



Anomalies in the early stages of plant transgenesis: interests and interpretations surrounding the first transgenic plants

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Abstract

The origins of plant transgenesis are discussed and the experiments that led to the first transgenic plants are analyzed. This process involved a series of actors, practices and interests specific to biotechnology. Consensus about the meaning of fundamental experiments was also at issue here. These events illustrate some of the conflicts related to genetically modified organisms, since scientists had different responses to plant transgenesis at the time of the first experiments, and opinions of the anomalies in those experiments varied. Thus, this article analyzes the interests and interpretations surrounding the first experiments involving transgenic plants.

Keywords: genetically modified organism; anomalies; transgenic plants; experiments; silencing.

Since the 1930s, genetics began to gain visibility at the expense of other biological disciplines – such as embryology and physiology – and more researchers became interested in studying genes and their effects (Fox Keller, 2002; Regal, 1996). Genetic research received a considerable boost from a series of events, most notably Watson and Crick’s description of DNA’s helix structure in 1953. Strictly speaking, the crucial moment for the start of modern biotechnology were the years 1972-1974, when a series of experiments carried out in American universities successfully cut a fragment of DNA from one species and integrated it into the genetic sequence of another.

Thus, around 1973 the first genetically modified organism in history was obtained: a bacteria (Wright, 1986). The first transgenic mouse was created in 1980, when a genetically manipulated sequence of DNA was microinjected into an embryo, which was then implanted in a female mouse (Gordon et al., 1980; Hanahan, Wagner, Palmiter, 2007). Plants, however, presented another set of difficulties.

One of the fundamental problems that needed to be solved in order to obtain a transgenic plant was how to introduce DNA into a plant cell. This was not difficult to achieve in the case of a bacteria, because all one had to do was to add salts to a test tube and to produce heat shock so that the pores in the bacteria’s membrane would open and the DNA could get in. But plant structure is completely different. The cell is the basic structural unit of biology, and there are basically two types: prokaryotic cells and eukaryotic cells. All bacteria are prokaryotic. Their cell structure is much simpler and older than the eukaryotic type. The prokaryotic cell has an outer layer called the cell wall, in which there is a membrane with a single compartment containing DNA, RNA, proteins and other small molecules. That is all. Eukaryotic cells, on the other hand, are much larger and more complex. They have intracellular structures like the nucleus, and various specialized organelles outside the nucleus. Also, plant cells in particular are covered with a rigid outer layer called the plant cell wall (Alberts et al., 1996).

To obtain a transgenic plant, the genetic construct to be inserted had to cross the cell wall, the plasma membrane and the nuclear membrane; of course, all this had to be done without destroying the plant cell, and a whole plant then had to be regenerated. Introducing a gene into the nucleus of a plant cell was much more complicated than for other types of cells. The solution was to use a biological system that would infect plant cells, and this tool was developed once there was interest in obtaining a transgenic plant.

In 1907, it was discovered that the tumors known as crown gall disease seen in some plants were caused by the presence of a bacteria (Smith, Townsend, 1907). The researchers who described this phenomenon named the oncogenic agent *Bacterium tumefaciens* (a bacteria that creates tumors), and this was later popularized as *Agrobacterium tumefaciens*. It was believed that the bacteria must transfer something to the plant, a sort of tumor-inducing principle, in order to cause the tumor, but it was not clear what that was (Braun, 1958).

At Ghent University in Belgium, Jeff Schell and Van Montagu’s group were studying the interactions between plants and soil bacteria. In 1974, they showed that an *Agrobacterium* plasmid, which they called the Ti plasmid, was responsible for causing the tumor in plants (Zaenen et al., 1974; Van Montagu, 2011).¹ They then tried to develop methods for altering *Agrobacterium* so as to use it as a vehicle for genetically modifying plants.

Whoever could control *Agrobacterium* would control plant transgenesis. So a kind of race developed to see who could manage to manipulate *Agrobacterium's* Ti plasmid (Vasil, 2008). Around that time, Mary-Dell Chilton (2011), a researcher at Washington University, started looking for the genes of the plasmid that caused the tumor, which was, incontrovertibly, the tumor-inducing principle for *Agrobacterium*.

Chilton put her entire laboratory to work on a single mission: finding out which areas of the plasmid were involved in producing the tumors. She found that a specific sequence of the plasmid, called T-DNA, was present in the tumors (Drummond et al., 1977). There was something new and peculiar about the way *Agrobacterium tumefaciens* worked: a bacteria transfers its DNA to the cells of a plant, which incorporates that DNA into its own genome and develops a tumor that produces nutrients for the bacteria. In other words, the result of the interaction between *Agrobacterium* and the plant is a plant that has naturally recombinant cells. *Agrobacterium* became the perfect tool for the emerging field of plant biotechnology. By redesigning the T-DNA (extracting the oncogenic genes and inserting different ones), it could be used as a vector for developing transgenic plants (De Framond, Barton, Chilton, 1983).

