion system of *Agrobacterium* is the best characterized in the class, although it differs in that a DNA element is also translocated to the host, where it becomes stably integrated in the genome. The harnessing of this trans-kingdom gene transfer mechanism has revolutionized plant biotechnology, and further elucidation of the plant factors involved in the process may be the key to future progress. Therefore, *Agrobacterium* is not a typical pathogenic bacterium but a sophisticated manipulator of its environment.

**T-DNA: THE STAMPED, ADDRESSED ENVELOPE**

Many features of T-DNA and the nature of its delivery and integration have made it an invaluable tool in plant biotechnology. Not least of these features is the fact that the actual transfer and integration do not rely on genes encoded by the T-DNA itself. In fact, the only necessary sequences on the T-DNA are the border sequences (right and left) that delineate it. Between the border sequences, genes of scientific interest can be cloned, allowing for great flexibility in what is transferred to the host cells.

An important role for both VirD2 and VirE2 has been demonstrated by analyzing the virulence of *Agrobacterium* with either of these genes deleted (Ott et al., 1984). The absence of either of these proteins abolishes virulence, indicating that their function is vital. Import of the VirE2 protein has been demonstrated by producing a fusion protein with Cre recombinase (Schrammeijer et al., 2003). Interestingly, VirE2 expressed in a host plant leads to restoration of virulence of the VirE2 deficient *Agrobacterium* strain (Citovsky et al., 1992). The indication from these plants was that the major role for VirE2 is in planta. The next step was to search for specific plant-interacting factors. Tzfira and coworkers used the yeast (*Saccharomyces cerevisiae*) two-hybrid system to identify Arabidopsis proteins that interact with VirE2. VIP1 (VirE2-interacting protein 1) was shown to be a bZIP domain-containing protein that is localized in the nucleus and appears to facilitate VirE2 nuclear import (Tzfira et al., 2001). A second protein, VIP2, was found to be homologous to fruitfly (*Drosophila melanogaster*) Rga. Rga is thought to interact with chromatin and, in so doing, control the transcription machinery (Tzfira et al., 2000). This second nuclear VIP found to have a role in the nucleus is indicative of a role for VirE2 in T-DNA integration.

Further research has established a role for VirD2 in the nuclear import of the T-DNA, confirming the functionality of the putative nuclear localization signals (Ballas and Citovsky, 1997; Ziemienowicz et al., 2001). Because VirD2 was known to be covalently attached to the T-DNA within the host nucleus, it was suggested that it may play a role in the integration process. This was investigated using in vitro ligation assays, which indicated that the presence of VirD2 itself was not sufficient to ligate T-DNA to a target template (Ziemienowicz et al., 2000). Then, it was demonstrated that plant extracts could catalyze the ligation, and more specifically purified Arabidopsis ligase I could ligate the T-complex to the template (Wu et al., 2001). Interestingly, the presence of VirD2 in the reaction actually lowered the efficiency of ligation, which suggests that its major role is in targeting the T-DNA to the host nucleus and not in the actual integration. These indications of the interactions between virulence factors and host factors have directed research toward identifying more host partners.

![Figure 1](image1.png)

**Figure 1.** The path that the T-DNA travels from the stage of T-DNA processing from the Ti plasmid (pTi) to stable integration into the plant genome (Rossi et al., 1998).

![Figure 2](image2.png)

**Figure 2.** T-DNA processing. The VirD2 remains covalently attached to the 5’ terminus of the T-DNA.
AGROBACTERIUM, ARABIDOPSIS, AND RATS

One of the major contributions of *Agrobacterium* research to plant research has been the use of T-DNA as a mutagen. To date, it has not been possible to target T-DNA to any particular locus in the genome with any great efficiency. This has the advantage that, in a library containing a large number of independently transformed seeds, the plant genome would be saturated with individual T-DNA integrations. In such a library, there is a high likelihood that there are T-DNA insertions in every open reading frame, and the resultant mutant plants can then be screened by phenotype or using reverse genetics to identify specific mutant line of interest. The first large-scale T-DNA-tagged library was made available by Krysan et al. (1999). It contained over 60,000 independent mutants and marked a change in the direction of Arabidopsis genetic research from chemical mutagenesis followed by chromosome walking and mapping to reverse genetics. Using T-DNA with a known sequence offers a myriad of possibilities for researchers such as using enhancer traps or genetic complementation. The sequence-independent nature of T-DNA integration means that identification of the sequences adjacent to the T-DNA became possible by cloning a bacterial vector between the border sequences and cloning the genome sequences by plasmid rescue. There is now a >75% chance of finding a plant with a T-DNA located in a gene of interest, with the obvious exception of insertions, which create a dominant lethal phenotype.

