**BIOTECHNOLOGY AND PLANT PRODUCTS:
QUESTIONS OF FOOD SAFETY**

Sue Tolin

Department of Plant Pathology, Physiology, and Weed Science
Virginia Polytechnic Institute and State University
Blacksburg, Virginia

The objective of this paper is to present some of my thoughts and experiences regarding the safety of foods that consist of, or in part of, plants and their products where biotechnology has been used in their development. Although I have no experience or credentials in food safety, I have worked with plants and viruses that infect them for a number of years. I have also been involved with biotechnology for over ten years and participated in some of the first discussions and approvals of the deliberate release of genetically engineered organisms into the environment. That was not very many years ago. We have seen in a very short time a transition from the experimental stage to plant products that are essentially ready to go to the market place.

My intent is to present an overview of the progress in that field, some of the science underlying that progress, and some of the issues that are addressed in getting these plants approved for the marketplace. I will also give you a little information on the processes that the federal government and groups of scientists, including groups that you are associated with, go through in addressing what has turned out to be a rather difficult question.

General Applications of Biotechnology To Plants

There has been a great deal of speculation of what an ideal genetically engineered plant might be. One design might be something that is all things to all people. It would produce fruits, vegetables, and grains in a single pot. We would no longer need gardens, orchards, or fields. The closest thing to this now is perhaps apple trees that have five different varieties grafted to the same stock.

Since vegetables are the plant foods that are consumed most directly by humans, I will emphasize them this morning. Interestingly enough some of the first major advances scientifically have been with tobacco.

In actuality, applications of biotechnology to food or feed plants fall into the areas in Table 1. The first, improving plant yields and processing characteristics, is a major area. Improving yield is probably the most difficult since this is influenced by many genes in plants, and only one or a few genes can at present be transferred into plants by recombinant DNA methods. A breakthrough has come, however, to improve the processing characteristics of tomato. Calgene, a biotech company in California, isolated a gene that normally caused tomato fruits to become soft during the normal ripening process. They inserted this gene backwards, or in the antisense orientation. The result was a blocking or inhibition of the enzyme. This caused the tomatoes, after they were picked from the vine, to turn red before they turn soft. Thus the tomatoes have a longer shelf life and should have a greater value to the producer. I was at a conference last year where this was reported and someone asked the speaker, "What did these tomatoes taste like?" The answer was, correctly at that time, "Well, no one has tasted them to see." Because of the regulations at that time, tasting and consumption by humans, or even animals, was not a part of the approved experiment. To my knowledge these tomatoes have not yet been tasted. The hard work in even finding out whether these tomatoes are safe for human consumption, and how and whether their use should be regulated, is yet ahead of us.

The second area in which biotechnology is applied to plants is in the development of new plant varieties. Although only rather simple traits conditioned by single genes might be introduced, this slight change is often enough to change the characteristics of that plant. Resistance to disease-causing agents, specifically viruses, is one example of success. Resistance to virus diseases has been achieved by a mechanism that is not too much different from a vaccination to protect humans from virus diseases. However, plants do not have an immune system and do not make antibodies. Thus resistance is introduced by a different mechanism which will be discussed later. There has been considerable success in this area.

Insect resistant plants (Table 1) have been developed by taking a gene from bacteria that produces a toxic compound that is present normally in the environment. It is the active ingredient in the biological control agent for certain insects called BT, for the bacterium Bacillus thuringiensis, and you can go to your local garden store and buy this bacterium to sprinkle in your garden. The bacterial species produces a protein that is toxic to certain types of insects, such as the big green worms often found on tomatoes. Scientists have isolated the specific gene that codes for that toxin and have engineered it into tomato plants. When insects chew on those plants, they do not live very long and thus do not chew up much of the tomato leaf. Thus the plants can be called resistant to the insect. This experiment, interestingly, is raising some issues because people ask, "What does that insect toxin do to me?" It's a very good question and one that is being addressed very actively. The gene has also been put into cotton where it is active against the boll weevil, and the concerns do not seem to be as great.

Plants resistant to herbicides (Table 1) have been developed and tested in a number of field tests. The first example was tobacco resistant to atrazine and glyphosate. Tobacco was used because it is the white mouse of the plant kingdom. It is very easily manipulated and plant physiologists know a great deal about it. In Virginia and a few surrounding states, tobacco is still an important farm crop. Tobacco is a close relative of tomato, and much of the initial work with tobacco has now also been done with tomato. Engineering plants for herbicide resistance came about first from studies of how herbicides work in plants and why they work specifically to kill some plants and not all plants. It was discovered that a single enzyme controls the pathway that detoxifies the herbicide. The gene was isolated, then inserted into a plant. A plant previously sensitive to the herbicide now detoxifies it and grows in the presence of at least low levels of that herbicide.