The first transgenic plant

Once the biological vector for transferring the relevant DNA to the plant had been defined, the next task was to extract the oncogenic genes from the T-DNA (because otherwise a mass of tumor cells would be produced instead of a plant) and insert genes from another species into it; then the modified T-DNA had to be introduced into the Ti plasmid, which would in turn be inserted into the *Agrobacterium tumefaciens*, which would finally be put in contact with the plant cell. Chilton managed to do this in an experiment that was crucial to the development of plant biotechnology: she inserted bacteria and yeast genes into the Ti plasmid, thereby obtaining a plant containing those new sequences in its genome (Barton et al., 1983). This transgenic plant was featured on the front cover of the prestigious scientific journal *Cell* in April 1983.

The feasibility of obtaining a transgenic plant lay in overcoming two problems that were present in the early 1980s, both of which Chilton's experiment successfully solved: on the one hand, researchers needed to obtain a whole plant after having infected it with *Agrobacterium*, and on the other, the plant had to express the foreign genes that had been inserted into it.

Obtaining a whole plant after exposing it to *Agrobacterium* was not a simple matter, precisely because *Agrobacterium* creates a cancerous growth in the plant. *Agrobacterium's* Ti plasmid has genes that code for proteins, which allow it to insert its T-DNA into the nucleus of the plant cell; it also has genes that express hormones, which cause the growth of a tumor in the plant's tissue; and it has genes that code for metabolites that function as nutrients for *Agrobacterium*. Thus it functions as a biological colonizer: it makes the plant's cells work to satisfy the needs of *Agrobacterium*. Therefore, using the Ti plasmid meant that the plant cells would turn into tumor tissue, not a whole plant. In this particular experiment, Chilton and her group inserted yeast and bacteria genes into the T-DNA, replacing the oncogenic genes in the sequences. Since this allowed a whole plant to be regenerated, the problem was solved.

On the other hand, getting the foreign gene to integrate successfully with the plant's cells was also a challenge. Some years earlier, Boyer and Cohen had shown that it was possible to express different species of genes in bacteria. But plants have much more complex systems for regulating and expressing genetic information than bacteria. Chilton inserted a yeast gene and a bacteria gene into the Ti plasmid that was introduced into *Agrobacterium tumefaciens*. This bacteria with the recombinant plasmid was placed in an in vitro culture along with cell tissue from a tobacco plant. From this tissue, the plant regenerated and from that plant, another was obtained by self-pollination. The second tobacco plant's genome contained the sequences for the yeast and bacteria genes. Published in 1983, this experiment showed that foreign DNA had been successfully introduced and had remained stable within the plant's genome. It also showed that those genes transcribed their corresponding RNA in the plants. But the experiment did not find any of the respective proteins – for yeast and bacteria – present in the plant's tissue. The authors state that “the apparent lack of expression is disappointing but not surprising”, since they admit that “precise requirements for expression of DNA sequences at various developmental stages in plants are not known” (Barton et al., 1983, p.1041). The article concludes by saying that these difficulties would be overcome if people continued doing the same type of experiments, in other words, as plant transgenesis progressed.²

Industry and biotechnology in the early stages of transgenesis

Transgenic plants were desirable because of the opportunities they represented. While the first organisms to be genetically modified were bacteria, they were only interesting as an initial model, since their potential commercial value was fairly limited. But transgenic plants opened the door to the world of agriculture, which promised a completely different scale of production. Commercial interest in biotechnology skyrocketed between the late 1970s and early 80s. Anyone who was an expert in genetic engineering in those years – especially in the United States – received generous funding from the private sector.³ Any scientist using recombinant DNA techniques had links with companies (Wright, 1994, p.107). Recombinant DNA opened up the possibility of a new world market and with it came an avalanche of contracts, money and offers to make a name for oneself in the development of biotechnology products. The explosion of resources happened mainly in the United States – and to a lesser extent in England – which is where research on recombinant DNA was concentrated and where there was funding available to devote to this new field.

In 1974, Stanford University filed a patent request on the methodology for recombinant DNA developed by Boyer and Cohen. In 1980, the patent was granted. Anyone wishing to use recombinant DNA for commercial purposes had to pay royalties. The patent covered everything from plasmids to the replication and expression of foreign DNA in microorganisms. In the first four months after Stanford University began enforcing the patent, 72 companies acquired licenses to use Boyer and Cohen's technology. The patent expired in 1997. During those years, recombinant DNA proved to be an authentic goldmine for Stanford: profits from the patent reached some 250 million dollars (Smith Hughes, 2001).

The scientists who developed the techniques for DNA splicing, the pioneers of recombinant DNA, had also become successful businessmen. One of the first companies to emerge in

the field was Cetus, in 1971. Soon, the company directors hired Joshua Lederberg and, in 1975, they recruited Stanley Cohen. Paul Berg helped found the company DNAX.⁴ Biogen boasted various leading scientists, such as Walter Gilbert, on its board of directors. Sydney Brenner – one of the major figures in molecular biology research in England – became a board member for the brand-new British company Celltech. Meanwhile, Herbert Boyer – together with Robert Swanson, a venture capitalist – founded the company Genentech in 1976. This company's startup budget was under a thousand dollars, but four years later, it was worth over five hundred million dollars.

Research on recombinant DNA also shot up. In 1975, the National Institutes of Health (NIH) funded just two projects on the topic, allotting them twenty thousand dollars. In 1982, it was funding 1,588 research projects for a total of 185 million dollars. Thus, along with recombinant DNA, a new market was being developed, and it was taking giant steps forward.