As more and more T-DNA insertion sites have been sequenced, a wealth of information has been generated that, upon analysis, can provide us with insights into the subtle preferences for T-DNA integration. Most recently, Alonso et al. (2003) analyzed 88,000 T-DNA insertions from the SALK library, revealing a bias at both the chromosome and gene levels. At the chromosomal level, fewer T-DNAs were found at the centromeric region, which is consistent with the observation that there is a correlation between T-DNA insertions and gene density. Specific preferences within a gene were also analyzed, with promoter and coding exons making up nearly 50% of all insertion sites. This result may be skewed, however, by the selection of kanamycin-resistant plantlets in the T1 generation, resulting in the analysis of insertion sites with transcriptionally active T-DNAs.

These observations are consistent with a study that looked specifically at the conservation of the T-DNA borders and the genomic sequences after integration (Brunaud et al., 2002). The authors concluded that there are microsimilarities involved in the integration of both the right and left borders. These similarities, however, need only occur over a stretch of 3 to 5 bp and can be between any T-DNA and genomic sequence. This basically allows T-DNA to integrate at any locus in the genome. It was also reported that a T-rich region was often found upstream of the insertion site, which would account for the high number of T-DNAs found in promoter regions and may be an indication of integration mechanism. Based on these findings, the current model for T-DNA integration is outlined in Figure 3.

To identify plant factors that are involved in the transformation process, T-DNA-tagged mutant Arabidopsis libraries were screened to identify plants which were resistant to *Agrobacterium* transformation (RAT; Nam et al., 1999; Zhu et al., 2003). It was important to devise an assay as close to the in vivo situation as possible; for this reason, the roots of Arabidopsis were inoculated with wild-type *Agrobacterium*, and tumor formation was observed. Mutants identified from the screen were tested using a second root assay which involved generating antibiotic resistant calli when stable transformation has occurred. This second assay confirmed that the RAT phenotype was linked to the original mutating T-DNA. Extensive screening resulted in the identification of many genes required for *Agrobacterium*-mediated transformation. Classification of these mutants has generated categories that could have been predicted such as cell wall attachment, nuclear import, and DNA metabolism. This was important in confirming the validity of the screen. There were also some surprising genes identified; for example, those coding for receptor-like protein kinases (ratT8 and ratT9), DEAD box RNA helicase (ratT7), and transcription factor DREB2A (ratT5).

All of the genes identified could feasibly have an impact on transformation of recalcitrant species, but one key target area is the stable integration of the T-DNA into the host genome. Table I summarizes the Arabidopsis mutants that have been identified either through the screen or by reverse genetics to be specifically involved in the integration step. The *rat5* mutant is the best characterized to date and was shown to be mutated in one of the histone genes (H2A-1; Mysore et al., 2000). The corresponding cDNA not only complements this mutant, but it was also shown to increase wild-type transformation rates. In a later study, it was also shown that expression levels of H2A-1 can be correlated with cell susceptibility to transformation (Yi et al., 2002). Both of

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**Figure 3.** The current model of T-DNA integration. The T-rich region [(T)n] and microsimilarities that were identified as being important for the integration mechanism are depicted. Modified from Brunaud et al. (2002).
Table 1. T-DNA integration/chromatin remodeling mutants

<table>
<thead>
<tr>
<th>Mutant Gene</th>
<th>Tumor Assay</th>
<th>Transient Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histone H2A-1 (rat5)</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Histone H2A-3</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Histone H2A-11</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Histone H4-3</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Histone H4-4</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>Histone deacetylase-1</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>Histone acetyl transferase-6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Histone acetylase complex HXA2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chromatin silencing group 1</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>ARID-containing protein (ratL3)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* a, <25%; ++, <50% of wild type; +++, <50%; +++++, >50%; ++++++, wild type.  
* b ND, Not determined.

these factors suggest a possible role for H2A-1 in improving transformation of recalcitrant species. The exact mechanism by which H2A-1 affects T-DNA integration is still unknown; whether it is through chromatin remodeling, direct interaction with VirD2 in targeting an integration site, or in recruiting other important proteins to the vicinity remains to be seen.