This type of genetic engineering of plants is very controversial right now. A bill was even introduced in the U.S. Senate to prevent any federal funds from support of this area of research. The fear is that having herbicide resistant plants will encourage the use of herbicides. It is being widely discussed right now whether this will actually occur. I will not discuss this further except to say that the presence of the particular enzyme which acts to degrade the herbicide is not thought to affect the safety of the plant for direct consumption or use by humans. The concern, instead, is of the environmental impact of increased herbicide usage and of the possibility of transfer of herbicide resistance genes to wild plants which then may become aggressive weeds.

The third major area in Table 1 is improving the nutritional value of plants. This area is certainly very promising and I think provides abundant possibilities. Specific examples, however, are not yet available. Possibilities include changing the amino acid content such as high lysine corn, as, of course, has also been done by conventional breeding. The possibilities also include improving the amino acid or vitamin content, or changing oil composition. Attempts are being made with oil seed rape, or cannola. It is in this area of food plant biotechnology where Data Bank information discussed at this Conference may really have a role in directing the plant biotechnologist. Now that it is possible to change a gene in a plant, you could provide direction to the biotechnologist with regard to what you would want to change to improve the nutritional value. This is certainly an area for the future and an area of interdisciplinary collaboration.

The Need for Virus Resistant Plants

I'm going to use an example from my own field of controlling viruses to explain the value of genetic engineering of plants. I use this example because I know more about it, and because the application of biotechnology to plant virology is a scientific reality. Although it is not common knowledge that plants have viruses, they do. Viruses are in plants you eat all the time. Viruses may even be contributing to some of the variability in the food constituency data since they are known to change protein content. I intend to show you the importance of this technology and why virologists around the country and the world are very excited about this new technology.

Because viruses are different than other plants and plant diseases, there are no effective chemical controls, and the use of biotechnology will not reduce the amount of pesticides. It is not an issue with virus-resistant plants because we don't use chemicals to eliminate viruses in nature right now. Preventative measures are widely used. We find ways to eliminate the source of the virus by producing virus-free plants as well as virus-free seeds, because plant propagules are often the source of the viruses. Viruses also exist in nature in weeds. Insects then carry the virus from the weeds or other plants to the crop plant that you are interested in. Thus a second major way for controlling viruses is to kill or block the activity of virus vectors. Sometimes this can be done successfully with insecticides, but it is seldom 100% effective. The ideal control method is to breed resistant varieties. I have worked in this area of research and collaborated with breeders. We have bred corn and soybeans and a few other crops to be resistant to some viruses.

Little progress has been made in getting virus resistance into vegetables, however. The 20 major vegetables consumed by humans are known to be commonly infected by at least 50 different viruses. The viruses often cause such severe diseases that the crop is unproductive or the produce is misshapen, discolored, or otherwise made undesirable for consumption or marketing. There is not a single one of these vegetables that has virus resistance bred into it that also has the proper characteristics of desirable traits. This is an area in which the use of biotechnology is really important.

The fourth method for controlling viruses is to use cross protection. This is accomplished by inoculating a plant with a mild strain of the virus which protects it from infection with more severe strains of the virus. A variation of this method has been used in biotechnology to make the resistant plants.

Examples of Viruses in Food Plants

Several slides were used to illustrate what viruses do to plants and describe something about the diseased plants in relation to their consumption by people. This brief review was intended to provide a perspective on why there is a need to find a way of protecting plants from virus infections. The illustrations included:

(1) *Grapes* grown in Virginia infected with tomato ringspot virus, which reduces the yield, makes smaller berries and may change the taste of the berry. The Japanese have a virus in grapes that they call "Ashinakika", which means tasteless. This virus renders the grapes totally tasteless and of course valueless for direct consumption for table grapes or for the making of wine. The virus in the vines probably came from infected planting material.

(2) *Tomatoes* with tobacco mosaic virus that might have been infected from two sources. The first is from the seed because the virus exists on the surface of the seeds. The control is to eliminate the virus from the seed. The second is one you may have heard about. The virus is present in tobacco products and someone who handles tobacco products and then tomato plants will infect those plants. In greenhouse-grown tomatoes, tobacco mosaic virus is extremely important because it survives on surfaces of benches and spreads just by contact. If you have one infected plant that you touch when you are pruning or picking fruit, the virus will eventually spread to all plants. It is a very contagious virus and is almost impossible to control. It causes a darkening of the fruit and causes tomato fruits to show various mottling symptoms. You might eat some of the infected tomatoes fresh or after processing. Others are so aesthetically undesirable that they would not be consumed fresh. Taste may be affected somewhat, but not so much that they are inedible. I am sure all of you

have eaten a lot of viruses through the course of getting your fresh food. To my knowledge, there has been no known documentation of a food safety problem.

