

TRANSGENIC PLANTS, THEN AND NOW: A PERSONAL PERSPECTIVE

David W. Ow¹

INTRODUCTION

Although I was asked to give a talk on transgenic plants and issues concerning genetically modified organisms (GMOs), I decided that this was too broad a topic. I would only be qualified to describe a few examples of the transgenic plants that have been a part of my research. I chose these examples only because of my familiarity with them, nothing more. Hence, this article should not be considered a comprehensive review on the achievements in this field or as a definitive view on GMO issues.

MAKING A PLANT GLOW

My first attempt at making a transgenic plant was in 1985, when I was a postdoctoral fellow with Stephen Howell at the University of California, San Diego (UCSD). Through collaboration with Don Helinski and Marlene DeLuca, we had obtained a gene from the firefly that encoded luciferase, the enzyme that makes fireflies glow. We found that the gene could produce functional luciferase in carrot protoplasts, which prompted us to test the tantalizing possibility of creating a plant that could emit light. At the time, plant gene transfer was in its infancy, with the first success reported in 1983. Following the sketchy descriptions of the technique in that report, we made our first transgenic plant. The tobacco plant was able to emit light with sufficient output to be captured by overnight exposure on film (Ow *et al.*, 1986). The photo had an eerie, “sci-fi” feel to it (Fig. 1). We managed to make four posters before UCSD copyrighted it, not that any of us would think of selling our posters. The news that a plant could glow lit a fire in the news. Today, this photo is found in numerous textbooks, and the luciferase gene has proved to be a valuable tool in research. The photo, being an icon of plant genetic engineering, also received its share of criticism. There was not much opposition to transgenic technology back then, but signs were emerging. I experienced my first quack call from someone claiming to be associated with a religious group that opposed genetic engineering and tobacco use. Apparently, that person saw the headlines of a newspaper that read “Tobacco that Lights Itself.” A year later, I was told that Jeremy Rifkin had shown the glowing-plant photo on *Good Morning America* to exemplify the wild creations that could emerge from tinkering with DNA.

¹ Plant Gene Expression Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710.

DELETING ANTIBIOTIC RESISTANCE GENES

The above work turned me into a *bona fide* plant genetic engineer. When I started my lab at the USDA, the Plant Gene Expression Center (PGEC) in 1987, I naturally continued with research related to plant biotechnology. I became interested in finding a way to remove antibiotic resistance genes used as selectable markers in genetic engineering. Genetic transformation was, and still is, a very inefficient process. Only a few cells in a million incorporate the foreign DNA into the plant genome. Genes that detoxify cytotoxic agents are used to find the rare transformed cells. When treated with an antibiotic, only the transgenic cells survive. However, being linked to the gene to be transferred, marker genes are nearly impossible to segregate from the transgene of interest. As a result, all transgenic plants would harbor selectable marker genes. There is no documented harm associated with them, but once transformed cells are identified by selection, there is no need for them. In fact, their presence in the cell line would mean that a second round of gene transfer could not be conducted using the same selection strategy. Even today, there are not many effective selectable marker genes for use in genetic transformation. So one rationale for removing the marker genes from transformed plants was to permit their use in subsequent transformations on the same plant.

Removing the drug-resistance genes would also eliminate possible concerns for food and environmental safety. These genes code for enzymes that are not natural food proteins. Even though we ingest some bacteria that make them, they are not normally consumed in large quantities. If produced in a wide variety of transgenic food, these proteins would become a standard dietary component—and one that has not been tested through human evolution. In all probability, these proteins would be broken down like any other, but to say that they are 100% safe is impossible. For instance, what if rare individuals find them allergenic. Aside from food safety considerations, there is also the concern that genes engineered into crop plants might find their way back to bacteria. If so, there would be the possibility, however slim, that they could compromise clinical effectiveness of the drugs. Already, there is growing medical concern on the rise in drug-resistant bacteria and the diminishing number of effective antibiotics. The possible worsening of an already bad situation would be a public relations disaster that will nip at the buds of this promising technology. So rather than face the prospects of countless debates, risk assessment studies, and lawsuits over these genes, we decided that a method had to be available to remove them. However infinitesimal their risk, we could reduce that risk to zero by removal of the marker genes.