**HOW DOES T-DNA INTEGRATE?**

The observation of the random, as opposed to targeted, nature of T-DNA integration has led to the suggestion that the likely mechanism is through non-homologous end joining (NHEJ). NHEJ is the DNA repair mechanism that joins double-stranded breaks (DSBs) irrespective of sequence. This is the process involved in well-characterized cases such as variable-diversity-junction (VDJ) joining in the mammalian immune system (Pastink et al., 2001). NHEJ is an unfaithful repair mechanism that often incorporates filler DNA, such as T-DNA, into the repair site. Further insights into mechanisms of T-DNA integration have been gained by using yeast as a model system. In contrast to the situation in plants, integration of T-DNA in yeast usually occurs by homologous recombination (HR), but, in the absence of any homology, T-DNA can also integrate using NHEJ. The use of yeast mutants has been very valuable in understanding the balance between HR and NHEJ. It has been shown that in a Yku70 mutant, which is deficient in NHEJ, T-DNA integrates solely by HR (van Attikum et al., 2001) and, conversely, in a rad52 mutant integration occurs only by NHEJ (van Attikum and Hooykaas, 2003). Finally, in the Yku70/rad52 double mutant, no T-DNA integration was detected. It will be interesting to discover whether the same balance between repair pathways is found in plants.

Ligase IV is the specific ligase responsible for NHEJ and has therefore been the focus of a great deal of recent research in plants. Two independent Arabidopsis ligase IV mutants have been characterized with respect to T-DNA integration and sensitivity to gamma radiation (DSB) and methyl methane sulfonate. Both mutants showed a loss in ability to repair DSBs, as indicated by the gamma plantlet phenotype. Less clear, however, was the role ligase IV might play in T-DNA integration. Friesner and Britt (2003) analyzed T-DNA integration by looking at the number of kanamycin-resistant seedlings germinated after transformation via flower dipping. This ligase IV mutant produced a slightly lower percentage of resistant seedlings indicating less T-DNA integration than the wild type, although there was still approximately 1% of seeds, compared with 1.4% with wild type, which had been transformed. In contrast, van Attikum et al. (2003) reported that when using the root tumor assay, there was no difference in the transformation rate between another ligase IV mutant and wild type. Together, these results infer a role for NHEJ in T-DNA integration, but there must be an alternative integration mechanism.

Recent research, using VirD2 as bait in a yeast two-hybrid assay, allows new insights into the alternative integration mechanism. VirD2 was shown to interact with Arabidopsis TATA-binding protein and CAK2M, a nuclear kinase (Bako et al., 2003). The CAK2M kinase also interacts with RNA polymerase II (C-terminal domain). These reactions together are suggestive of a role for transcription and transcription-coupled repair in T-DNA integration. The interaction of VirD2 with proteins involved in this second DNA repair mechanism may explain the T-DNA integration events observed in the mutant ligase IV experiments. As a pathogen, Agrobacterium is both resourceful and flexible in achieving its aim.