(3) *Peanuts* are severely stunted by a virus we named peanut stunt virus. The yield is decreased and the pods and the seeds are affected so you certainly would not eat them at a cocktail party. We learned that this virus lives in nature in clover. Insects carry this virus to all kinds of beans as well. Green bean pods, particularly desirable varieties like Bush Blue Lake, are smaller, curled, and often have light-green blotches on them. Infected beans can often be found in the grocery stores or local produce markets. Except for reducing the yield by more than half, no adverse effects on the quality of the beans is known.

(4) *Cucumbers* often show mottling and discoloration caused by virus. Cucumber mosaic virus in known to cause a more bitter taste, but is not known to be hazardous. Viruses are also common in other cucurbits like squash and pumpkin. They may distort the fruit into bizarre shapes and make them less desirable, but little is known of their effect on quality.

(5) *Papaya* fruit grown on papaya ringspot-infected plants shows light and dark green rings and is stunted. A researcher in New York state has developed a mild strain of this virus that he has taken to China and to the Philippines and shown that infection with a mild virus can protect it from infection.

Biotechnology to Make Virus Resistant Plants: The Science

The biotechnology method that has been used most successfully is called coat protein-mediated protection. It takes a brief lesson in virology to see exactly what is meant by this and what the implications are with respect to food safety.

The Virus. The starting point is the particles of tobacco mosaic virus (TMV), visible only with the electron microscope. They are straight rods with a total length of 300 nanometers. The complete molecular structure of this particle is known. In each particle, there are some 6,000 or so molecules of a single protein of 17,500 daltons, each consisting of 158 amino acids. In a model of the TMV particle, this protein is shaped like a wooden shoe, and it covers the nucleic acid or genetic information of the virus. Thus it is called the "coat" protein. It is a very specific structure and a very well defined structure in terms of composition. Of the 158 amino acids, there are no unusual amino acids, none that you wouldn't find in any normal cells or any normal food stocks.

The point I want to make is that virus coat protein is a chemically defined molecule that is present in the plant at concentrations up to 1% of the total protein in infected leaf cells. The genetic information of the virus is quite limited and contains codes for only the sequence for the coat protein and for three other proteins. Two of these are enzymes that are essential for making more virus, and the other enables the virus to move better in the plant. The genetic engineering steps take only the nucleic acid coding for the coat protein, about 500 bases. The information to make the rest of the virus is not used. If you have the genetic information only for the coat protein then you have no chance of reforming the virus. The information for the other proteins is missing.

The Method. Insertion of a gene into a plant is done by using a bacterium, Agrobacterium tumefaciens, that infects the plant cell and moves a piece of nucleic acid somewhere into the nuclear chromosome of the plant. This is called the T-DNA or Ti plasmid system. Very simplistically, one takes the gene of interest, such as the TMV coat protein gene, and uses recombinant DNA methods to insert it into the TDNA. The bacterium then is used to move the TDNA-insert into the chromosome of a plant cell. Then a transformed plant cell results which can be grown up into a whole plant. This technology was first reported in 1983. Thus there are seven years of success in transforming plants. The induction of resistance in tobacco and tomato to tobacco mosaic virus via the coat protein method was first reported in 1986 (Powell-Abel et al., 1986). There is now five years experience in the laboratory, and no unexpected adverse effects have been noted.

The size of the coat protein at 17,500 daltons and its particular antigenicity were used to determine that this is the only new protein that is present in the genetically engineered plant. The amount of protein from a genetically engineered plant is considerably less than that present in a normal virus-infected plant. It is the same protein that is not changed at all from what it would be if it were virus infected. When you inoculate virus to non-engineered plants, all plants, after about five days, are showing symptoms. The engineered or protected plants don't begin to show symptoms until nearly two weeks after infection and then just a very small proportion of the plants succumb. This is still useful resistance since often in the field, if you can protect plants from infection when they are young and small, the overall affect of the virus is much less. Interestingly, if you select resistant plants, save the seeds from them and grow them up and then analyze the progeny of those plants in the second generation, those that are expressing coat protein and are therefore resistant to the virus and those that are not expressive come out to a 3 to 1 ratio. In accordance with Mendelian genetics, this segregates as a single gene trait. Thus the gene appears to be totally incorporated into the genome and transferred as a normal genetic trait.