The first biotechnology product to be marketed was recombinant insulin, produced by Genentech in 1978, using a genetically modified bacteria. By that time, recombinant DNA had conquered bacteria. Profits from those first biotechnology experiments, however, did not lie in recombinant proteins themselves so much as in the expectations they generated about new biotechnology markets. In fact, for example, human recombinant insulin was more expensive to obtain than the kind previously produced using pig glands. The new biotechnology companies' success was due to lucrative contracts obtained from the big multinational corporations that had grown up in the field of chemistry and pharmaceuticals and that were now starting to invest in an area that was unfamiliar for them. Biotechnology's sudden commercial success stemmed, therefore, from the expectations it raised.

The big multinationals had absolutely no experience in these new genetic engineering issues. So they gave contracts to the new startup companies being created by scientists and entrepreneurs, so as not to be left behind in the potential new market generated by biotechnology and to start acquiring some basic knowledge of the area. To that end, they also set up agreements with the universities, where expertise on recombinant DNA had been developed. Thus, during those years, multinational corporations like DuPont, Monsanto, Lilly, Merck or Upjohn poured millions and millions of dollars into academic genetic engineering laboratories and into the new biotechnology companies that had been created. Little by little, these big industrial chemical, oil and pharmaceutical corporations started absorbing information about recombinant DNA. Around 1982, the scene started to shift. The multinational corporations could now handle recombinant DNA technologies by themselves and did not need to keep sending their resources elsewhere. DuPont announced the opening of its own agricultural research department to the record sum of \$85 million (Wright, 1994). From then on, a few companies began to emerge as the leaders in the biotechnology market, and the number of new startups so typical of the sector a few years earlier began to decline. The explosion of resources lasted only a short while, but it was very intense.

For a few years, genetic engineering laboratories in American universities were swimming in dollars and the scientists who had started biotechnology companies became millionaires overnight. But that would not happen again, at least not in this area of biotechnology: the market had opened up and the major players were already defined. In this new phase, the biotechnology race was being run by the big multinational corporations.

Why were businesses so interested in plant transgenesis? It is not a trivial question, because for a large part of the twentieth century work on new improvements to plants was carried out by the public sector. In particular, in the United States, agricultural research depended heavily on the efforts of the public sector, particularly after the Second World War. Private companies only worked on innovation in specific niche areas, such as, in particular, the development of hybrid sweet corn.⁵ Then, in the 1960s, a legal framework was set up to provide powerful legal protection for the new plant varieties, and this spurred crop enhancement research in the private sector (Murphy, 2007).

During these years of exploding opportunities for recombinant DNA (the late 1970s and early 1980s), people became interested in developing genetically modified plants. Mary-Dell Chilton was the first to obtain a transgenic plant, but there were also two other leading groups in the field of plant genetic engineering. In early 1983, at a conference on genetic engineering in plants, Schell and Van Montagu (of the University of Ghent), Rob Horsch and Fraley (of Monsanto) and Chilton (from Washington University) all announced similar results: all three groups had successfully expressed antibiotic genes in plant cells (Newton, 2010; Fraley et al., 1983; Herrera-Estrella et al., 1983; Chilton, 2001). All three groups had set out to find a method for transferring genes to plants, and they had succeeded.⁶

Fraley and Horsch continued working for Monsanto, and both became vice-presidents of the company. Schell and Van Montagu created their own company in Ghent, Belgium, called Plant Genetic Systems Inc., which was absorbed years later by Bayer CropScience. Meanwhile, Mary-Dell Chilton was soon recruited by Ciba-Geigy, the company that would later become Syngenta, one of the biggest plant biotechnology companies in the world.

All the scientists who have contributed to the field of recombinant DNA since the 1970s have gone on to important positions in biotechnology firms. Contributing to knowledge in the growing field of biotechnology was also a way to ensure one's place in the industry that was beginning to emerge.

Needless to say, the fact that a new scientific field was linked to the growth of an associated industry was nothing new. Indeed, it would be surprising if such links did not exist. An example of this occurred in the chemical industry in the second half of the nineteenth century in Germany, where there were a number of famous scientists, at a time when many chemical compounds were being discovered and companies like Bayer, Basf and Agfa (Aftalion, 2001) were created. But the comparison would be too general, since needless to say these were very different contexts and it would be unreasonable to compare the number of scientists and the amount of capital circulating in the chemical industry in the nineteenth century to the biotechnology industry at the end of the twentieth century.

In any case, in the late 1970s and early 1980s, information about recombinant DNA commanded enormous interest in the industrial sector, and that translated into new biotechnological companies, contracts and massive investments. Of course, a great number of scientists and a considerable amount of the companies' capital came from the chemical and pharmaceutical industry, so the market for biotechnological products expanded rapidly. Subsequently, a small number of companies emerged as the lead players, and then the flurry of contracts, investments and startup companies began to die down. Thus, the links between

knowledge and production made biotechnology happen, but those links were never as strong and extensive as in that initial stage.