**TRANSGENIC CROPS IN THE FIELD: THE POLITICAL BOTTLENECK**

With rapid advances being made in Agrobacterium and transgenic plant research, the possibilities for crop improvement are numerous. One recent advance epitomizes the current situation: coffee (Coffea canephorea) plants that have been genetically modified to contain less caffeine (Ogita et al., 2003). The caffeine biosynthesis gene, coding for obromine synthase, was targeted for down-regulation using the concept of RNA interference. The transgenic coffee plantlets obtained after Agrobacterium-mediated transformation had a 50% to 70% reduction in caffeine. These decaffeinated coffee plants will, in theory, bring an end to the expensive, industrial decaffeination that also results in a loss of taste. One problem remains. Even though the traditional breeding time of 25 years has been reduced to one year, the plants that were transformed were C. canephorea and not Coffea arabica. The difficulties in transforming C. arabica, which is the commercially valuable species, could be addressed by understanding the mechanics of Agrobacterium-mediated transformation and using the knowledge to improve the transformation rate.
Perhaps the most difficult hurdle facing the advance of *Agrobacterium* and transgenic plant research is not a technical but a political one. Decisions that will shape the future of plant research are about to be made as countries all over the world are preparing to draft new legislation to control the development of transgenic crops. There are many different parties contributing to the debate, each with their own concerns and goals. Many have obvious merit, but concessions will have to be made to find solutions and ensure the future of plant research. At a naïve level, there are the large corporations on one side and environmental groups on the other. Scientists are largely placed on the side of big business, and the farmers are portrayed as sharing concerns with the environmentalists. These boundaries are blurred, however, as is apparent with the farmers in India who crossed transgenic pest-resistant cotton (*Gossypium hirsutum*) with another variety to increase their yields, an act that would appear to be in violation of the patent on the resistance gene held by Monsanto (Jayaraman, 2001). This demonstrates that the ultimate driving force in agricultural progress is economics, whether it is in the hands of multinational companies or small-scale farmers.

Another major driving force for the progression toward the use of GM crops is the pressure imposed by the world’s growing population. It is estimated that in the next 50 years, the world’s population will grow from 6 to 9 billion, where it will hopefully stabilize (Evans, 1998). This argument is often portrayed by environmentalists as lip service that ultimately hides industrially driven greed. Another aspect is that even with enough food per capita to feed the world, the problems with distribution, corruption, and the suppression of developing countries also need to be addressed. In her recent publication, Jennifer Thomson (Thomson, 2002) discussed the prospective gains for Africa in the utilization of GM crops. The main benefits described for Africa are: (a) Crops could be engineered to be resistant to indigenous pests, (b) Indigenous crops could be modified so that not only crops of value to developed countries are available, and (c) The possibility of economic self-sufficiency would be made available to small-scale farmers and, eventually, the nations themselves.

Tackling the problem of world hunger requires a multifaceted approach involving not only a country’s transport infrastructure, its political transparency, and external industrial motives but also incorporation of biotechnological advances.

It is certainly true to say that industrial companies will always have to make a profit and environmentalists might claim that feeding the world may not be their first priority, but this does not change the fact that feeding 9 billion people remains our future. It should also be noted that in the case of “Golden Rice,” rice (*Oryza sativa*) enriched for the vitamin A precursor B-carotene, the farmers need only pay proceeds to the industrial producers when annual profits exceed $10,000 (Thomson, 2002). In this case, nutritionally enhanced GM rice could benefit the consumer, the small-scale farmer, and the company that funded the development of the technology.

**GM CROP DISTRIBUTION**

To understand the implications of GM crops on the agricultural and economical future of the world and, in particular, developing countries, the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) report by James (2002) provides valuable insight to the current situation and trends. In the 6-year period since the first commercial GM crop was introduced, growth in the amount of global coverage has been observed. A sustained growth rate of over 10% per annum has resulted in 145 million acres of land growing GM crops by the end of 2002. As can be seen in Figure 4A, the United States has by far the greatest impact by growing 66% of the world total. Perhaps surprisingly, Argentina is the second biggest grower of GM crops with 23%. This is a developing nation with a recent history of economic hardship that has enthusiastically embraced the biotechnological advances and will continue to be a model for developing nations with respect to the benefits or problems associated with these crops.

It is also of interest to investigate which countries have increased their GM output compared with the previous year. Figure 4B shows that in 2002, South Africa and China were the two countries whose output increased the most compared with the previous output figures. Is this an indication of the need in developing countries for this type of technology? It is certainly an indication that developing countries are likely to play a major role in the future of GM crops worldwide.