Table 2 summarizes the experimental approach that one undergoes to genetically engineer plants. For any selected gene of interest, you construct the recombinant DNA plasmids that contain the selected DNA sequences. This has to be edited a bit to put the right start and stop signals on the gene to make it work well in the plant. The Agrobacterium system is used to introduce the DNA into the plant cells and transform them. Whole plants are regenerated, then tested to see whether the gene is expressed in the plant. You then select particular lines, increase the seed and then take plants into the field. Field experiments are needed to see if it actually performs in the field. Scientists are now in their fourth season of testing genetically engineered plants for virus resistance in the field. There are tests throughout this country and throughout the world. The next step will be to have commercial production of virus protected plants.

The Mechanism. In engineered protection, it is believed that the coat protein simply blocks the ability of the challenge virus to disassemble so that the nucleic acid becomes active and is read by the plant. Success is not just with tomato and tobacco for TMV resistance. In only four years, this biotechnology method has enabled resistant cultivar development for 15 viruses in five plants: tobacco, tomato, alfalfa, squash, and cucumber (Beachy et al. 1990). Thus the area is moving along extremely rapidly and plants will soon be ready for use in agricultural production.

Controversies

An experiment in California to field test a bacterium on plants in the spring of 1987 touched off a controversy as to whether these research plots were doing anything that was dangerous to the general public. This controversy was led by Jeremy Rifkin and various environmental groups, both being great opponents of this technology. Thus, the field tests conducted thus far have required approval by the Federal Government agencies. The issues that have been faced so far have really been more from the environmental standpoint and the research standpoint, than from the human consumption and the food safety standpoint. But now, with the successes in hand and demonstration of the benefits of this technology, issues of food safety are being raised. One of the reasons for the delay is that people haven't been willing to address such a controversial issue. It has been such a sensitive area because it is felt that more of a research base is needed.

Background. The controversy on recombinant DNA dates back more than fifteen years. The "track record" thus far is excellent. We have had the technology to move genes around for fifteen years and during that time there has not been a single laboratory-related or application-related accident that can be attributed to the use of biotechnology. By all standards of comparison to other technologies, it is as safe as anything else. Many people think it is safer because it is much more defined in terms of knowing exactly what you are doing, whereas in making traditional genetic crosses you don't. In traditional genetics one plant is crossed with another, and 50% of the genes from one and 50% of the genes from the other are combined and the resulting phenotypes are observed. With genetic engineering, one variety that may already be in commercial production is used and one gene is inserted. The change then is much less than by traditional crossing, but the exact site of insertion and what effect this has on the plant's genes is uncertain.

Regulation of Biotechnology

Issues are now emerging not just with plant genetic engineering but also with transgenic animals and human gene therapy. The control of this research and the ability to proceed in research was allowed in 1976 by the National Institutes of Health, which established guidelines for conducting recombinant DNA research. NIH went through the government process of filing an environmental impact statement to assess the effect on the environment of issuing guidelines for doing this type of research. What we have seen in the course of these fifteen years is a progression of authorities and procedures for getting materials into the environment. Agencies other than NIH have come into play at the research level. At the U.S. Department of Agriculture, the Office of Agriculture Biotechnology and the Agricultural Biotechnology Research Advisory Committee (ABRAC), on which I serve as a member were established. NBIAP, the National Biological Impact Assessment Program, was also formed. This is a computer bulletin/board information system that contains a wealth of information on biotechnology, including current regulatory schemes. Anyone in the country can tap in to by dialing 1-800-NBIAPBD; the computers for it are actually located here at VPI. Thus far, the NBIAP is focused primarily on the environmental and the agricultural information and not on the human safety viewpoint.

U.S. Government regulatory agencies are quite active in the regulation of biotechnology. The USDA Animal and Plant Health Inspection Service (APHIS), when there is a product, gives permits and licenses. In practice, this means they have given permits for every field test of genetically engineered plants. APHIS interacts with the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), depending on the product and its use. The overall coordination has been by an interagency committee called the Biotechnology Science Coordinating Committee. This committee met regularly and essentially coordinated the use of existing rules and regulations for looking at biotechnology.