The silence inside plants

Chilton's experiment gave rise to a new age of plant biotechnology. Could the results of her experiment be interpreted to mean that the first transgenic plant had been created? They certainly could, but it was also possible to read things very differently. The fact that the foreign genes were not expressed in proteins could be seen as demonstrating the complex system of regulation and expression of plant genes, and as a result, one could conclude that it was very difficult to obtain a transgenic plant that would function the way one wanted. This is not an anachronistic interpretation: ever since 1941 it had been known that each gene specifies the structure of a protein, and from the 1950s on it was clear that the sequence involved DNA transcribing RNA and then proteins.⁷ Chilton's plant had DNA and RNA from the transgenes, but not the proteins. This could be seen either as an illustration of the problem facing transgenesis, or as a step towards solving it. The fact that the scientific community had adopted the latter position was not obvious.

Just as the first transgenic plant obtained by Chilton's laboratory in Washington failed to express recombinant protein, similar attempts by other research groups ran into the same sort of difficulties. In many subsequent experiments, researchers managed to express the recombinant protein, but the levels varied greatly. Often, the transgenic plants showed very little recombinant protein, much less than expected. In such cases, the transgenic plants were abandoned in favor of ones that presented more acceptable expression levels (Matzke, Matzke, 1995). Some years would go by before this anomaly (the lack of protein expression in the transgenes introduced into plants) turned into a research problem in its own right and then became a whole research field. In fact, this happened when other players with other interests appeared on the scene, players able to redefine the difficulties of protein expression in transgenic plants as a research problem. Two of these new players were, however, Chilton's disciples.

Marjori and Antonius Matzke met when they were working as post-doctoral researchers in Chilton's laboratory, from 1980-1982. In fact, Antonius was named as one of the co-authors of the article about the first transgenic plant published in the journal *Cell*. After they finished their post-doctoral studies, the Matzkes went to Austria, where they set up their own laboratory, and they continued working together from then on. In Austria, their research differed from Chilton's because they were trying to explore the anomalies of plant transgenesis, convinced that those anomalies would help shed light on issues related to genetics in general. Antonius claims that he was always interested in using plant transgenesis for basic research; in other words, he was not interested in obtaining a transgenic plant as a final result, but rather in discovering how genes functioned in plants (Matzke, 2010). So, once they got to Austria, the Matzkes focused on the problems of transgene expression in plants. They showed that T-DNA, with which they were experimenting, underwent a small chemical modification within the plant, which might explain why the gene was not expressed (Matzke et al., 1989).

Shortly afterwards, another research group confirmed Matzke's results with a similar experiment, although with one exception: they introduced a gene into a plant that already possessed a gene from that family. The goal was to overexpress that protein. But the result was the opposite: the inserted gene was not expressed, and neither was the gene the plant already had. This was called co-suppression (Napoli, Lemieux, Jorgensen, 1990).

Another researcher who would become famous for studying these phenomena was Hervé Vaucheret. His doctoral research in a cellular biology laboratory at the Institut National de la Recherche Agronomique (Inra) in France clearly involved basic research: to characterize a gene – nitrate reductase – belonging to plants. Within that framework, he used some transgenes of nitrate reductase, but did not manage to get them to express. After finishing his doctoral dissertation in 1989, he developed his own line of research on the phenomena he had observed while working on his thesis. Thus, Vaucheret set about characterizing various plant proteins involved in suppressing gene expression. He described how a plant can systematically silence a gene, transmitting small RNA sequences to the plant cells. He also specialized in characterizing a set of proteins, the Argonauts, involved in post-transcriptional gene silencing.

It soon became clear that plants have a number of different mechanisms to help them avoid expressing a transgene. A general term was invented to refer to this range of mechanisms: gene silencing. On the one hand, the transcription of a gene can be inhibited, so that silencing works by blocking DNA. This is called transcriptional gene silencing. But silencing can also work on RNA, breaking it down (which is known as post-transcriptional gene silencing). Small RNA sequences play an important role by bonding with the RNA messenger that is subsequently broken down. Researchers also began to identify various proteins involved in silencing phenomena. They thought that plants normally use these mechanisms to defend themselves from infection by a virus.

Research studies multiplied. The ways in which plants can silence a gene are extremely varied. Different proteins and other molecules are involved in these mechanisms. Then it was discovered that gene silencing was not exclusive to plants: it also occurs in animals and bacteria. The study of mechanisms inhibiting gene expression began to develop into a research field. All of this stemmed from the difficulties of expressing recombinant proteins in plant transgenesis.

While plant transgenesis was seeking to produce genetically modified plants, a discipline began to emerge involving the very problems that genetic engineering researchers had seen as uninteresting. Indeed, these other scientists who focused on gene silencing saw the first transgenic plant very differently. For them, Chilton's plant in 1983 was not proof of the feasibility of transgenic plants, but of something else: the importance of supragenetic phenomena operating on transgenes. In their view, the first transgenic plant was not 'really' transgenic, because it could not express the transgenes due to the gene silencing mechanisms at work.

The usual interpretation of Chilton's 1983 experiment was that transgenic plants were an imminent fact, and that the small technical difficulties still remaining would be overcome by further genetic engineering. Chilton's 1983 plant was proof of plant transgenesis. But another reading was starting to emerge. In this reading, that plant did not represent transgenesis, but its own internal silencing.