The method we developed is based on site-specific recombination (Dale and Ow, 1991). It is not patented and is available for public use. The method is efficient but requires incorporating certain features into the initial molecular construct. That means transgenic plants must be constructed with these features built in. By the early 1990s, many biotechnology firms had already made transgenic plants that were at various steps toward product development. Our intent in developing this technology was to advance the improvement of future products. Unfortunately, a dominant segment in industry viewed it as an impediment to future product development. This led to extensive debates in the scientific circles (e.g., Flavell *et al.*, 1992; Leather & Bryant, 1992)

that migrated into the regulatory arena. Over the years, this issue proved to be a weak link in the controversy over genetically engineered plants. Opponents of the technology found the antibiotic resistance gene an easy target. In 1997, while the various European Union states were indecisive on whether these genes should be approved, Norway, being outside of the EU, took the bold move to ban all antibiotic resistance genes from transgenic products. That tipped the balance. Since then, the EU has been tilting toward the Norwegian position. As a result, there is now a *de facto* ban on the use of these genes, along with an apparent victory for the opponents of transgene technology.

HERBICIDE-RESISTANT COTTON

In 1987, Gerry Still, then director at the Plant Gene Expression Center (PGEC), and Jerry Quisenberry, then director of the ARS laboratory at Lubbock, Texas, brought a group of their scientists together to explore areas of mutual interest. Initially, nothing came out of that meeting, but a year later, Norma Trolinder and I found common ground and established a collaboration to engineer cotton for 2,4-D tolerance. The use of this herbicide for other crops has been a problem for cotton growers, and damage to cotton from herbicide drift has led to a number of lawsuits. A bacterial gene that degrades this herbicide was reported. It was just a matter of getting the gene and figuring out how to transform cotton. Getting the gene from the authors of the report was more complicated than expected. Consequently, we cloned an analogous gene from a different bacterial strain. While we were making the 2,4-D gene construct, Norma worked out the transformation protocol for cotton with the luciferase gene. As soon as we completed the 2,4-D resistance gene clone, she transformed cotton with it. The plants tolerated not only drift levels of the herbicide, but also levels sufficient for weed control (Bayley *et al.*, 1992).

The plants were provided to the cotton-breeding community. Jim Becton of UAP (United Agricultural Products) conducted a series of field studies that showed the plants were tolerant to direct spraying of the herbicide (Fig. 2). Since 2,4-D is much less expensive than patented herbicides, there was interest in its use in weed control in cotton. Unfortunately, due to the drift problem, this would be possible only if all cotton varieties had the 2,4-D resistance gene. Otherwise, it would only exacerbate the very problem our research was designed to solve. As for providing a solution for 2,4-D drift damage, it was a scientific success that has yet to impact the cotton community. The plants generated could not be grown commercially because the plant was engineered with the 35S gene promoter, which was thought to be in the public domain. It was not known until later that Monsanto was awarded the claim for its commercial use. Since the plant might compete with Roundup Ready cotton, UAP was unable to negotiate a license from Monsanto. Nonetheless, the concept was proven and the commercial sector could reconstruct similar plants using a different promoter.

During this time, opposition to genetic engineering had gained momentum. There was intense criticism of the commercial interest in herbicide resistance traits. It was argued that this technology was perpetuating a reliance on chemical herbicides. It did not take long for someone to find in the government database that we were doing re-

search on this topic. A freelance reporter contacted the USDA, which then sent her to me. She said she only wanted to get an idea of the research conducted at the PGEC. I spent over an hour explaining our various research projects. Only in the final minutes did I touch upon our collaborative work on how 2,4-D-tolerant cotton would help farmers fend off accidental drift damage. Months later, my picture appeared with a lengthy story that described how tax dollars were used to help corporations profit from environmentally damaging herbicides (*Bay Guardian*, September, 1990). Since nothing was mentioned of anything else I had told her, the interview must have been a setup from the start.