The ABRAC is advisory to the Assistant Secretary for Science and Education in USDA. We have drafted research guidelines that have to do with how to view the engineered organisms and what attributes are important with respect to biosafety, and are awaiting their publication in the Federal Register. The basic premise is that the risk of field research with a genetically modified organism is judged by comparing it to the parental organism from which the modification was made. A number of traits that have potential importance from the environmental standpoint have been identified upon which one can design the particular experiment so it can be conducted safely in small scale field tests. The guidelines do not yet address the further development for commercial use, including food use of genetically engineered plants. We think that will come with time. The ABRAC's main intent has been to establish relations with the regulatory agencies and describe a scientific basis for their activities.

Approaches to Examining The Biosafety of Engineered Food

In looking at some of the things that are coming out now in relation to foods, the product safety assessment of the foods from genetically engineered plants, several points emerge. Kent Stewart and other food chemists have been involved in developing information and concepts that are being provided to the Federal Government. The recommended approach is to compare the "new" product to the traditional product, i.e. take the engineered product and look at its attributes in comparison with the traditional product. Quite possibly this will involve some of the data that you are collecting and organizing. As a plant biologist, I think the comparison to the traditional product is a bit too broad. I lean to the ABRAC proposal of comparing it to the actual parental plant. There is enough varietal variation that the comparison, I think, should first of all be made to the same plant without the introduced trait.

A key point is going to be to identify natural toxic constituents that are in the particular parental plant as well as in related plants, and to evaluate these before you get to the stage of testing the foods. Plant breeders use research plots to evaluate the performance of an organism and judge whether a product of it might have an application. I think what is needed now is to encourage researchers, as they are choosing genes to introduce into the plants and developing ways to introduce them, to make them as safe as possible. They may then to be able to demonstrate to the consumer that the engineered product has the same level of safety, the same utilization characteristics, and the nutritional impact as does the traditional product unless these are the exact things that are changed and are an improvement.

Table 3 is a compilation of some information from a report (IFBT, 1990) that is coming out soon, that included identification of toxicants in plants that are harmful to humans. I was surprised at how few there actually are and their sources. Out of 200 or so toxicants on the list, only 10% or 21 are directly harmful to humans and most of these are in honey. Toxicants may also be concentrated in local diets, depending on whether certain legume crops are eaten. The cyanogenic glycoside compounds, linamarin and lotaustralin, are quite toxic to humans and documented a number of times in instances of human illness and death. Solanine in potatoes often is used as an example of a toxicant that has come out of the normal plant breeding program. It is a neurotoxin that shows up on the green skins of potatoes. A few years ago a variety was released that was very high in solanine. It was very rapidly taken off the market. But this is being used as the example of why plant breeding should be looked at very carefully. In squash and cucumber, cucurbitacin occurs sometimes when wild species are used in crosses, but the cultivated varieties do not have these compounds. Various leafy vegetables grown under improper fertility regimes will have high nitrates and cause the same problems that nitrates do when they are added to foods.

The issues that are involved in looking at the safety of engineered foods are many and involve large numbers of people and disciplines. In looking at the issue from a nutrient and toxicological standpoint, there are three principle questions that I think should be asked, and must be linked in addressing the safety of the engineered products as foods:

1) What is the genetic origin of the gene that is put into the new food?
2) What does this genetic modification do in relation to the traditional counterpart?
3) What is the expected intake pattern of or the exposure to the new food?

References

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TABLE 1. APPLICATIONS OF BIOTECHNOLOGY IN PRODUCTION OF FOOD/FEED PLANTS

o Improved plant yields/processing characteristics

o New plant varieties

- Disease resistant plants
- Insect resistant plants
- Herbicide resistant plants

o Improved nutritional value of plants

TABLE 2. EXPERIMENTAL APPROACH FOR APPLYING BIOTECHNOLOGY IN PRODUCING GENETICALLY ENGINEERED PLANTS

1. Select gene of interest
2. Construct recombinant plasmids with selected DNA sequences
3. Insert cloned DNA into Agrobacterium tumefaciens Ti plasmid transformation system
4. Transform plant cells; select transformants
5. Regenerate whole plants
6. Assay for presence of and expression of insert
7. Select lines with highest expression and increase seed
8. Test performance in field experiments

TABLE 3. PLANT TOXICANTS HARMFUL TO HUMANS

o Honey Toxicants
Flowers of Azalea family, jasmin, tutu tree

o Toxicants from Legumes in Local Diets
- Ackee fruit (immature) - Hypoglycin A
- Lima beans - Linamarin
- Cassava root - Lotaustralin
- Chick pea - B-N-Oxalylamino-L-alanine

o Plant Genetic Factors/Handling
- Potato - solanine
- Squash, cucumber - cucurbitacin
- Celery, Spinach, Lettuce - high nitrates