One final aspect of the current situation that is likely to eventually differ within developing countries is the type of GM crop grown. At present, there are four main types of GM crops currently grown worldwide (soybean [*Glycine max*], maize [*Zea mays*], cotton, and canola [*Brassica napus*]; Fig. 4C). It is also interesting to note that there are two main genetic modifications within the crop species being grown currently: crops that produce the *Bacillus thuringiensis* protein, which is toxic to insect pests; and herbicide-resistant plants, which allow selective growth of the crop at lower levels of herbicide usage. The impact on yield has been reported to be a 10% increase with the soybean in Argentina and 514,000-metric ton increase in cotton in China (James, 2002). To the small-scale farmer, the value of these increases cannot be underestimated. Future improvements in plant transformation technology will allow the genetic modification of indigenous crops by local scientists in developing countries and lead to the desired self-sufficiency.
With political and economical issues to one side, the most pertinent issue remaining is that of human health. It is obviously in nobody’s interest to introduce a GM crop that will lead to illness. Steps have since been taken to ensure minimal foreign DNA content in the final GM plants. One of the major obstacles in this area of the debate is the need to define acceptable risk. GM food is discussed in the realms of risk free, but it would be more constructive to compare it with food consumption risks in general. As much as 1 g of bacterial, viral, animal, and plant DNA is consumed every day, most of which is destroyed by stomach acid or by enzymes within the digestive system (Doerfler and Schubbert, 1997). Adding a small percentage (<0.001%) of GM DNA to a diet should pose no greater risk to health than non-GM DNA. It should also be considered that many countries worldwide have rigorous safety trials followed by field trials before general release into the environment is allowed. To date, no GM crops have been shown to be detrimental to mammalian health, although conscientious tests in the future remain of central importance.

TO TRANSFER OR NOT TO TRANSFER: THAT IS THE ETHICAL QUESTION

The ethical debate is centered around the acceptability of taking genes from one species and moving them into another species, with species boundaries (ability to produce fertile offspring) being used to define “normal” and “natural.” The question of “naturalness” is discussed in a recent review (Verhoog, 2003) where the differences between intrinsic (for example, playing God) and extrinsic (such as health risks) moral concerns are defined. Verhoog postulates that one of the biggest divides in the ethical debate is what an individual understands “nature” to be. If “nature” is considered to be neutral, then GM technology is viewed as neutral, in essence something that can be used for either good or evil. On the other hand, if “nature” is viewed as good, then doing anything to change it is seen as evil. Obviously, the ability of Agrobacterium for interkingdom DNA transfer opens the debate as to what is “natural.” This is an organism that has evolved a mechanism of securing its own environment by genetically modifying another organism. Would using Agrobacterium to produce GM crops be morally acceptable?

WHAT DOES THE FUTURE HOLD?

At present, the field of Agrobacterium research is increasing our understanding of the bacterium itself; in particular, the mechanism by which the T-DNA is translocated into the plant cell and exactly which bacterial virulence proteins accompany it. This understanding is not only vital for the biotechnological application of Agrobacterium, but it also has implica-
tions in the understanding of human pathogens with the type IV secretion system.

There is a rapidly increasing wealth of knowledge available (http://www.bio.purdue.edu/about/faculty/gelvin/gelvinweb/main.html) about the plant factors used by *Agrobacterium* to ensure transport across the plant wall, membrane, and cytoplasm, nuclear import, and finally integration of the T-DNA.

The application of this knowledge to improve transformation rates will bring gene technology to species that, at present, are recalcitrant to *Agrobacterium* and not transformation competent using a biolic approach. The improvement of transformation protocols is a practical advance, but the really exciting advances will be in the types of modification and the application therein. Current research foci include biodegradable plastics in plants (Mittendorf et al., 1998), vaccination against common human diseases administered by eating the plant (Staub et al., 2000), and plants as indicators of environmental toxins (Kovalchuk et al., 2003).

With so much promise, *Agrobacterium* could be the key to future agricultural progress. It can only be hoped that regular, constructive debate can lead to legislative solutions for the ethical, health, and political issues that are likely to play such an influential role in the development of our society.

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**LITERATURE CITED**


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