GOSSYPOL-FREE COTTONSEED

Prior to the work on 2,4-D resistance, I had not shown much interest in cotton as it was not a model system for molecular biology. However, with the generous support of Cotton Incorporated, and encouragement from Gay Jividen, we took on the challenge to make gossypol-free cottonseed. For every pound of fiber, there are 1.67 pounds of seed, a rich source of protein and oil. The annual worldwide cottonseed yield could supply the dietary protein needs of some 240 to 350 million people (Lusas and Jividen, 1987). Ruminant animals tolerate cottonseed meal, but it is toxic to monogastric animals because of the compound gossypol. If gossypol were not present, cottonseed oil could be made more economically, and cottonseed meals could be processed for food and feed for a wider range of domesticated animals. The use of glandless cotton will produce gossypol-free cottonseed, but the lack of gossypol makes the plant highly susceptible to insect predation. To keep gossypol in the plant but away from seed, we proposed engineering the seed-specific breakdown of this compound. We reasoned that gossypol in cottonseed, like all organic matter, must get recycled into the basic building blocks. That would mean there should be microbial enzymes that breakdown this compound.

Heather Koshinsky, a postdoctoral associate in my laboratory, began the work by screening microorganisms from gin trash. To make a long story short, we didn't make much progress for a number of years. If this project were funded by the National Science Foundation, funding would have run dry long ago. But our persistence paid off, and in our fifth year, we finally identified an enzyme that could degrade gossypol. This led to the gene and the construct for expression in plants. As a proof-of-concept experiment, we engineered transgenic cotton using a promoter that expresses throughout the plant. For the past year, we have obtained several transgenic cotton plants in which the size and density of gossypol-containing glands were reduced in the leaves. This is consistent with an expectation that the gene caused a reduction of the toxic compound. Our current challenge is confining the expression of this gene to seed.

CONCLUDING REMARKS

I would like to conclude by thanking the organizers for inviting me to share these stories with you. We are all aware of the current hurdle of the European public that is skeptical of transgenic food. Some of those feelings are also spreading over to the

United States. I feel that work from my lab has addressed some of the consumer concerns. A number of companies are publicly stating that antibiotic resistance genes will not be in future products. The industry has also been polishing its image in other respects. Most companies are stating that they are moving toward an emphasis on quality traits, as opposed to traits that give the appearance of promoting chemical sales. The next wave of products would benefit the consumer directly, such as a healthier composition of lipids in oil, a more complete blend of vitamins in cereals, and disease-preventing vaccines in fruit. In this regard, our current work on developing gossypol-free cottonseed would fit into the category of quality traits. If successful, it could provide a source of protein to help meet the needs of the world's burgeoning population.

LITERATURE CITED

- Bayley, C., N. Trolinder, C. Ray, M. Morgan, J.E. Quisenberry, and D.W. Ow. 1992. Engineering 2,4-D resistance into cotton. *Theoretical & Applied Genetics* 83:645-649.
- Bryant, J. and S. Leather. 1992. Removal of selectable marker genes from transgenic plants: needless sophistication or social necessity. *Trends in Biotechnology* 10:274-275.
- Dale, E.C. and D.W. Ow. 1991. Gene transfer with subsequent removal of the selection gene from the host genome. *Proceedings of National Academy of Sciences USA* 88:10558-10562.
- Flavell R.B., E. Dart, R.L. Fuchs, and R.T. Fraley. 1992. Selectable marker genes: Safe for plants? *Bio/Technology* 10:141-144.
- Lusas, E.W. and G.M. Jividen. 1987. Glandless cottonseed: A review of the first 25 years of processing and utilization research. *JAOCS* 64:839-854.
- Ow, D.W., K.V. Wood, M. DeLuca, J.R. de Wet, D.R. Helinski, and S.H. Howell. 1986. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* 234:856-859.



Figure 1. Tobacco plant expressing the firefly luciferase gene photographed in light (top) or after long exposure in the dark (below). Photo excerpted from *Time* magazine.



Figure 2. Field test of 2,4-D-resistant cotton plants. Transgenic plants survive herbicide treatment compared to non-transgenic plants that failed to grow. Photo provided by Jim Becton, United Agricultural Products.