Reinterpreting transgenesis

The competence of Chilton's experiment was never an issue. This was because there was a consensus about what type of experiments could work and what results to expect. This is what, according to Harry Collins (1995), defines an experiment as competent.⁸ In fact, ever since the early 1970s it was clear that a gene from one species could be inserted into the genome of another, and that this gene could remain in the new genome and express itself. As we saw earlier, this had already happened in bacteria, and it was expected that it would be achieved for other organisms. Chilton was notable for being among the first researchers to come up with a method for introducing genes into the genome of a plant cell, and for getting those genes to stabilize so that the plant could grow. Thus her experiment was a success and the journal *Cell* celebrated it as such on its front cover. It was expected that a transgenic plant would be achieved and Chilton managed to do it. It is true that she did not manage to get the transgenes to express themselves in proteins and that this constituted an 'anomaly'. Chilton acknowledged that this did not match her expectations, but she stressed that the major step of obtaining a complete plant whose genome contained genes from another species had been performed, and that with a little more practice researchers would be able to get those genes to express themselves too. The anomaly, from Chilton's perspective, would be resolved within the framework of transgenesis as it developed.

But other researchers began to explore this anomaly from another perspective. Some felt that it was the result of a mechanism unforeseen by transgenesis: gene silencing. Furthermore, the anomaly could be extended to explain the whole of Chilton's transgenic plant, thereby displacing transgenesis. Whether or not Chilton's plant was transgenic was something that gene silencing called into question.

Hervé Vaucheret, who, as I said earlier, started specializing in gene silencing mechanisms in the late 1980s, thought that studies of gene silencing in plants provided a logical explanation for the absence of gene expression in Chilton's plant. In fact, Vaucheret (2010) argued that Chilton had managed to obtain a complete plant thanks to the action of gene silencing.

It is possible to distinguish two types of argument in this position. On the one hand, I would say that explaining the absence of expression in transgenes as a product of gene silencing is the 'strong' argument, whereas attributing the plant's ability to regenerate to gene silencing is a 'weak' argument.

The explanation that gene silencing provoked the absence of expression of transgenes in Chilton's plant is a 'strong' argument because there is no alternative explanation. Chilton acknowledged the anomaly of the absence of expression of transgenes in 1983, but she offered no explanation, merely trusting that the issue would be resolved with more experimentation. But no alternative explanations for the anomaly appeared subsequently; developments in plant genetic engineering simply discounted plants with low levels of expression and concentrated on the ones with satisfactory results, suggesting that some genetic constructs led to good levels of protein expression.

Vaucheret (2010), on the other hand, argues that in order to transform Chilton's plant, a non-disarmed T-DNA was used, which therefore contained sequences that coded for growth

hormones that generate undifferentiated tissues. As a result, he maintains that the plant will only be able to regenerate if those genes are inactivated. We could say that this is a 'weak' argument, in that Chilton does offer an explanation for the plant's regeneration: she shows that she did disarm the T-DNA by inactivating the oncogenes. In fact, other gene silencing researchers do not share Vaucheret's position. Matzke (2010), who studies gene silencing but also took part in Chilton's experiment, believes that the plant regenerated because the gene for resistance to kanamycin was inserted into the sequence of an oncogene of T-DNA, thereby inactivating it.

Since gene silencing emerged as a research problem thanks to anomalies in the first genetically modified plants, perspectives on the first transgenic plant became a scene of symbolic conflict: what should be recognized in that first plant, the possibility of plant transgenesis or the importance of gene silencing?

In this sense, Vaucheret's second argument contains a high-impact image in this symbolic conflict: if the transgenic plant regenerated because the gene silencing mechanisms blocked expression of the T-DNA oncogenes, it implies that if the transgenic cell had not been silenced it would only have generated a tumor. Needless to say, a mass of plant tumor cells would not have made it to the front cover of the journal *Cell*. As a result, according to this line of reasoning, the value of this experiment lies in gene silencing and not in transgenesis. Or at least, the value of transgenesis was limited to showing the phenomena of gene silencing. The rhetorical basis for this argument is understandable but, as we said, it has no supporters, nor does it have a solid empirical basis; on the contrary, the very methodology used for transgenesis could account for the plant's ability to regenerate.

We have only to consider Vaucheret's 'strong' argument in order to understand that a new discipline arose from the anomaly in the first transgenic plants. The different levels of expression of transgenes in the first transgenic plants constituted a problem to which there was no solution except to ignore the examples that showed low expression levels. On the other hand, gene silencing provided an answer to those problems, but it meant using a series of assumptions that differed from the ones underlying transgenesis experiments. Gene silencing research assumed that scientists should focus on what surrounds the genes, not the genes themselves.

Interests involving plants: industry and research niches

How can the same experiment be interpreted as proof of the success of transgenesis and also give rise to a different theory based on the problems of transgenesis? According to Collins, heterodox interpretations of data arise when there is a lack of consensus about what results to expect from an experiment (Collins, 1992). So is it that there was no consensus about whether proteins in transgenic plants would be expressed or not? Certainly there was an agreement. It had been known since the 1940s that the vast majority of genes were expressed in proteins, and these particular transgenes fell within that majority group. So there was firm agreement about the results to be expected in the plant and yet, nevertheless, differing interpretations ensued about the experiment. The point is that there was no one level of predictable results. It may be too simplistic to assume that scientists were only expecting 'one' result from

an experiment. At least in this case, the team that developed the experiment had several expectations about the results. On the one hand, they hoped that by introducing the transgene, it would be transcribed to RNA and expressed in proteins. They acknowledged that this did not occur. But they did manage to introduce the transgene and get it to remain inside the plant genome and transcribe to RNA. Because of this, the experiment was considered a success and was featured on the front cover of the journal *Cell*.

The key lies in the different assessments of that phenomenon. For Chilton, the fact that the proteins were not expressed was unexpected, but definitely something minor, a marginal piece of data that did not affect the experiment overall; it was a technical issue that would be resolved with a little more practice in transgenesis. However, for those who developed the theory of gene silencing, the absence of protein expression in the transgenic plant was an extremely important phenomenon that allows us to rediscover what biology tells us about gene functioning. The question, then, is why this phenomenon was interpreted in such disparate ways. What was influencing the conceptual framework of these scientists so that some saw it as an incredibly important issue while others saw it as largely insignificant?

One perspective within social studies of science holds that learning to distinguish what is meaningful from what is not involves a cultural acquisition that could be called tacit knowledge (Collins, 1992). From this perspective, the fact that certain people tend to evaluate things the same way can be explained as a matter of socialization: the transmission of certain codes that make us treat some things as significant and others not. On the other hand, there are other perspectives that seek to explain the conceptual frameworks used by scientists using the notion of 'interest' (Barnes, 1977; MacKenzie, 1978). Thus, 'cognitive interests' refers to the possible uses of a theory and the way this affects scientists' attitude to evaluating and developing the theory. The explanatory power of the notion of 'interest' lies in providing an interpretive framework for the origins and goals of scientific practices by linking them to a broader social context.

In the case we are considering here, there was a clear objective which some laboratories had been seeking since the early 1970s: to develop transgenic plants. Multinational chemical corporations poured millions of dollars into recombinant DNA research, seeking their place in the new biotechnology market. Applying transgenesis to agricultural crops was one of these companies' prime goals (Kloppenborg, Kenney, 1984). Just as the scientists involved in developing the first transgenic bacteria soon ended up in important positions in genetic engineering companies, the outlook for those who developed the first transgenic plant was no different. At a point when companies were investing huge sums of money in the hope of developing biotechnological products, since no one dominated this particular field yet, anyone who managed to develop a transgenic plant was guaranteed a coveted position in terms of commercial prospects.⁹ But it was also a position full of symbolic rewards: now that the first genetically modified bacteria had been obtained, there would be major recognition for whoever who could obtain a transgenic plant. The journal *Cell* crowned Chilton the winner by publishing her transgenic plant on their front cover, and she therefore became known as the leading figure in the field. This reputation was also built on other events such as the 1983 symposium in which Chilton, Schell and Horsch were seen as the pioneers of plant biotechnology.¹⁰

Bourdieu (1976) shows that scientists routinely turn scientific capital into economic capital. For those who developed the first transgenic plants, both forms of capital appeared simultaneously as potential rewards: the prestige of having made a scientific contribution so important that it opened up a new field (plant biotechnology) and the material rewards bestowed by companies (that sought to hire whoever knew how to use the techniques of this up-and-coming field).

These interests explain not only the creation of a research agenda (the attempt to obtain a transgenic plant) but also the way that experimental results were evaluated. I should stress that I do not attribute these interests to Chilton personally, but in a general way to the whole set of players linked to the topic. Clearly, it was her peers who recognized the value of the experiment by placing it on the front cover of one of the most prestigious journals in the field, and indeed no one – at that time – questioned the experiment. In other words: the scientific field saw the results as Chilton saw them, and that is precisely why she received recognition.

We should remember that although the goal was to obtain a transgenic plant, the challenge was to design a way of transferring foreign DNA into the nucleus of the plant cell and to obtain a plant with the transgene. Chilton had made a substantial contribution to this project by designing a vehicle for the transgene (characterizing the Ti plasmid), and now she took a crucial step by showing that it was possible to obtain a whole plant (not just a single cell) that had the transgene in its genome. Why was the fact that proteins were not expressed not considered relevant? On the one hand, because those who belonged to this niche field – the first prestigious plant biotechnology scientists and the companies awaiting the first transgenic plants – had some decades-old conceptual baggage, mostly from biology, telling them that the important thing was the gene, and that the protein was merely a byproduct. On the other hand, their goal was to obtain a transgenic plant, not to describe any particular phenomenon in plants; it was not proteins but the transgenic plant that would allow them to embark on a new phase of research and development. Clearly, the intended uses for the plant (as a way of gaining prestige in the scientific field and leading to a stage of productive development that many companies eagerly awaited) affected the reading of the experimental results: the important thing was that a plant with a transgene had been obtained, whereas what was happening to the proteins was a minor issue.

Why then did other researchers decide to focus on what was happening to the proteins in transgenic plants? First of all, it is important to remember the time lapse, the years that passed before this was seen as a research problem. Obtaining a transgenic plant became a goal in the 1970s and it was achieved in early 1983. However, the first studies of gene silencing were published in 1989 and developed in the years after that. There is a reason for this difference of a few years between one research topic and the other. Once the first transgenic plants were developed, the corresponding honors were bestowed. The first scientists to reach this goal received the symbolic and material prizes they were hoping for. The next round of researchers working on transgenic plants could not expect the same rewards. The scientific and material capital accrued to the scientists who pioneered the area. This does not mean that the emerging field of plant biotechnology was saturated; on the contrary, there was and still is plenty of room for research and development of transgenic plants. But the rewards those researchers could aspire to would not be the same. It was hard for someone

developing a transgenic plant to garner the same intellectual recognition and commercial interest as the early experiments. At that particular point in time, developing a transgenic plant established those who had achieved it as possessing a skill and expertise that no one had managed to acquire before.

What could the next set of plant biotechnology researchers do? Only build on the expertise that had already been celebrated. In other words, in Bourdieu's terms, they had to adopt a subversive strategy and go outside the accepted boundaries of the field, which involved some risks, but also the possibility of acquiring major recognition. Finding a completely new biological mechanism in something that was merely a small anomaly to the pioneers of plant transgenesis would allow them to open up a new space for recognition.

Does gene silencing present a different scientific paradigm compared to plant transgenesis? That depends on whom you ask. For those who develop transgenic plants, gene silencing was just one more tool. Knowing about these biological mechanisms allows researchers to stop the transgenes being blocked and thus to obtain desirable levels of protein expression. On the other hand, for those who sought to be leaders in gene silencing, there really were different paradigms involved. For them, the conceptual approach is different, the research topics are different, and the research environment is different. In order to achieve significant scientific capital, one has to show that what one is doing is completely new. Even though all these researchers (both those developing transgenic plants and those working on gene silencing) were trained in the same molecular biology techniques and had even, in some cases, worked in the same laboratories, the two disciplines were starting to look different. This divergence involved both conceptualizing gene silencing in terms of a 'scientific revolution' or a 'new paradigm' (Matzke, Matzke, 2004), and a long scientific tradition opposed to genetic engineering biotechnology. In this search to demonstrate a different tradition, with its own hidden history and origins, it was even claimed that a 1928 publication on tobacco plant infection was the first article on silencing, even though its authors did not know it at the time (Baulcombe, 2004). Thus, gene silencing was included in epigenetics, which had been seen as a different area when genetics was starting up, but had not taken off after that. Epigenetics studies changes in gene expression due to phenomena external to genes. When gene silencing was affirmed as a field in its own right, it was given its own institutional space, with research centers, publications and funding to study epigenetics. A new niche was emerging for these researchers, but only on condition that it was clearly distinguished from that of the biotechnologists. Matzke, a disciple of Chilton and a pioneer in gene silencing, defined the differences between the two fields thus:

While companies are struggling to find ways to avoid silencing, a small cadre of basic scientists has become fascinated by the phenomenon and is analyzing a variety of silencing systems. To this latter group, the phenomenon of silencing represents more than just an unwanted response to foreign genes; rather, it has opened a door that might lead to a deeper understanding of previously unsuspected ways that plants naturally use homologous or complementary nucleic acid sequences ... as a means to control excess production of mRNA or replication of RNA pathogens (Matzke, Matzke, 1995, p.679).

Besides providing a description of biotechnology and gene silencing, these comments show the researchers' need to differentiate their area. Clearly, whether or not silencing is seen as

anything more than an undesired response to exogenous genes depends on who uses it and what they use it for, since for those seeking to produce transgenic plants, silencing effectively blocks transgenes, and biotechnology companies use knowledge about gene silencing to avoid this happening, proof that silencing was also an important contribution for them. Thus, the differences or overlap between biotechnology and epigenetics depend on the point of view of the person evaluating them.

In the early 1980s, finding a transgenic plant meant recognition for something that many people had longed for but none had yet achieved. By the end of that decade, finding a biological mechanism hidden in transgenesis meant gaining recognition for a totally new discovery.

Final considerations

Once the first transgenic plants had been obtained, biotechnology companies refined the transformation techniques, focusing on the plants that were commercially viable for them. At the beginning, the difficulty of expressing proteins in transgenic plants was partially overcome by discarding plants with little expression and preserving the ones that presented more acceptable expression levels, in a simple selection and rejection mechanism. When gene silencing research provided an explanation for these difficulties, ways to avoid silencing and thus obtain good expression levels for the transgenes were designed. But in the early stages of plant transgenesis, there were a series of key factors that help explain some of the trends in the research and development of transgenic plants in subsequent decades.

Firstly, there was a significant difference between the uses for plant transgenesis, whether as a way to gain prestige and academic status or to obtain genetically modified plants that could then be put to productive use. In other words, there were different cognitive interests at work. When no one had yet managed to obtain a transgenic plant, both uses were presented as valuable to scientists. But when producing a transgenic plant was no longer a novelty, cognitive interests within the scientific field of transgenesis began to diverge. This was most extreme in the early stages of gene silencing research, when establishing an academic niche for investigating the anomalies of transgenesis was at loggerheads with the practical uses of genetic engineering to obtain transgenic plants.

On the other hand, the ability to transform a plant relegated issues such as the protein expression level or the particular variety of the plant to second place.

Lastly, there is a paradox that deserves to be highlighted. Before transgenic plants existed, the conviction that they were feasible meant that anomalies such as the lack of protein expression were not considered relevant. However, years later, when transgenic plants existed in laboratories and field trials (and were being marketed in numerous countries from 1996 on), the anomalies of these plants were frequently considered proof that they were not viable. Needless to say, the first transgenic plant did not meet all expectations: it could not express the transgene's proteins. In real terms, the plant was slightly faulty. But what made the cover of the journal *Cell* in 1983 was something else: it was really the idea of a transgenic plant that was being recognized. This was because the idea that a transgenic plant was entirely feasible and close had so much support that the anomalies were seen as something minor. Chilton

showed that the idea of a transgenic plant was now demonstrably real. By this I do not mean to suggest that nature plays no role in the production of scientific knowledge. The experiment on the first transgenic plant does not allow us to interpret transgenesis any way we like, but it does allow us to draw some conclusions. What I am trying to point out is that natural phenomena are not represented univocally and that conflicts start to emerge precisely because there are alternative systems of representation (Hacking, 1983, p.139). What made it possible to design and interpret the results of the experiments was the coming of age of a system of representation for biological phenomena (which found the determining unit in genes), together with the tools for managing those biological units (restriction enzymes, plasmids, *Agrobacterium*). But when other forms of representation began to be imposed, the meaning of transgenic plants changed. Chilton's experiment could be considered a success because it achieved a whole plant containing a transgene, or a failure because it did not express the transgene's proteins. If at the time it was seen as the former, it was because scientists' prime interest lay in obtaining the scientific capital linked to obtaining a transgenic plant. And when theories of gene silencing were developed a few years later, it was because the scientific capital in transgenesis had already been won by other researchers, so that scientists new to the field needed to differentiate themselves if they wished to gain significant recognition. And when in the 1990s alternative forms of representing transgenesis emerged in the research and development field, it was because transgenic plants – which had now become actual merchandise circulating on the international market – had begun to affect and involve a broader range of interests.

NOTES

¹ Besides chromosomal DNA, bacteria have a round DNA molecule called a plasmid. Plasmids usually contain genes that give the bacteria resistance to some antibiotics. Besides, bacteria can pass plasmids among themselves.

² The article states that “the general approach used here to insert the ADH I DNA into the genome of intact plants should afford access to such information” (Barton et al., 1983, p.1041).

³ ‘Genetic engineering’ is a broad term that refers to any form of DNA manipulation of an organism. The technology of recombinant DNA, in particular, involves introducing a DNA sequence from one species into the genome of another.

⁴ Lederberg received the Nobel Prize in 1958 for demonstrating that genetic information is exchanged between bacteria. Paul Berg was the first to obtain a hybrid DNA molecule (DNA from two different origins).

⁵ ‘Hybrid vigor’ allows the enhancement of certain qualities in crops. However, not all plants can be crossed to create hybrid varieties. Sweet corn can, with the result that seed companies put a great deal of innovation effort into generating new hybrid varieties of this particular crop. The key to this in business terms lies in the fact that hybrid vigor wanes in subsequent generations. This forces farmers to buy new hybrid seeds every year. Thus there is no need for a legal framework to make farmers dependent on companies' seed, since this happened anyway due to the characteristics of hybridized sweet corn.

⁶ These three groups announced their results at a symposium in Miami in January 1983. A few months later, in April of that year, a group from the University of Wisconsin announced that they had successfully introduced a bean gene into sunflower cells (Schlegel, 2007).

⁷ In 1941, Beadle and Tatum proposed the hypothesis that every gene had a corresponding enzyme, although it was not yet known how that link was regulated nor how genes worked. In 1958, Francis Crick formulated a fundamental basis for genetics by establishing the ‘central dogma of biology’, according to which genetic information is stored in DNA, which is then transcribed to RNA, which in turn translates into proteins (Crick, 1958). Over time, some objections or complements were formulated for this scheme when it was

discovered that not all DNA sequences are transcribed, and that proteins play a fundamental role in the flow of genetic information.

⁸ Collins believes that if there is disagreement about what counts as a competently-run experiment, a debate ensues about what the appropriate result of the experiment is. On the other hand, an anomalous result can give rise to a range of different interpretations. The debate only ends when the interpretation is tested. See Collins (1992) on this issue. This did not happen with Chilton's experiment, or at least not in that order. For a while, there was a uniform way of interpreting her experiment. Then, after a few years, that changed.

⁹ In 1983, almost as soon as the 'first transgenic plant' was announced, Mary-Dell Chilton was recruited by the company that came to be known as Syngenta. After a few years, Syngenta would become one of the few companies in the world to market its own transgenic seed. In recognition of Chilton's contributions, in 2002, the company named one of its facilities after her: the Agricultural Biotechnology Research Center, Syngenta's innovation facility in North Carolina, in the Research Triangle Park's technology hub.

¹⁰ Chilton also received numerous forms of institutional recognition, such as the Benjamin Franklin Medal, the John Scott Award, and membership of the US National Academy of Sciences and the American Academy of Arts and Sciences. The University of Washington, where Chilton developed the first transgenic plant, created an endowed professorship named the Mary-Dell Chilton Distinguished Professor.

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